

# ***Cerebral Blood Flow in the Non-Human Primate. An In Vivo Model and Drug Interventions***

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## Abstract

Cerebral blood flow dynamics is an essential component for preserving cerebral integrity. Cerebral blood flow abnormalities are often seen in patients with central nervous system pathologies such as epilepsy, migraine, Alzheimer's Disease, vascular dementia, stroke, and even HIV/AIDS. There is increasing clinical and experimental evidence implicating cerebral hypoperfusion during ageing. The determination of cerebral perfusion has therefore become an important objective in physiological, pathological, pharmacological, and clinical investigations. The knowledge of regional cerebral blood flow further provides useful diagnostic information and/or data for a better understanding of the complex clinical presentations in patients with neurological and psychiatric disorders. Several cerebrovasoactive drugs have found application in the clinical setting of cerebrovascular diseases such as migraine and dementia.

Due to the similarities between humans and non-human primates with respect to their brains, both structurally and behaviourally, numerous studies have been conducted and several non-human primate models have been developed for physiological, pathological, pharmacological, and clinical studies, amongst others in Parkinson's disease and diabetes. The relatively large size of the Cape baboon *Papio Ursinus* with a weight of 27-30 kg for a large male, makes this primate especially suitable for *in vivo* brain studies using radiotracers and Single Photon Emission Computed Tomography (SPECT).

The main aim of the current study was therefore to develop a suitable radiotracer ( $^{99m}\text{Tc}$ -hexamethylpropylene amine oxime (HMPAO) or  $^{99m}\text{Tc}$ -ethyl-cysteinatedimer (ECD) or  $^{123}\text{I}$ -iodoamphetamine (IMP)) for adapted *in vivo* cerebral blood flow measurements in a non-human primate (*Papio ursinus*) as an investigative model. The model was to be validated and applied in various drug studies for the evaluation of pharmacological interventions. The study design made use of split-dose methodology, whereby the radiopharmaceutical (radiotracer) was administered twice during each study. The first administration

was injected soon after the induction of the anaesthesia, and was followed by the first SPECT data acquisition. The second administration of the radioligand, a double dose of radioactivity with respect to the first radioligand injection, was done at a specific time during the study, which took into account the pharmacodynamics of the drug. A second SPECT data acquisition followed subsequently. The drugs that were included in the study were acetazolamide, a carbonic acid anhydrase inhibitor (often used in nuclear medicine to determine cerebral reserve); sumatriptan, a 5-HT (serotonin) agonist used for treatment of migraine; sodium valproate (an anti-epileptic drug); nimodipine, a calcium channel blocker and nitroglycerine, a vasodilator used for angina. Arterial blood pressures were recorded from a catheter in the femoral artery and heart rates were concurrently monitored.

The split-dose method was successfully applied to develop a non-human primate cerebral blood flow model under anaesthesia. The model showed differences in cerebral perfusion of the different anaesthesia regimes. These anaesthesia data sets were suitable as control/baseline results for drug intervention studies. Acetazolamide evaluation through the split-dose method in the baboon confirmed the sensitivity of the model by presenting comparable perfusion. This result compared to those already familiar prompted the model to be applied in pharmacological intervention studies. Subsequent results of these investigations showed increases in perfusion for single drug nimodipine treatment (25%). However, nimodipine attenuated the increases in perfusion when administered in combination with acetazolamide. Sumatriptan was able to decrease and normalise the increased perfusion after long duration anaesthesia. Decreased cerebral blood flow was observed for combinations of nimodipine with sodium valproate suggesting drug-drug interaction with important clinical implications. Similar decreases were found also for sumatriptan and nitroglycerine when administered in combination with nimodipine.

Studies with the various tracers ( $^{99m}\text{Tc}$ -HMPAO or  $^{99m}\text{Tc}$ -ECD or  $^{123}\text{I}$ -IMP) showed clear differences in the perfusion data, confirming variation in the biochemical performance of the tracers. These differences, if not taken into

consideration, caution for inappropriate clinical conclusions and subsequent erroneous therapeutic decisions. Improvement of radiotracer efficacy was subsequently attempted through application of the cyclodextrine complexation approach. Although cyclodextrine technology did not markedly improve the brain disposition of the  $^{99m}\text{Tc}$ -ECD, protection of the tracer against degradation was demonstrated. This study encouraged further exploration of this method for protection of the tracer against chemical and metabolic degradation.

The current study was aimed to develop and effectively apply a non-human primate model with nuclear medicine technology for cerebral blood flow determinations after pharmacological interventions. This was achieved through the split-dose method and dedicated computer programming, which yielded a successful model with the non-human primate under anaesthesia. The model was validated with the application of acetazolamide to confirm familiar cerebrovascular reserve results, indicating that the model is sensitive to CBF changes. The model was also effectively applied in several pharmacological intervention studies, whereby cerebropharmacodynamics of selected drugs were investigated and established.

This unique model of a non-human primate, *Papio ursinus* for cerebral blood flow determinations has served pharmacological research successfully during the past 12 years and could do so in the future, with scope to investigate new frontiers with improved technologies.

**KEY WORDS:** *Cerebral blood flow, SPECT, split-dose method, cerebrovascular diseases, non-human primate model, pharmacological interventions, anaesthesia, radiopharmaceuticals.*

## Opsomming

Serebrale bloedvloedinamika is noodsaaklik vir normale brein fisiologiese instandhouding. Serebrale bloedvloeiafwykings word algemeen by pasiënte met sentrale senuweestelselpatologie, soos byvoorbeeld epilepsie, migraine, Alzheimer se siekte, vaskulêre demensie en selfs MIV/VIGS, waargeneem. Daar is toenemend eksperimentele en kliniese bewyse dat serebrale hipoperfusie tydens veroudering voorkom. Die bepaling van serebrale bloedvloei is dus 'n baie belangrike oogmerk in fisiologiese, patologiese, farmakologiese en kliniese ondersoeke. Die kennis van regionale serebrale bloedvloei verskaf voorts nuttige diagnostiese inligting wat bydra tot beter insig in die komplekse kliniese beeld van pasiënte met neurologiese en psigiatriese siektetoestande. Verskeie serebrovaso-aktiewe geneesmiddels het gevolglik kliniese toepassing by die behandeling van serebrovaskulêre siektes, soos migraine en demensie, gevind.

Die strukturele en gedragsooreenkomste tussen die breine van die mens en die nie-menslike primate, het verskeie studies moontlik gemaak. Terselfdertyd het dit tot die ontwikkeling van fisiologiese, patologiese, farmakologiese en kliniese modelle vir onder andere Parkinson se siekte en diabetes, gelei. Die relatiewe grootte van die Kaapse bobbejaan, *Papio ursinus* met 'n massa van tussen 27 en 30 kg vir 'n volwasse manlike bobbejaan, maak hierdie primate veral geskik vir *in vivo* breinstudies met gebruikmaking van radiofarmaseutiese middels en Enkel Foton Emissie Berekeningstomografie (Single Photon Emission Computed Tomography (SPECT)).

Die primêre doelstelling van hierdie navorsing was om 'n *in vivo* nie-menslike primate (*Papio ursinus*) model, vir serebrale bloedvloei-metings tydens farmakologiese intervensies te ontwikkel. Vir hierdie doel is daar gebruik gemaak van gepaste radiofarmaseutiese middels ( $^{99m}\text{Tc}$ -heksametielpropileenamienoksiem (HMPAO) of  $^{99m}\text{Tc}$ -etielsisteïendimeer (ECD) of  $^{123}\text{I}$ -iodoamfetamien (IMP)). Die model se geldigheid is vervolgens bevestig en in verskeie geneesmiddelstudies aangewend. Die studie-ontwerp

het van die verdeelde dosis (split-dose) metode gebruik gemaak, waar die radiofarmaseutiese middel twee keer tydens die studie toegedien is. Die eerste toediening het kort na die induksie van narkose plaasgevind en is opgevolg deur SPECT-dataversameling. 'n Tweede toediening, dubbel die radioaktiewe dosis van die eerste toediening, het op 'n spesifieke tyd wat die farmakodinamika van die betrokke geneesmiddel in berekening bring, plaasgevind. Die tweede SPECT-dataversameling het daarna gevolg. Die geneesmiddels wat gebruik is, sluit in: asetasoolamied, 'n koolsuursuuranhidrase inhibeerder (gebruik in kerngeneeskunde om serebrale reserwe te bepaal); sumatriptan, 'n 5-HT (serotonien) agonis in die behandeling van migraine; natriumvalproaat ('n anti-epileptiese middel); nimodipien, 'n kalsiumkanaalblokkeerder en nitrogliserien, 'n vasodilator in die behandeling van angina. Die arteriële bloeddrukke is in die femorale arterie bepaal en die hartslag is vir ondersteunende inligting gemonitor.

Die verdeelde dosis metode is suksesvol aangewend in die ontwikkeling van die nie-menslike primate serebrale bloedvloeimodel onder narkose. Die model was in staat om tussen die verskille in serebrale perfusie, as gevolg van die onderskeie narkose middels, te onderskei. Die resultate van hierdie narkose studies was geskik en noodsaaklik om as kontrole/basislyn data te dien vir die opvolgstudies. Asetasoolamiedtoediening volgens die verdeelde dosis metode, het vergelykbare bloedvloeieffekte soos in mense waargeneem, tot gevolg gehad en dus die sensitiwiteit van die model in die bobbejaan bevestig. Hierdie resultaat het die aanwending van die model in verdere farmakologiese studies gesteun. Opvolgstudies met 'n enkele dosis nimodipien het 'n verhoging (25%) in bloedvloeimeebring. Nimodipien het egter in 'n geneesmiddelkombinasie studie die verhoogde asetasoolamied-geïnduseerde bloedvloeie onderdruk. Geneesmiddel-geneesmiddel interaksies met belangrike kliniese implikasies is na studies van nimodipien in kombinasie met valproaat, nitrogliserien en sumatriptan, aangedui.

Verskille tussen die onderskeie radiofarmaseutiese beeldmiddels ( $^{99m}\text{Tc}$ -HMPAO or  $^{99m}\text{Tc}$ -ECD or  $^{123}\text{I}$ -IMP) se opname in die brein, het variasies in die biochemiese hantering van hierdie beeldmiddels bevestig. Potensiëel

onvanpaste kliniese afleidings met moontlik verkeerde terapeutiese besluite kan gemaak word indien die verskille tussen hierdie radiofarmaseutiese middels nie in ag geneem word nie. In die lig van hierdie bevindinge is gepoog om met behulp van siklodekstrienkompleksering die effektiwiteit en breinopname van die middels te verbeter. Hierdie benadering het nie 'n beduidende verbeterde biobeskikbaarheid vir  $^{99m}\text{Tc}$ -ECD meegebring nie, maar wel duidelike beskerming teen sistemiese afbraak bewerkstellig wat in verdere studies positief aangewend kan word.

Die studies opgeneem in hierdie proefskrif, het die ontwikkeling en effektiewe aanwending van 'n nie-menslike primate model in kerngeneeskundige tegnologie vir die bepaling van serebrale bloedvloei tydens farmakologiese behandeling, ten doel gehad. Hierdie studie het, deur middel van die verdeelde dosis metode en spesifiek ontwerpte rekenaarprogrammatuur, daarin geslaag om 'n suksesvolle model vir die nie-menslike primate onder narkose daar te stel. Die model is met behulp van asetamoolamied en vergelykbare serebrale reserwe resultate in die mens bevestig. Die model is voorts effektief in verskeie serebrofarmakodinamiese studies van geselekteerde geneesmiddels aangewend.

Hierdie unieke nie-menslike primate, *Papio ursinus* model vir serebrale bloedvloei-bepalings is die afgelope twaalf jaar suksesvol in farmakologiese navorsing gebruik en kan ook in die toekoms aangewend word om nuwe horisonne met behulp van verbeterde tegnologie te ondersoek.

**SLEUTELWOORDE:** *Serebrale bloedvloei, SPECT, verdeelte dosis metode, serebrovaskulêre siektes, nie-menslike primate model, farmakologiese intervensies, narkose, radiofarmaseutiese middels.*

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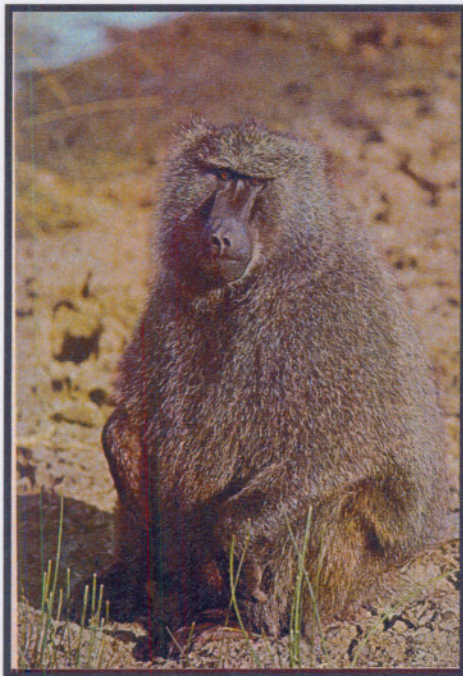
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*We know nothing at all. All our knowledge is but the knowledge of schoolchildren. The real nature of things we shall never know.*  
**Albert Einstein**

*Science is not a scared crow. Science is a horse. Don't worship it. Feed it.*  
**Aubrey Eben**

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# Introduction Aims and Study Design

## Chapter 1

### 1.1 Background

Cerebral blood flow dynamics play an integral part in the maintenance of cerebral integrity with respect to physiological and biochemical processes in the central nervous system. Abnormalities in cerebral perfusion are often seen in pathological disorders of the central nervous system (Lassen and Ingar *et al.*, 1963; Waldemar *et al.*, 1991, Catafau *et al.*, 1994a; Kuikka *et al.*, 1994; Menzel *et al.*, 1994; Catafau *et al.*, 1994b). The determination of cerebral perfusion has therefore become an important objective in physiological, pathological, pharmacological, and clinical investigations, in order to establish relevance and importance of cerebral blood flow in normal and patient subjects. The assessment of regional cerebral blood flow provides useful diagnostic information and/or data for a better understanding of the complex clinical presentations in patients with neurological and psychiatric disorders. Abnormalities in cerebral blood flow are found amongst others in patients with epilepsy, migraine, Alzheimer's disease, vascular dementia, stroke, and even HIV/AIDS (Holman *et al.*, 1992; Markus *et al.*, 1995; Harris *et al.*, 1994; Herring *et al.*, 2002; Lojkowska *et al.*, 2002; Sagiuchi *et al.*, 2001; Lindahl *et al.*, 2002; Lee *et al.*, 2003; Van Paesschen *et al.*, 2003). There is increasing clinical and experimental evidence implicating cerebral hypoperfusion during ageing, and cerebrovascular insufficiency is furthermore a vital component of the neuropathological progression of dementia (Kawamura *et al.*, 1991; Farkas *et al.*, 2002). Several cerebrovasoactive drugs have found application in the clinical setting, e.g. in migraine, cerebrovascular diseases such as dementia and in ageing (Dormehl *et al.*, 1999). Recently it was demonstrated that anti-HIV/AIDS treatment show beneficial effects on cerebral perfusion (Herring *et al.*, 2001), indicating that improvement in pathology is often accompanied by changes in cerebral perfusion.

The non-human primate has already been used for pharmacological and medical research for more than 100 years, since the documentation of studies in which monkeys was used by Robert Koch to investigate the pattern of infection of human trypanosomiasis. Non-human primates are morphologically and often functionally similar to man, and this is of particular relevance with respect to the brain, hands and uterus. The susceptibility of the non-human primate to pharmacological and toxicological effects often resembles that of man, and several examples exist where drugs are relatively toxic to other laboratory animals e.g. the rat and dog in comparison to primates and man. The similarities and importance of the non-human primate in pharmacological/toxicological studies have been shown in dependence studies, as early as in the 1960s (Wagenen, 1966; Wilson, 1966). Subsequent recent studies on alcohol and metamphetamine dependence further supported the unique similarities between the human and the non-human primate (Davidson *et al.*, 2001; Schmidt *et al.*, 2001). Similarities as discussed above have prompted the development of several non-human primate models for physiological, pathological, pharmacological, and clinical studies. The models have successfully been applied, amongst others, in the fields of Parkinson's disease and diabetes. The relatively large size of the Cape Baboon *Papio Ursinus* with a weight of 27-30 kg for a large male, makes this primate especially suitable for *in vivo* studies (Hill, 1970; Dormehl *et al.*, 1997). This aspect prompts the use of nuclear medical instrumentation and techniques for non-invasive *in vivo* investigations (Connolly, 1960; Dormehl *et al.*, 1997). The non-human primate is therefore almost ideally suited for *in vivo* cerebral blood flow investigations using Single Photon Emission Computed Tomographic (SPECT) imaging and brain perfusion radiopharmaceuticals and may thus be used with considerable confidence for neuroSPECT purposes.

Radiopharmaceutical technology has developed significantly, to play an integral part in the diagnosis, prognosis, explanation, as well as monitoring and treatment of several disease states (Dormehl *et al.*, 1997). Several radiopharmaceuticals are used in brain studies in nuclear medicine including amongst others, technetium labelled ligands (HMPAO and ECD) and iodine labelled compounds (amphetamine). SPECT technologies using such brain

imaging agents have been used for the assessment of cerebral perfusion in neurological and psychiatric diseases, proving that abnormalities are indeed present in several diseases.

## 1.2 Aim and Objectives

The main aim of this study was to develop a radiotracer based *in vivo* cerebral blood flow model in the non-human primate, specifically the baboon *Papio ursinus*, to confirm the model and to apply the model, in various drug studies for the evaluation of pharmacological interventions.

### 1.2.1 Specific Objectives

The specific objectives of this study present the course of the research program, its aim and its eventual fruition:

1. The development and understanding of a cerebral perfusion baboon model under anaesthesia, specifically induction with ketamine and maintenance with a barbiturate;
2. The determinations in the model of cerebral perfusion after various pharmacological interventions, such as studies using single drug or drug combinations in order to measure;
  - 2.1. The effects of the selected drugs on cerebral blood flow during anaesthesia in order to determine drug own effects and drug interaction effects that may be of clinical importance;
  - 2.2. The differences and similarities in drug effects using different brain perfusion agents in order to compare the performances of the different agents and thereby contribute to optimal choice of specific brain perfusion agents in conjunction with specific drugs;
  - 2.3. The effect of cyclodextrine technology on the disposition and therefore the performance of the brain imaging agents in order to improve the bioavailability of brain imaging agents, which has beneficial cost implications.

### 1.3 Study Design

The study design made use of radiotracers, such as technetium labelled radiopharmaceutical ligands, i.e.  $^{99m}\text{Tc}$  -hexamethylpropylene amine oxime (HMPAO) or  $^{99m}\text{Tc}$  -ethylcysteinatedimer (ECD) and iodine labelled  $^{123}\text{I}$  - iodoamphetamine, and SPECT with a dedicated gamma camera system in the non-human primate adult male, the baboon *Papio ursinus* under anaesthesia. The study design also followed the Split-Dose protocol where the radiopharmaceutical (radiotracer) was administered twice during each study (Wyper *et al.*, 1991; Pantano *et al.*, 1992). The first injection of the radioligand was soon after the induction of the anaesthesia, followed by the first SPECT data acquisition. The second administration of the radioligand, double the first radioactive dose, was injected at a specific time during the study, to accommodate the pharmacological properties of the drug, e.g. the response time of the drug. Subsequently the second SPECT data acquisition followed. The drugs included in this study as pharmacological interventions of CBF are amongst others acetazolamide, a carbonic anhydrase inhibitor (used to determine cerebral reserve); sumatriptan, a 5-HT agonist used for treatment of migraine; sodium valproate (an antiseizure drug); nimodipine, a calcium channel blocker; and nitroglycerine, a vasodilator used for angina (Hardman, Limbird and Gillman 2001). The arterial blood pressures were recorded from a catheter in the femoral artery and heart rates were monitored. Drug results were compared with controls, amongst themselves and with combinations.

These studies were performed after approval by the Ethics Committee of the University of Pretoria, according to the guidelines of the National Code for Animal Use in Research, Education and Testing of Drugs and Related Substances in South Africa. These guidelines are in line with international standards.

### 1.4 Presentation of thesis

The reader is reminded that this thesis is presented in the publication format, whereby the methods, results and discussions of this thesis were incorporated

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into the ten papers (Chapters 4 – 13). Attention is drawn to the fact that the ten papers have already been published internationally.

## References

- CATAFAU, A.M., PARELLADA, E., LOMENA, F.L., BERNADO, M., PAVIA, J., ROS, D., SETOAIN, J. & GONZALEZ-MONCLUS, E.** 1994a. Prefrontal and temporal blood flow in schizophrenia: resting and activation technetium-99m-SPECT patterns in young neuroleptic-naïve patients with acute disease. *Journal of nuclear medicine*, 35:935-941.
- CATAFAU, A.M., SOLA, M., LOMENA, F.L., GUEJAR, A., MIRO, J.M. & SETOAIN, J.** 1994b. Hyperfusion and early technetium-99m-HMPAO SPECT appearance of central nervous system toxoplasmosis. *Journal of nuclear medicine*, 35:1041-1043.
- CONNOLLY, C.I.** 1950. (*In: External morphology of the primate brain.* Springfield, USA: Charles C. Thomas Co. p. 3.)
- DAVIDSON, C., GOW, A.J., LEE, T.H. & ELLINWOOD, E.H.** 2001. Methamphetamine neurotoxicity: necrotic and apoptotic mechanisms and relevance to human abuse and treatment. *Brain research reviews*, 36(1):1-22.
- DORMEHL, I.C., JORDAAN, B. & OLIVER, D.W.** 1999. SPECT Cerebral blood flow improvement of elderly patients on long-term treatment with nootropic drugs. *Clinical nuclear medicine*, 24:29-34.
- DORMEHL, I.C., OLIVER, D.W. & HUGO, N.** 1997. The Primate Model in Neuropharmacology for Cerebral Blood Flow Determinations with HMPAO SPECT. (*In De Deyn, P.P., Drieckx, R.A., Alavi, A. & Pickut, B.A., eds. SPECT in Neurology and Psychiatry.* Amsterdam, the Netherlands: John Libbey and Company. Chapter 62. pp 521-535).
- FARKAS, E., DE WILDE, M.C., KILIAAN, A. & LUITEN, P.** 2002. Chronic cerebral hypoperfusion-related neuropathologic changes and compromised cognitive status: window of treatment. *Drugs of today (Barcelona, Spain : 1998)*, 38(5):365-376.

**HARDMAN, J.G., LIMBIRD, L.L. & GILMAN, A.G.** 2001. (In Goodman and Gilman's The Pharmacological Basis of Therapeutics. New York, USA: McGraw-Hill. pp 278; 325; 508; 763; 845; 853).

**HARRIS, G.J., PEARLSON, G.D., MCARTHUR, J.C., ZEGER, S. & LAFRANCE, N.D.** 1994. Altered cortical blood flow in HIV-seropositive individuals with and without dementia: a single photon emission computed tomography study. *AIDS*, 8(4):495-499.

**HERNING, R.I., BETTER, W.E., TATE, K. & CADET, J.L.** 2001. Antiviral medications improve cerebrovascular perfusion in HIV+ non-drug users and HIV+ cocaine abusers. *Annals of the New York Academy of Sciences*, 939:405-412.

**HERNING, R.I., TATE, K., BETTER, W. & CADET, J.L.** 2002. Cerebral blood flow pulsatility deficits in HIV+ poly substance abusers: differences associated with antiviral medications. *Drug and alcohol dependence*, 65(2):129-135.

**HILL, W.C.O.** 1970. (In Primates: comparative anatomy and taxonomy, volume 8: cynopithecinae. New York: Wiley Interscience. p. 5)

**HOLMAN, B.L., JOHNSON, K.A., GERADA, B., CARVALHO, P.A. & SATIN, A.** 1992. The scintigraphic appearance of Alzheimer's disease: a prospective study using technetium-99m-HMPAO SPECT. *Journal of nuclear medicine*, 33:181-185.

**KAWAMURA, J., MEYER, J.S., TERAYAMA, Y. & WEATHERS, S.** 1991. Cerebral hypoperfusion correlates with mild and parenchymal loss with severe multi-infarct dementia. *Journal of the neurological sciences*, 102(1):32-38.

**KUIKKA, I.T. & BERKOVIC, S.F.** 1994. Localization of epileptic foci by single-photon emission tomography with new radiotracers. *European Journal of nuclear medicine*, 21:1173-1174.

**LEE, Y.C., LIU, R.S., LIAO, Y.C., SUN, C.M., WANG, P.S., WANG, P.N. & LIU, HC.** 2003. Statistical parametric mapping of brain SPECT perfusion

abnormalities in patients with Alzheimer's disease. *European neurology*, 49(3):142-145.

**LINDAHL, A.J., ALLDER, S., JEFFERSON, D., ALLDER, S., MOODY, A. & MARTEL, A.** 2002. Prolonged hemiplegic migraine associated with unilateral hyperperfusion on perfusion weighted magnetic resonance imaging. *Journal of neurology, neurosurgery, and psychiatry*, 73(2):202-203.

**LOJKOWSKA, W., RYGLEWICZ, D., JEDRZEJCZAK, T., SIENKIEWICZ-JAROSZ, H., MINC, S., JAKUBOWSKA, T. & KOZLOWICZ-GUDZINSKA, I.** 2002. SPECT as a diagnostic test in the investigation of dementia. *Journal of the neurological sciences*, 203-204:215-219.

**MARKUS, H.S., LEES, A.J., LENNOX, G., MARSDEN, C.D. & COSTA, D.C.** 1995. Patterns of regional cerebral blood flow in corticobasal degeneration studied using HMPAO SPECT; comparison with Parkinson's disease and normal controls. *Movement Disorders*, 10(2):179-187.

**MENZEL, C., GRÜNWARD, F., PAVICS, L., HUFNAGEL, A., STAWOVY, B., REICHMANN, K., ELGER, C.E. & BIERSACK, H.J.** 1994. Brain single-photon emission topography using technetium-99m bicisate (ECD) in a case of complex partial seizure. *European journal of nuclear medicine*, 21:1243-1246.

**PANTANO, P., DI PIERO, V., RICCI, M., FIESCHI, C., BOZZAO, L. & LENZI, G.L.** 1992. Motor stimulation response by technetium-99m hexamethylpropylene amine oxime split-dose method and single photon emission tomography. *European journal of nuclear medicine*, 19(11):939-945.

**SAGIUCHI, T., ISHII, K., ASANO, Y., AOKI, Y., KIKUCHI, K., JINGUJI, K., YANAIHARA, H., WOODHAMS, R. & HAYAKAWA, K.** 2001. Interictal crossed cerebellar hyperperfusion on Tc-99m ECD SPECT. *Annals of nuclear medicine*, 15(4):369-372.

**SCHMIDT, K., NOLTE-ZENKER, B., PATZER, J., BAUER, M., SCHMIDT, LG. & HEINZ.** 2001. Psychopathological correlates of reduced dopamine receptor

sensitivity in depression, schizophrenia, and opiate and alcohol dependence. *Pharmacopsychiatry*, 34(2):66-72.

**VAN PAESSCHEN, W., DUPONT, P., VAN DRIEL, G., VAN BILLOEN, H. & MAES, A.** 2003. SPECT perfusion changes during complex partial seizures in patients with hippocampal sclerosis. *Brain*, 126(Pt 5):1103-1111.

**WAGENEN, G.** USA. 1966. Proceedings: Conference on nonhuman primate toxicology. U.S. Gov: Print Office. p 103.

**WALDEMAR, G., HASSELBALCH, S.G., ANDERSEN, A.R., DELECLUSE, F., PETERSEN, P., JOHNSEN, A. & PAULSON, O.B.** 1991. <sup>99m</sup>Tc-d, I-HMPAO and SPECT of the brain in normal ageing. *Journal of cerebral blood flow and metabolism*, 11:508-521.

**WILSON, J.G.** USA. 1966. Proceedings: Conference on nonhuman primate toxicology. U.S. Gov: Print Office, p. 114.

**WYPER, D.J., HUNTER, R., PATTERSON, J., GOODWIN, G. & McCULLOCH J.** 1991. A split-dose technique for measuring changes in cerebral blood flow patterns. *Journal of cerebral blood flow and metabolism*, (Suppl 2) 11:S449.

# **Literature Review of Primates in Medical Research and Cerebral Blood Flow Linked Diseases**

## **Chapter 2**

### **2.1 Introduction**

In view of the importance, extensive use and suitability of primates in medicinal research for human diseases and treatments, this chapter focuses on the primate models and their application in disease and treatment investigations with particular attention given to cerebral blood flow related conditions. Some of the work for this study has extensively been covered in publications presented in this thesis (Chapters 4-13), as well as in a chapter of a book (Dormehl, Oliver, & Hugo, 1997, See Appendix) and the reader is consequently referred to those. Therefore, the literature review in this chapter and the information in Chapter 3 on the methodology for the development of the experimental primate cerebral blood flow model mainly focus on those details generally not necessary in the publications as such.

### **2.2 Animal Models in Medical and Pharmacological Research**

Since the earliest of times, health and disease have been part of human and other life forms on earth. Improving health or attempts to cure diseases by man also played a significant role in human endeavours for not only survival but also improvement in the quality of life. To this day, as was the case with early humans, man exhibits unique observation skills and abilities to observe his environment, i.e. the human, animal, plant, marine, and sky kingdoms. It is also of importance to note that the creativity of humans lead to significant improvements not only for observation but also to plan, organise, design, manufacture, analyse, evaluate, predict, in the quest for a better life and sometimes also for a better environment. These investigations have indeed excelled and accelerated during the last century and in particular the last two

decades with the dawn of computer technologies. Man's desire to investigate its own species has always characterised his creativity as illustrated by early recordings. The pre-Middle Age period has seen serious investigations into human biology and anatomy from initial dissections on human corpses and cadavers, until these fell into disfavour and were subsequently banned. The research emphasis in medical sciences thereafter moved towards the use of animals, increasing particularly during the last century with the advent of modern medicine.

Blood transfusions in animals were a focus in medical science research, with Richard Lower successfully performing transfusions on dogs in the middle of the 17<sup>th</sup> century. France experienced the first blood transfusion on human when Jean Babtiste Denis transfused sheep's blood into two boys. Both boys died subsequent to the transfusion. It was nearly two centuries later that the importance and impact of early animal research was confirmed and established with the sound scientific research of the Nobel Prize winner, Karl Landsteiner (1868-1943) on blood transfusions and as early as the 1800s, the brain functions were localised by David Ferrier through stimulation of the cortex in animal brain.

The twentieth century was indeed characterised by an array of animal models being specifically developed to investigate the pharmacology of the enormous arsenal of novel structures yielded by synthetic medicinal chemistry. These animal models in general proved to be useful in drug efficacy and drug safety (toxicology) evaluations. The earlier models were primarily based on normal animals and therefore lacked the feature to supply accurate information on pharmacological efficacy and safety for the pathology in question. Presently more and more animal models are being developed which would simulate pathologies in man, in order to give deeper understanding of possible molecular mechanisms of such disorders. Such models of pathology further assisted drug development efforts to yield efficacy and toxicology data. Biotechnology, molecular biology and genomics have found increasing application in animal models, due to the increasing evidence of the genetic basis of diseases, resulting in a surge of gene knockout and transgenic cell

cultures, cell lines and animals. Although animal models on species other than primates have contributed significantly to the field of pharmacology and medicine as described above, the dissimilarities between such species and humans necessitate the development of models from non-human primate species.

The renewed interest in developing primate models, strongly support the notion that non-human primate models are of essential importance in investigations of physiology, pathology and in subsequent drug development for the treatment of human diseases. Recent developments in non-human primate models are described in paragraph 2.2.1 below for selected medicinal fields and in particular those relating to central nervous system disorders that are often accompanied by changes in cerebral perfusion dynamics.

## **2.2.1 Motivation for Non-human Primates in Pharmacological Research**

### ***2.2.1.1 Pharmacology and physiology in non-human primates in relation to humans***

Of all the animals, non-human primates are morphologically and functionally the most similar to man. The similarities between human and non-human primates are reflected in various areas that include superiority of humans and non-human primates as presented by amongst others their hands, brains and uteri. The kidney structures of humans and non-human primates are further both of multi-papillar nature and microscopic examination in rhesus monkeys with nephritis showed identical results to those in humans. Pharmacological and toxicological drug response studies on non-human primates reveal susceptibility that resembles those in humans, which may not always be seen in other species (Davidson and co-workers, 2001; Heinz, 2002). The importance of the non-human primate studies in the field of dependence has been well documented (Davidson and co-workers, 2001; Heinz, 2002).

In the middle 1980s Harwerth and Smith (1985) proposed that the rhesus monkey could serve as a model for normal human vision. Siegel and Anderson

(1988) showed that the rhesus monkey is able to detect 3-D structures from motion in the same way as human subjects. They showed furthermore that information is integrated both spatially and temporally for this higher visual function, being dependent in both species on specific parameters for vision.

The previous century has in particular seen numerous non-human primate pathological models being developed, with Robert Koch using monkeys in a human trypanosomiasis model in the early 20<sup>th</sup> century. The last three decades have experienced a surge in new models for a wide variety of human diseases and drug interventions ranging from HIV/AIDS, to central nervous system disorders. Selected fields and models are presented below to illustrate the importance of non-human primates in medical and pharmacological research.

### **2.2.1.2 Dependence**

Pathogenesis of schizophrenia as well as drug and alcohol dependence is associated with the dysfunction of central dopaminergic neurotransmission in primates (Heinz, 1999; Schmidt *et al.*, 2001). Reinforced drug consumption is often accompanied by different drugs of abuse that stimulate dopamine release in the ventral striatum, whereas the increased subcortical dopamine release has also been associated with the pathogenesis of positive symptoms in schizophrenia. It was concluded that the role of dopaminergic neurotransmission in reward anticipation and its dysfunction in different neuropsychiatric diseases is similar in both humans and non-human primates (Heinz, 2002). In a recent paper, Davidson and co-workers (2001) reviewed the neurotoxicity after metamphetamine administration and suggested that animal models using experimental one-day metamphetamine dosing regimen protocols also present the acute overdose pathologies seen in humans. The authors came to the conclusion that both the human and non-human primate studies show long-term losses in DA (dopamine) and 5-HT (serotonin) function after chronic metamphetamine dosing.

### **2.2.1.3 Parkinson's Disease**

Parkinson's disease is a neurodegenerative disorder that is accompanied by a loss of nigral dopaminergic neurons leading to a depletion of dopamine in the striatum. Several models using mice and non-human primate species are available.

Kolata (1983) and Burns and co-workers (1983) reported monkey models for Parkinson's disease. A mouse model was developed soon afterwards by Heikkila *et al.* (1984a) and Heikkila and Sonsalla (1987). Strategies aimed at neuroprotection, *via* inhibition of the monoamine oxidase enzyme based on the information from these studies, followed soon (Heikkila *et al.*, 1984b). Currently there are two neurotoxic compounds available for inducing animal models of Parkinson's disease, i.e. 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). A third compound rotenone, which was recently introduced, still needs further investigations.

Feger and co-workers (2002) recently used MPTP with progressive unilateral nigrostriatal lesioning on green african monkeys (*Cercopithecus aethiops sabaesus*) and provided a reliable model of the idiopathic disease of Parkinson's Disease. It is concluded from the studies that MPTP administration results in biochemical and histological changes in mice and monkeys similar to those reported in humans and that similar effects are also seen in Parkinson's Disease in humans.

### **2.2.1.4 Stroke**

Kemper and co-workers (1999) investigated microinfarctions due to hypertension in cynomolgus monkeys in a cerebrovascular disease primate model. The autopsies of the animals revealed microinfarcts that did not correspond to usually described lesions in the human brain in hypertension or in other animal models of hypertensive cerebrovascular disease. The authors proposed that the infarcts represent the early changes in hypertensive neuropathology (Kemper *et al.*, 1999).

Kito and co-workers (2001) reported a model aimed at addressing thromboembolic stroke in cynomolgus monkeys without intracranial surgery. This was achieved by delivering an autologous blood clot to the middle cerebral artery (MCA) via the internal carotid artery in cynomolgus monkeys. Several parameters and effects were observed post the intervention, such as cerebral blood flow using positron emission tomography (PET) and hypotonia of the contralateral upper and lower limbs, as well as mild to severe incoordination. The authors proposed that this model is applicable to thromboembolic stroke in humans in neurologic dysfunction and histopathologic brain damage.

Earlier this year, a baboon model for acute stroke was presented that can be used for the evaluation of potential neuroprotective therapeutic agents for stroke prior to clinical trials (Mack *et al.*, 2003). The model involves an occlusion in the middle cerebral artery followed by reperfusion, and the animals were evaluated for behaviour and motor function. The model may serve to increase the functional predictive value of pre-clinical stroke studies in the future.

### ***2.2.1.5 Learning, Memory, Ageing and Dementia***

Several studies aiming at developing non-human primate models to investigate the physiological and biochemical processes, receptor systems involved and factors detrimentally affecting the cognitive function, have contributed to our understanding of, amongst others, mechanisms of cognitive processes. The pathological disorder of dementia is very complex since the pathophysiological development of dementias can be of different origins. Dementia that are currently especially in focus for investigation are Alzheimer's Disease (AD) dementia, vascular dementia and HIV/AIDS dementia. Early, accurate detection of these degenerative neurological disorders is essential for therapies that are to be designed to slow disease progression.

The studies of Taffe and co-workers (2002) on rhesus monkeys support the use of this non-human model. Their study involved a touch-screen mediated visuo-spatial paired-associates learning (vsPAL) task performance test that is sensitive to Alzheimer's disease in humans, using two pharmacological models of cognitive impairment.

Similarities and differences between the incidence distribution, progression and chemical composition of amyloid-beta deposits in the brains of young (5 years) and old (25-30 years) rhesus monkeys were investigated by Sani *et al.*, (2003). Their findings display many similarities to those observed in the elderly human and Alzheimer's disease, suggesting that the old rhesus monkey shows promise as a model for research of the pathological effects of amyloid-beta in the primate brain.

The squirrel monkey (*Saimiri sciureus*) proved to be a valuable model to study pathogenesis of Alzheimer's disease as well as the mechanisms involved in cerebrovascular beta amyloid angiopathy (Walker *et al.*, 1990; Walker, 1997; Bading *et al.*, 2002). This monkey model has for more than a decade significantly contributed to improving our understanding and knowledge of this important neurodegenerative disorder affecting millions of people over the world.

#### **2.2.1.6 HIV/AIDS infection and AIDS dementia**

Non-human primates have been the focus of several studies in the field of HIV/AIDS in view of the proportions the epidemic has taken. These studies and models aim at investigating the pathogenesis of the infection, the progress of pathologies that may develop subsequent to the infection and the development of therapeutic strategies, i.e. drug investigation and vaccine development (Rausch *et al.*, 1999).

Rhesus monkeys have proved to be an indispensable animal model of experimental HIV infection, to assess the pathogenesis, to validate therapeutic approaches and to develop vaccination strategies against HIV infection in humans (Sauerman, 2001).

Tracey *et al.* (1997) showed in macaques by using proton magnetic resonance spectroscopy in brain extracts of control and SIV-infected animals, that neuronal damage occur upon infection with simian immunodeficiency virus (SIV), a retrovirus closely related to the human immunodeficiency virus (HIV). Their results support the applicability of the SIV macaque model to study the mechanisms for the neuropathogenesis of HIV. Mankowski and co-workers (2002) recently developed another model using macaques. These monkeys develop clinical manifestations similar to those of HIV-infected humans upon infection with simian immunodeficiency virus (SIV). This model proved to be ideal in tracking the viral, cellular, and immunological changes in the brain during acute and asymptomatic infection and during the progression of the infection and development of SIV encephalitis. A recent paper addressed AIDS dementia focusing on potential mechanisms implicated in the pathophysiology of the AIDS dementia complex (Galicia *et al.*, 2002). This study involved the HIVgp120-induced behavioural changes as observed in animals and the correlation with clinical symptoms observed in the HIV infected subjects. Macaques have also been used to study the effects of HIV in brain disease by applying an accelerated, consistent simian immunodeficiency virus macaque infection model (Mankowski, 2002, Zink & Clements, 2002). This model is unique in that it presents a high incidence of CNS disease, giving an opportunity to correlate host and viral events in the CNS during acute infection, with the later development of CNS disease. It was found in the SIV-macaque model of AIDS from studies on the type and severity of cellular damage in the brain, that neuronal injury occurs soon after infection. The study further suggested a link between the early response of the central nervous system and later development of dementia (Gonzales *et al.*, 2002). Furthermore, protection was recently observed in an immunization study of macaques with live simian human immunodeficiency virus vaccines (Kumar *et al.*, 2002).

Sopper and co-workers (2002) concluded that the SIV-infection of macaques currently provides the best animal model to study pathogenesis, therapy and prevention of HIV-infection based on the high concordance with the human system.

### **2.2.1.7 Diabetes Mellitus**

Insulin-dependent diabetes mellitus was studied in juvenile cynomolgus monkeys (1999). Diabetes mellitus is accompanied by diabetic nephropathy and Birrell et al. (2002) investigated primary prevention thereof with aminoguanidine in a nonhuman primate model of Type 1 diabetes over a period of 4 years using male baboons (*Papio hamadryas*). They found that the structural and functional changes in the kidney of the baboons were similar to those seen in the early stages of diabetes in human and that aminoguanidine showed beneficial effects. Based on their findings, it can be concluded that the non-human primate model can effectively be applied in investigating the pathophysiology of diabetes and potential drug therapies.

Bodkin *et al.* (2003) studied the mortality and morbidity of more than 100 rhesus monkeys over a period of approximately 25 years in an intensive dietary controlled investigation. The results indicated that controlled dietary intake is an essential factor contributing to an increase in the average age at death in these primates when compared with the non-controlled dietary intake animals. This increase in the average age at death was associated with the prevention of hyperinsulinemia and the mitigation of age-related disease (Bodkin *et al.*, 2003).

### **2.2.1.8 Summary**

There is abundant evidence in the literature, supporting the believe that non-human primates due to their similarities to humans, are essential in the investigation of human physiology, pathology, and pharmacology aiming at development of effective and safe drug therapies. The examples indicated above, although limited in number, strongly support this concept of similarity in behavioural and non-behavioural, healthy and non-healthy situations between unique human and non-human primates. A large number of non-human primate models exists that is currently effectively being used to contribute to the well-being of man.

## 2.3 Cerebral blood flow in neurological and neuropsychiatric disorders

Cerebral perfusion measurements, utilizing SPECT and PET technologies in several neurological and psychiatric diseases, as well as with ageing, demonstrated abnormalities in cerebral blood flow. These abnormalities have been assessed using various brain imaging SPECT agents, i.e. Xenon-133 ( $\text{Xe}^{133}$ ), technetium labelled ligands (HMPAO and ECD) and  $^{123}\text{I}$ -iodoamphetamine (Veall and Mallett, 1965; Warkentin *et al.*, 1990; Iidaka *et al.*, 1997; Noda *et al.*, 2002). Diagnostic information can thus be deduced from regional cerebral blood flow (rCBF) determinations, and/or provide scientists and clinicians with data for a better understanding of unique and complex clinical indications observed from patients with such disorders. Selected disorders and their cerebral perfusion abnormalities are described below.

### 2.3.1 Cerebral Blood Flow Dynamics and Cerebrovascular Reserve

Maintaining regional cerebral blood flow (rCBF) within physiological limits is regulated by an autoregulation dynamics through cerebrovascular reserve (CVR) (Buell *et al.*, 1997). The rCBF and regional cerebral metabolic rate for glucose (rCMRGlc) are associated with functional activity of the cerebral cells. The cerebrovascular reserve is regulated by the need to supply in the metabolic demands of cerebral cells. CVR is thus under the control of a multitude of factors, i.e. carbon dioxide ( $\text{CO}_2$ ) tension, perfusion pressure, cerebral blood volume, rheological parameters (e.g. hematocrit), metabolic demands, transneuronal depression, ageing and pathology. With respect to ageing, a study of Bentourkia *et al.* (2000) showed a decline in rCBF- and rCMRGlc-values as a function of age and that CBF and CMRGlc is interdependent.

SPECT is only one of several methods (PET, transcranial Doppler sonography, Xenon enhanced X-ray computed tomography, magnetic resonance imaging) to investigate cerebral perfusion and CVR (Yamashita *et al.* 1992; Buell *et al.*, 1997; Rempp *et al.*, 1994).

CBF-value during the resting state is 50 ml/min/100g and can be increased by up to 60% depending on the particular stimuli applied (Buell *et al.*, 1997). Stimuli that have been used in SPECT studies to determine cerebrovascular reserve include acetazolamide (the carbonic anhydrase inhibitor with a diuretic action used for the treatment of glaucoma) and carbondioxide (CO<sub>2</sub>). These agents significantly increase cerebral perfusion under various conditions, also depending on the different radiotracers for perfusion measurements (Buell *et al.*, 1997). In particular, acetazolamide has been used to measure CVR in the presence of pathology. Determination of CVR is a powerful tool for functional brain imaging in the clinical setting where comparison of CVR from normal control volunteers to CVR from patients, could have predictive value for therapeutic outcomes. Abnormalities in CVR and vasoreactivity are often seen in pathologies (Stoppe *et al.*, 1995; De Reuck *et al.*, 1999; Pavics *et al.*, 1999; Casadevall *et al.*, 2002). The recent report of Casadevall *et al.* (2002) suggests that the loss of cerebrovascular reserve may be implicated as one of the possible mechanisms for the development of vascular dementia. CVR determinations are indicated to assess vasoreactivity, haemodynamic balance and rheologic penumbra in patients. Furthermore, CVR may be employed amongst others for detection of cerebrovascular disease in the early stages, evaluation of subarachnoid haemorrhage and differentiation of Alzheimer's dementia from vascular dementia.

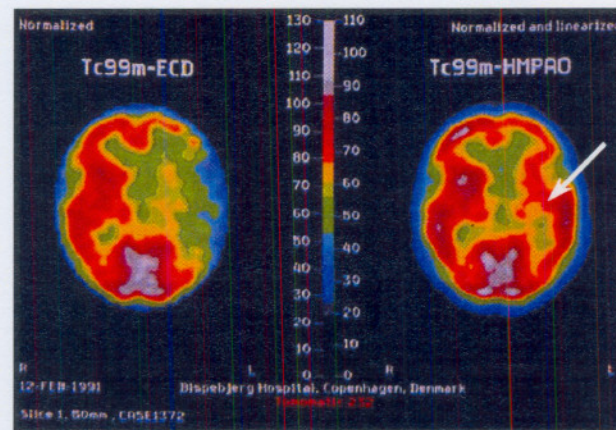
### **2.3.1.1 Cerebrovascular Ischaemic Diseases (Stroke)**

A reduced CBF has already been observed in the 1980s for patients with chronic cerebrovascular disease (Buell *et al.*, 1984). Inadequate perfusion of tissues and organs, such as heart and brain with subsequent development of ischaemia, triggers a cascade of events in the body. The pathophysiology of ischaemic diseases is very complex and the processes as well as endogenous substances involved during the development of ischaemic disorders have not yet been fully elucidated. The ischaemia itself may cause damage to the affected tissues, and the development of shock where there is a widespread reduction of tissue perfusion may result in death. Events during ischaemia involve a significant decrease in the ATP concentration to as low as 30% of the

baseline value. Several ATPases as well as ATP-dependent flipase are important in maintaining ATP concentrations for cell viability (Post *et al.*, 1988; Musters *et al.*, 1993; Musters *et al.*, 1995; Post *et al.*, 1995). Various intracellular changes occur, amongst others increased sodium, calcium and hydrogen ion concentrations that influence the electrical and pH balance of the cell leading to irreversible damage (Piper *et al.*, 1998). These events eventually lead to mitochondrial damage while it was also recently suggested that an increased sodium influx occurring with ischaemia, may induce irreversible damage to mitochondria, with decreased ATP-generation activity which may lead to the development of ischaemia injury in perfused rat hearts (Iwai *et al.*, 2002). Allopurinol was shown to reduce cell death in ischaemia/reperfusion studies (Post *et al.*, 1998). Melatonin is another substance that protected against ischaemic-reperfusion myocardial damage (Lagneux *et al.*, 2000; Salie *et al.*, 2001; Szarszoi *et al.*, 2001).

Understanding the pathophysiology of stroke is not only important in the pre-stroke phase, but also during and in the post-stroke phases. Detailed knowledge thereof will aid in the management of the post-stroke recovery phase. Nuclear medicine imaging has played an important role in stroke management (Mountz *et al.*, 2003; Baron, 2002). Physiological imaging of middle-cerebral artery (MCA) stroke allows the nuclear clinician to differentiate between the pathological tissue subtypes: i) irreversibly damaged ("core"); ii) severely hypoperfused ("penumbra"), which represents the main target for therapy; iii) mildly hypoperfused ("oligaemia"), not at risk of infarction unless secondary complications arise; and iv) reperfused/hyperperfused. The importance of  $^{99m}\text{Tc}$ -ECD SPECT was demonstrated in ischaemic stroke as it successfully localized the infarct core and peri-infarct ischaemia in all lesions in both the acute and the sub-acute stages (Kim *et al.*, 2002).  $^{123}\text{I}$ -isopropylidoamphetamine ( $^{123}\text{I}$ -IMP) SPECT revealed in patients with unilateral cerebral infarction, hypoperfusion ipsilaterally in the thalamus and contralaterally in the cerebellum, while the vasoreactivity is maintained in both the thalamus and the cerebellum (Sakashita *et al.*, 1993). The difference in the tracer uptake properties of these brain imaging agents are clearly evident.

Sperling and Lassen (1997) investigated the application of SPECT in ischaemic stroke with respect to the CBF measurements and the perfusion tracers being used, as well as the pathophysiology. The study addressed the challenges in the choice of tracer in view of the different mechanisms involved in the retention of the tracer in the brain and the subsequent interpretation of the SPECT results. Figure 2-1 illustrates the difference in the HMPAO and ECD SPECT of the same patient showing a masking of the infarct by HMPAO (arrow) but not by ECD.



**Figure 2-1** HMPAO (right) masking the infarct and ECD SPECT (left) showing the infarct with reduced perfusion (taken from Sperling and Lassen 1997)

Another disorder presenting amongst others with stroke-like episodes, MELAS, (mitochondrial myopathy, encephalopathy, lactic acidosis) showed increases in CBF in two siblings during a post stroke-like episode with HMPAO SPECT (Peng *et al.*, 2000). It was suggested that the particular mechanism in the pathology of the disease that leads to a decrease in pH due to the increase in lactic acid, may be implicated in the increased CBF.

### **2.3.1.2 Migraine**

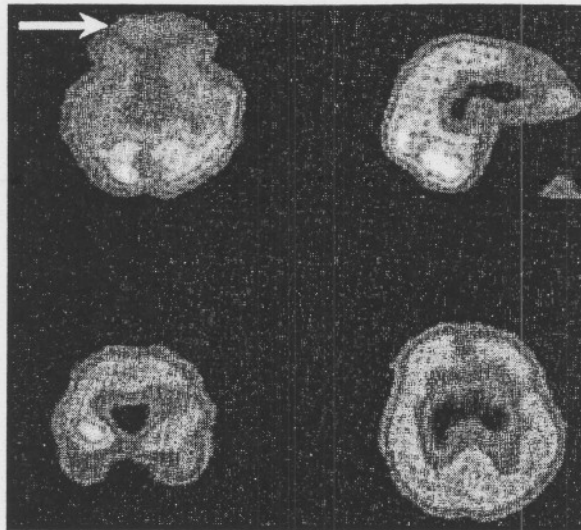
Several observations have been made on abnormalities in cerebral perfusion in patients suffering from migraine (Cutrer *et al.*, 2001; Barbour *et al.*, 2001; Masuzaki *et al.*, 2001; Calandre *et al.*, 2002). Magnetic Resonance (MR) angiography and perfusion MR imaging on a young girl suffering from migraine, revealed unilateral dilation of branches of both the middle and posterior cerebral arteries and hyperperfusion of the ipsilateral hemisphere (Masuzaki *et*

cerebral arteries and hyperperfusion of the ipsilateral hemisphere (Masuzaki *et al.*, 2001). Both magnetic resonance imaging and SPECT on a patient with hemiplegic migraine during pregnancy showed hyperperfusion of the implicated hemisphere (Barbour *et al.*, 2001). The authors concluded that hemiplegia was caused and sustained by hyperperfusion and the case revealed supportive evidence to a vasodilatory mechanism and hyperperfusion in such hemiplegic migraine headaches. In a study designed to investigate the effect of treatment of migraine attacks with a non steroidal anti-inflammatory drug, piroxicam (complexed with beta-cyclodextrin), it was found that abnormalities in cerebral perfusion for the parietal or parieto-occipital areas were reversed with piroxicam treatment (Trucco *et al.*, 1994).

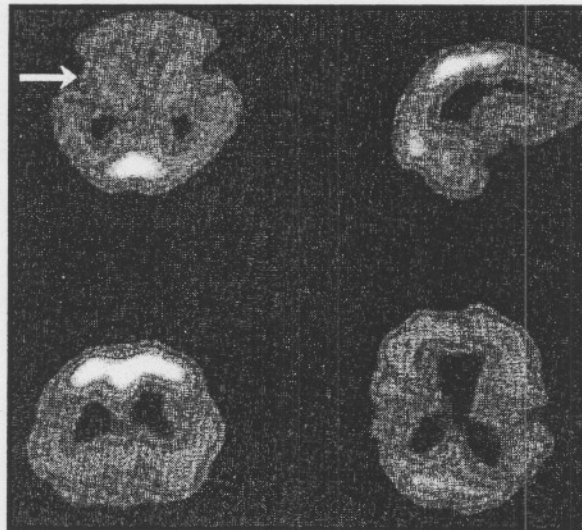
### **2.3.1.3 Vascular Dementia and Alzheimer's disease**

In all the different types of dementias, cerebrovascular disease is one of the major causes resulting in vascular dementia (VD) (Starkstein & Vázquez, 1997, Korszyn, 2002). VD is also more prevalent in men and the prevalence increases with age.

Several studies showed abnormalities in cerebral perfusion in patients suffering from Alzheimer's disease (Egger *et al.*, 1992; Holman *et al.*, 1992; Wyper *et al.*, 1993; Soinenen *et al.*, 1995; Sabbagh *et al.*, 1997; Starkstein & Vázquez, 1997; Arbizu *et al.*, 1999). Reduction in CBF in Alzheimer's disease (AD) was found in the early phase of AD. Hypoperfusion has specifically been established in the temporo-parietal cortex. The reduction in neuronal activity due to neuronal cell loss through atrophy and neurodegeneration of synapses could account for decreased metabolic demand thus contributing to local hypoperfusion (Holman *et al.*, 1992; Starkstein & Vázquez, 1997 See Figures 2-2 and 2-3). HMPAO SPECT by Sabbagh *et al.*, (1997) revealed decreased perfusion of left frontal, parietal, and temporal regions relative to right regions in patients with Alzheimer's disease.



**Figure 2-2** SPECT scan with vascular dementia showing hypoperfusion in the frontal region (taken from Starkstein and Vázquez 1997)



**Figure 2-3** SPECT scans with Alzheimer's disease showing hypoperfusion in the temporo-parietal regions (taken from Starkstein and Vázquez 1997)

Due to the heterogeneity of Alzheimer's disease (AD) identifying AD subtypes is of importance to devise specific treatment strategies. One aim is the design of studies to examine regional cerebral blood flow (rCBF) in AD subtypes. Two subgroups of AD patients were identified, using a cluster analysis study design, which includes performance on memory, language, visuospatial, praxic, and executive functions, as well as rCBF measurement by HMPAO SPECT (Soininen *et al.*, 1995). The authors also showed several significant correlations between decreased rCBF and impairment of memory and other cognitive functions. In a late 1990s study Arbizu and co-workers

(1999) reported data from a SPECT imaging and neuropsychology study, which indicated that controls can be distinguished from AD patients with mild and moderate grades of dementia, presenting strong correlation from the early stages of AD. In a HMPAO SPECT study by Nobili *et al.*, (2002) the effects of acetylcholine esterase inhibitor treatment (donepezil and rivastigmine) were investigated in AD patients with respect to regional cerebral blood flow. The results showed that the CBF was decreased in the cortical regions in those patients who presented with further cognitive deterioration, as was also observed in the untreated patients. However, for those patients with stabilised cognitive performance during therapy, cerebral perfusion was maintained at the pretreatment level (Nobili *et al.*, 2002). The maintenance or improvement of CBF therefore plays an important role in stabilizing AD patients.

#### **2.3.1.4 HIV/AIDS**

Investigation of the pathologies surrounding AIDS receives significant attention due to the world-wide impact the epidemic has on many millions of people. Several studies on cerebral perfusion and HIV infection and its secondary pathologies have been conducted (Tran Dinh *et al.*, 1990; Holman *et al.*, 1992; Catafau *et al.*, 1994b; Rubbert *et al.*, 1994; Szeto *et al.*, 1998; Ernst *et al.*, 2002). More than a decade ago Tran Dinh and co-workers (1990) observed abnormalities in CBF in a  $^{133}\text{Xenon}$  SPECT study in HIV patients. This study also included psychometric tests, magnetic resonance imaging (MRI) and electroencephalography (EEG). The frontal areas were affected in almost all the patients by decreased perfusion, indicating changes due to metabolic or vascular lesions. The study also demonstrated defects in CBF at a very early stage of the HIV infection (Tran Dinh *et al.*, 1990). Perfusion defects were noted in HMPAO SPECT in HIV patients with the presence of anticardiolipin antibodies (Rubbert *et al.*, 1994). This finding may implicate autoimmune mechanisms in CBF abnormalities in HIV patients. AIDS dementia complex (ADC) is another complication of the infection with HIV. SPECT investigation has shown hypoperfusion in the frontal and parietal lobes while the degree of the hypoperfusion significantly correlates with the severity of the dementia complex (Maini *et al.*, 1990; Szeto *et al.*, 1998). Szeto and co-

workers (1998) reported in a pilot study that the CBF abnormalities in HIV patients were reversed upon treatment with atevirdine, suggesting that CBF monitoring of HIV patients assists in assessing therapeutic responses in HIV/AIDS dementia complex.

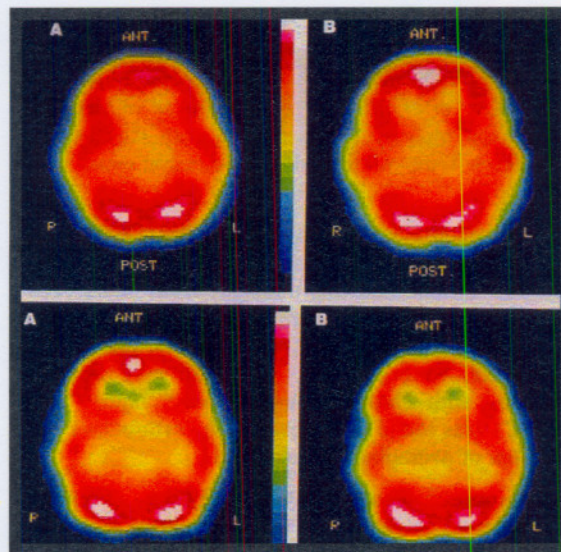
It is well established that HIV infection may also be a result of intravenous drug use and that abnormal cerebral perfusion has resulted from cocaine use. Comparing the HMPAO SPECT of cocaine users to patients with HIV/AIDS dementia complex, showed that the perfusion patterns for these groups are indistinguishable (Holman *et al.*, 1992). The authors however cautioned against specific diagnosis for these patient cases.

One of the common opportunistic CNS infections that AIDS patients encounter is cerebral toxoplasmosis. The infection is neuropathologically characterized by inflammatory infiltrates with polymorphonuclear leucocytes, lymphocytes, and histocytes. The later stages of the infection include the development of cysts and areas of necrosis. AIDS patients present with abnormal HMPAO SPECT CBF patterns which may indicate early stage development of cerebral toxoplasmosis lesions (Catafau *et al.*, 1994b).

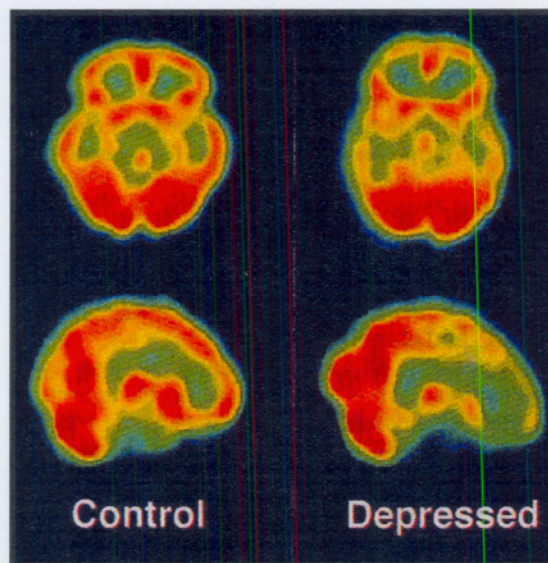
### **2.3.1.5 Mood Disorders (Schizophrenia, Depression and Obsessive Compulsive Disorder)**

During an Wisconsin Card Sorting (WCST) activation test on a schizophrenic patient and representative control, the HMPAO SPECT showed (See Figure 2-4) an increase in prefrontal rCBF in the control patient during WCST, while for the patient hyperperfusion occurred prefrontally at rest and a decrease in rCBF during the test in the same region (Catafau *et al.*, 1994a).

Although the psychiatric rating scales correlated poorly with HMPAO SPECT CBF studies, the investigations detected hypoperfusion in the paralimbic region of depressed patients (Mayberg *et al.*, 1994) (See figure 2-5).



**Figure 2-4** HMPAO SPECT of schizophrenic patient (bottom) and control (top) during resting (left – A) and Wisconsin Card Sorting Test (right – B) (taken from Catafau et al. 1994a)



**Figure 2-5** Transaxial (upper) and sagittal (lower) views of HMPAO SPECT images from a depressed patient and an age-, sex- matched non depressed control (taken from Mayberg et al. 1994)

Iidaka *et al.* (1997) investigated a group of patients with bipolar disorder and major depression for CBF patterns using SPECT. Their findings revealed decreased mean and regional CBF with respect to the controls and although the correlation on the severity of the symptoms were not sufficient for clinical use, this non-invasive technique showed good inter- and intra-observer reliability. They further reported significant increases in CBF upon drug

treatment. D'haenen (1997) reviewed numerous SPECT studies in mood disorders. Although the results in these studies are non-consistent, there are less discrepancies in the unipolar depression, showing regional hypoperfusion particular in the frontal and temporal regions. A very recent study in South Africa on the effects of electroconvulsive therapy on rCBF demonstrated an improvement in frontal and temporal hypoperfusion (Vangu *et al.*, 2003) in those patients who responded to the treatment. It appears from this study that hypoperfusion may serve as a marker in depression. In the field of treatment-resistant depression, the research of Hornig and co-workers (1997) suggested that functional abnormalities in limbic circuitry, as noted from the increased perfusion in hippocampus-amygdala, may contribute to the pathophysiology of treatment-resistant depression (Hornig *et al.*, 1997).

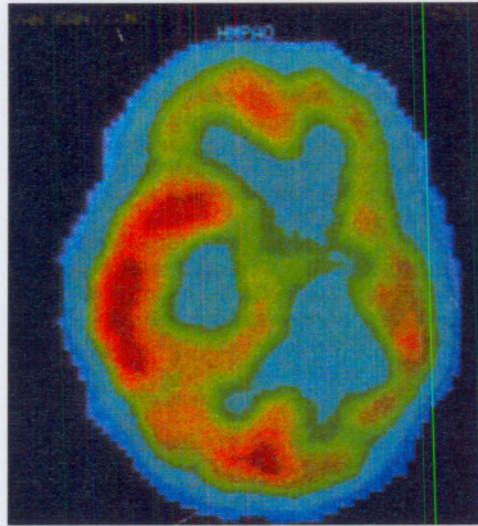
Obsessive compulsive disorder (OCD) has a complex neurological basis and is characterized by the presence of either obsessions and/or compulsions. Chierichetti and co-workers (1997) reported reduced frontal CBF in OCD patients and concluded that perfusion SPECT assisted in the pathophysiological understanding of OCD. Turkish patients with OCD were recently compared with controls in a HMPAO SPECT study on cerebral perfusion properties (Alptekin *et al.*, 2001). The study revealed that the right thalamus, left frontotemporal cortex and bilateral orbitofrontal cortex showed significant hyperperfusion in patients with OCD.

### **2.3.1.6 Epilepsy**

The functional status of the brain is assessed with SPECT and PET techniques, and these approaches provide useful information in epileptic patients under consideration for surgery (Sadzot *et al.*, 1997). In contrast to the anatomical precision of Magnetic Resonance Imaging (MRI), SPECT and PET are able to reveal changes in localized function due to the epileptic focus.

Epilepsy is characterized by different states, i.e. ictal, interictal and post-ictal, with major differences being observed in functional images of epileptic patients (Kuikka & Berkovic, 1994; Menzel *et al.*, 1994). It was found that in most patients experiencing unilateral temporal lobe seizures, hyperperfusion occur in

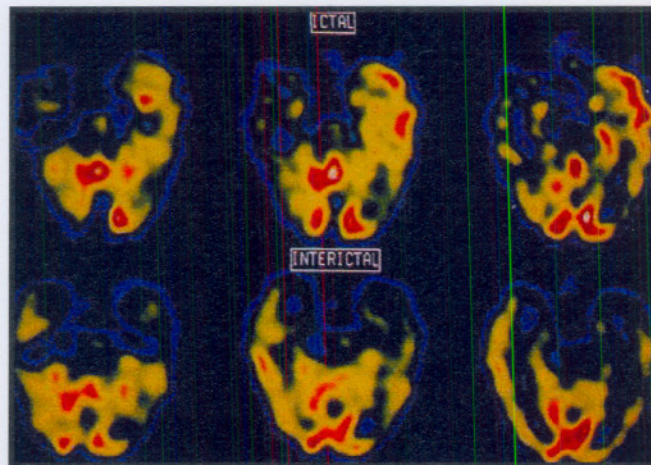
the particular temporal lobe during the ictal state of epileptic patients (Rowe *et al.* 1997). Perfusion studies in epileptic children have shown hyperperfusion during the ictal SPECT, showing temporal hyperperfusion in a patient with complex partial seizures in the area of the epileptogenic focus (Denays, 1997) (See Figure 2-6).



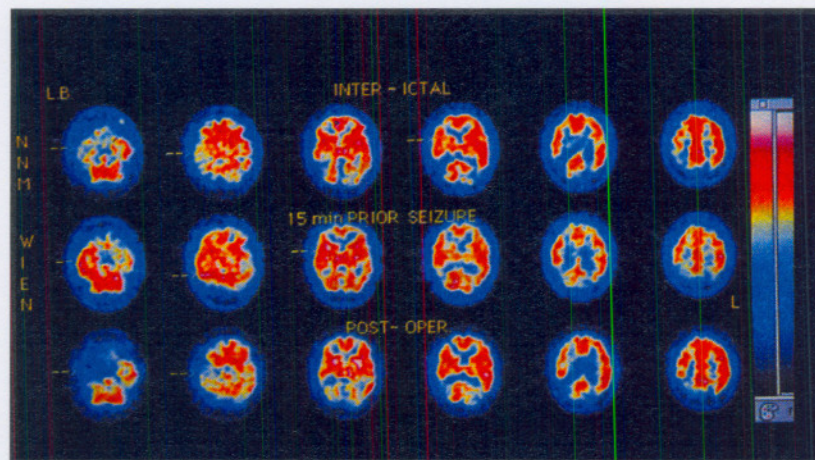
**Figure 2-6** Ictal SPECT showing right temporal hyperperfusion in a child with complex partial seizures (taken from Fenays 1997)

In Figure 2-7 the ictal and interictal patterns are shown of a patient with suspected left temporal sclerosis and suffering from bilateral foci (Grünwald *et al.*, 1994). The pattern clearly shows decreased perfusion interictally in the left temporal lobe with marked hyperperfusion during the epileptic seizure in the left temporolateral and temporopolar regions.

Pedreka and co-workers (1997) reviewed their findings of video - EEG and SPECT studies on epileptic patients who were candidates for surgery. Figure 2-8 shows the differences in perfusion interictally, 15 min prior to seizure and postoperatively. An increase in perfusion is clearly visible for the 15 min prior to the seizure set (middle set) when compared with the interictal findings (top set). A significant decrease in CBF was observed postoperatively (bottom set) in comparison with the pre-seizure data, in closer resemblance to the patterns of the interictal state.



**Figure 2-7** Ictal and interictal perfusion patterns in a 38 year- old woman with bilateral EEG foci and suspected left temporal sclerosis (taken from Grünwald et al. 1994)



**Figure 2-8** HMPAO SPECT interictally (top), 15 min before seizure onset (middle) and post operatively (bottom). Arrows indicate increases (middle) and decreases (bottom) in perfusion (taken from Pedreka et al. 1997).

It is clear that SPECT and other techniques have provided important information on marked changes in CBF in epileptic patients for diagnosis, localization, the different stages of the disease, pathogenesis and subsequent management of the epilepsy.

### **2.3.1.7 Summary on cerebral blood flow diseases**

From the information on the variety of diseases indicated above, as well as other data from the literature, it follows that cerebral blood flow is an important

indicator of several brain pathologies and other disorders such as addiction (Miller *et al.*, 1992; Jacobs *et al.*, 1994; Ichise *et al.*, 1994; Matsuda *et al.*, 1988; Lill *et al.*, 1994; Ichise *et al.*, 1993). SPECT has furthermore diagnostic potential and along with pharmacological interventions, is an important tool in the assessment of brain disorders and their therapies. Several studies, some of which have been referred to above, unequivocally show that reversal of abnormal CBF occurs upon drug treatment. The importance of these described abnormalities in cerebral perfusion for such diseases also suggest that models for cerebral blood flow determinations during pharmacological intervention would indeed contribute to our understanding of drug therapies, drug interactions and mechanisms, and treatment progress.

### **2.3.1.8 Chapter Summary**

Animals and their pharmacological and toxicological models have significantly contributed to medical sciences to the benefit of *Homo sapiens* in assuring the health of mankind. Non-human primates have played their part in the past and will do so in the future to the advancement of human health mainly because of our marked similarities. The large number of diseases that show cerebral perfusion abnormalities necessitates the development of animal models, and in particular non-human primate models, for the measurement of cerebral perfusion. In the following chapters, the thesis focuses on the development of such a non-human primate cerebral blood flow model and its application.

## References

- ALPTEKIN, K., DEGIRMENCI, B., KIVIRCIK, B., DURAK, H., YEMEZ, B., DEREBEK, E. & TUNCA, Z. 2001. Tc-99m HMPAO brain perfusion SPECT in drug-free obsessive-compulsive patients without depression. *Psychiatry research*, 107(1):51-56.
- ARBIZU, J., LARUMBE, R., GAMEZ, C., MARTI, J., MARTINEZ-LAGE, J. & RICHTER, J. 1999. Correlations between brain SPECT and neuropsychology assessments in mild and moderate stages of Alzheimer's disease. *Revista espanola de medicina nuclear*, 18(4):252-260.
- BADING, J.R., YAMADA, S., MACKIC, J.B., KIRKMAN, L., MILLER, C., CALERO, M., GHISO, J., FRANGIONE, B. & ZLOKOVIC, BV. 2002. Brain clearance of Alzheimer's amyloid-beta40 in the squirrel monkey: a SPECT study in a primate model of cerebral amyloid angiopathy. *Journal of drug targeting*, 10(4):359-368.
- BARBOUR, P.J., CASTALDO, J.E. & SHOEMAKER, E.I. 2001. Hemiplegic migraine during pregnancy: unusual magnetic resonance appearance with SPECT scan correlation. *Headache*, 41(3):310-316.
- BARON, J.C. 2002. Stroke: imaging and differential diagnosis. *Journal of neural transmission*, Suppl(63):19-36.
- BENTOURKIA, M., BOL, A., IVANOIU, A., LABAR, D., SIBOMANA, M., COPPENS, A., MICHEL, C., COSNARD, G. & DE VOLDER, A.G. 2000. Comparison of regional cerebral blood flow and glucose metabolism in the normal brain: effect of aging. *Journal of the neurological sciences*, 181(1-2):19-28.
- BIRRELL, A.M., HEFFERNAN, S.J., KIRWAN, P., MCLENNAN, S., GILLIN, A.G. & YUE, D.K. 2002. The effects of aminoguanidine on renal changes in a baboon model of Type 1 diabetes. *Journal of diabetes and its complications*, 16(4):301-309.

**BODKIN, N.L., ALEXANDER, T.M., ORTMEYER, H.K., JOHNSON, E. & HANSEN, B.C.** 2003. Mortality and morbidity in laboratory-maintained Rhesus monkeys and effects of long-term dietary restriction. *The journals of gerontology. Series A, Biological sciences and medical sciences*, 58(3):212-219.

**BUELL, U., MOSER, E.A., SCHMIEDEK, P., LEINSINGER, G., KREISIG, T., KIRSCH, C.M. & EINHAUPL, K.** 1984. Dynamic SPECT with <sup>133</sup>Xe: regional cerebral blood flow in patients with unilateral cerebrovascular disease. *Journal of nuclear medicine*, 25(4):441-446.

**BUELL, U., STRECKENBERGER, M., SABRI, O. & CREMERIUS, U.** 1997. The Cerebral Reserve (CVR): Access by SPECT. (In De Deyn, P., Dierckx, R.A., Alavi, A. & Pickut, BA, eds. *A Textbook of SPECT in Neurology and Psychiatry*. London: John Libbey. p307-315).

**BURNS, R.S., CHIUEH, C.C., MARKEY, S.P., EBERT, M.H., JACOBOWITZ, D.M. & KOPIN, I.J.** 1983. A primate model of parkinsonism: selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Proceedings of the National Academy of Sciences of the United States of America*, 80(14):4546-4550.

**CALANDRE, E.P., BEMBIBRE, J., ARNEDO, M.L. & BECERRA, D.** 2002. Cognitive disturbances and regional cerebral blood flow abnormalities in migraine patients: their relationship with the clinical manifestations of the illness. *Cephalalgia : an international journal of headache*, 22(4):291-302.

**CASADEVALL, T., SANTOS, S., RAZOLA, P., TEJERO, C., LARRODE, P., INIGUES, C., PASCUAL, L.F., MOSTACERO, E., BANZO, J. & MORALES, F.** 2002. Acetazolamide Vasoreactivity in Vascular Dementia. (In Korczyn, A.D., ed. *Proceedings of the 2<sup>nd</sup> International Congress on Vascular Dementia*, Bologna: Monduzzi Editore. p89-93).

**CATAFAU, A.M., PARELLADA, E., LOMENA, F.L., BERNADO, M., PAVIA, J., ROS, D., SETOAIN, J. & GONZALEZ-MONCLUS, E.** 1994a. Prefrontal and

temporal blood flow in schizophrenia: resting and activation technetium-99m-SPECT patterns in young neuroleptic-naïve patients with acute disease. *Journal of nuclear medicine*, 35:935-941.

**CATAFAU, A.M., SOLA, M., LOMENA, F.L., GUEJAR, A., MIRO, J.M. & SETOAIN, J.** 1994b. Hyperfusion and early technetium-99m-HMPAO SPECT appearance of central nervous system toxoplasmosis. *Journal of nuclear medicine*, 35:1041-1043.

**CHIERICCHETTI, F., BIANCHIN, G.L., VALLERINI, A., SIATTA, B., RUBELLO, D. & FERLIN, G.** 1997. Reduced Frontal Cerebral Blood Flow in Drug-Free Obsessive-Compulsive Disorder (OCD): Preliminary Data of a SPECT Study. (In De Deyn, P., Dierckx, R.A., Alavi, A. & Pickut, BA, eds., A Textbook of SPECT in Neurology and Psychiatry. London: John Libbey. p219-224).

**CONNOLLY, C.I.** 1950. (In 'External morphology of the primate brain', Springfield, USA: Charles C. Thomas Co. p. 3).

**CUTRER, F.M., O'DONNELL, A. & SANCHEZ DEL RIO, M.** 2000. Functional neuroimaging: enhanced understanding of migraine pathophysiology. *Neurology*, 55(9 Suppl 2):S36-45.

**D'HAENEN, H.A.H.** 1997. SPECT Imaging in Primary Mood Disorders. (In De Deyn, P., Dierckx, R.A., Alavi, A. & Pickut, BA, eds., A Textbook of SPECT in Neurology and Psychiatry. London: John Libbey. p219-224).

**DAVIDSON, C., GOW, A.J., LEE, T.H. & ELLINWOOD, E.H.** 2001. Methamphetamine neurotoxicity: necrotic and apoptotic mechanisms and relevance to human abuse and treatment. *Brain research reviews*, 36(1):1-22.

**DE REUCK, J., DECOO, D., HASENBROEKX, M.C., LAMONT, B., SANTENS, P., GOETHALS, P., STRIJCKMANS, K. & LAMAHIEU, S.** 1999. Acetazolamide reactivity in vascular dementia: a positron emission tomographic study. *European neurology*, 41:31-36.

**DORMEHL, I.C., OLIVER, D.W. & HUGO, N.** 1997. The Primate Model in Neuropharmacology for Cerebral Blood Flow Determinations with HMPAO SPECT. (In De Deyn, P., Dierckx, R.A., Alavi, A. & Pickut, BA, eds., A Textbook of SPECT in Neurology and Psychiatry. Amsterdam, The Netherlands: John Libbey and Company. Chapter 62, pp 521-535).

**EAGGER, S., SYED, G.M., BURNS, A., BARRETT, J.J. & LEVY, R.** 1992. Morphologic (CT) and functional (rCBF-SPECT) correlates in Alzheimer's disease. *Nuclear medicine communications*, 13(9):644-647.

**ERNST, T., ITTI, E., ITTI, L. & CHANG, L.** 2000. Changes in cerebral metabolism are detected prior to perfusion changes in early HIV-CMC: A coregistered (1)H MRS and SPECT study. *Journal of magnetic resonance imaging : JMRI*, 12(6):859-865.

**FEGER, J., PESSIGLIORE, M., FRANCOIS, C., TREMBLAY, L. & HIRSCH.** 2002. Experimental models of Parkinson's disease. *Annales pharmaceutiques francaises*, 60(1):3-21.

**GALICIA, O., SANCHEZ-ALAVEZ, M., MENDEZ DIAZ, M., NAVARRO, L. & PROSPERO-GARCIA.** 2002. HIV glycoprotein 120: possible etiological agent of AIDS-associated dementia. *Revista de investigacion clinica*, 54(5):437-452.

**GONZALEZ, R.G., CHENG, L.L., WESTMORELAND, S.V., SAKAIE, K.E., BECERRA, L.R., LEE, P.L., MASLIAH, E. & LACKNER, A.A.** 2000. Early brain injury in the SIV-macaque model of AIDS. *AIDS*, 14(18):2841-2849.

**GRÜNWARD, F., MENZEL, C., PAVICS, L., BAUER, J., HUFNAGEL, A., REICHMANN, K., SAKOWSKI, R., ELGER, C.E. & BIRSACK, HJ.** 1994. Ictal and interictal brain SPECT imaging in epilepsy using technetium-99m-ECD. *Nuklear Medizin/Nuclear Medicine* 35(12):1896-1901.

**HARWERTH, R.S. & SMITH, E.L.** 1985. Rhesus monkey as a model for normal vision of humans. *American journal of optometry and physiological optics*, 62(9):633-641.

**HEIKKILA, R.E., HESS, A. & DUVOISIN, R.C.** 1984a. Dopaminergic neurotoxicity of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine in mice. *Science*, 224(4656):1451-1453.

**HEIKKILA, R.E., MANZINO, L., CABBAT, F.S. & DUVOISIN, R.C.** 1984b. Protection against the dopaminergic neurotoxicity of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine by monoamine oxidase inhibitors. *Nature*, 311(5985):467-469.

**HEIKKILA, R.E. & SONSALLA, P.K.** 1987. The use of the MPTP-treated mouse as an animal model of Parkinsonism. *The Canadian journal of neurological sciences*, 14(3 Suppl):436-440.

**HEINZ, A.** 1999. Psychopathological correlates of dopaminergic dysfunction in alcoholic and schizophrenic patients. *Der Nervenarzt*, 70(5):399-407.

**HEINZ, A.** 2002. Dopaminergic dysfunction in alcoholism and schizophrenia--psychopathological and behavioral correlates. *Psychiatry*, 17(1):9-16

**HILL, W.C.O.** (*In Primates: comparative anatomy and taxonomy, volume 8: cynopithecinae.* New York: Wiley Interscience. 1970, p. 5).

**HOLMAN, B.L., GARADA, B., JOHNSON, K.A., MENDELSON, J., HALLGRING, E., TEOH, S.K., WORTH, J. & NAVIA, B.** 1992. A comparison of brain perfusion SPECT in cocaine abuse and AIDS dementia complex. *Journal of nuclear medicine*, 33(7):1312-1315.

**HOLMAN, B.L., JOHNSON, K.A., GERADA, B., CARVALHO, P.A. & SATIN, A.** 1992. The scintigraphic appearance of Alzheimer's disease: a prospective study using technetium-99m-HMPAO SPECT. *Journal of nuclear medicine*, 33:181-185.

**HORNIG, M., MOZLEY, P.D. & AMSTERDAM, J.D.** 1997. HMPAO SPECT brain imaging in treatment-resistant depression. *Progress in neuro-psychopharmacology & biological psychiatry*, 21(7):1097-1114.

**ICHISE, M., TOYAMA, H. & FORNAZZARI, L. BALLINGER, JR. & KIRSH, JC.** 1993. Iodine-123-IBZM dopamine D2 receptor and technetium-99m-HMPAO brain perfusion SPECT in the evaluation of patients with and subjects at risk for Huntington's disease. *Journal of nuclear medicine* 34:1274.

**ICHISE, M., CHUNG, D-G. & WANG, P. WORTSMAN,G., GRAY, BF. & FRANKS, W.** 1994. Technetium-99m- HMPAO SPECT, CT and MRI in the evaluation of patients with chronic traumatic brain injury: a correlation with neuropsychological performance. *Journal of nuclear medicine*, 35:217.

**IIDAKA, T., NAKAJIMA, T., SUZUKI, Y., OKAZAKI, A., MAEHARA, T., SHIRAIISHI, H. & MATSUDA, H.** 1997. Cerebral blood Flow Measured by <sup>99m</sup>Tc-HMPAO SPECT in Mood Disorder. (In De Deyn, P., Dierckx, R.A., Alavi, A. & Pickut, BA, eds., A Textbook of SPECT in Neurology and Psychiatry. London: John Libbey. p219-224).

**IWAI, T., TANONAKA, K., INOUE, R., KASAHARA, S., MOTEGI, K., NAGAYA, S. & TAKEO, S.** 2002. Sodium accumulation during ischemia induces mitochondrial damage in perfused rat hearts. *Cardiovascular research*, 55(1):141-149.

**JACOBS, A., PUT, E., INGELS, M. & BASSUYT.** 1994. An evaluation of technetium-99m-HMPAO SPECT in mild and moderate traumatic brain injury. *Journal of nuclear medicine*, 35(6):942-947.

**KEMPER, T., MOSS, M.B., HOLLANDER, W. & PRUSTY, S.** 1999. Microinfarction as a result of hypertension in a primate model of cerebrovascular disease. *Acta neuropathologica*, 98(3):295-303.

**KIM, H.S., KIM, D.I., LEE, J.D., JEONG, E.K., CHUNG, T.S., YOON, P.H., LEE, S.K., KIM, E.J., YOON, Y.K., SUH, B.C. & LEE, BI.** 2002. Significance of 99mTc-ECD SPECT in acute and subacute ischemic stroke: comparison with MR images including diffusion and perfusion weighted images. *Yonsei medical journal*, 43(2):211-222.

**KITO, G., NISHIMURA, A., SUSUMU, T., NAGATA, R., KUGE, Y., YOKOTA, C. & MINEMATSU, K.** 2001. Experimental thromboembolic stroke in cynomolgus monkey. *Journal of neuroscience methods*, 105(1):45-53.

**KOLATA, G.** 1983. Monkey model of Parkinson's disease. *Science*, 220(4598):705.

**KORCZYN, A.D.** 2002. (In: Proceedings of the 2<sup>nd</sup> International Congress on Vascular Dementia, Ed. Korczyn AD, Monduzzi Editore, Bologna, preamble).

**KUIKKA, I.T., BERKOVIC, S.F.** 1994. Localization of epileptic foci by single-photon emission tomography with new radiotracers. *Journal of nuclear medicine*, 21: 1173-1174.

**KUMAR, A., MUKHERJEE, S., SHEN, J., BUCH, S., LI, Z., ADANY, I., LIU, Z., ZHUGE, W., PIATAK, J.R., LIFSON, J., MCCLURE, H. & NARAYAN, O.** 2002. Immunization of macaques with live simian human immunodeficiency virus (SHIV) vaccines conferred protection against AIDS induced by homologous and heterologous SHIVs and simian immunodeficiency virus. *Virology*, 301(2):189-205.

**LAGNEUX, C., JOYEUX, M., DEMENGE, P., RIBUOT, C. & GODIN-RIBUOT, D.** 2000. Protective effects of melatonin against ischemia-reperfusion injury in the isolated rat heart. *Life sciences*, 66(6):503-509.

**LE GROS CLARK, W.E.** 1960. (In The antecedents of man: an introduction to the evolution of the primates. Chicago, USA: Quadrangle Books. p. 262).

**LILL, D.W., MOUNTZ, J.M. & DARJI, J.T.** 1994. Technetium-99m- HMPAO brain SPECT evaluation of neurotoxicity due to manganese toxicity. *Journal of nuclear medicine*, 35(5):863-866.

**MACK, W.J., KING, R.G., HOH, D.J., COON, A.L., DUCRUET, A.F., HUANG, J., MOCCO, J., WINFREE, C.J., D'AMBROSIO, A.L., NAIR, M.N., SCIACCA, R.R. & CONNOLLY, E.S.** 2003. An improved functional neurological examination for use in nonhuman primate studies of focal reperfused cerebral ischemia. *Neurological research*, 25(3):280-284.

**MAINI, C.L., PIGORINI, F., PAU, F.M., VOLPINI, V., GALGANI, S., ROSCI, M.A. & NARCISO, P.** 1990. Cortical cerebral blood flow in HIV-1-related dementia complex. *Nuclear medicine communications*, 11(9):639-648.

**MANKOWSKI, J.L., CLEMENTS, J.E. & ZINK, M.C.** 2002. Searching for clues: tracking the pathogenesis of human immunodeficiency virus central nervous system disease by use of an accelerated, consistent simian immunodeficiency virus macaque model. *The Journal of infectious diseases*, 186 (Suppl 2):S199-208.

**MASUZAKI, M., UTSUNOMIYA, H., YASUMOTO, S. & MITSUDOME, A.** 2001. A case of hemiplegic migraine in childhood: transient unilateral hyperperfusion revealed by perfusion MR imaging and MR angiography. *American journal of neuroradiology*, (9):1795-1797.

**MATSUDA, H., HIGASHI, S., ASLI, I.N., EFTEKHARI, M., ESMAILI, J., SEKI, H., TSUJI, S., OBA, H., IMAI, K. & TERADA, H.** 1988. Evaluation of cerebral collateral circulation by technetium 99m-HMPAO brain SPECT during Matas test: report of three cases. *Journal of nuclear medicine*, 29(10):1724-1729.

**MAYBERG, H.S., LEWIS, P.J., REGENOLD, W., WAGNER, H.N. JR.** 1994. Paralimbic hypoperfusion in unipolar depression. *Journal of nuclear medicine*, 6:929-934.

**MENZEL, C., GRÜNWARD, F., PAVICS, L., HUFNAGEL, A., STAWOVY, B., REICHMANN, K., ELGER, C.E. & BIERSACK, H.J.** 1994. Brain single-photon emission tomography using technetium-99m bicisate (ECD) in a case of complex partial seizure. *Journal of nuclear medicine*, 2 1: 1243-1246.

**MILLER, B.L., MENA, I., GIOMBETTI, R., VILLANUEVA-MEYER, J. & DJENDEREDJIAN, A.H.** 1992. Neuropsychiatric effects of cocaine: SPECT measurements. *Journal of addictive diseases*: 11(4):47-58.

**MOUNTZ, J.M., LIU, H.G. & DEUTSCH, G.** 2003. Neuroimaging in cerebrovascular disorders: Measurement of cerebral physiology after stroke and assessment of stroke recovery. *Seminars in nuclear medicine*, 33(1):56-76.

**MUSTERS, R.J.P.H., OTTEN, E., BIEGELMANN, E., BIJVELT, J., KEIZER, J.J.H., POST, J.A., OP DEN KAMP, J.A.F. & VERKLEIJ, A.J.** 1993. Loss of phosphatidylethanolamine transbilayer asymmetry in the sarcolemma of the isolated neonatal rat cardiomyocyte during simulated ischemia. *Circulation research*, 73: 514-523.

**MUSTERS, R.J.P.H., PRÖBSTL-BIEGELMANN, E., VAN VEEN, T.A.B., HOEBE, K.H.N., OP DEN KAMP, J.A.F., VERKLEIJ, A.J. & POST, J.A.** 1996. Sarcolemmal phospholipid reorganization during simulated ischemia: reversibility and ATP dependency. *Molecular membrane biology*, 13: 159-164.

**NOBILI, F., KOULIBALY, M., VITALI, P., MIGNECO, O., MARIANI, G., EBMEIER, K., PUPI, A., ROBERT, P.H., RODRIGUEZ, G. & DARCOURT, J.** 2002. Brain perfusion follow-up in Alzheimer's patients during treatment with acetylcholinesterase inhibitors. *Journal of nuclear medicine*, 43(8):983-990.

**NODA, A., OHBA, H., KAKIUCHI, T., FUTATSUBASHI, M., TSUKADA, H. & NISHIMURA, S.** 2002. Age-related changes in cerebral blood flow and glucose metabolism in conscious rhesus monkeys. *Brain research*, 936(1-2):76-81.

**PAVICS, L., GRÜNWARD, F., REICHMAN, K., HORN, R., KITECHENBERG, A., HARTMANN, A., MENZEL, C., SCHOMBURG, A., OVERBEECK, B., CSERNAY, L. & BIERSACK, H.J.** 1999. Regional cerebral blood flow single-photon emission tomography with 99m Tc-HMPAO and acetazolamide test in the evaluation of vascular and Alzheimer's dementia. *European journal of nuclear medicine*, 26:239-245.

**PENG, N.J., LIU, R.S., LI, J.Y., TSAY, D.G., KONG, K.W., KWOK, C.G. & STRAUSS, H.W.** 2000. Increased cerebral blood flow in MELAS shown by Tc-99m HMPAO brain SPECT. *Neuroradiology*, 42(1):26-29.

**PIPER, H.M., GARCIA-DORADO, D. & OVIZE, M.** 1998. A fresh look at reperfusion injury. *Cardiovascular research*, 38:291-300.

**PODREKA, I., BAUMGARTNET, C., OLBRICH, A., RELIC, A., PIETRZYK, U., SELES, W., NOVAK, K., WIMBERGER, D., ALULL, A., LINDINGER, G.,**

**LURGER, S., BRUCKE, T., PUNZ, E. & STELLAMOR, V.** 1997. HMPAO SPECT and Video – EEG Monitoring in Candidates for Surgical Treatment of Epilepsy. (In De Deyn, P., Dierckx, R.A., Alavi, A. & Pickut, BA, eds., A Textbook of SPECT in Neurology and Psychiatry. London: John Libbey. p219-224).

**POST, J.A., VERKLEIJ, A.J. & LANGER, G.A.** 1995. Organization and function of sarcolemmal phospholipids in control and ischemic/reperfused cardiomyocytes. *Journal of molecular and cellular cardiology*, 27: 749-760.

**POST, J.A., SCHNEIJDENBERG, C.T.W.M., DE HINGH, Y.C.M., VAN ROOIJEN, R., LAHPOR, J.R., OP DEN KAMP, J.A.F. & VERKLEIJ, A.J.** 1998. Lipid peroxidation in myocardial cells under ischemia and oxidative stress. The effect of allopurinol and oxypurinol. (In Op den Kamp, J.A.F., ed. Biological membranes: Lipid and protein traffic pathways and molecular mechanisms. Berlin: Springer Verlag. p. 343-356).

**POST, J.A., LANGER, G.A., OP DEN KAMP, J.A.F. & VERKLEIJ, A.J.** 1988. Phospholipid asymmetry in cardiac sarcolemma. Analysis of intact cells and "gas-dissected" membranes. *Biochimica et biophysica acta*, 943: 256-266.

**RAUSCH, D.M., MURRAY, E.A. & EIDEN, L.E.** 1999. The SIV-infected rhesus monkey model for HIV-associated dementia and implications for neurological diseases. *Journal of leukocyte biology*, 65(4):466-474.

**REMPF, K.A., BRIX, G., WENZ, F., BECKER, C.R., GUCKEL, F. & LORENZ, W.J.** 1992. Quantification of regional blood flow and volume with dynamic susceptibility contrast-enhanced MR imaging. *Radiology*, 193(3):63741.

**ROWE, C., BOUNDY, K., KITCHENER, M., BARNDEN, L., KASSIOU, M., KATSIFIS, A. & LAMBERT, R.** 1997. Ictal <sup>99m</sup>Tc-SPECT and <sup>123</sup>I-Iododexetimide SPECT in Temporal Lobe Epilepsy. (In De Deyn, P., Dierckx, R.A., Alavi, A. & Pickut, BA, eds. A Textbook of SPECT in Neurology and Psychiatry. London: John Libbey. p219-224).

**RUBBERT, A., BOCK, E., SCHWAB, J., MARIENHAGEN, J., NUSSLEIN, H., WOLF, F. & KALDEN, J.R.** 1994. Anticardiolipin antibodies in HIV infection: association with cerebral perfusion defects as detected by 99mTc-HMPAO SPECT. *Clinical and experimental immunology*, 98(3):361-368.

**SABBAGH, M.N., LYNN, P., JHINGRAN, S., MASSMAN, P., VILLANUEVA-MEYER, J., OLUP, J. & DOODY, R.S.** 1997. Correlations between SPECT regional cerebral blood flow and psychometric testing in patients with Alzheimer's disease. *The Journal of neuropsychiatry and clinical neurosciences*, 9(1):68-74.

**SADZOT, B., DEBETS, R.M.C. & FRSNCK, G.** 1997. Biochemical and Functional Imaging for Adult Partial Epilepsy: PET or SPECT? (*In De Deyn, P., Dierckx, R.A., Alavi, A. & Pickut, BA, eds. A Textbook of SPECT in Neurology and Psychiatry. London: John Libbey. p207-217*).

**SAKASHITA, Y., MATSUDA, H., KAKUDA, K. & TAKAMORI, M.** 1993. Hypoperfusion and vasoreactivity in the thalamus and cerebellum after stroke. *Stroke; a journal of cerebral circulation*, 24(1):84-87.

**SALIE, R., HARPER, I., CILLIE, C., GENADE, S., HUISAMEN, B., MOOLMAN, J. & LOCHNER, A.** 2001. Melatonin protects against ischaemic-reperfusion myocardial damage. *Journal of molecular and cellular cardiology*, 33(2):343-357.

**SANI, S., TRAU, D., KLINK, A., NIARAKI, N., GONZALO-RUIZ, A., WU, C.K. & GEULA, C.** 2003. Distribution, progression and chemical composition of cortical amyloid-beta deposits in aged rhesus monkeys: similarities to the human. *Acta neuropathologica*, 105(2):145-156.

**SAUERMAN, U.** 2001. Making the animal model for AIDS research more precise: the impact of major histocompatibility complex (MHC) genes on pathogenesis and disease progression in SIV-infected monkeys. *Current molecular medicine*, 1(4):515-522.

**SCHMIDT, K., NOLTE-ZENKER, B., PATZER, J., BAUER, M., SCHMIDT, L.G. & HEINZ.** 2001. Psychopathological correlates of reduced dopamine receptor sensitivity in depression, schizophrenia, and opiate and alcohol dependence. *Pharmacopsychiatry*, 34(2):66-72.

**SIEGEL, R.M. & ANDERSEN, R.A.** 1988. Perception of three-dimensional structure from motion in monkey and man. *Nature*, 331(6153):259-261.

**SOININEN, H., HELKALA, E.L., KUIKKA, J., HARTIKAINEN, P., LEHTOVIRTA, M. & RIEKKINEN, P.J. SR.** 1995. Regional cerebral blood flow measured by 99mTc-HMPAO SPECT differs in subgroups of Alzheimer's disease. *Journal of neural transmission. Parkinson's disease and dementia section*, 9(2-3):95-109.

**SOPPER, S., KOUTSILIERI, E., SCHELLER, C., CZUB, S., RIEDERER, P. & TER MEULEN, V.** 2002. Macaque animal model for HIV-induced neurological disease. *Journal of neural transmission*, 109(5-6):747-766.

**SPERLING, B. & LASSEN, N.A.** 1997. Cerebral Blood Flow by SPECT in Ischaemic Stroke. (In De Deyn, P., Dierckx, R.A., Alavi, A. & Pickut, BA, eds. A Textbook of SPECT in Neurology and Psychiatry. London: John Libbey. p299-305).

**STARKSTEIN, S.E. & VÀZQUES, S.** 1997. SPECT Findings in Vascular Dementia and Alzheimer's Disease. (In De Deyn, P., Dierckx, R.A., Alavi, A. & Pickut, BA, eds. A Textbook of SPECT in Neurology and Psychiatry. London: John Libbey. p219-224).

**STOPPE, G., SCHUTZE, R., KOGLER, A., STAEDT, J., MUNZ, D.L., EMRICH, D. & RUTHER, E.** 1995. Cerebrovascular reactivity of acetazolamide in (senile) dementia of Alzheimer's type. *Dementia*, 6(2):73-82.

**SZARSZOI, O., ASEMU, G., VANECEK, J., OST'ADAL, B. & KOLAR, F.** 2001. Effects of melatonin on ischemia and reperfusion injury of the rat heart. *Cardiovascular drugs and therapy*, 15(3):251-257.

- SZETO, E.R., FREUND, J., BREW, B.J., LODER, A. & GRIFFITHS, M.R.** 1998. Cerebral perfusion scanning in treating AIDS dementia: a pilot study. *Journal of nuclear medicine*, 39(2):298-302.
- TAFFE, M.A., WEED, M.R., GUTIERREZ, T., DAVIS, S.A. & GOLD, L.H.** 2002. Differential muscarinic and NMDA contributions to visuo-spatial paired-associate learning in rhesus monkeys. *Psychopharmacology*, 160(3):253-262.
- TRACEY, I., LANE, J., CHANG, I., NAVIA, B., LACKNER, A. & GONZALEZ, R.G.** 1997. <sup>1</sup>H Magnetic resonance spectroscopy reveals neuronal injury in a simian immunodeficiency virus macaque model. *Journal of acquired immune deficiency syndromes and human retrovirology*, 15(1):21-27.
- TRAN DINH, Y.R., MAMO, H., CERVONI, J., CAULIN, C. & SAIMOT, A.C.** 1990. Disturbances in the cerebral perfusion of human immune deficiency virus-1 seropositive asymptomatic subjects: a quantitative tomography study of 18 cases. *Journal of nuclear medicine*, 31(10):1601-1607.
- TRUCCO, M., CANANZI, C., SALVADORI, P.R. & BADINO, R.** 1994. Piroxicam-beta-cyclodextrin in induced migraine attacks: a SPECT study with Tc-99m-HM-PAO split-dose method. *Functional neurology*, 9(5):247-257.
- VANGU, M.D., ESSER, J.D., BOYD, I.H. & BERK, M.** 2003. Effects of electroconvulsive therapy on regional cerebral blood flow measured by 99mtechnetium HMPAO SPECT. *Progress in neuro-psychopharmacology & biological psychiatry*, 27(1):15-19.
- VEALL, N. & MALLETT, B.L.** 1965. The two-compartment model using <sup>133</sup>Xe inhalation and external counting. *Acta neurologica Scandinavica*, 14:83-84.
- WALKER, L.C.** 1997. Animal models of cerebral beta-amyloid angiopathy. *Brain research reviews*, 25(1):70-84.
- WALKER, L.C., MASTERS, C., BEYREUTHER, K. & PRICE, D.L.** 1990. Amyloid in the brains of aged squirrel monkeys. *Acta neuropathologica*, 80(4):381-387.

**WARKENTIN, S., NILSSON, A., RISBERG, J., KARLSON, S., FLEKKOY, K., FRANZEN, G., GUSTAFSON, L. & RODRIGUEZ, G.** 1990. Regional cerebral blood flow in schizophrenia: repeated studies during a psychotic episode. *Journal of psychiatric research*, 35(1):27-38.

**WYPER, D., TEASDALE, E., PATTERSON, J., MONTALDI, D., BROWN, D., HUNTER, R., GRAHAM, D., MCCULLOCH, J.** 1992. Abnormalities in rCBF and computed tomography in patients with Alzheimer's disease and in controls. *The British journal of radiology*, 66(781):23-27.

**YAMASHITA, T., HAYASHI, M., KASHIWAGI, S., NAKANO, S., EGUCHI, Y., TAKASAGO, T., URAKAWA, M. & ITO, H.** 1992. Cerebrovascular reserve capacity in ischaemia due to occlusion of a major arterial trunk: studies by Xe-CT and acetazolamide test. *Journal of computer assisted tomography*, 16(5):750-755.

**ZINK, M.C. & CLEMENTS, J.E.** 2002. A novel simian immunodeficiency virus model that provides insight into mechanisms of human immunodeficiency virus central nervous system disease. *Journal of neurovirology*, Suppl(8) 2:42-48.

## Methodology Cerebral Blood Flow Model

## Chapter 3

### 3.1 Introduction

This chapter will address aspects of the methodology, the study design and model development as applied in the thesis. The experimentation involved in the thesis, which was deemed necessary for full comprehension but which was not fully covered in the different published studies was also included. Components that will be addressed include radiotracers in cerebral blood flow SPECT studies, the *Papio ursinus* baboon model as such, and split-dose procedures. In essence these all together constitute the “Cerebral Blood Flow Model” of the title as applied in cerebral blood flow studies used in drug research, and discussed here.

### 3.2 SPECT and PET

Both the technologies SPECT and PET (Positron Emission Tomography, utilizing the positron emission radionuclides such as  $^{18}\text{F}$ ,  $^{11}\text{C}$ ,  $^{13}\text{N}$  or  $^{15}\text{O}$ ) are well established in nuclear medicine with a wide variety of medical and medicinal applications. PET is a three-dimensional imaging technique designed to show detailed images of structures and to measure the level of metabolic activity within the cell using fluorine-18-labeled fluorodeoxyglucose ( $^{18}\text{F}$ -FDG) for brain studies, whereas SPECT is useful to determine functional cerebral perfusion information. An on-site cyclotron with researchers to produce PET radiotracers or a distribution centre in close proximity to the PET facility is a prerequisite in view of the short half-lives (2 min to 2 h) of PET radiotracers. This adds to the difficult logistics and cost of PET procedures.

### 3.3 Radiotracers in Cerebral Perfusion SPECT Studies

Several radiotracers are currently in use in cerebral perfusion investigations with SPECT, e.g.  $^{99m}\text{Tc}$ -hexamethylpropylene amine oxime ( $^{99m}\text{Tc}$ -HMPAO),  $^{99m}\text{Tc}$ - ethyl cysteinate dimer ( $^{99m}\text{Tc}$ -ECD),  $^{123}\text{I}$  Iodoamphetamine, and  $^{133}\text{Xe}$  ( $^{133}\text{Xe}$  as an inhalation). These radioactive brain perfusion agents differ in chemical and metabolic properties, their application and brain disposition.

Although these tracers are not ideal, they have been used extensively in research and nuclear medicine with a significant degree of success. Ongoing research is continuously searching to optimize the properties of the current tracers and designing new potential agents. Important characteristics of the tracers were addressed in the research papers in this thesis and are well covered in several of the studies. Selected physico-chemical characteristics of the brain perfusion tracers used in the thesis are presented in Table 3.1. The poor chemical stability of HMPAO limits the design possibilities of brain studies and HMPAO must be used within 30 minutes after preparation. For IMP, where chemical stability is pH dependent, the preparation is thus also critical. The newer brain agent ECD exhibits improved characteristics.

Table 3-1 Characteristics of brain perfusion tracers

Characteristics	$^{99m}\text{Tc}$ -HMPAO	$^{99m}\text{Tc}$ -ECD	$^{123}\text{I}$ -IMP
Radio half-life	6 hr	6 hr	13.2 hr
Photon energy (keV)	140	140	159
Chemical stability	30 min	6 hr	12 hr*

\*dependent on pH

#### 3.3.1 General considerations and properties of CBF tracers

Ideally a brain perfusion agent should have one of the following characteristics (Amershamhealth 2003):

- Trapped by the perfused tissue in proportion to perfusion,
- Remain strictly intravascular, or

- Diffuse freely throughout the tissue of interest.

Delivery of drugs and other substances are challenged by the presence of the blood brain barrier. This physiological barrier acts as a diffusion barrier for amongst others hydrophilic compounds and allows the diffusion of lipophilic compounds through the endothelium of the parenchymal vessels. The administered brain perfusion tracers  $^{99m}\text{Tc}$ -HMPAO,  $^{99m}\text{Tc}$ -ECD,  $^{123}\text{I}$  Iodoamphetamine give a 'snapshot' of the cerebral perfusion at the time of injection due to their delivery reaching steady state as a first-pass perfusion agent (Abbott *et al.* 1999; Arano 2002; Amershamhealth 2003).

### **3.3.1.1 $^{99m}\text{Tc}$ -HMPAO and $^{99m}\text{Tc}$ -ECD**

Both the ligands HMPAO and ECD (See Figure 3-1) are easy to label with technetium, with HMPAO being chemically less stable and more susceptible to radiolysis than ECD. *In vivo* instability of the tracers follows upon contact with blood, and this effect together with tracer changes in circulating blood, results in relatively poor brain extraction for the purpose of CBF measurement.

$^{99m}\text{Tc}$ -HMPAO is a lipophilic neutral complex obtained after reduction of  $^{99m}\text{Tc}$  with  $\text{Sn}^{2+}$  ion during the labelling procedure. The  $^{99m}\text{Tc}$ -generator loaded with molybdenum-99 ( $^{99}\text{Mo}$ ), a radioisotope produced either in a reactor or by a cyclotron, provides  $^{99m}\text{Tc}$  continually, through the  $^{99}\text{Mo}$  decaying process.  $^{99}\text{Mo}$  decays, with a half-life of 66 hours, to  $^{99m}\text{Tc}$  which is easily extracted for use. The presence of radiochemical impurities such as free  $^{99m}\text{TcO}_4^-$  and hydrolysed  $^{99m}\text{Tc}$  in other  $^{99m}\text{Tc}$ -labelled complexes in the  $^{99m}\text{Tc}$ -HMPAO result in poor quality images due to the high background from the surrounding tissues and blood emphasizing the importance of quality assurance of these tracer products.

$^{99m}\text{Tc}$ -ECD labelling involves an initial reduction of  $^{99m}\text{Tc}^{7+}$  to a lower oxidation state and conversion to  $^{99m}\text{Tc}$ -EDTA-complex. A ligand exchange procedure subsequently yields a trans-chelation reaction between EDTA and ECD producing  $^{99m}\text{Tc}$ -ECD. The first-pass uptake of  $^{99m}\text{Tc}$ -ECD by the brain after intravenous administration is fast with peak activity occurring between 1 to

2 minutes. Of the injected dose between 5 - 6 % is retained within the brain with very slow washout (Gopal 1998).

### 3.3.1.1.1 Quality control of technetium labelled radiotracers

As with all products and in particular for medicinal products, quality assurance (QA) is an integral part of good manufacturing practice (GMP) to ensure that the product complies with the specifications of the product. Control measures are of essence in radiopharmacy and QA of technetium labelled radiopharmaceuticals has been reported in depth and are well described (Forsyth 1994, Venter 2003). Typically the QA at the final phase of the preparation of the technetium labelled HMPAO and ECD requires thin layer chromatography and is performed to ensure that the product is used with a labelling efficiency of more than 80 % (manufacturer's specifications). Radiochemical impurities arise from decomposition due to the action of solvent, change in temperature or pH, light, presence of oxidising or reducing agents, and radiolysis.

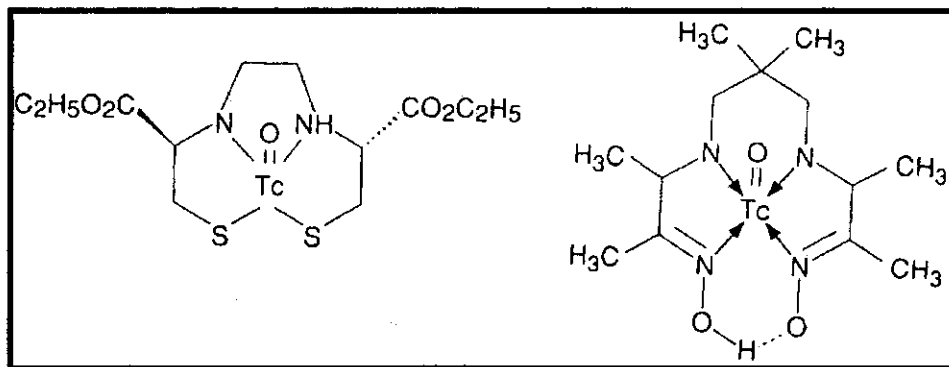
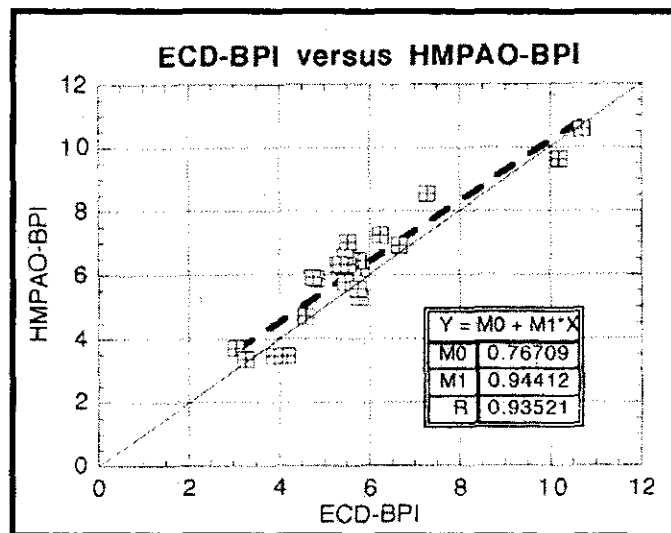


Figure 3-1 Chemical Structures of <sup>99m</sup>Tc-HMPAO (right), <sup>99m</sup>Tc-ECD (left)

Figure 3-2 illustrates the comparison of <sup>99m</sup>Tc-HMPAO and <sup>99m</sup>Tc-ECD with respect to brain perfusion indices (BPI) (See Figure 3.2; Matsuda *et al.* 1995) These brain perfusion indices, which describe the extraction ratios of the brain tracer with respect to the brain and peripheral dispositions, showed in a patient study for HMPAO and ECD a highly significant correlation ( $r = 0.935$ ), illustrating a marked performance similarity between these two tracers. Groiselle and co-workers (2002) presented an approach for improving the measurement of the BPI by temporal analysis whereas the method of Matsuda

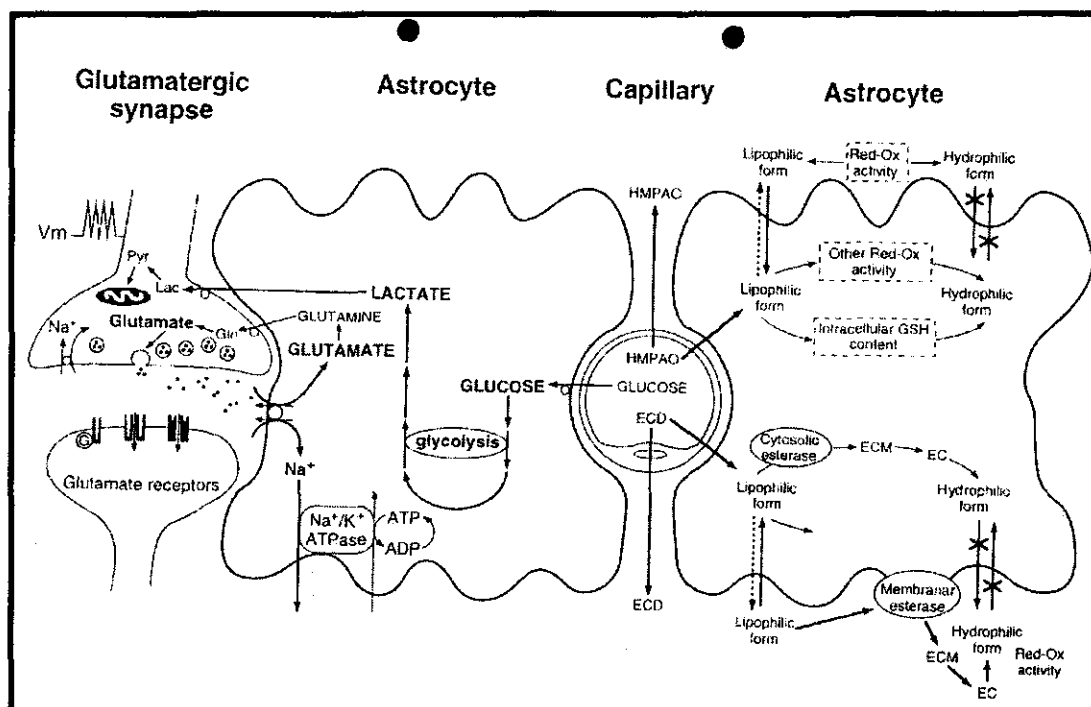
computed the ratio of the cumulated counts in the cerebral hemispheres and aortic arch. It was concluded that this method has advantages over that of Matsuda with respect to reproducibility particularly for naive observers. A recent radionuclide angiographic study for the estimation of absolute cerebral blood flow using  $^{99m}\text{Tc}$ -ECD by Van Laere and co-workers (2001), provided reference values for normal perfusion indices assessed by graphical (Patlak-Gjedde,  $K_{pat}$ ) and spectral analysis.



**Figure 3-2** The Brain Perfusion Indices (BPI) of HMPAO and ECD in patient study (taken from Matsuda *et al.* 1995)

A schematic representation of the mechanism for the uptake and metabolism of HMPAO and ECD are given in Figure 3.3. The brain first-pass extraction rate for both HMPAO and ECD is cerebral flow-dependent. The retention of the extracted tracer in brain parenchyma is associated with the enzymatic reactions with GSH (glutathione) for HMPAO and stereospecific deesterification to acid derivatives for ECD. The products from both tracers are hydrophilic, resulting in their trapping or retention in the brain with limited back diffusion (Lo *et al.* 2001). A study by Zerarka *et al.* (2001) provided evidence that the astrocytes is the primary site for the retention of HMPAO by a factor of approximately 2.5 over neurons. Furthermore, HMPAO's glial metabolism may account for flow-independent changes in  $^{99m}\text{Tc}$ -HMPAO retention as observed by SPECT in some pathologies. Pathologies influence the conversion due to changes in the metabolic states of cerebral cells and changes in pH. The

outcome of this may lead to significant deviations in the trapping of the tracer, and subsequently the uptake of the tracer is not necessarily representative of the cerebral blood flow (Moretti *et al.* 1995; Koyama *et al.* 1997; Siennicki-Lantz *et al.* 1999). Furthermore there are also cases where the uptake of  $^{99m}\text{Tc}$ -HMPAO and  $^{99m}\text{Tc}$ -ECD differs significantly due to the difference in the conversion mechanisms of the tracers (Rieck *et al.* 1998; Huyn *et al.* 2001). Some of these differences between  $^{99m}\text{Tc}$ -HMPAO and  $^{99m}\text{Tc}$ -ECD SPECT are in fact opposite findings. Rieck *et al.* (1998) reported recently in patients with herpes simplex encephalitis that  $^{99m}\text{Tc}$ -HMPAO presented unilateral regional increases whereas  $^{99m}\text{Tc}$ -ECD presented a decrease in uptake in the affected temporal lobe, and may lead to masking of the pathology.



**Figure 3-3** Schematic representation of the mechanism for the uptake and metabolism of HMPAO and ECD (taken from Slosman and Magistretti 1997).

### 3.3.1.2 $^{123}\text{I}$ Iodoamphetamine (IMP)

$^{123}\text{I}$ -IMP is a lipophilic amino compound and has a high first-pass brain extraction fraction (96%), with a linear relationship between tissue activity and cerebral blood flow. The retention of IMP in the brain follows a stereo selective retention mechanism that may involve conversion to hydrophilic metabolites,

and an affinity to high capacity and relatively non-specific binding sites. The blood clearance of IMP is very rapid with protein binding less than 10 % and the brain uptake is about 6-9% of the injected dose with total brain activity peaking within 20 minutes. The pH of the blood influences the uptake of IMP with a decreased uptake following a decreased pH, due to amongst others local lactate production.

$^{123}\text{I}$ -IMP is prepared by an isotope exchange method by heating isopropyl amphetamine and  $^{123}\text{I}$ -NaI at 150 °C for 30 min. Ether extraction procedure and purification by washing with dilute hydrochloride acid follow to yield an  $^{123}\text{I}$ -IMP containing solution. The pH of the  $^{123}\text{I}$ -IMP solution is critical to ensure chemical stability and should be between 4 to 6.

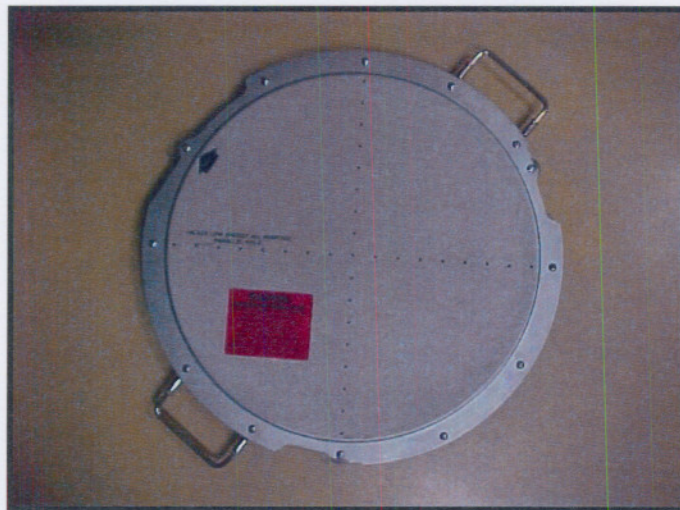
### 3.4 Drug Delivery and Cyclodextrin

A major challenge in developing drugs is to ensure the delivery of the substance through the membranes of the living body. These barriers are lipophilic in nature with the blood brain barrier showing even higher lipophilicity than other bio membranes. Furthermore, the delivery is compounded by the chemical and physical properties of the drugs themselves. Poor bioavailability of drugs is frequently a problem and researchers are continuously searching for novel strategies to improve drug delivery. This also applies to radiopharmaceuticals. One approach that has successfully been applied involves the complexation of a substance with a cyclodextrin to improve the bioavailability of the substance in SPECT studies (Yaksh *et al.* 1991; Trucco *et al.* 1994; Camargo *et al.* 2001; Brewster *et al.* 2002).

Due to the chemical and metabolic instability of technetium tracers for CBF measurements as well as their relatively poor brain disposition, a study was conducted using cyclodextrin for complexation with the technetium brain perfusion tracer, ECD. Detailed methodology for this investigation is described in Chapter 13.

### 3.5 SPECT Instrumentation

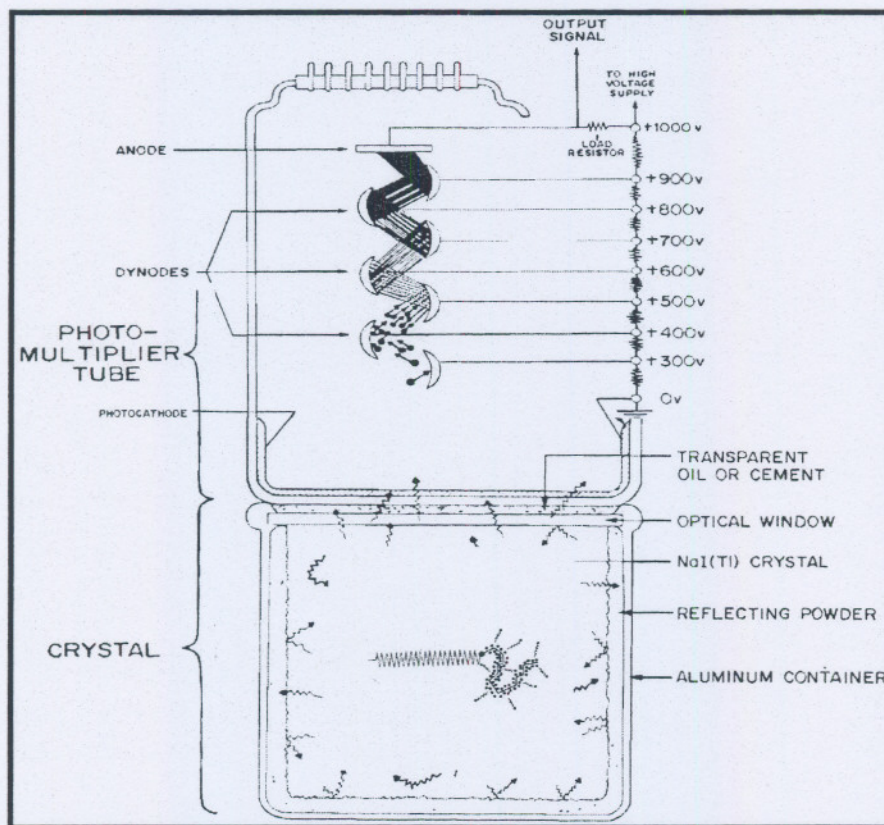
Since the first reports of scintillation cameras and emission tomography (Jaszczak *et al.* 1997; Keyes *et al.*, 1977), SPECT gamma camera systems have become abundant both for research or clinical purposes. The basic principle of SPECT technology involves the administration of a gamma emitter tracer to the subject under investigation, followed by *in vivo* measurement of the gamma radiation. The determination or counting of the gamma radiation from the subject by the gamma camera is achieved by stepwise processes which involve the radiation striking the face of the collimator, which is mounted on the detector head of the camera. Different collimators are available, depending on the particular application. A parallel hole collimator (typically low energy high resolution; LEHR)) allows only those gamma rays which are travelling in a direction approximately perpendicular to its face to pass through and be counted. The collimator septa stops most other gamma rays. Parallel hole collimators consist of a lead shield with a series of holes (or channels) typically running in parallel straight through it (See Figure 3-4).



**Figure 3-4** The parallel hole collimator (LEHR) used in the current study.

Unrejected gamma rays traveling through the collimator produce scintillations in the NaI(Tl) crystal in a position reflecting a corresponding position in the subject under investigation (Siemens 1988). These photons interact through photoelectric process with the crystal and reach the surface of the crystal facing the adjacent array of photomultiplier tubes (up to 72 tubes),

where about 30% of them will reach the photocathodes of the multiplier tubes (See figure 3-5). These scintillations or light photons strike the photocathode, and photoelectrons will be emitted. The photoelectrons are accelerated to the immediately adjacent dynode due to the voltage difference between the electrodes. The accelerated electrons strike the dynode and in addition, more secondary electrons are emitted, and further accelerated along the photomultiplier tube. This process of multiplication of secondary electrons continues until the last dynode is reached, with a subsequent pulse of  $10^5$  to  $10^8$  electrons being produced. This pulse is finally delivered to the preamplifier and amplifier after final collection on the anode (Gopal 1998).



**Figure 3-5** Schematic representation of scintillation detection processes in the crystal and photomultiplier tube (Designed from Bernard *et al.* 1976)

The pulse information is then converted by an analogue to digital converter to binary digits, i.e. pixels, which can be displayed through appropriate computer hardware and software as an image of the subject. Such an image therefore represents a visualisation of the distribution of the gamma emitter in the subject, which can by means of mathematical algorithms and computer

programmes be reconstructed as distinct focal planes or slices through the subject in three dimensions. SPECT systems generally consist of a typical gamma camera (See Figure 3.6) with one to three NaI(Tl) detector heads mounted on a gantry, that rotates around the long axis of the subject at small angle increments for 180° or 360° angular sampling. For data acquisition and processing an on-line computer and a display system is used. The data are stored in a 64 x 64 or 128 x 128, up to 512 x 512 matrixes for later reconstruction of the images into the planes (slices) of interest presented as transaxial, sagittal, and coronal images (Gopal 1998).



**Figure 3-6** Gamma camera used in the current study.

### 3.6 The Cape Baboon, *Papio ursinus*, in NeuroSPECT

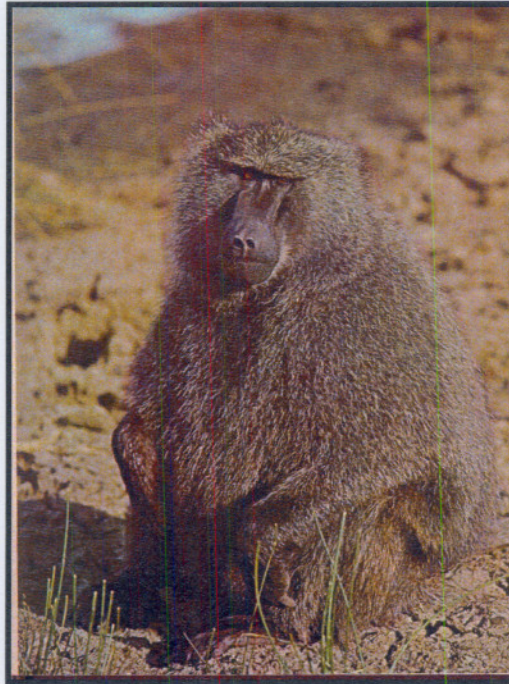
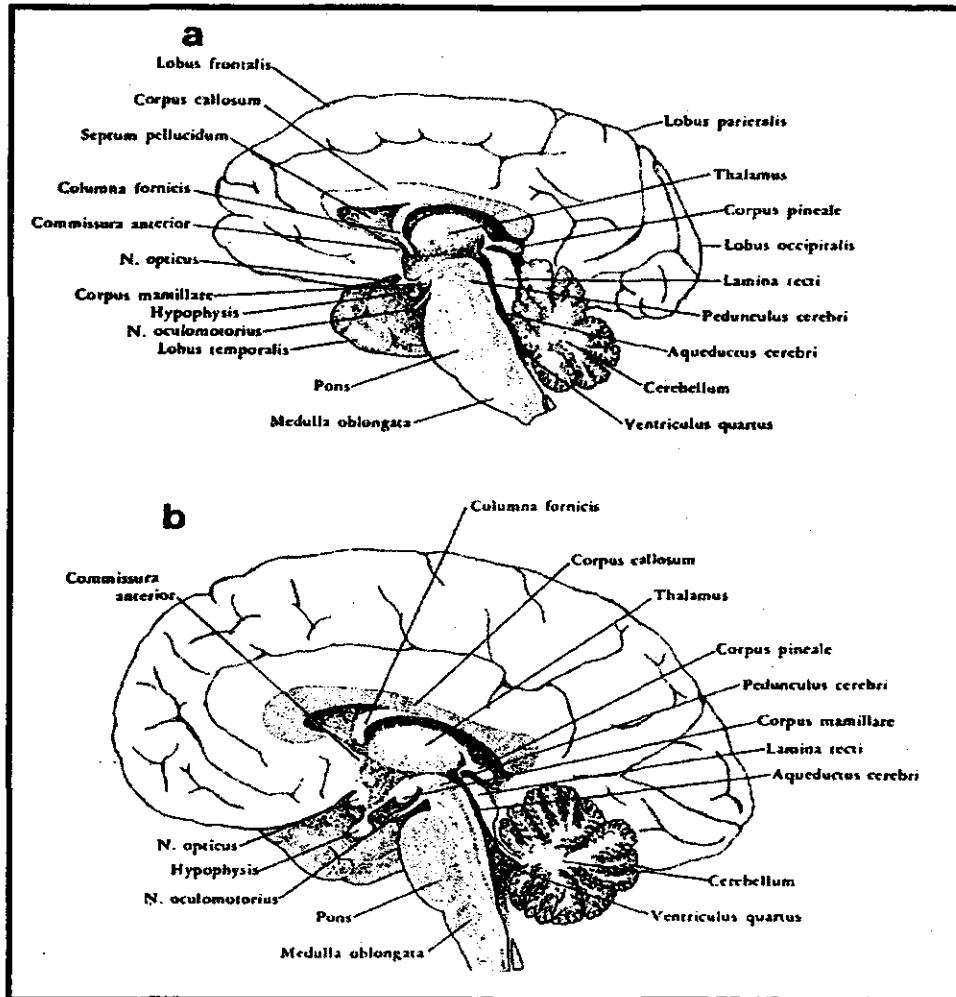


Figure 3-7 The Cape baboon *Papio ursinus*

The Cape baboon, *Papio ursinus* is a relatively large non-human primate with a weight of 27-30 kg for an adult male. Its large size makes the Cape baboon suitable for *in vivo* investigations, using nuclear medical technologies (See Figure 3-7).

The baboon's brain is likewise relatively large when compared to its weight, and it is therefore almost the ideal model for cerebral blood flow studies using SPECT imaging and perfusion radiotracers. The relative sizes of the cerebral hemispheres, medulla, pons and cerebellum of the Cape baboon are comparable (Figure 3-8) to those of man (Hill 1970).



**Figure 3-8** The brain of a, *Papio* and b, *Homo* (from an Atlas of Primate Gross Anatomy, Doris R Swindler and Charles D Wood, 1982)

The cerebral hemispheres of the baboon brain are incompletely separated from each other by a longitudinal fissure. The cerebral cortex spreads over the surface of the hemisphere, and its surface area is increased by the presence of gyri, separated by sulci. Several important sulci demarcate the brain into the four brain lobes, i.e. frontal, parietal, temporal and occipital. The configuration of the convolutions shows a general increase in complexity in man when compared to those of the baboon. The relation between the degree of fissuration and the size of the brain, is further dependant on body weight (Connolly 1950). Greater detail of fissuration is seen in man due to the development of secondary and tertiary sulci, yielding the major difference between baboon and man. The cerebellum of the baboon, similar to man, consists of three main lobes, i.e. the two floccular lobes and the petrosal lobe. The anatomical differences between the baboon and human brain are more of

a quantitative nature, e.g. cell densities, rather than qualitative. Le Gross Clark (1960) stated that "there is no neomorphic element" that distinguishes man from baboon.

It is therefore clear from the comparisons between the baboon and the human brain that the Cape baboon can be used with confidence during *in vivo* neuropharmacological SPECT investigations.

### **3.7 Split-dose Studies in SPECT**

The first reports of the split-dose approach in SPECT investigations have been presented more than 10 years ago in the literature. Wyper and co-workers (1991) reported the technique to measure cerebral blood flow changes and this was followed soon after by Pantano *et al.* (1992).

#### **3.7.1 Split-dose method**

The split-dose SPECT method involves the administration of two consecutive injections of the brain perfusion agent, before and after the expected induced CBF change, thus allowing the measurement and evaluation of the change. The method is based on the unique chemical and brain disposition properties of the tracer that succeeds to cross the blood brain barrier due to a significant degree of the lipophilicity that these tracers display. The cerebro-distribution of these tracers is proportional to the regional blood flow and retention of hydrophilic products that enables snapshots of CBF, as measured from the two consecutive injections, e.g. before and after the intervention. The split-dose method can therefore effectively be applied for intervention studies.

The method is further designed to improve correction for the radioactivity from background remaining from the first injection by using 2 or 3 times the original dosage for the second tracer injection. The two injections of the tracer are each followed by SPECT data acquisition (SPECT-1 and SPECT-2), and this data will represent the slice dependent rCBF during the conditions at the time of injection.

The effect of an intervention on the CBF is measured by the second SPECT (SPECT-2) data acquisition, based on second tracer injection at such times as to appropriately reflect at the required response time of the particular intervention. The data from both the SPECT acquisitions are used to determine ratios ( R ) based on the count rate data (counts/pixel from the images) obtained from SPECT-2 with respect to SPECT-1, after the subtraction of background from SPECT-1, corrected for radioactive decay.

$$R = \frac{(SPECT - 2) - (SPECT - 1) *}{(SPECT - 1)}$$

\*decay corrected

It could therefore be expected that ideally an R ratio value of 2 would be observed when a dose twice that of the first tracer dose was injected, in the absence of any CBF changes due to an intervention. Deviations from this value would imply a change in CBF, either as an increase ( $R > 2$ ) or a decrease ( $R < 2$ ) in CBF. Deviations from the R ratio value of 2 in non-intervention control studies as have been reported in this thesis are explained for amongst others due to back diffusion of the tracers, and the cardiovascular effects of ketamine. Very recently it was shown that the performance of the neuroreceptor radioligands was influenced by several anaesthetics (Elfvig *et al.* 2003). Decreases and increases for the target-to-background ratios of neuroreceptor tracers were found for ketamine/xylazine and halothane anaesthetics, respectively.

### 3.8 The Baboon Cerebral Blood Flow SPECT Model

The main aim of this study involved the design, development and application of a non-human primate model for CBF determinations. It is of necessity that the baboon animal model would be under anaesthesia. The conscious baboon would present logistical problems, including reproducibility and safety and would be difficult to handle under conscious procedures. It is well known that anaesthesia in itself may influence cerebral blood flow and would therefore

have to be taken into consideration and be embedded in the model. The split-dose method described was ideally suited to incorporate the effects of the anaesthetic regime on the CBF.

### 3.8.1 General procedure

Adult male baboons (n=6) (*Papio ursinus*, average weight 25 kg) were included in each study. The animals were obtained from Mr. E. Venter, Vaalwater, Limpopo Province (Republic of South Africa). The studies were performed after approval by the Ethics Committee of the University of Pretoria, according to the guidelines of the National Code for Animal Use in Research, Education and Testing of Drugs and Related Substances in South Africa. These guidelines are in line with international standards.

A typical time (minutes) schedule of a study is presented in Figure 3-9 with halothane en thiopentone (table below) anaesthesia.

For the control study (Procedure A), each baboon was sedated with ketamine hydrochloride (10 mg/kg intra-muscular) (Anaket-V<sup>®</sup>, Centaur Labs., Bryanston, Gauteng, SA.). This was followed immediately by a maintained and controlled infusion of thiopentone sodium (70 ml/h of 0.5% solution) (Intraval<sup>®</sup>, Rhone- Poulenc Rorer SA, Midrand, Gauteng, SA.) or halothane or a barbiturate alternative. After a 12 minute stabilization period under anaesthesia, the control study (Procedure A), started at t = 0 with an intravenous injection of <sup>99m</sup>Tc-hexamethylpropylene amine oxime (HMPAO) or <sup>99m</sup>Tc-ethyl cysteinyl dimer (ECD) or <sup>123</sup>I-iodoamphetamine (IMP). The latter was obtained from the National Accelerator Centre, Faure, South Africa. The HMPAO or ECD was labeled according to the manufacturer's directions. Five minutes after the tracer injection for Procedure A, the first SPECT acquisition (SPECT-1) with a Siemens Orbiter gamma camera followed, using 32 projections of 20 seconds per view during a 360° rotation. A period of 5 minutes is sufficient to ensure that the tracer reaches steady state.

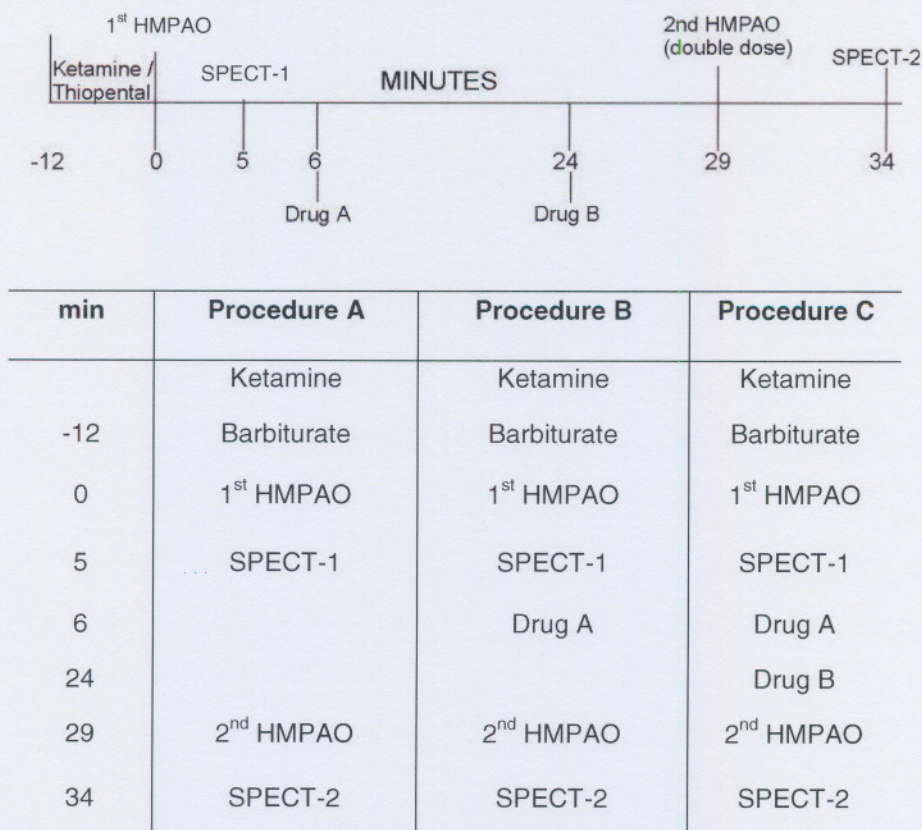


Figure 3-9 A typical time schedule illustrating the split-dose procedure

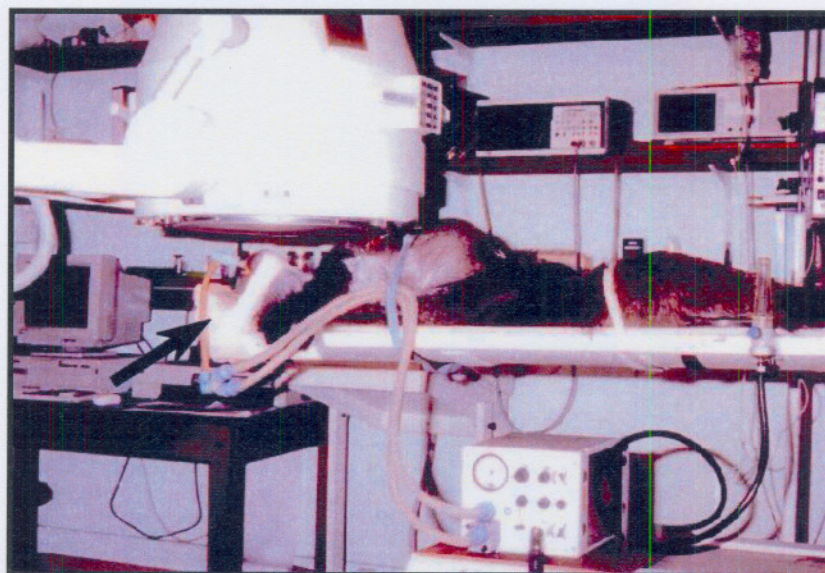
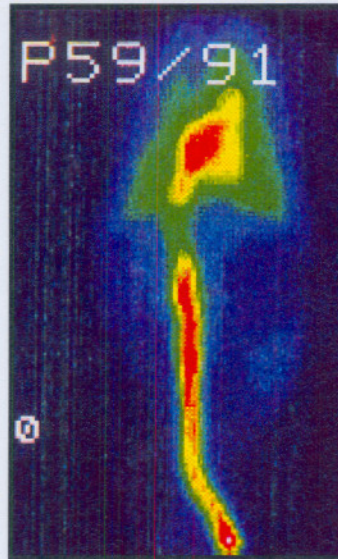


Figure 3-10 Baboon Supine Positioning for Cerebral blood Flow Studies

The baboons were positioned in the supine position with a special headrest (Figure 3-10 arrow) to ensure reproducible and comparable tomographic slices for all procedures (See Figure 3-10). Figure 3-11 shows the typical tracer peripheral biokinetics via the femoral artery to the heart and lungs, immediately after the intravenous injection of the radiotracer (i.e. first-pass transit). Note

the tracer presents itself primarily in the peripheral blood compartment during this first-pass transit and radioactivity is shown in the heart and lungs and the vascular system.



**Figure 3-11** Typical first-pass transit of the tracer from the femoral artery to the heart and lungs

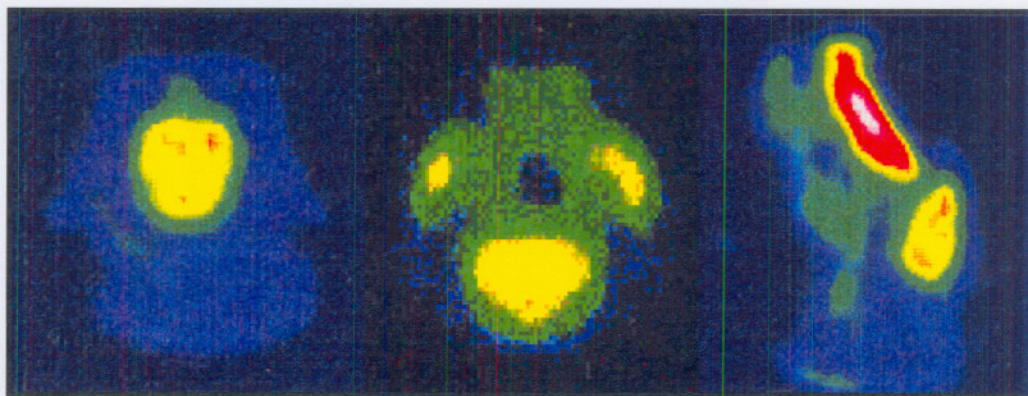
SPECT- 1 was followed by a second intravenous administration of radiotracer of double the dose of radioactivity when compared with the first administration at  $t = X$  minutes. The time  $t = X$  minutes depended on the particular intervention, as several aspects may influence this, e.g. the pharmacokinetic and pharmacodynamic properties of a particular test drug. After another 5 minutes (at  $t = X+5$  minutes) a similar SPECT acquisition, SPECT- 2 followed (the split dose method), which, for Procedure A, measured the anaesthesia related cerebral blood flow (CBF) changes taking place in between the two radiotracer administrations, without any intervention. Procedure A became the valuable control study of the model which largely incorporated anaesthesia effects on CBF. See Figure 3-8 for a typical time scale for the tracer and intervention procedures, with Procedure A the control, Procedure B a single drug intervention and Procedure C a drug-combination intervention. The intervention procedures allowed for the administration of the drugs with identical time scales as the controls to make comparisons meaningful. After back-projection and reconstruction of SPECT-1 and SPECT-2 data, the brain images in all procedures consisted of transaxial, sagittal and

coronal slices, representing global and regional CBF (rCBF) information. Four to eight slices of two to one pixel thickness respectively, each represented the brain in all three views, as mentioned above (Figures 3-12, 3-13).



**Figure 3-12** Cerebral blood flow slices of the baboon in the coronal view which resemble transaxial slices of the human brain

The coronal slices of the baboon brain were not aligned to brain axis, so that different cerebral regions were not properly defined. rCBF in this model refers to areas defined by particular slices. The coronal slices resembled transaxial slices of the human brain. Transaxial and coronal slices were aligned perpendicularly to each other. Regions of interest were placed over the total brain, in each slice.



**Figure 3-13** Typical sagittal (right), transaxial (middle) and coronal (left) views of the baboon brain

**Left:** Sagittal slices measured from right to left of the brain.

**Middle:** Transaxial slices measured from the occipital to the frontal lobes.

**Right:** Coronal slices measured from the cerebellum to the dorsal slice of the cerebrum (see Figure 3-12)

During all above procedures, arterial blood pressures were recorded from a catheter in the femoral artery. Heart rates were monitored as well as blood gasses (CO<sub>2</sub> and O<sub>2</sub>).

In conclusion therefore, the model is a non-human primate under anaesthesia subjected to CBF measurements using the SPECT split-dose modified methodology to assess pharmacological interventions.

### 3.9 Statistical methods

The R-values for eight slices in transaxial, sagittal and coronal views of the SPECT studies covered in this thesis, were compared between control and drug interventions and between the various drug interventions themselves, and in single drug and drug combination studies. A two-tailed Student's *t*-test for paired variables was used, with a 5% level of confidence, to establish statistical significant differences.

### 3.10 Studies conducted and discussed in this thesis

- **DORMEHL, I., REDELINGHUYS, F., HUGO, N., OLIVER, D.W. & PILLOY, W.** 1992. The Baboon model under anaesthesia for in vivo cerebral blood flow studies using single photon emission computed tomographic (SPECT) techniques. *Journal of medical primatology*, 21:270-274. **Chapter 4**
- **DORMEHL, I., OLIVER, D.W. & HUGO, N.** 1993. Dose response from pharmacological interventions for CBF changes in a baboon model using <sup>99</sup>Tc<sup>m</sup>-HMPAO and SPECT. *Nuclear medicine communications*, 14: 573-577. **Chapter 5**
- **OLIVER, D.W., DORMEHL, I., REDELINGHUYS, F., HUGO, N., & PILLOY, W.** 1993. Drug Effects of Cerebral Blood Flow in the Baboon Model - Acetazolamide and Nimodipine. *Nuklear Medizin/Nuclear Medicine*, 32:292-298. **Chapter 6**

- OLIVER, D.W., DORMEHL, I. & HUGO, N. 1994. Effect of Sumatriptan on Cerebral Blood Flow in the Baboon Model. *Arzneimittel Forschung/Drug Research*, 44, 925-928. **Chapter 7**
- DORMEHL, I.C., OLIVER, D.W. & HUGO, N. 1995. Cerebral Blood Flow effects of Sumatriptan in Drug Combinations in the Baboon Model. *Arzneimittel Forschung/Drug Research*, 45 (9):952-956. **Chapter 8**
- DORMEHL, I.C., OLIVER, D.W., HUGO, N. & ROSSOUW, D. 1995. A Comparative Cerebral Blood Flow Study in a Baboon Model with Acetazolamide Provocation:  $^{99m}\text{Tc}$ -HMPAO vs.  $^{123}\text{I}$ (IMP). *Nuclear medicine and biology*, 22(3):373-378. **Chapter 9**
- DORMEHL, I.C., OLIVER, D.W. HUGO, N., LANGEN, K-J. & CROFT, S. 1997. Technetium-99m-HMPAO, Technetium-99m-ECD and Iodine-123-IMP Cerebral Blood Flow Measurements with Pharmacological Interventions in Primates. *The journal of nuclear medicine*, 38(12):1897-1907. **Chapter 10**
- OLIVER, D.W. & DORMEHL, I.C. 1998. Cerebral Blood Flow Effects of Sodium Valproate and its Drug Combinations in the Baboon Model. *Arzneimittel Forschung/Drug Research*, 48(11):1058-1063. **Chapter 11**
- OLIVER, D.W. & DORMEHL, I.C. 1999. Cerebral Blood Flow Effects of the Nitric Oxide Donor, Nitroglycerin and its Drug Combinations in the Non-Human Primate Model. *Arzneimittel Forschung/Drug Research*, 49:732-739 (1999). **Chapter 12**
- OLIVER, D.W. & DORMEHL, I.C., LOUW, W., MORETTI, J-L. & KILLIAN, E. 2000. Effect of Cyclodextrin Complexation on the *in vivo* Disposition of the Brain Imaging Radiopharmaceutical,  $^{99m}\text{Tc}$  Ethyl Cysteinate Dimer ( $^{99m}\text{Tc}$ -ECD). *Arzneimittel Forschung/Drug Research*, 50:75-81. **Chapter 13**

Apart from the studies listed above, and others listed in the Appendix using the split-dose method, there were several human and non-human studies that followed this approach since this baboon model was developed. These

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studies include Dormehl *et al.* (1993); Hashikawa *et al.* (1994); Trucco *et al.* (1994); Ohnishi *et al.* (1997); Van Laere *et al.* (2000); Imaizumi *et al.* (2002); and Clauss *et al.* (2002). These studies were mainly performed in humans, except the studies from Dormehl *et al.* (1993) and Clauss *et al.* (2001, 2002), which were performed in non-human primates.

The reader is also referred to the thesis of Jordaan (1998), and dissertations of Forsyth (1994), Venter (2003) and Cruywagen (2003) for further reading on the topics addressed in this chapter.

## References

**ABBOTT, N., CHUGANI, D.C., ZAHARCHUK, G., ROSEN, B.R. & LO, E.H.** 1999. Delivery of imaging agents into brain. *Advanced Drug Delivery Reviews*, Apr 5;37(1-3):253-277.

Amershamhealth.com 2003: <http://www.amershamhealth.com> 30 June 2003

**ARANO, Y.** 2002. Recent advances in 99mTc radiopharmaceuticals. *Annals of Nuclear Medicine*, 16(2):79-93.

**ASENBAUM, S., BRUCKE, T., PIRKER, W., PIETRZYK, U. & PODREKA, I.** 1998. Imaging of cerebral blood flow with technetium-99m-HMPAO and technetium-99m-ECD: a comparison. *Journal of nuclear medicine* 39(4):613-618.

**BREWSTER, M.E. & LOFTSSON, T.** 2002. The use of chemically modified cyclodextrins in the development of formulations for chemical delivery systems. *Pharmazie*, 57(2):94-101.

**CAMARGO, F., ERICKSON, R.P., GARVER, W.S., HOSSAIN, G.S., CARBONE, P.N., HEIDENREICH, R.A. & BLANCHARD, J.** 2001. Cyclodextrins in the treatment of a mouse model of Niemann-Pick C disease. *Life Sciences*, 70(2):131-142.

**CLAUSS, R.P., DORMEHL, I.C., OLIVER, D.W., NEL, W.H., KILIAN, E. & LOUW, W.K.A.** 2001. Measurement of cerebral perfusion after zolpidem administration in the baboon model. *Arzneimittel Forschung/Drug Research*, 51 (8), 619-622.

**CLAUSS, R.P., DORMEHL, I.C., KILIAN, E., LOUW, W.K., NEL, W.H. & OLIVER, D.W.** 2002. Cerebral blood perfusion after treatment with zolpidem and flumazenil in the baboon. *Arzneimittel-Forschung/Drug Research*, 52(10):740-744.

**CONNOLLY, C.I.** 1950. (*In* External morphology of the primate brain. Springfield, USA: Charles C. Thomas Co. p. 3).

- DORMEHL, I.C., LIPP, M.D., HUGO, N., DAUBLAENDER, M. & PICARD, J.A.** 1993. Influence of intravenously administered lidocaine on cerebral blood flow in a baboon model standardized under controlled general anaesthesia using single-photon emission tomography and technetium-99m hexamethylpropylene amine oxime. *European journal of nuclear medicine*, 20(11):1095-1098.
- ELFVING, B., BJØRNHOLM, B. & KNUDSEN, F.** 2003. Interference of anaesthetics with radioligand binding in neuroreceptor studies. *European Journal of Nuclear Medicine and Molecular Imaging*, 30(6):912-915.
- FORSYTH, O.** 1995. Dissertation University of Pretoria "The quality assurance of Technitium labelled radiopharmaceuticals"
- GOPAL, B.S.** 1998. Fundamentals of nuclear pharmacy. 4<sup>th</sup> ed. New York: Springer p36-45
- GROISELLE, C., ROCCHISANI, J.M. & MORETTI, J-L.** 2000. Improving the measurement of the 99Tc(m)-ECD brain perfusion index by temporal analysis. *Nuclear medicine communications* 21(9):811-816.
- HASHIKAWA, K., MATSUMOTO, M., MORIWAKI, H., OKU, N., OKAZAKI, Y., UEHARA, T., HANDA, N., KUSUOKA, H., KAMADA, T. & NISHIMURA, T.** 1994. Split dose iodine-123-IMP SPECT: sequential quantitative regional cerebral blood flow change with pharmacological intervention. *Journal of nuclear medicine*, 35(7):1226-1233.
- HILL, W.C.O.** 1970. (*In Primates: comparative anatomy and taxonomy, volume 8: cynopithecinae.* New York: Wiley Interscience. p. 5).
- HOLM, S., MADSEN, P.L., SPERLING, B. & LASSEN, N.A.** 1994. Use of 99mTc-bicisate in activation studies by split-dose technique. *Journal of cerebral blood flow and metabolism*, 14 Suppl 1:S115-120.
- HYUN, Y., LEE, J.S., RHA, J.H., LEE, I.K., HA, C.K. & LEE, D.S.** 2001. Different uptake of 99mTc-ECD and 99mTc-HMPAO in the same brains: analysis by statistical parametric mapping. *European journal of nuclear medicine*, 28(2):191-197.

**IMAIZUMI, M., KITAGAWAM K., HASHIKAWAM K., OKUM N., TERATANIM T., TAKASAWA, M., YOSHIKAWA, T., RISHU, P., OHTSUKI, T., HORI, M., MATSUMOTO, M. & NISHIMURA, T.** 2002. Detection of misery perfusion with split-dose <sup>123</sup>I-iodoamphetamine single-photon emission computed tomography in patients with carotid occlusive diseases. *Stroke*, 33(9):2217-2223.

**JASZCZAK, R.J., MURPHY, P.H. & HUARD, D,** et al. 1977. Radionuclide emission computed tomography of the head with <sup>99m</sup>Tc and a scintillation camera. *Journal of nuclear medicine*, 18:373-380.

**JORDAAN, B.** 1996. Thesis PU vir CHO "The in vivo effects of piracetam, pentifylline and nicotinic acid on the cerebral blood flow in a primate model and assessment in humans"

**KEYES, J.W. JR., ORLANDEA, N. & HEETDERKS, W.J.** et. al. 1977. The humongotron: a scintillation-camera transaxial tomograph. *Journal of nuclear medicine* , 18:381-387.

**KOYAMA, M., KAWASHIMA, R., ITO, H., ONO, S., SATO, K., GOTO, R., KINOMURA, S., YOSHIOKA, S., SATO, T. & FUKUDA, H.** 1997. SPECT imaging of normal subjects with technetium-<sup>99m</sup>-HMPAO and technetium-<sup>99m</sup>-ECD. *Journal of nuclear medicine*, 38(4):587-592.

**LE GROS CLARK, W.E.** 1960. (*In* The antecedents of man: an introduction to the evolution of the primates. Chicago, USA: Quadrangle Books. p. 262).

**LO, J-M., HUANG, W-T., KAO, C-H. & YANG, C-S.** 2001. Mechanism of <sup>99m</sup>Tc-d,l-HMPAO Retention in Brain Cells. *Annals of nuclear medicine*, 14 215-222.

**MACK, W.J., KING, R.G., HOH, D.J., COON, A.L., DUCRUET, A.F., HUANG, J., MOCCO, J., WINFREE, C.J., D'AMBROSIO, A.L., NAIR, M.N., SCIACCA, R.R. & CONNOLLY, E.S. JR.** 2003. An improved functional neurological examination for use in nonhuman primate studies of focal reperfused cerebral ischemia. *Neurology research*, 25(3):280-284.

**MATSUDA, H., YAGISHITA, A., TSUJI, S. & HISADA, K.** 1995. A quantitative approach to technetium-99m ethyl cysteinate dimer: a comparison with technetium-99m hexamethylpropylene amine oxime. *European journal of nuclear medicine*, 22(7):633-637.

**MORETTI, J.L., CAGLAR, M. & WEINMANN, P.** 1995. Cerebral Perfusion Imaging Tracers for SPECT: Which One to Choose? *Journal of nuclear medicine*, 36: 359-363.

**OHNISHI, T., YANO, T., NAKANO, S., JINNOUCHI, S., NAGAMACHI, S., FLORES, L.I., NAKAHARA, H. & WATANABE, K.** 1997. Acetazolamide challenge and technetium-99m-ECD versus iodine-123-IMP SPECT in chronic occlusive cerebrovascular disease. *Journal of nuclear medicine*, 38(9):1463-1467.

**OPPENHEIM, B.E. & BECK, R.N.** 1976. (In Gottschalk, A., Potchen, E.J. eds. Golden's Diagnostic Radiology Section 20: Diagnostic Nuclear Medicine. Baltimore, USA: The Williams and Wilkins Company. p66-73)

**PANTANO, P., DI PIERO, V., RICCI, M., FIESCHI, C., BOZZAO, L. & LENZI G.L.** 1992. Motor stimulation response by technetium-99m hexamethylpropylene amine oxime split-dose method and single photon emission tomography. *European journal of nuclear medicine*, 19(11):939-945.

**RIECK, H., ADELWOHRER, C., LUNGENSCHMID, K. & DEISENHAMMER, E.** 1998. Discordance of technetium-99m-HMPAO and technetium-99m-ECD SPECT in herpes simplex encephalitis. *Journal of nuclear medicine*, 39(9):1508-1510.

**SIEMENS.** 1988. Operating instructions ORBITER™ detector stands model 6601. Publication #55 51 304 Revision B.

**SIENNICKI-LANTZ, A., LILJA, B. & ELMSTAHL, S.** 1999. How to interpret differing cerebral blood flow patterns estimated with 99Tcm-HMPAO and 99Tcm-ECD SPET in a healthy population. *Nuclear medicine communications*, 20(3):219-226.

**SLOSMAN AND MAGISTRETTI** 1997. Biological Basis of Cerebral Perfusion SPECT. (In De Deyn, P., Dierckx, R.A., Alavi, A. & Pickut, BA, eds. A Textbook of SPECT in Neurology and Psychiatry. Amsterdam, The Netherlands: John Libbey and Company. Chapter 62. p513-519).

**TRUCCO, M., CANANZI, C., SALVADORI, P.R. & BADINO, R.** 1994. Piroxicam-beta-cyclodextrin in induced migraine attacks: a SPECT study with Tc-99m HM-PAO split-dose method. *Functional neurology*, 9(5):247-257.

**VAN LAERE, K., VAN DER LINDEN, C., SANTENS, P., VANDEWALLE, V., CAEMAERT, J., IR, P.L., VAN DEN ABBEELE, D. & DIERCKX, R.** 2000. <sup>99</sup>Tc(m)-ECD SPET perfusion changes by internal pallidum stimulation in Parkinson's disease. *Nuclear medicine communications*, 21(12):1103-1112.

**VAN LAERE, K., DUMONT, F., KOOLE, M. & DIERCKX, R.** 2001. Non-invasive methods for absolute cerebral blood flow measurement using <sup>99m</sup>Tc-ECD: a study in healthy volunteers. *European journal of nuclear medicine* 28(7):862-872.

**VENTER, R.** 2003 Dissertation PU vir CHO "Haemodynamic changes induced by L-NAME and L-arginine in rats and primates measured with SPECT and planar scintigraphy"

**WYPER, D.J., HUNTER, R., PATTERSON, J., GOODWIN, G. & McCULLOCH, J.** 1991. A split-dose technique for measuring changes in cerebral blood flow patterns. *Journal of cerebral blood flow and metabolism*, (Suppl 2) 11:S449.

**YAKSH, T.L., JANG, J.D., NISHIUCHI, Y., BRAUN, K.P., RO, S.G. & GOODMAN, M.** 1991. The utility of 2-hydroxypropyl-beta-cyclodextrin as a vehicle for the intracerebral and intrathecal administration of drugs. *Life Sciences*, 48(7):623-633.

**ZERARKA, S., PELLERIN, L., SLOSMAN, D. & MAGISTRETTI, P.J.** 2001. Astrocytes as a predominant cellular site of (<sup>99m</sup>Tc)-HMPAO retention.

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*Journal of cerebral blood flow and metabolism*, 21(4):456-468.

# The Baboon model under anaesthesia for in vivo cerebral blood flow studies using single photon emission computed tomographic (SPECT) techniques \*

## Chapter 4

### Summary

*Single photon emission computed tomography of the brain could be useful in animal experimentation directed toward cerebral conditions. A well-established and understood baboon model, necessarily under anaesthesia, could be especially valuable in such investigations. Six normal baboons were studied under various anaesthetic agents and their combinations: ketamine, thiopentone, pentobarbitone, and halothane. Cerebral blood flow (CBF) studies were performed with  $^{99m}\text{Tc}$ -HMPAO. CBF effects from various anaesthesia were detected, requiring careful choice of the anaesthesia for cerebral investigations.*

**KEY WORDS:** primate model, CBF, SPECT imaging

### 4.1 Introduction

Single photon emission computed tomographic (SPECT) imaging of the brain to establish cerebral blood flow (CBF) patterns could provide valuable adjunctive information in those neurological diseases where blood flow imaging has diagnostic and prognostic value [3,7]. Patients with ischemic brain lesions, dementia, psychiatric disorders, or other neurological signs or symptoms may

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\* DORMEHL, I., REDELINGHUYS, F., HUGO, N., OLIVER, D.W. & PILLOY, W. 1992. *The Baboon model under anaesthesia for in vivo cerebral blood flow studies using single photon emission computed tomographic (SPECT) techniques. Journal of medical primatology, 21:270-274.*

be followed up with SPECT for disease progression and for monitoring the efficiency of pharmacological interventions [10]. Additionally, results of cerebral blood flow studies and metabolic studies can be matched [4].

The radiopharmaceutical hexamethylpropylene amine oxime ( $^{99m}\text{Tc}$ -HMPAO) is taken up rapidly in the brain tissue. It exhibits prolonged retention in the brain because of intracellular conversion to a hydrophilic compound that diffuses poorly across cell membranes [8, 9]. It has been validated as a marker of regional CBF (rCBF) although it is not linearly so dependent, and provides high-resolution static imaging of brain perfusion [1, 6, 13]. The pattern of its distribution is representative of the blood flow conditions during its injection, with no redistribution taking place.

The purpose of this study was the standardization of a baboon model for *in vivo* CBF studies using SPECT and  $^{99m}\text{Tc}$ -HMPAO. The baboon model necessitates the use of anaesthesia for the duration of the investigation. First and foremost then would be to establish the influence of various forms of anaesthesia on CBF patterns, bearing in mind the essential procedure of initial darting with ketamine hydrochloride and subsequent maintenance of the animal on long- or short-acting anaesthesia depending on the duration of the study. Ketamine, producing so-called dissociative anaesthesia, does not serve the last purpose well because of ensuing hallucinations, which result in undesirable involuntary movement. An established baboon model with the effects of anaesthesia determined and understood can be used to assess surgical and pharmacological interventions by SPECT imaging.

## 4.2 Materials and methods

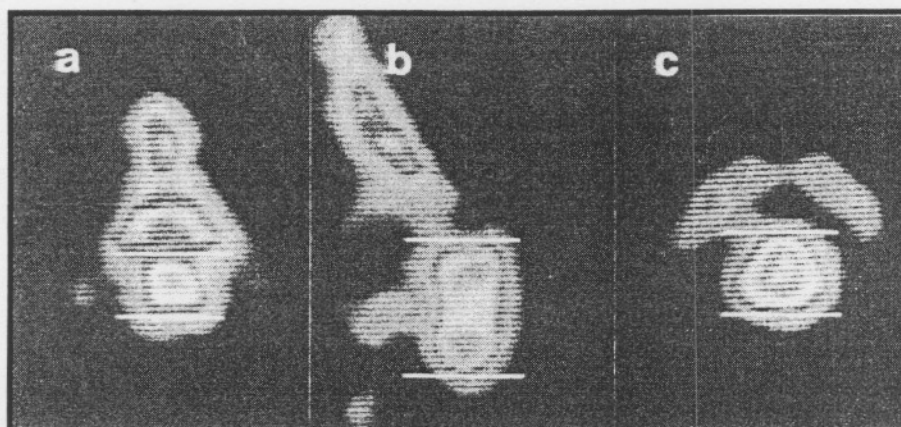
Six adult male baboons (*Papio ursinus*, average weight 27kg) were selected for this study. Anaesthesia was induced in each by darting with ketamine hydrochloride (Ketalar<sup>®</sup>, Parke-Davis, S.A.; 10mg/kg) and was followed immediately by an intravenous injection of  $^{99m}\text{Tc}$ -HMPAO (148 MBq), the distribution of which would then represent the effect of ketamine. Five minutes later the baboon was intubated, maintained, and controlled for the duration of the study under a second anaesthetic agent, which was alternatively

thiopentone sodium (Intraval<sup>®</sup>, Maybaker, S.A.), pentobarbitone sodium (Sagatal<sup>®</sup>, Maybaker, S.A.), or halothane (Fluothane<sup>®</sup>, Maybaker, S.A.).

The subsequent SPECT acquisition to obtain the HMPAO distribution in the brain during ketamine was done with a Siemens Orbiter gamma camera coupled to an A<sup>3</sup> MDS computer using 32 views and 360° (10 sec / view). Following the first acquisition, the baboon was reinjected with <sup>99m</sup>Tc-HMPAO (296 MBq) and tomographed to detect the radionuclide distribution during the anaesthesia of the second agent. Care was taken to administer the two HMPAO injections at its latest within 30 minutes after reconstitution with <sup>99m</sup>Tc. Baboons were viewed in a supine position with a special headrest to ensure a reproducible position for comparable tomographic slices.

Tomographic procedures took place for all six baboons, with the following combinations of anaesthesia. A) thiopentone-thiopentone, B) ketamine-thiopentone, C) ketamine-pentobarbitone, and D) ketamine-halothane, with thiopentone (in A) and ketamine (in B, C and D) being designed as baseline studies and thiopentone (in A and B), pentobarbitone (C) and halothane (D) as interventions. In the case of thiopentone-thiopentone the anaesthesia was first induced by darting with ketamine and was then immediately followed by, and maintained by a controlled i.v. infusion of thiopentone (70ml/hr of a 0.5% solution) using an administration (drip) set. After 30 minutes when the thiopentone blood levels predominated, <sup>99m</sup>Tc-HMPAO was injected to obtain, through SPECT, a distribution in the brain under the influence of thiopentone. The baboon remained on thiopentone anaesthesia for the second HMPAO administration and subsequent tomography. This study (A) formed a baseline reference to evaluate the effect of ketamine as would occur in procedures B, C, and D.

For procedure B thiopentone was maintained as described above. The pentobarbitone was maintained during procedure C with an infusion pump (30ml/hr of a 9 mg/ml solution). For the halothane procedure (D) 2% halothane / oxygen (Boyle's machine) was used for maintenance of anaesthesia.



**Figure 4-1** Typical tomographic brain slices in the coronal (a), sagittal (b), and transaxial (c) view, indicating the position of the regions of interest (ROI), i.e., the total brain between solid lines.

During all procedures the blood pressures (BP), heart rates (HR) and blood gases were monitored.

After backprojection and reconstruction the brain images consisted of transaxial, sagittal, and coronal slices representing CBF information during conditions prevailing under the various forms of anaesthesia. Sixteen transaxial slices represented the whole brain, of which every second slice was considered for count rate evaluation by the region of interest (ROI) feature. Similarly six sagittal slices and five coronal slices were selected to cover all of the brain. In subsequently placing the respective ROI's (Fig. 4-1, a, b, c) for count/pixel values of the brain slices care had to be taken to avoid the baboon sinus cavities and salivary glands.

Count rate data were then inserted into the following equation to obtain the ratio R

$$R = \frac{(Intervention\ 2nd\ anaesthesia) - (Baseline\ 1st\ anaesthesia)}{Baseline(1st\ anaesthesia)}$$

which is an indication of the level change of the rCBF during the second anaesthesia ("intervention") with respect to that during the first anaesthesia ("baseline") allowing for subtraction of retained activity from the first anaesthesia. For all three views graphs were plotted of R versus slice numbers starting at the occipital to frontal lobes transaxially, from right to left sagittally,

and from the cerebellum to the dorsal slice of the cerebrum coronally (see Fig. 4-2, a, b, c). The coronal slices were not aligned to the axis of the brain (Fig. 4-3), and they largely resemble transaxial slices of the human brain. The brain was then divided into four equal, not anatomically specific, regions or segments using the curves described in Figure 4-2 (divided into four segments along the abscissa) as guidelines. Relating to these representatives, regional blood flow values were obtained. Radioactive decay corrections were made throughout.



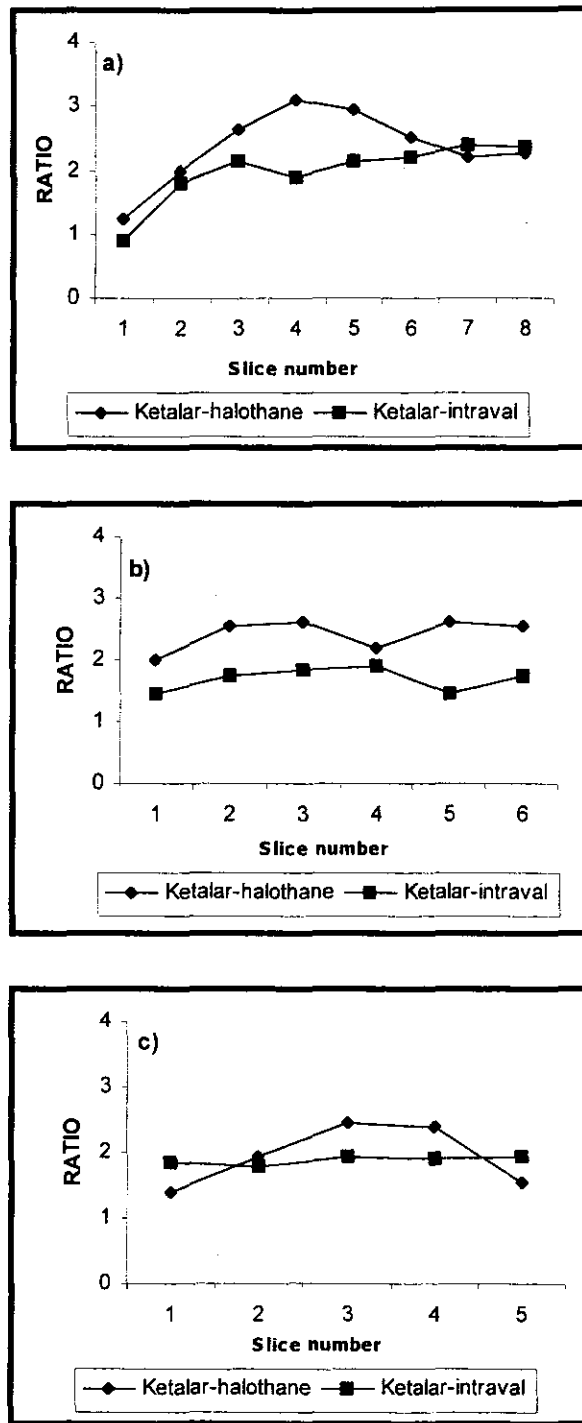
**Figure 4-2** The position of the four coronal slices of the baboon brain, in this study not aligned to brain axis, and resembling transaxial slices of the human brain.

### 4.2.1 Statistical methods

Mean ratios and standard deviations (SD) were evaluated for similar regions and for the total brain in the various projections as obtained from the baboons for the different procedures, and these were compared for procedural as well as regional effects. The comparisons were assessed for significant differences using Student's two-tailed *t*-test for paired observations.

## 4.3 Results

Tables 4-1, 4-2, and 4-3 present the mean ( $n = 6$ ) ratios (R) and SD obtained from the four brain regions as from Figure 4-2, and the total brain viewed respectively transaxially, sagittally, and coronally under the various conditions of anaesthesia.



**Figure 4-3** A set of typical curves of ratio (R) versus slice number starting at the occipital lobes to the frontal lobes transaxially (a), from right to left of the brain sagittally (b), and from the cerebellum to the dorsal slice of the cerebrum coronally (c).

The total brain ratios for the anaesthesia procedures A, B, and C to a large degree tend to approach, but not reach, the value 2, which would correspond with the second double dosage of  $^{99m}\text{Tc}$ -HMPAO and also agree to small CBF changes due to intraprocedural anaesthesia variations. For these three

procedures there are, furthermore, no statistically significant differences between the intraprocedural regional ratios ( $P > 0.05$ ), thus indicating no influence from A, B, and C on rCBF.

The values from ketamine-thiopentone and ketamine-pentobarbitone are significantly similar ( $P > 0.05$ ) as is to be expected since only the pharmacokinetic properties of thiopentone and pentobarbitone differ, with the latter the long acting barbiturate.

Procedure A ratios tend to be larger regionally and also for the total brain than those from procedures B and C, but the difference do not reach statistical significance ( $P > 0.05$ ). The prolonged maintenance (30 minutes) under thiopentone during this last procedure could possibly account for the ratios distinctly larger than 2. The barbiturates (BP 95, HR 108, pCO<sub>2</sub> 36, pO<sub>2</sub> 96) accumulate in the muscle and subsequently in the fatty tissue and an enhanced pCO<sub>2</sub> effect could result from their release during a prolonged study, leading to increased cerebral blood flow from additional vasodilatation (seen in the interventional phase of procedure A) in the absence of controlled ventilation [4].

**Table 4-1**, Mean ( $\pm$  SD) ratios from transaxial views of four equal cerebral regions and from the total brain region as obtained from different anaesthesia procedures

	Region 1	Region 2	Region 3	Region 4	Total Brain
<b>Procedure A</b>	2.27 $\pm$ 0.62	2.17 $\pm$ 0.36	2.16 $\pm$ 0.33	2.13 $\pm$ 0.22	2.18 $\pm$ 0.06
<b>Procedure B</b>	1.73 $\pm$ 0.87	1.88 $\pm$ 0.19	1.86 $\pm$ 0.37	1.95 $\pm$ 0.36	1.86 $\pm$ 0.09
<b>Procedure C</b>	1.82 $\pm$ 0.52	2.06 $\pm$ 0.35	1.92 $\pm$ 0.29	2.18 $\pm$ 0.36	2.00 $\pm$ 0.16
<b>Procedure D</b> <sup>a,b</sup>	2.21 $\pm$ 0.50	3.04 $\pm$ 0.18	2.82 $\pm$ 0.28	2.74 $\pm$ 0.31	2.70 $\pm$ 0.35

<sup>a</sup> Statistically significant changes ( $P < 0.05$ ) between region 1, and regions 2 and 3 for procedure D.

<sup>b</sup> Statistically significant changes ( $P < 0.05$ ) between procedure D, and procedures A, B, and C.

Ketamine hydrochloride, however, is known and was shown here to increase arterial blood pressure and heart rate (BP 145, HR 130, pCO<sub>2</sub> 38, pO<sub>2</sub> 61), leading to augmented CBF [5, 11], which in this study is indicated by ratios lower than 2 as obtained from procedure B and C (see tables).

**Table 4-2** Mean ( $\pm$  SD) ratios from sagittal views of four equal cerebral regions and from the total brain region as obtained from different anaesthesia procedures <sup>a</sup>

	Region 1	Region 2	Region 3	Region 4	Total Brain
<b>Procedure A</b>	2.35 $\pm$ 0.83	2.25 $\pm$ 0.36	2.30 $\pm$ 0.29	2.56 $\pm$ 0.67	2.37 $\pm$ 0.14
<b>Procedure B</b>	1.84 $\pm$ 0.29	2.10 $\pm$ 0.18	1.97 $\pm$ 0.39	1.83 $\pm$ 0.54	1.94 $\pm$ 0.13
<b>Procedure C</b>	2.06 $\pm$ 0.49	2.06 $\pm$ 0.42	1.82 $\pm$ 0.55	1.62 $\pm$ 0.38	1.89 $\pm$ 0.21
<b>Procedure D <sup>b</sup></b>	2.43 $\pm$ 0.70	2.90 $\pm$ 0.22	2.99 $\pm$ 0.19	2.86 $\pm$ 0.32	2.80 $\pm$ 0.25

<sup>a</sup> No regional statistical significant differences ( $P > 0.05$ )

<sup>b</sup> Statistically significant changes ( $P < 0.05$ ) between procedure D, and procedures A, B, and C.

Procedure D yields markedly higher ratios for the total brain and mostly also regionally, especially with respect to procedure B and C ( $P < 0.05$ ), but also with respect to procedure A, especially for the transaxial viewing. In addition, there is a decidedly regional influence from the halothane with statistically significantly increased ratios ( $P < 0.05$ ), between the first, second and third regions transaxially, and the first, second and third regions coronally. The transaxial results point to increased blood flow under halothane to the temporal lobes of the cerebrum compared to the frontal lobes.

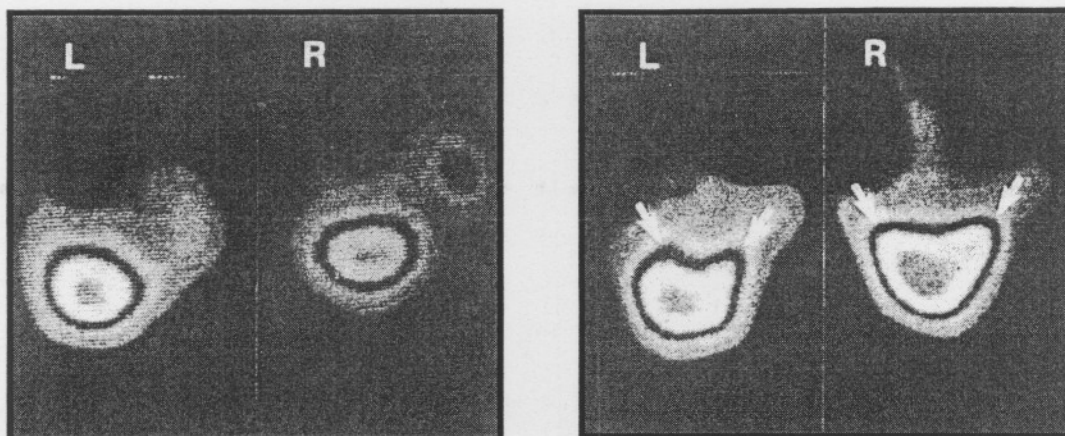
**Table 4-3** Mean ( $\pm$  SD) ratios from coronal views of four equal cerebral regions and from the total brain region as obtained from different anaesthesia procedures

	Region 1	Region 2	Region 3	Region 4	Total Brain
<b>Procedure A</b>	2.18 $\pm$ 0.48	2.08 $\pm$ 0.27	2.71 $\pm$ 0.90	2.47 $\pm$ 0.48	2.36 $\pm$ 0.29
<b>Procedure B</b>	1.91 $\pm$ 0.32	1.93 $\pm$ 0.23	1.92 $\pm$ 0.28	1.73 $\pm$ 0.59	1.87 $\pm$ 0.10
<b>Procedure C</b>	1.96 $\pm$ 0.24	2.04 $\pm$ 0.35	2.09 $\pm$ 0.45	2.30 $\pm$ 0.52	2.10 $\pm$ 0.15
<b>Procedure D <sup>a,b</sup></b>	2.20 $\pm$ 0.37	2.99 $\pm$ 0.34	2.99 $\pm$ 0.22	2.66 $\pm$ 0.51	2.71 $\pm$ 0.37

<sup>a</sup> Statistically significant changes ( $P < 0.05$ ) between region 1, and regions 2 and 3 for procedure D.

<sup>b</sup> Statistically significant changes ( $P < 0.05$ ) between procedure D, and procedures A, B, and C.

This redistribution of activity with procedure D is clear in Figure 4-4, which compares transaxial slices of the procedures B and D. In the coronal viewing the result also confirms an increase in CBF in the temporal lobes predominantly visible in the region 2 compared to the cerebellum, pons and medulla oblongata, which are viewed in region 1.



**Figure 4-4** Corresponding transaxial slices indicating the baseline HMPAO distribution (L) and the HMPAO distribution due to only the second anaesthesia (R), i.e., after baseline subtraction, in (a) for the ketamine-Intraval<sup>®</sup> combination (procedure B), and in (b) the same for the ketamine-halothane combination (procedure D). Note the changed distribution in (b) especially with respect to the enhanced temporal lobes (see arrows).

Hypotension and negative chronotropy are known often to result from halothane [2, 12] as also observed in this study (BP 100, HR 95, pCO<sub>2</sub> 33.3, pO<sub>2</sub> 454). Inhibition of the autoregulation of CBF in response to blood pressure changes leads to the vasodilatory effects of halothane with changed blood flow distribution to various organs and specifically decreased cerebral vascular resistance, which explains the increase CBF measured here.

#### 4.4 Conclusion

Brain SPECT with <sup>99m</sup>Tc-HMPAO in the baboon model confirms, and is therefore sensitive to, the effects of anaesthesia on CBF. In any animal experimentation involving cerebral drugs and/or surgical intervention, it is therefore imperative to do baseline investigations with the animals maintained only under the chosen anaesthetic agent for a time equal to experimental duration as part of the protocol. The effects of the ketamine-barbiturate combinations on cerebral blood flow have been explained in this experiment, and the conclusion is that these could be good selections of anaesthesia for the baboon model in cerebral experimentation.

## 4.5 References

1. **ANDERSON AR, FRIBERG HH, SCHMIDT JF, HASSELBACH SG.** 1988. Quantitative measurements of cerebral blood flow using SPECT AND [99m-Tc]-d,1-HM-PAO compared to xenon-133. *Journal of Cerebral Blood Flow and Metabolism* 8:S69-S81.
2. **DEUTSCH S, LINDE HW, DIRPPS RD, PRICE HL.** 1962. Circulatory and respiratory actions of halothane in normal man. *Anaesthesiology* 23:631-638.
3. **ELL PJ, JARRITT PH, COSTA DC, CULLUM ID, LUI D.** 1987. Functional imaging of the brain. *Sem Nuc Med* 17:214-229.
4. **KUSCHINSKY W.** 1991. Physiology of cerebral blood flow and metabolism. *Arzneimittel-Forschung* 41(1):284-288.
5. **LASSEN NA, CHRISTENSEN MS.** 1976. Physiology of cerebral blood flow. *British Journal of Anaesthesia* 48:719-734.
6. **LEONARD JP, NOWOTNIK DP, NIERINCKX RD.** 1986. A new radiopharmaceutical for imaging regional brain perfusion using SPECT – A comparison with iodine-123 HIPDM. *Journal of Nuclear Medicine* 27:1819-1823.
7. **LEVEILLE J, DEMOCEAU G, DE ROO M, RIGO P, TALLIEFER R, MORGAN RA, KUPRANICK D, WALOVITCH RC.** 1989. Characterization of 99m-Tc 1,1-ECD for brain perfusion imaging Part 2: Bio distribution and brain imaging in humans. *Journal of Nuclear Medicine* 30.
8. **NIERINCKX R, CANNING L, PIPER I, NOWOTNIK DP, PICKETT RD, HOLMES RA, COLKERT WA, FORSTER AM, WEISNER PS, MARRIOT JA, CHAPLIN SB.** 1987. Technetium-99m-d,1-HM-PAO. A new radiopharmaceutical for SPECT imaging of region cerebral blood perfusion. *Journal of Nuclear Medicine* 28:191-202.

9. **NIERINCKX RD, BURKE JF, HARRISON RC, FORSTER AM, ANDERSON AR, LASSEN NA.** 1988. The retention mechanism of technetium-99m-HM-PAO; Intracellular reaction with glutathione. *Journal of Cerebral Blood Flow Metabolism* 8:S4-S12.
10. **WALOVITCH RC, WILLIMAS FJ, LAFRANCE ND.** 1990. Radio-labeled agents for SPECT imaging of brain perfusion. *Nuclear Medicine and Biology* 17:77-83.
11. **WOOD M, WOOD AJJJ.** 1990. Inhalational anaesthetic agents. *Drugs Anaesth* 2:179-223
12. **WOOD M, WOOD AJJJ.** 1990. Inhalational anaesthetic agents. *Drugs Anaesth* 2:225-270.
13. **YONEKURA Y, NISHIZAWA S, MYKAI T, FUJITA T, FUKUYAMA H, ISHIKAWA M, KIKUCHI H, KONISHIM J, ANDERSON AR, LASSEN NA.** 1988. SPECT with [99mTc]-d,1-hexamethyl-propylene amine oxime (HM-PAO) compared with regional cerebral blood flow measured by PET: Effects of linearization. *Journal of Cerebral Blood Flow Metabolism* 8: S82-S89.

**Dose response from  
pharmacological interventions  
for CBF changes in a baboon  
model using  $^{99}\text{Tc}^m$ -HMPAO and  
SPECT\***

**Chapter  
5**

## Summary

*This study assesses the sensitivity of the baboon model under anaesthesia to determine by single photon emission computed tomography (SPECT) and  $^{99m}\text{Tc}$ -hexamethylpropylene amine oxime (HMPAO) dose responses from drugs (acetazolamide) with known regional cerebral blood flow (rCBF) effects on humans. Three dosages of acetazolamide were chosen: 250, 500 and 750 mg. The effects of these were studied by conventional SPECT 5 min after intravenous (i.v.) administration and compared to previous studies of rCBF with the baboons under anaesthesia only. An additional study concerned the effect of 500 mg acetazolamide at 15 min after administration. Haemodynamic parameters and blood gases were also monitored. No statistically significant regional effects were noted ( $P > 0.05$ ). The largest increase in CBF (39%) was observed from 500 mg acetazolamide after 5 min. This was statistically significantly different from control values only at a 10% level of confidence; then followed a 27% increase above control values after 750 mg (5 min). At 15 min 500 mg yielded values lower by 10% than the high dose. No effects were observed from 250 mg acetazolamide; only  $p\text{O}_2$  showed changes which largely confirm the CBF findings. The model did not give significant results at a 5% level of confidence but large fluctuations were observed, also in the haemodynamic and blood gas values. At a 10% level a significant dose response was confirmed for acetazolamide.*

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\* DORMEHL, I., OLIVER, D.W. & HUGO, N. 1993. Dose response from pharmacological interventions for CBF changes in a baboon model using  $^{99}\text{Tc}^m$ -HMPAO and SPECT. *Nuclear medicine communications*, 14: 573-577.

## 5.1 Introduction

A baboon (*Papio ursinus*) model has been developed to assess *in vivo* the effect of drugs on cerebral blood flow (CBF) and to an extent on regional CBF (rCBF) using  $^{99m}\text{Tc}$ -hexamethylpropylene amine oxime (HMPAO). This model [1], of necessity under anaesthesia, confirmed the influence of anaesthetic drugs on CBF, and prompted the use of anaesthesia by induction with ketamine hydrochloride and subsequent maintenance on a long- or short-acting barbiturate in a prerequisite control study for each experimental brain blood flow investigation. The success of the model to detect, over and above the anaesthesia, the effects of drugs with known CBF influences in humans, (acetazolamide, nimodipine) has likewise been established [2]. For pharmacological investigations the sensitivity of the model for allowing dose response determinations becomes important. This study therefore concerns the efficacy of the model for yielding a sensitive dose response to the administration of acetazolamide (Diamox<sup>®</sup>, S.A. Cyanamid (Pty) Ltd), a drug chosen for its present day wide application as a provocative intervention in nuclear medicine to facilitate diagnosis of stroke in patients and for its therapeutic use in patients with glaucoma and to a limited extent epilepsy [3]. In cases of glaucoma, acetazolamide inhibits the carbonic anhydrase to reduce the intraocular pressure [4]. Three dosages of acetazolamide were chosen for the investigation: 250, 500 and 750 mg. The effects of these were studied 5 min after intravenous (i.v.) administration. An additional study concerned the effect of 500 mg acetazolamide at a time interval of 15 min after administration [5].

## 5.2 Materials and methods

Six adult male baboons (average weight 27 kg) were used for the investigation. Each animal was subjected to five different procedures (A to E) with at least a 6-week interval between the consecutive procedures. Procedure A was the control study under anaesthesia only [1] in which induction was with ketamine hydrochloride ( $10 \text{ mg kg}^{-1}$  i.v) (Ketalar<sup>®</sup>, Parke-Davis, Cape Town) followed immediately by an i.v. injection of 148 MBq  $^{99m}\text{Tc}$ -HMPAO. Five

minutes later the animal was put on an infusion of thiopentone sodium ( $70 \text{ ml h}^{-1}$  of a 0.05% solution) (Intraval<sup>®</sup>, Sandos S.A., Randburg) with the first single photon emission computed tomography (SPECT) acquisition (SPECT-1) following after five more minutes. The data of SPECT-1 represent the HMPAO distribution (uptake and retention) in the brain resulting from CBF during ketamine sedation [6, 7]. This acquisition, using a Siemens Orbiter gamma camera with 32 projections during a  $360^\circ$  rotation and allowing 10 s per view, was immediately followed by a second i.v. administration of  $^{99}\text{Tc}^{\text{m}}$ -HMPAO (296 MBq, i.e. double the first dosage), the split-dose method [8, 9] and 5 min later by the second SPECT acquisition (SPECT-2) as before. These data represent a CBF pattern of HMPAO under thiopentone anaesthesia, but with a background from the first HMPAO distribution under ketamine.

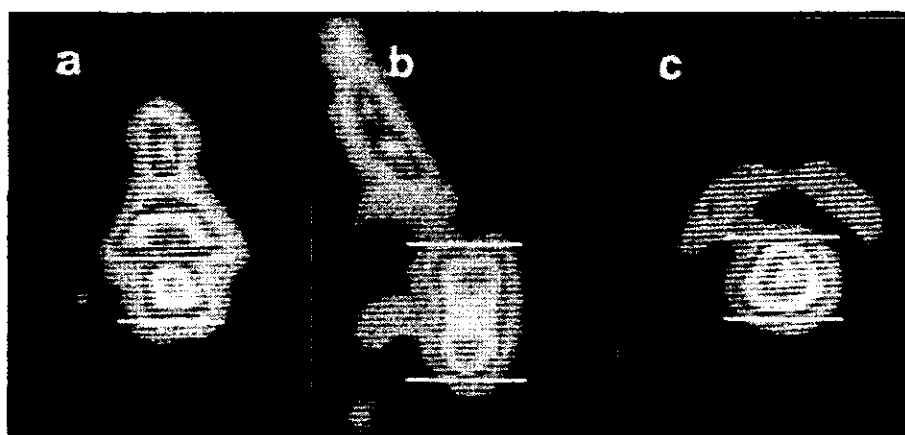
Procedures B to D followed the same protocol until the completion of the first SPECT acquisition (SPECT-1), but this was immediately followed by an i.v. injection of acetazolamide. For procedure B, 250mg acetazolamide was administered, for procedures C and D, 500 and 750mg, respectively. The protocol was continued for all three procedures 5 min later by a second HMPAO injection (296 MBq), to be followed after another 5 min by SPECT-2, of which the acquired data now also represent the effect on CBF patterns of the various doses of acetazolamide at a time 5 min after their administration [6-9]. Procedure E differed from the above acetazolamide procedures in that the second HMPAO injection (296 MBq) was administered 15 min after the acetazolamide injection (500 mg). The SPECT-2 data here represent the CBF pattern of HMPAO 15 min after the administration of 500 mg acetazolamide.

The split-dose method is based on the chemical properties of the tracer that crosses the blood-brain barrier and is trapped in brain cells. Radiochemical purity of HMPAO was consequently checked before its first application (within 5 min of preparation with a fresh eluate) for each procedure, and the lipophilic complex was never found to be below 90%. The second injection of HMPAO following the first by 15 to 30 min (depending on the procedure) was accompanied on a count down by a dynamic data acquisition (15 s per image for 4 min). The shape of the time-activity curve reflected the reduction of

lipophilic  $^{99}\text{Tc}^m$ -HMPAO, and was a determining factor for proceeding with SPECT-2.

Reproducible positioning of the animal for SPECT procedures allowing eventual comparisons was ensured by a special headrest with the baboon in the supine position.

Arterial blood pressure (BP), heart rates (HR) and blood gases ( $\text{pCO}_2$  and  $\text{pO}_2$ ) were also measured during each procedure (i.e. immediately before the administration of the acetazolamide ant then again 5 min thereafter for procedures B, C and D, and after 15 min for E).



**Figure 5-1** Typical tomographic brain slices in the (a) coronal, (b) sagittal and (c) transaxial views indicating the position of the regions of interest, i.e. the total brain between solid lines.

Following backprojection and reconstruction the brain images consisted of two sets each of the following compacted slices: eight transaxial slices, six sagittal slices and five coronal slices, the two sets representing rCBF patterns, respectively, from SPECT-1 (ketamine related) and SPECT-2 (related to thiopentone without or with the various dosages of acetazolamide). Regions of interest (ROIs) were placed on the brain slices from SPECT-1 and SPECT-2 data, in each view, for all the procedures, and count per pixel obtained from each slice (Fig. 5-1). These values for each slice were inserted into the following equation.

$$R = \frac{[SPECT - 2](counts / pixel) - [SPECT - 1] * (counts / pixel)}{SPECT - 1(counts / pixel)}$$

where R represents the level of change of rCBF during the second anaesthesia, thiopentone (without or with acetazolamide), with respect to that during ketamine (the first anaesthesia) after subtraction of the background (indicated by \*), having corrected for decay.

Not much anatomical structure was noted from the various slices. Further compacting of the slices led to their consolidation into four equal brain regions, 1 to 4, in each view, and to subsequent R-values relating to the regions. From the individual baboon values mean regional and total brain ratios with standard deviations (SD) were evaluated in each view for each of the procedures A to E, and compared for possible regional as well as procedural effects (Fig. 5-2). The comparisons were assessed for significant differences using Student's two-tailed *t*-test at 5 and 10% levels of confidence.

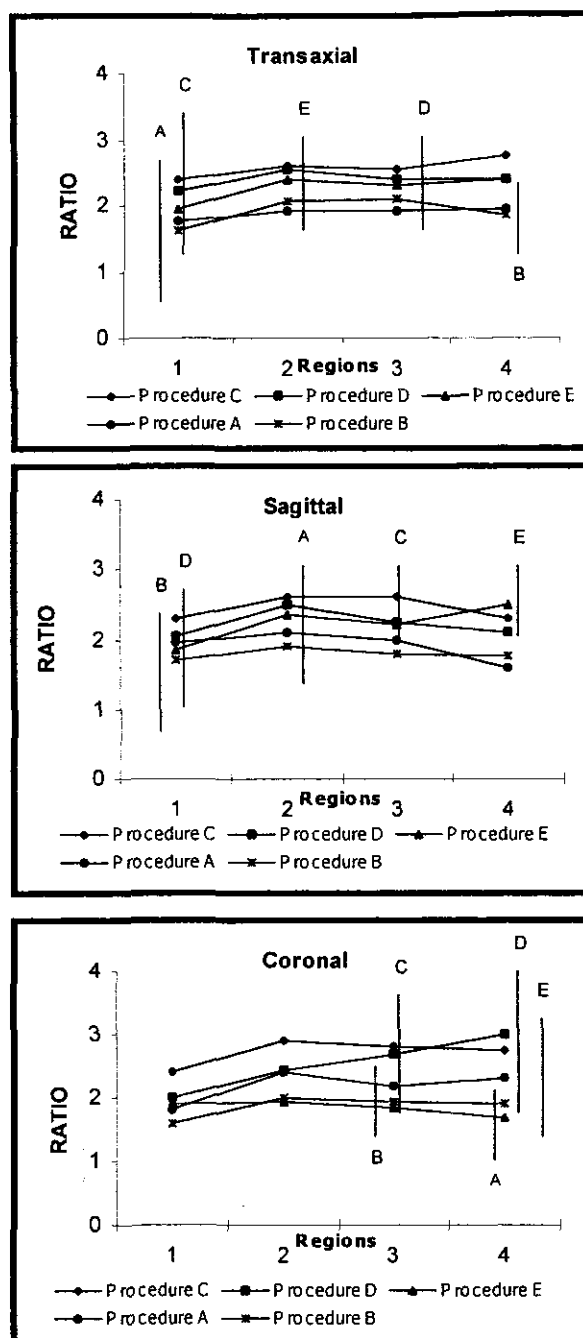
### 5.3 Results and discussion

The results are summarized in Tables 5-1 to 5-3 and in Fig. 5-2. Table 5-1 presents mean regional and total brain ratios,  $R \pm \text{SD}$  in each view for all the procedures. In Table 5-2 the mean percentage increases ( $\pm \text{SD}$ ) in R-values are given between the various procedures, indicating percentage CBF changes because of the drug interventions.

No statistically significant regional effects were noted in any of the procedures ( $P > 0.05$ ). This is well illustrated in Fig. 5-2 where none of the curves showed any notable shape change.

Values of two (i.e. 2) for R would indicate no difference in rCBF observed between SPECT-1 and background corrected SPECT-2 data, assuming that CBF is the largely dominant factor in the HMPAO cerebral distribution [6, 9, 10].

The average total brain values of R approximating 1.9 in all views for the procedure A control study indicate possibly only a slight increase in CBF because of the ketamine hydrochloride [1]. This does not change with the low dose of acetazolamide (procedure B, Fig. 5-2) confirming no statistically significant effect ( $P > 0.10$ ) on CBF from such a low dose measured 5 min after its injection (Table 5-2).



**Figure 5-2** Curves of ratio (R) versus region number starting at frontal lobes to the occipital lobes transaxially, from right to left of the brain sagittally, and from the cerebellum to the dorsal slice of the cerebellum coronally. The largest standard deviations for each procedure are correspondingly indicated.

Increased CBF as indicated by values of R larger than 2 was observed from procedure C (500 mg acetazolamide at 5 min post injection; Table 5-1, Fig. 5-2). The average percentage increase measured from the total brain in all views was  $39 \pm 7\%$  with respect to the control study values. This result is in accordance with our previous observations [2]. Although substantial, this

increase in CBF was only statistically significant ( $P < 0.05$ ) in the coronal view. At a 10% level of confidence, however, all three views transaxial, sagittal and coronal, had R-values for procedure C significantly higher than the control values. This observation is also true for comparisons between effects of procedure C with the low dose procedure B, where the average increase because of the 500 mg acetazolamide amounts to 37% (Table 5-2).

**Table 5-1** Mean (n = 6) regional and total brain ratios and standard deviations (R ± SD) obtained in the three tomographic views from the various procedures.

		Transaxial regions					Sagittal regions				
Procedure		1	2	3	4	Total	1	2	3	4	Total
		brain					brain				
A	R	1.73	1.88	1.86	1.94	1.85	1.84	2.10	1.97	1.63	1.88
	SD	0.87	0.19	0.31	0.36	0.36	0.29	0.80	0.39	0.51	0.41
B	R	1.52	2.04	2.03	1.94	1.87	1.58	1.89	1.82	1.72	1.80
	SD	0.28	0.54	0.41	0.53	0.33	0.72	0.62	0.55	0.58	0.46
C	R	2.37	2.58	2.50	2.71	2.57	2.23	2.58	2.56	2.23	2.40
	SD	0.94	0.72	0.58	0.61	0.66	0.57	0.57	0.64	0.53	0.49
D	R	2.09	2.59	2.41	2.42	2.37	1.99	2.45	2.24	2.12	2.30
	SD	0.05	0.39	0.51	0.44	0.40	0.92	0.74	0.74	0.64	0.59
E	R	1.93	2.36	2.26	2.43	2.24	1.90	2.29	2.13	1.70	2.00
	SD	0.46	0.60	0.48	0.50	0.45	0.33	0.60	0.56	0.67	0.33

		Coronal regions				
Procedure		1	2	3	4	Total
		brain				
A	R	1.91	1.93	1.92	1.73	1.87
	SD	0.32	0.23	0.28	0.59	0.30
B	R	1.61	1.94	1.98	1.80	1.88
	SD	0.58	0.46	0.67	0.29	0.41
C	R	2.23	2.80	2.72	2.66	2.63
	SD	0.52	0.83	0.84	0.34	0.51
D	R	1.93	2.36	2.67	2.90	2.46
	SD	0.72	0.50	0.66	0.99	0.59
E	R	1.80	2.41	2.20	2.29	2.21
	SD	0.56	0.56	0.66	0.77	0.51

**Table 5-2** Mean percentage cerebral blood flow (CBF) (total brain changes) and (SD) due to the various procedures.

With respect to baseline		Dose -dependent effects		Time and dose-dependent effects	
Procedures	%	Procedures	%	Procedures	%
A to B	2.0±1.7	B to C	37.0±1.5	B to E	20.0±3.0
A to C	39.0±7.0	B to D	27.6±2.0	C to E	-13.0±3.2
A to D	27.0±4.5	C to D	-8.6±1.5	E to D	10.3±5.0
A to E	18.3±10.7				

High R-values were also observed for procedure D (750 mg acetazolamide at 5 min; Table 5-1, Fig. 5-2). These were likewise elevated with respect to the control values pointing to an average CBF increase of  $27 \pm 4.5\%$ , i.e. not to the same extent as caused by procedure C. The CBF changes induced by procedure D do not differ statistically significantly from either control values, procedure B values or procedure C values ( $P > 0.05$ ). However, at a 10% level of confidence the increase of 27% in the CBF above the control values as obtained from procedure D was statistically significant, as was the difference with regard to the low dose values.

**Table 5-3** Mean (n = 6) haemodynamic and blood gas values (±SD) obtained from the various procedures respectively before (1) drug administration and as close to 5 min (or to 15 min for procedure E) after administration (2).

	Heart rate	Blood pressure	pCO <sub>2</sub>	PO <sub>2</sub>	
A <sub>1</sub>	106±10	112±42	46.73±2.31	69.50±2.33	(n = 6)
A <sub>2</sub>	107±12	118±39	45.17±2.33	75.93±6.77	
B <sub>1</sub>	121±23	114±31	45.83±4.63	70.07±8.50	
B <sub>2</sub>	122±27	122±38	44.67±5.15	76.41±11.08	
C <sub>1</sub>	117±13	117± 4	41.60±2.07	56.35±4.91	(n = 4)
C <sub>2</sub>	112±17	110± 5	40.08±4.87	76.43±7.47	
D <sub>1</sub>	117±21	131±32	45.47±3.84	67.82±4.24	
D <sub>2</sub>	116±24	133±39	47.95±2.05	70.75±9.86	
E <sub>1</sub>	118±10	111±27	41.23±6.70	66.83±6.65	
E <sub>2</sub>	114±20	110±15	42.00±4.50	82.37±3.29	

Although the effects from 500 mg acetazolamide at 15 min post injection (procedure E) were lower by 13% than those from procedure C, i.e. when measured at 5 min, the changes were not statistically significant ( $P > 0.10$ ). These effects were also not statistically significantly different from control values (+18%;  $P > 0.10$ ), and lower than procedure D values (10%) from the high dose of acetazolamide (750 mg) 5 min post injection.

The effects of acetazolamide used during the different procedures on the heart rate, blood pressure,  $\text{pCO}_2$  and  $\text{pO}_2$  in the arterial line are summarized in Table 5-3. The heart rate and  $\text{pCO}_2$  values remained constant for all the procedures. Previously it was observed that acetazolamide initially induced a decrease in blood pressure followed by a subsequent increase after 25 min of acetazolamide administration [2]. In the present study no significant changes were observed for procedure E either (the late, 15 min, study). Acetazolamide increased the arterial  $\text{pO}_2$  parameter. This increase was, however only statistically significant for procedure C (500 mg, 15 min) ( $P < 0.05$ ) and procedure E (500 mg, 5 min) ( $P < 0.05$ ). At a time 5 min after administration no significant differences in the  $\text{pO}_2$  were observed for procedures B and D. These results support previous suggestions that acetazolamide may have an effect on local cerebral oxygen metabolic rate [11, 12]. The  $\text{pO}_2$  effects are also dose dependent when the effects of the different dosages are compared. It was previously [5] reported that the maximum increase in CBF of humans was achieved 25 min after acetazolamide administration. However, the present study in the baboon indicates a maximum increase in CBF after 5 min, although the effect on the  $\text{pO}_2$  was found to last much longer. This further strengthens the suggestion that the vasodilatory properties of acetazolamide are unrelated to the inhibition of carbonic anhydrase [11, 12].

## 5.4 Conclusion

In the current study the baboon model previously described by us was evaluated for time-dose effect sensitivity on CBF using the drug acetazolamide. Although acetazolamide, an acidic drug, binds strongly to plasma albumin (85-95%), no measurable displacement of any albumin bound HMPAO is foreseen,

so as to change the availability of the lipophilic  $^{99m}\text{Tc}$ -HMPAO [5]. Acetazolamide is further tightly bound to carbonic anhydrase. Cells that are rich in carbonic anhydrase such as the erythrocytes and glial cells contain a higher concentration of acetazolamide than the plasma. Any possible acetazolamide-induced changes in blood cell labelling can only lead to small changes in blood radioactivity in the brain [13] since the volume of blood in the brain is small with respect to the total blood volume. Furthermore, no changes in blood-brain barrier permeability and cardiac output have been reported for acetazolamide that could account for the observed increase of HMPAO level in the cerebrovascular system. The study is therefore describing a model sensitive to monitoring dose effect interventions of CBF. In particular it was noted for acetazolamide that a low dosage (250 mg) show no effect while the high dosage (750 mg) induced changes in the CBF lower than those observed for a 500 mg dosage at 5 min after administration. The model also confirmed that this last procedure has an optimal effect since 15 min post injection the effect from 500 mg acetazolamide was again reduced.

## 5.5 References

1. **DORMEHL IC, REDELINGHUYS IF, HUGO N, OLIVER D, PILLOY W.** 1992. The baboon model under anaesthesia for *in vivo* cerebral blood flow studies using SPECT techniques. *Journal of Medical Primatology* 21:270-274.
2. **OLIVER DW, DORMEHL IC, REDELINGHUYS IF, HUGO N, BEVERLY G.** 1993. Drug effects on cerebral blood flow in the baboon model. Acetazolamide and Nimodipine. *Nuklear Medizin* 32:292-298.
3. **GOODMAN LS, GILMAN AG.** 1990. The pharmacological basis of therapeutics. (8<sup>th</sup> Edition. *In: Gilman AG, Rall Tw, Nies AS, Talor P, eds.* New York: Pergamon, 716-717).
4. **MITHOEFER J, MAYER P, STOCKS J.** 1957. Effect of carbonic anhydrase inhibition of the cerebral circulation of the anaesthetized dog. *Fed Proc* 16: 88-89.

5. **BONTE FJ, DEVONS MD, REISCH JS.** 1988. The effect of acetazolamide on regional cerebral blood flow in normal human subjects as measured by single photon emission computed tomography. *Investigative Radiology* 23: 564-568.
6. **BACCIOTTINI L, PUP A, FORMICONI AR, DE CRISTOFARO MTR, MELDOLESI U.** 1989. Regional cerebral blood flow measurement in rat with HM-PAO and the indicator fractionation technique. *Journal of Cerebral Blood Flow Metabolism* 9:S414.
7. **PUPI A, BISI G, SIAGRA R, SANTORO D, DE CRISTOFARO MTR, FORMICONI AR, MELDOLESI U.** 1989. The comparison of brain distribution of HM-PAO and microspheres in humans. *Journal of Cerebral Blood Flow Metabolism* 9:S411.
8. **WYPER DJ, HUNTER R, PATTERSON J, GOODWIN G, McCULLOCH J.** 1991. A split-dose technique for measuring changes in cerebral blood flow patterns. *Journal of Cerebral Blood Flow Metabolism* (Suppl 2) 11 :S449.
9. **HOLM S, MADSEN PL, RUBIN P, SPERLING B, FIRBERG L, LASSEN N.** 1991. Tc-99m HM-PAO activation studies: validation of the split-dose, image subtraction approach. *Journal of Cerebral Blood Flow Metabolism* (Suppl 2) 11 :S766.
10. **PANTANO P, DI PIERO V, RICCI M, FIESCHI C, BOZZAO L, LENZI GL.** 1992. Motor stimulation response by technetium-99m HMPAO split-dose method and single photon emission tomography. *European Journal of Nuclear Medicine* 19:939-945.
11. **VORSTRUP S, HENRICKSEN L, PAULSON OB.** 1984. Effect of acetazolamide on cerebral blood flow and cerebral metabolic rate for oxygen. *Journal of Clinical Investigation* 74:1634-1639.
12. **LAUX BE, RAICHLE ME.** 1978. The effect of acetazolamide on cerebral blood flow and oxygen utilization in the rhesus monkey. *Journal of Clinical Investigation* 62:585-592.

13. NEIRINCKX RD, CANNING LR, PIPER IM, NOWOTNIK DP, PICKETT RD, HOLMES RA, VOLKERT WA, FORSTER AM, WEISNER PS, MARRIOTT JA, CHAPLIN SB. 1987. Technetium-99m d,l-HM-PAO; a new radiopharmaceutical for SPECT imaging of regional cerebral blood perfusion. *Journal of Nuclear Medicine* 28:191-202.

## Drug Effects of Cerebral Blood Flow in the Baboon Model - Acetazolamide and Nimodipine \*

## Chapter 6

### Summary

*The sensitivity of the baboon model under anaesthesia for single photon emission computed tomography (SPECT) of the brain with  $^{99m}\text{Tc}$ -HMPAO, as recently developed by us to study cerebral blood flow patterns, was investigated using drugs that are known to increase cerebral blood flow, e.g. acetazolamide, the carbonic anhydrase inhibitor and nimodipine, the calcium channel blocker. Increases in cerebral blood flow for both acetazolamide and nimodipine were observed that correspond well with other studies. Statistically significant regional specificity was noted for acetazolamide and nimodipine. Interestingly, a combination of these drugs did not enhance cerebral blood flow but rather decreased it in comparison with the individual drug responses. The results were correlated with arterial blood pressure, heart rate,  $\text{pCO}_2$  and  $\text{pO}_2$ . A blood pressure decrease was noted for both drugs, while acetazolamide had a marked influence on  $\text{pO}_2$ . The results indicate that the baboon model is sensitive for evaluation of drug effects on cerebral blood flow.*

**KEY WORDS:** SPECT, acetazolamide, nimodipine, blood flow, baboon model

### 6.1 Introduction

Single photon emission computed tomography (SPECT) has fully established itself as a useful technique for brain perfusion imaging [21].  $^{99m}\text{Tc}$ -labelled radiopharmaceuticals are experiencing an ever-increasing role in the

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non-invasive diagnosis of cerebral diseases (cerebral ischemia, dementia, epilepsy) and find application for subsequent monitoring of pharmacological interventions [18,21].

The design and development of new pharmaceuticals that cross the blood brain barrier and show cerebral vascular smooth muscle and regional selectivity still pose a challenge. Animal models have been useful in the evaluation of promising novel structures for efficacy and toxicological information, particularly those that act on the central nervous system influencing cerebral blood flow (CBF) patterns. Results from such in vivo animal studies can provide important information in the development of new pharmaceuticals. Non-human primates are phylogenetically close to man and consequently facilitate meaningful extrapolation of results [8]. A baboon model for SPECT brain imaging with  $^{99m}\text{Tc}$ -HMPAO has been developed and found to be sensitive to the effects of anaesthesia on CBF [4]. Anaesthesia is necessary for restraining the baboon during prolonged scintigraphy. The combination of ketamine and a barbiturate, with the ketamine used for darting of the animal, proves to be the anaesthesia of choice in CBF and regional CBF (rCBF) studies [4].

The purpose of the current study was to evaluate the sensitivity of the baboon model under anaesthesia using drugs that are known to increase cerebral blood flow, e.g. Acetazolamide [1-3, 7, 16] and nimodipine [6, 11, 12, 19]. Additionally, with the effects from these established, the cerebrovascular dilatory response from a combination of acetazolamide and nimodipine was evaluated for possible beneficial implications.

## 6.2 Materials and methods

Six adult male baboons (*Papio ursinus*, average weight 27kg) were selected for this study. Anaesthesia was induced in each by darting with ketamine hydrochloride (Ketalar<sup>®</sup>, Parke-Davis, S.A.; 10 mg/kg) and was followed immediately by an intravenous injection of  $^{99m}\text{Tc}$ -HMPAO (148 MBq), the cerebral distribution of which would then represent the effect of ketamine with no redistribution taking place. Five minutes later the baboon was intubated, maintained and controlled for the duration of the study under sodium

thiopentone (Intraval<sup>®</sup>, Maybaker, S.A.; 70 ml/h of a 0.5% solution). The subsequent SPECT acquisition to obtain the HMPAO distribution in the brain during ketamine was done with a Siemens Orbiter gamma camera coupled to an A<sup>3</sup> MDS computer using 32 views and 360° (10 sec/view). Following the first acquisition the baboon was reinjected with <sup>99m</sup>Tc-HMPAO (296 MBq) and tomographed to detect the radionuclide distribution attained during what was then thiopentone anaesthesia. Care was taken to administer the two HMPAO injections at its latest within 30 min after reconstitution. Baboons were viewed in a supine position with a special headrest to ensure a reproducible position for comparison of tomographic slices.

The above procedure of two successive tomographic scans representing the effects of the two different forms of the anaesthesia in each baboon constitutes the control studies, and is called procedure A.

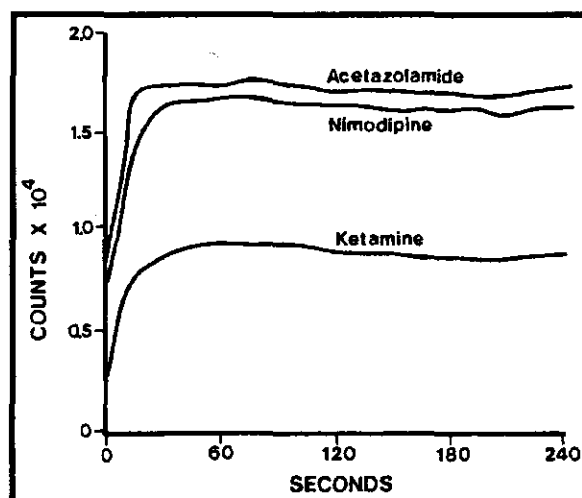
The effects of acetazolamide (Diamox<sup>®</sup>, S.A.; Cyanamid (Pty) Ltd) on cerebral blood flow were investigated (procedure B) in the following manner. The directions for the anaesthesia follow those for procedure A but the first SPECT acquisition was followed by an intravenous injection of 5 ml of acetazolamide (100 mg/ml), and only after another 5 min was the second double dose (296 MBq) administration of <sup>99m</sup>Tc-HMPAO given, allowing adequate blood levels of acetazolamide to be reached. The animal was subsequently tomographed, according to procedure A, to obtain a tracer distribution representative of the effect of acetazolamide when compared to procedure A.

Procedure C tested the effects of nimodipine (Bayer, Leverkusen) on the CBF of the baboon in a similar way as performed in procedure B, except that the first SPECT acquisition of the ketamine related cerebral distribution of <sup>99m</sup>Tc-HMPAO (148 MBq) was followed by a very slow infusion (over 15 min) of nimodipine (1 µg/kg/min) [15] taking care to use only PVC-free tubing or a stainless steel needle in the administration to avoid any absorption resulting in a decrease in the concentration of nimodipine [10]. After 10 min of nimodipine infusion the second injection of <sup>99m</sup>Tc-HMPAO (296MBq) followed, and

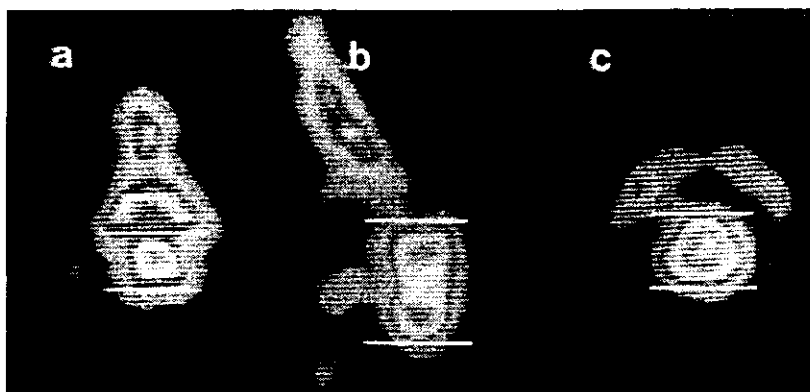
tomography started after another 5 min at the time that the infusion was terminated [14, 15].

Procedure D investigated the effect of a combination administration of 5 ml acetazolamide (100 mg/ml) and nimodipine (1  $\mu$ g/kg/min). The first SPECT acquisition was, as with procedure C, followed by a very slow infusion (over 15 min) of nimodipine. Five minutes after starting the infusion the acetazolamide was injected, and the  $^{99m}\text{Tc}$ -HMPAO injection followed after yet another 5 min. Tomography began 5 min later when the infusion was stopped, and the HMPAO distribution then is, as in procedure B, representative of a combination situation 5 min after the injection of acetazolamide, and 10 min after the start of the nimodipine (see procedure C). The arterial blood pressures were recorded during all the procedures from a catheter in the femoral artery. Heart rates were also monitored as well as blood gases from an arterial line.

In order to check the possible influence of the two drugs on the  $^{99m}\text{Tc}$ -HMPAO input, a dynamic scintigraphic study was done on a count-down of isotope administration during ketamine anaesthesia (the control), again after acetazolamide, and eventually after nimodipine, before the above described SPECT acquisitions in each case were performed. Sixteen images of 15 sec were acquired in 64 x 64 word mode, and the subsequent time activity curves compared for differences (Fig. 6-1).



**Figure 6-1** Time-activity curves from the dynamic acquisition on count-down indicating uptake of  $^{99m}\text{Tc}$ -HMPAO under anaesthesia only (control), under acetazolamide, and nimodipine (the latter two from double dosages of HMPAO).



**Figure 6-2 a,b,c** Typical tomographic brain slices in the coronal (a), sagittal (b) and transaxial (c) view indicating the regions of interest (ROI) i.e. the total brain between solid lines

### 6.2.1 Statistical methods

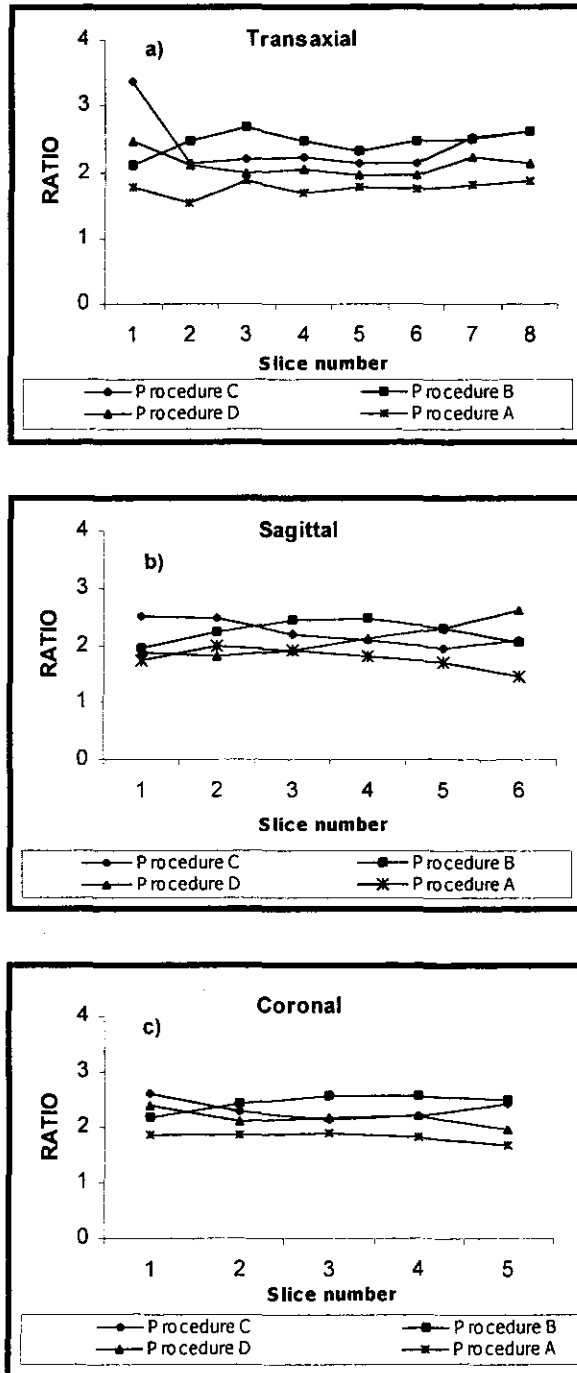
Mean ratios ( $n = 6$ ) and standard deviations (SD) were calculated per procedure for similar regions and for the total brain in the various projections. These were compared for interprocedural effects as well as regional effects for a particular procedure. The comparisons were assessed for significant differences using Student's two-tailed  $t$ -test.

### 6.3 Results

After backprojection and reconstruction the brain images consisted of transaxial, sagittal and coronal slices representing CBF and rCBF information during conditions prevailing under the procedure of anaesthesia only and under various drug interventions. Eight transaxial slices represented the whole brain, each of which was considered for count rate evaluation by the ROI (region of interest) feature. Similarly six sagittal slices and five coronal slices covering all of the brain were selected and analysed. In subsequently placing the respective ROIs (Fig. 6-2a, b, c) for count rate/pixel values of the brain slices, care had to be taken to avoid the baboon sinus cavities and salivary gland. Count rate data were then inserted into the following equation to obtain the ratio  $R$ ,

$$R = \frac{[Intervention](2^{nd} \text{ tracer injection}) - [Baseline](1^{st} \text{ tracer injection})}{Baseline(1^{st} \text{ tracer injection})}$$

which is an indication of the level change of the CBF during the drug intervention with respect to that attained during ketamine anaesthesia (baseline). The equation allows for subtraction of retained activity originating from the baseline study, (after decay correction [\*]).

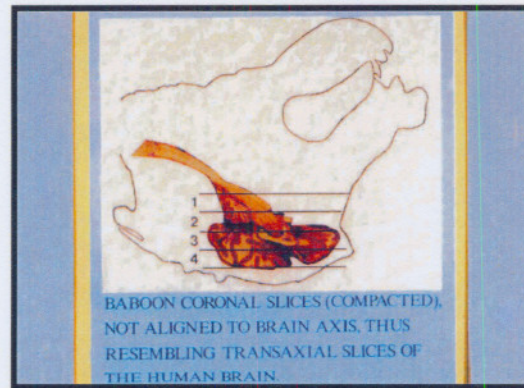


**Figure 6-3a,b,c** Curves of mean ratios (n = 6) vs. slice number starting at the occipital lobes to the frontal lobes transaxially (a), from the right to the left of the brain sagittally (b) and from the cerebellum to the dorsal slice of the cerebrum coronally (c).

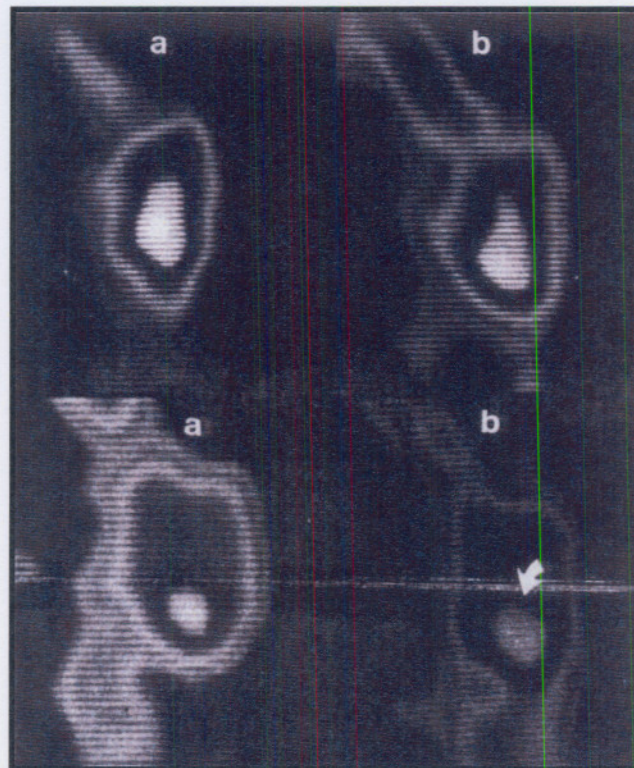
For all three views graphs were plotted of R vs. slice numbers starting at the occipital lobes to the frontal lobes transaxially, from right to left sagittally, and from the cerebellum to the dorsal slice of the cerebrum coronally (see curves in Fig. 6-3a, b, c). Contrary to human data the coronal slices were not aligned to the axis of the brain (Fig. 6-4). The brain was subsequently divided into four equal regions using the curves (Fig. 6-3) and equal segments taken along the abscissa. Representative regional blood flow ratios were thus obtained for these regions for each procedure. Ratios thus became available for the control (anaesthesia only) study (procedure A) and for the pharmacological procedures B, C and D.

The input functions for  $^{99m}\text{Tc}$ -HMPAO, from the dynamic studies under ketamine, acetazolamide and nimodipine do not differ (Fig. 6-1). Tables 6-1, 6-2 and 6-3 present the mean ( $n = 6$ ) ratios (R) and SD obtained for the 4 brain regions as derived from Fig. 6-3, and for the total brain viewed respectively transaxially, sagittally and coronally under anaesthesia only, and after the various drug interventions.

The effects of acetazolamide (carbonic anhydrase inhibitor), nimodipine (calcium channel blocker) and a combination on the heart rate, blood pressure,  $\text{pCO}_2$  and  $\text{pO}_2$  in the arterial blood are summarized in Table 5 for pre-injection and post injection (10 min) values. The heart rate remained constant for all procedures. Table 5 indicates a decrease in blood pressure from acetazolamide ( $P < 0.05$ ) and nimodipine ( $P > 0.05$ ) but the combination showed no effect. The  $\text{pCO}_2$  values indicate no influence from any of the procedures. Acetazolamide and the drug combination procedure significantly increased the arterial  $\text{pO}_2$  ( $P < 0.05$ , for both).



**Figure 6-4** The position of the coronal regions (slices) of the baboon brain in this study not aligned to the brain axis, and resembling transaxial slices of the human brain.



**Figure 6-5** Corresponding sagittal slices for baseline (a) and intervention (b) acquisition obtained from the anaesthesia only experiment (procedure A: top) and (bottom) from the nimodipine experiment (procedure C). Note for the latter procedure enhanced cerebellum (arrow) after nimodipine administration. This effect is not shown with procedure A (top).

## 6.4 Discussion

The dynamic  $^{99m}\text{Tc}$ -HMPAO results (Fig4. 6-1) clearly show a consistency in the uptake function which allows us to draw conclusions on the isotope distribution in the brain and cerebral blood flow under the various drug interventions.

Failure of the total brain and regional brain ratios to yield a value 2, which would correspond to the second double dose of  $^{99m}\text{Tc}$ -HMPAO point to CBF and rCBF changes due to intraprocedural anaesthesia variations as in procedure A, and additionally to the effect of the drugs as in procedures B, C and D.

Ketamine hydrochloride is known and was shown here to increase arterial blood pressure and heart rate (Table 6-4) leading to augmented CBV [4], which is in this study indicated by lower ratios than 2 as were obtained from procedure A (see Tables 6-1 to 6-3).

For procedures A and B there are no statistically significant differences between the intraprocedural regional ratios ( $P > 0.05$ ), thus indicating no influence from procedure A and B on rCBF. Procedure B ratios tend to be larger regionally as well as for the total brain than those from procedure A (Tables 6-1 to 6-3); differences do not reach statistical significance ( $P > 0.05$ ) except for region 4 in the coronal view which is mainly a dorsal representation. The percentage increases (Table 6-4) corresponded well with Bonte *et al.* [2], but in addition a high percentage increase in coronal region 4 was noted. Procedure C demonstrates a significantly higher ratio ( $P < 0.05$ ) for the cerebellum, represented in the transaxial region 1, than for any other region (Table 6-1).

**Table 6-1** Mean (SD) ratios from transaxial views of four equal cerebral slices and from total brain

	Region 1	Region 2	Region 3	Region 4	Total brain
Procedure A	1.73 ± 0.87	1.88 ± 0.19	1.86 ± 0.37	1.95 ± 0.36	1.86 ± 0.45
Procedure B	2.37 ± 0.94	2.58 ± 0.72	2.56 ± 0.64	2.23 ± 0.61	2.44 ± 0.73
Procedure C	3.30 ± 0.73	2.31 ± 0.63	2.33 ± 0.60	2.55 ± 0.77	2.62 ± 0.68
Procedure D	2.51 ± 0.96	2.14 ± 0.47	2.13 ± 0.46	2.50 ± 0.71	2.32 ± 0.65

*Statistically significant differences for procedure C between region 1 and regions 2,3,4. Also between procedure A and C for region 1, ( $P < 0.05$ )*

Ratios from procedure C also tend to be consistently higher regionally, and for the total brain than for the anaesthesia only procedure A. However only

region 1 transaxially (cerebellum) show a significantly and quite dramatically higher value ( $P < 0.05$ ) (Tables 6-1 to 6-4, and Fig. 6-3 and 6-5).

**Table 6-2** Mean (SD) ratios from sagittal views of four equal cerebral slices and from total brain

	Region 1	Region 2	Region 3	Region 4	Total brain
Procedure A	1.84 ± 0.29	2.10 ± 0.18	1.97 ± 0.39	1.83 ± 0.54	1.94 ± 0.35
Procedure B	2.23 ± 0.57	2.58 ± 0.57	2.56 ± 0.64	2.23 ± 0.53	2.40 ± 0.58
Procedure C	2.73 ± 0.88	2.46 ± 0.63	2.30 ± 0.53	2.25 ± 0.92	2.44 ± 0.75
Procedure D	2.06 ± 1.47	1.99 ± 0.47	2.31 ± 0.67	2.89 ± 1.87	2.31 ± 1.12

No statistically significant differences

**Table 6-3** Mean (SD) ratios from coronal views of four equal cerebral slices and from total brain

	Region 1	Region 2	Region 3	Region 4	Total brain
Procedure A	1.91 ± 0.32	1.93 ± 0.23	1.92 ± 0.28	1.73 ± 0.59	1.8 ± 0.36
Procedure B	2.35 ± 0.52	2.80 ± 0.83	2.75 ± 0.84	2.66 ± 0.34	2.64 ± 0.63
Procedure C	2.68 ± 1.00	2.34 ± 0.60	2.40 ± 0.53	2.69 ± 0.78	2.53 ± 0.73
Procedure D	2.24 ± 1.00	1.99 ± 0.63	2.10 ± 0.76	1.96 ± 0.73	2.07 ± 0.78

Statistically significant differences between procedures A and B, region 4 ( $P < 0.05$ )

No significant differences could be demonstrated between procedures B and C ( $P > 0.05$ ). Procedure D ratios can be seen from Tables 6-1 to 6-3 and from the curves (Fig. 6-3) to be predominantly depressed with respect to procedures B and C, occasionally taking on a value in between.

**Table 6-4** Mean percentage (%) changes of ratios from the four brain regions (n = 4) for the three views, comparing the different procedures with each other

Procedures	Transaxial	Sagittal	Coronal
Procedures A-B	32	24	41 (4:54%)
Procedures A-C	42 (1:91%)	26	36
Procedures A-D	25	20	11
Procedures B-C	9	2	-3
Procedures B-D	-4	-3	-21
Procedures C-D	-10	-4	-18

The parentheses contains the region number which had statistically significantly changed ( $P < 0.05$ ); the corresponding percentage change is also indicated. Negative values point to a CBF decrease.

Although the regional ratios from procedure D are higher than from the anaesthesia only (procedure A), indicating some effect, no statistically significant difference can be demonstrated ( $P > 0.05$ ).

The percentage changes in Table 6-4 indicate that the three drug procedures increase the mean regional ratios, less so for the combination drug, and that amongst themselves the drug procedures do not produce different results. None of these regional and total brain values differ statistically significantly, but it is interesting that the combination drug does not enhance CBF or rCBF at all, or proportionally to the effects of the drugs individually. Rather, the R-values from the combination drug seem to follow the regional pattern obtained from nimodipine only, which is again different from the regional distribution pattern obtained from procedure B (Fig. 6-3).

**Table 6-5** Effects of procedures B (acetazolamide), C (nimodipine) and D (combination) on heart rate, blood pressure, pCO<sub>2</sub> and pO<sub>2</sub> in arterial blood in baboons

Measurement	Heart rate	Blood pressure	pCO <sub>2</sub>	pO <sub>2</sub>
B <sub>1</sub>	116.25 ± 12.74(4)	118.00 ± 2.58(4)	41.10 ± 2.37(4)	56.35 ± 4.91(4)
B <sub>2</sub>	111.50 ± 16.84(4)	110.25 ± 4.50(4)	40.08 ± 4.87(4)	76.43 ± 7.47(4)*
C <sub>1</sub>	115.33 ± 22.38	132.50 ± 29.93	42.85 ± 7.55	61.12 ± 4.39
C <sub>2</sub>	116.33 ± 24.58	112.86 ± 20.26	40.50 ± 1.27(2)	68.50 ± 5.37(2)
D <sub>1</sub>	113.83 ± 18.95	108.50 ± 24.40	42.85 ± 7.55	61.12 ± 4.39
D <sub>2</sub>	117.83 ± 16.29	106.50 ± 32.49	38.90 ± 3.35	75.06 ± 9.23(5)*

Each value indicates the mean ± SEM of six experiments unless where indicated in parentheses. Subscripts 1 and 2 refer to the measurements respectively before and 10 min after injection of the drug. \*  $P < 0.05$  for post injection vs. corresponding pre-injection values.

Nimodipine was previously reported to increase the cerebral blood flow without significant influence on the blood pressure and arterial blood gases [14] and it was suggested that the cerebrovascular autoregulation is resistant to nimodipine [14]. Niashikibe *et al.* [17] showed that the blood pressure is dose-dependently decreased by nimodipine (1 – 10 µg/kg).

It was previously shown that neither nimodipine nor acetazolamide significantly influence the pCO<sub>2</sub> [7, 10, 14, 17]. Acetazolamide is known to produce metabolic acidosis due to its carbonic anhydrase activity, giving rise to

increased CO<sub>2</sub> tensions in the expired gas [5]. However, the current dose and time scale of the determination of pCO<sub>2</sub> showed no significant change in the intravascular CO<sub>2</sub> tension, indicating that the increase in the cerebral blood flow observed for acetazolamide is unrelated to the principal local effect from a raise in the intravascular CO<sub>2</sub> tension. In accordance with other authors nimodipine showed no significant effect on the pO<sub>2</sub> ( $P > 0.05$ ); only sporadically was the pO<sub>2</sub> increased in some animals [14, 17]. Table 6-5 indicates that the increase in pO<sub>2</sub> due to acetazolamide alone (35%) is greater when compared to the acetazolamide-nimodipine combination (23%) suggesting that nimodipine attenuates the effect of acetazolamide. Hyperventilation was observed under acetazolamide treatment that could account for the higher Po<sub>2</sub> values.

It is interesting to note that the increased cerebral blood flow observed for acetazolamide and nimodipine alone individually is greatly diminished when the drugs are combined. These results strongly support the suggestion that acetazolamide may have an effect on the local cerebral metabolic rate for oxygen [13, 20]. They may further indicate that the cerebral vasodilatory properties of acetazolamide are unrelated to its carbonic anhydrase inhibition. Also interesting is that although there is an initial decrease in blood pressure with acetazolamide the blood pressure was subsequently observed to increase to a maximum after 25 min. This time response was also observed for the Po<sub>2</sub>, coinciding with the maximum increase in cerebral blood flow preciously reported [2].

## 6.5 Conclusion

The current study has shown that the baboon model previously described [4] is sensitive to monitoring pharmacological interventions and to evaluate drug candidates for cerebral diseases. Some of the observations of this study will even add to the information available for the two drugs under consideration. It also becomes clear that a combination of acetazolamide and nimodipine used under the currently described time scheduling has no beneficial effects on cerebral blood flow pattern. The combination actually diminishes the positive

effects of the individual drugs via a mechanism that cannot be readily explained at this time.

## 6.6 References

1. **BAETHMANN A, SCHURER L, UTERBERG A, WAHL W, STAUB F, KEMPSKI O.** 1991. Mediator substanzen des Hirnödems bei der zerebralen Ischämie. *Arzneimittel-Forschung* 41:310-315.
2. **BONTE FJ, DEVOUS MD, REISCH JS.** 1988. The effect of acetazolamide on regional cerebral blood flow in normal human subjects as measured by single photon emission computed tomography. *Investigative Radiology* 23:564-568.
3. **BURT RW, REDDY RV, MOCK BM, WELLMAN HN, SCHANWECKER DS, WITT R.** 1986. Acetazolamide enhancement of HIPDM brain blood flow distribution imaging. *Journal of Nuclear Medicine* 27: 1627-1631.
4. **DORMEHL IC, REDELINGHUYS IF, HUGO N, OLIVER D, PILLOY W.** 1992. The baboon model under anaesthesia for *in vivo* cerebral blood flow studies using SPECT techniques. *Journal of Medical Primatology* 21:270-274.
5. **GOODMAN LS, GILMAN AG.** 1990. The pharmacological basis of therapeutics. (8<sup>th</sup> Edition. In: Gilman AG, Rall Tw, Nies AS, Talor P, eds. New York: Pergamon, 716-717).
6. **HARPER AM, CRAIGEN L, KAZDA S.** 1981. Effect of calcium antagonist, nimodipine, on cerebral blood flow and metabolism in the primate. *Journal of Cerebral Blood Flow and Metabolism* 1:349-356.
7. **HAUGE A, NICHOLAYSEN G, THORESEN M.** 1983. Acute effects of acetazolamide on cerebral blood flow in man. *Acta Physiologica Scandinavica* 117:233-239.
8. **HERMAN CLU, McKEE AE, CHILLING PW, DICKSON LG, HÖRWITZ DL, CORAN AG, CRYER PE, CAPRIVA CJ, FORSCHER BK, LILLEHEI**

- RC, STUBBS S.** 1972. Baboon as a sub-human primate shock model in shock, high and low flow states. *Amsterdam Exepta Medica* 42-48.
9. **HÖLLERHAGE H-G, GAAB MR, ZUMKELLER M, WALTER GF.** 1988. The influence of nimodipine on cerebral blood flow autoregulation and blood brain barrier. *Journal of Neurosurgery* 69:919-922.
10. **JAKOBSEN R, MIKKELSEN EO.** 1986. Determination of nimodipine by gas chromatography using electron-capture detection: external factors influencing nimodipine concentrations during intravenous administration. *Journal of Chromatography* 374:383-387.
11. **KRIEGLSTEIN J, PERUCHE B.** 1991. Pharmakologische Grundlagen der Therapie der zerebralen Ischämie. *Arzneimittel-Forschung* 41:303-309.
12. **LANGLEY MS, SORKIN EM.** 1989. Nimodipine. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic potential in cerebrovascular disease. *Drugs* 37: 669-699.
13. **LAUX BE, RAICHLE ME.** 1978. The effects of acetazolamide on cerebral blood flow and oxygen utilization in the rhesus monkey. *Journal of Clinical Investigation* 62:585-592.
14. **McGALDEN TA, NATH RG, THIELE K.** 1984. The effects of a calcium antagonist (nimodipine) on basal cerebral blood flow and reactivity to various agonists. *Stroke* 15(3):527-530.
15. **McGALDEN TA, NATH RG.** 1989. Cerebrovascular autoregulation is resistant to calcium channel blockade with nimodipine. *Experientia* 45:305-306.
16. **MITHOEFER J, MAYER P, STOCKS J.** 1957. Effect of carbonic anhydrase inhibition of the cerebral circulation of the anaesthetized dog. *Fed Proc* 16: 88-89.

17. **NIASHIKIBE M, KAMEI K, NAGURA J, SUZUKI K.** 1989. Effect of the newly synthesized calcium antagonist isopropyl methyl 2-carbamoyl-osymethyl-6-methyl-4-(2,3-dichlorophenyl)-1,4-dihydrophyridine-3,5-dicarboxylate on cerebral venous outflow in dogs. *Arzneimittel-Forschung* 39:678-681.
18. **NOWOTNIK DP, CANNING LR, CUMMING SA, HARRISON RC, HIGLEY B, NECHVATAL G, PIKETT RD, PIPER IM, BAYNE VJ, FORSTER AM, WEISNER PS, NEIRINCKS RD, VOLKERT WA, TRUTNER DE, HOLMES RA.** 1985. Development of a <sup>99m</sup>Tc-labelled radiopharmaceutical for cerebral blood flow imaging. *Nuclear Medicine Communications* 6:499-506.
19. **POZZILLI C, DIPIERO V, PANTANO P, LENZI GL.** 1989. Influence of nimodipine on cerebral blood flow in human cerebral ischemia. *Journal of Neurology* 236:199-202.
20. **VORSTRUP S, HENRICKSEN L, PAULSON OB.** 1984. Effect of acetazolamide on cerebral blood flow and cerebral metabolic rate for oxygen. *Journal of Clinical Investigation* 74:1634-1639.
21. **WALOVITCH RC, WILLIAMS SJ, SAFRANCE ND.** 1990. Radiolabelled agents for SPECT imaging of brain perfusion. *Nuclear Medicine and Biology* 17(1):77-93.

## Effect of Sumatriptan on Cerebral Blood Flow in the Baboon Model\*

## Chapter 7

### Summary

Changes in cerebral blood flow are implicated to be important in the pathophysiology of migraine. Furthermore, serotonin (5-HT) is known to be the most important substance in the etiology of migraine. Sumatriptan (CAS 103628-46-2), a 5-HT<sub>1D</sub> receptor agonist was recently introduced in the treatment of migraine. In the present study a baboon model was used to investigate the changes in cerebral blood flow due to anaesthesia and pharmacological interventions using <sup>99m</sup>Tc-labelled hexamethylpropylene amine oxime (<sup>99m</sup>Tc-HMPAO) and single photon emission computed tomography (SPECT). The effect of sumatriptan on cerebral blood flow was investigated after 10 min and again after 23 min, with the animal under anaesthesia, i.e. induction with ketamine and maintenance on thiopental. Sumatriptan did not alter the cerebral blood flow during the 10 min procedure. However, sumatriptan reversed the increased cerebral blood flow due to the prolonged anaesthesia (23 min), lowering the cerebral blood flow by more than 20%. No significant changes in the biochemical parameters (blood pressure, heart rate, pO<sub>2</sub> and pCO<sub>2</sub>) were observed. These results also suggest that sumatriptan reverses the increased cerebral blood flow most likely via 5-HT<sub>1D</sub> receptor stimulation.

**KEY WORDS:** Antimigraine drugs, CAS103628-46-2, Cerebral blood flow, Serotonin, Sumatriptan, pharmacology.

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\* OLIVER, D.W., DORMEHL, I. & HUGO, N. 1994. Effect of Sumatriptan on Cerebral Blood Flow in the Baboon Model. *Arzneimittel Forschung/Drug Research*, 44, 925-928.

## 7.1 Introduction

Migraine significantly affects the lives and productivity of about 10 to 15% of adult population [1]. The development of effective treatment of a migraine attack has for many years been the focus of many researchers. An aura consisting of transient visual, sensory or motor symptoms may precede a migraine attack or may be absent. A focal reduction of regional cerebral blood flow (rCBF) initiates a migraine attack associated with an aura while the neurological symptoms or the aura have been implicated in the reduction of tissue perfusion [2 – 8]. Serotonin (5-HT), a mono-amine, exerts its physiological effects via stimulation of several 5-HT receptors. The role of serotonin in the pathogenesis of migraine attacks is now well accepted [9, 10; 11]. Sumatriptan (CAS 103628-45-2) a new 5-HT<sub>1D</sub> receptor agonist has recently been added to the therapeutic arsenal in the treatment of acute migraine [12]. The antimigraine effect of sumatriptan appears to be via its stimulation of the 5-HT<sub>1D</sub> receptors. Friberg *et al.* recently reported that sumatriptan reversed the migraine pain that is associated with middle cerebral artery dilatation [13]. Diener *et al.* further reported that sumatriptan did not significantly change the blood flow and velocities of the middle cerebral and basilar arteries and suggested that its action is mediated via mechanisms other than the well-established vasoconstrictor actions on cerebral arteries [14]. Furthermore, sumatriptan was shown to selectively constrict the carotid arterial bed of anaesthetized dogs and cats [15].

We recently developed a baboon model under anaesthesia to study the effects of drugs on the cerebral blood flow [16]. In continuation of our interest in drugs that exhibit cerebroconstrictor and dilatory effects we here report the study of the effects of sumatriptan on the cerebral blood flow in the baboon model using photon emission computed tomography (SPECT) and the radiopharmaceutical hexamethylpropylene amine oxime (<sup>99m</sup>Tc-HMPAO).

## 7.2 Materials and methods

### 7.2.1 The animal study

Six adult male baboons (*Papio ursinus*, average weight 27 kg) were selected for this study. Each baboon was subjected to four different procedures on different days, at least one month apart. Because of the necessity of handling the animals under anaesthesia, the first procedure, A, concerns a control CBF study with  $^{99m}\text{Tc}$ -HMPAO and SPECT under only the standard anaesthetic conditions of anaesthesia induction with ketamine hydrochloride (Ketalar<sup>®</sup>, Parke-Davis, S.A.; 10 mg/kg), followed by maintenance on thiopental sodium as previously described by us (Intraval<sup>®</sup>; 10 ml/h Maybaker, S.A.).

Procedures B and C investigated the CBF changes due to the sumatriptan (Imigran<sup>®</sup>, Glaxo, S.A.; 6 mg subcutaneous) at 10 and 23 min, respectively, after administration. Procedure D was a second control to evaluate the CBF during prolonged thiopental sodium anaesthesia after the ketamine blood levels had reached insignificant values, which was the case during procedure C.

### 7.2.2 Procedure A (control study)

After anaesthesia induction with ketamine chloride, each baboon received an i.v. injection of  $^{99m}\text{Tc}$ -HMPAO (148 MBq), the cerebral distribution of which would then represent the effect of ketamine on CBF. During the subsequent 5 min waiting time until the start of SPECT the baboon was intubated and the anaesthesia changed to a thiopental i.v. infusion (70 ml/h of a 0.5% solution), using an administration (drip) set. The following SPECT acquisition to obtain the HMPAO distribution in the brain during ketamine (SPECT-1) was done with a Siemens Orbiter gamma camera, coupled to a Sophy 250G computer, using 32 views, 360° (10s/view), in 64 x 64 word mode. Following the first acquisition, which with camera and computer readjustment took approximately 8 min, and in addition a waiting time of 5 min, the baboon was reinjected with  $^{99m}\text{Tc}$ -HMPAO (296 MBq) and tomographed as above (SPECT-2) to detect the radionuclide distribution after 18 min of thiopentone anaesthesia. This is the split-dose method of HMPAO application in CBF studies [17]. Ketamine,

producing so-called dissociative anaesthesia is inappropriate during SPECT acquisitions because of undesirable involuntary movements of the animals. Procedure A is therefore the approach to expedite the start of an experiment and to allow correction for the influence that the ketamine might still have of CBF. The baboons were viewed in a supine position with a special headrest to ensure a reproducible position for comparable tomographic slices.

### **7.2.3 Procedure B (sumatriptan effect after 10 min)**

The procedure B is similar as for procedure A, except for a delayed switch to thiopental anaesthesia after the first HMPAO injection in order to attain an 18 min duration of thiopental infusion at 10 min after the administration of sumatriptan immediately after SPECT-1, when the second (double dose) HMPAO injection is administered. This distribution of HMPAO as obtained from SPECT-2 will reflect on the effects of 18 min of thiopental anaesthesia as in the control study as well as the effect of sumatriptan after 10 min. Control ketamine-thiopental conditions were therefore maintained.

### **7.2.4 Procedure C (sumatriptan effect after 23 min)**

Measuring the effect of sumatriptan after 23 min required a separate experiment as well as a changed procedure in order not to exceed the maximum interval of 30 min between reconstitution of  $^{99m}\text{Tc}$ -HMPAO and its i.v. injection because of degradation of the radiopharmaceutical. The problem was overcome by comparing the effect of sumatriptan after 23 min to that after 10 min which follows from procedure B. After darting with ketamine the baboon is immediately placed on thiopental sodium as before and thus maintained for 20 min before the administration of sumatriptan (6 mg, s.c.). 10 min later (i.e. after 30 min of thiopental) the first HMPAO injection (148 MBq) was given and SPECT-1, as previously, followed 5 min later. These results will represent CBF influenced by 30 min of thiopental anaesthesia and after 10 min of sumatriptan. The second double dose of HMPAO (296 MBq) was injected directly at the completion of SPECT-1; this was 23 min after the sumatriptan injection and the subsequent distributions from SPECT-2 reflected on the effects of 43 min of thiopental and after 23 min of sumatriptan. Procedure C can obviously not yield

results which could be compared to the control study procedure A, because of changed anaesthesia conditions: Therefore a second control study, was devised to take these changes into account (procedure D).

### 7.2.5 Procedure D (control study with prolonged thiopental sodium)

Immediately after the ketamine induction the baboon was placed on sodium thiopental for 30 min at which stage the thiopental blood levels predominated.  $^{99m}\text{Tc}$ -HMPAO was injected at this stage to obtain, through SPECT-1, a distribution in the brain under influence of thiopental (30 min duration, as in procedure C). The baboon remained only on thiopental anaesthesia for the second double dose of HMPAO (after SPECT-1) at 43 min of thiopental. Thus the subsequent SPECT-2 would yield results according to the time scale of procedure C.

### 7.2.6 Data processing

After backprojection and reconstruction the brain images in all procedures consisted of transaxial, sagittal and coronal slices representing CBF and rCBF related information during conditions prevailing during various forms of anaesthesia and due to time dependent effects of sumatriptan. Eight slices of two pixels thickness represented the brain in all three views. Regions of interest (ROIs) were placed on the total brain (Fig. 7-1) and count rate data (counts/pixel) thus obtained were inserted into the following equation to obtain the ratio R:

$$R = \frac{(SPECT - 2) - (SPECT - 1)^*}{(SPECT - 1)}$$

where \* refers to decay corrected data from SPECT-1, present during SPECT-2 and which has to be subtracted from the SPECT-2 data, as background; and R is an indication of the level change of rCBF due to the changed conditions prevailing during the second HMPAO injection with respect to that of the first injection.



**Figure 7-1** Typical tomographic brain slices in the transaxial, coronal and sagittal views, with the position of the regions of interest (ROI), i.e. the total brain indicated within the rectangles.

For procedure A the ratio  $R$  will reflect the change in rCBF during the anaesthesia change from ketamine to thiopentone. A value of  $R = 2$  (due to the double second dose of  $^{99m}\text{Tc-HMPAO}$ ) will indicate no rCBF change during procedure A due to changed anaesthesia.  $R$  for procedure B will additionally reflect on changes due to the sumatriptan 10 min after administration and was compared to  $R$  (procedure A) to assess the effects of the sumatriptan. For procedure C the ratio  $R$  will compare the effects of sumatriptan after 10 min and 23 min if the  $R$ -value for prolonged thiopental anaesthesia prevailing during procedure C is known. This value was available from procedure D. Comparisons of  $R$ -values between procedures A and B and between procedures C and D were done by a Student's two-tailed  $t$ -test on a 5% level of confidence.

Blood pressure, heart rate and blood gases ( $\text{pO}_2$  and  $\text{pCO}_2$ ) were measured before each HMPAO injection during each of the procedures to determine the effects of the different interventions and anaesthetic procedures on these parameters.

### 7.3 Results

The  $R$ -values for each of the eight slices in each view are presented in Fig. 7-2 and 7-3: Fig. 7-2 (a, b, c) compares the control  $R$ -values of procedure A and the  $R$ -values of sumatriptan at 10 min after the injection, and Fig. 7-3 (a, b,

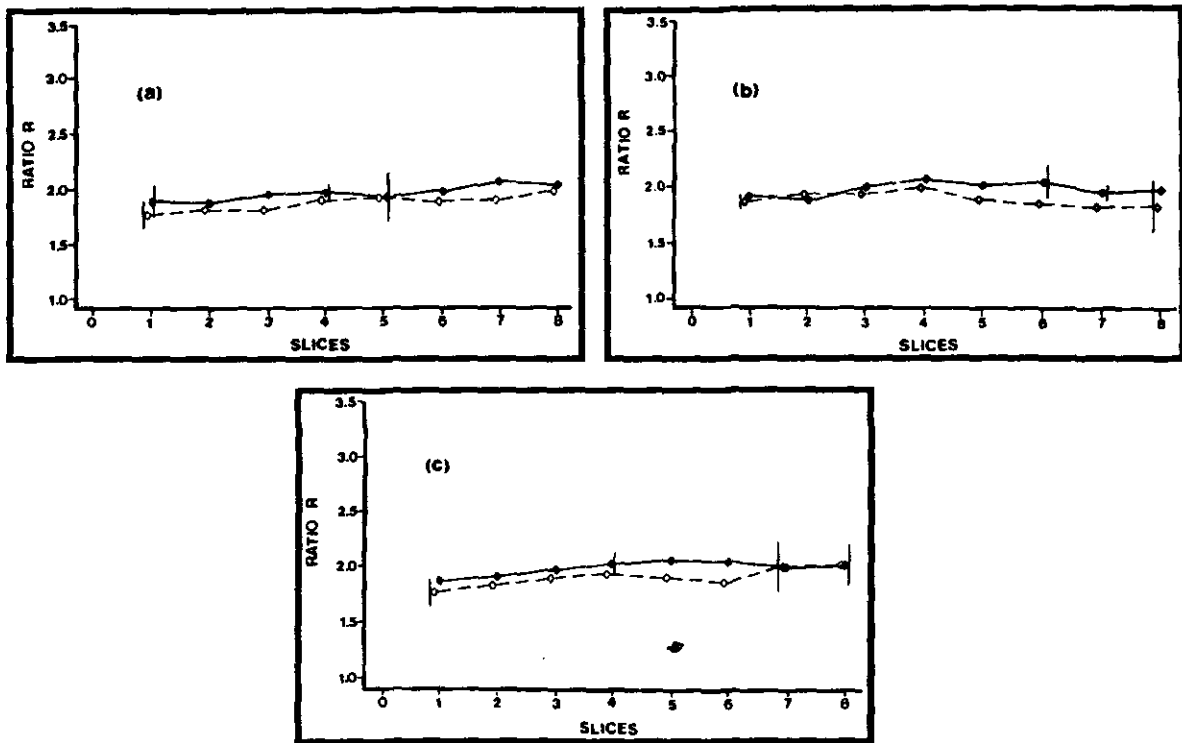
c) compares the R-values from procedure C (23 min post sumatriptan) with those from the prolonged thiopentone anaesthesia, procedure D. From the maximum and minimum standard deviation bars indicated in the figures no statistically significant ( $P > 0.05$ ) differences were found between the various slices in any one particular procedure, indicating no obvious regional effect on CBF during any of the four procedures. The values for the three views also do not differ statistically significantly, which confirms a lack of regional influence on the CBF. It therefore becomes sufficient to measure the effects of the four procedures by global or total brain CBF changes (rather than the slices), in which manner the information will also improve statistically.

Table 7-1

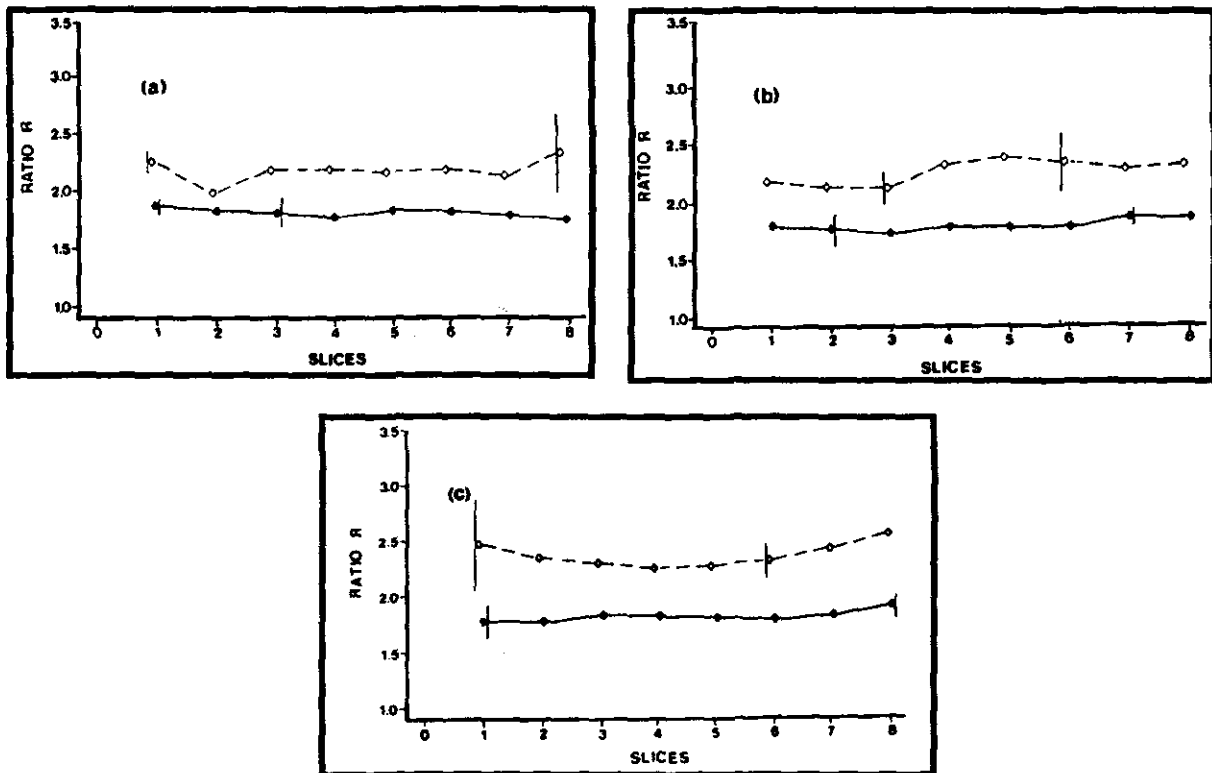
Procedure	Mean CBF and SD (n = 6)
A	1.89 ± 0.12
B	1.96 ± 0.10
C	1.82 ± 0.08
D	2.30 ± 0.27

Global CBF as a mean (n = 6) and SD for each procedure appears in Table 1. No statistically significant difference is found between procedures A and B ( $P > 0.05$ ), but procedure C significantly lowers the R-values which otherwise follow from the control study under prolonged anaesthesia (procedure D) ( $P < 0.05$ ).

The blood pressure, heart rate and blood gases ( $pO_2$  and  $pCO_2$ ) were monitored for any influence during the different procedures. Transient marginal increases were observed for blood pressure, heart rate and  $pCO_2$  at the 10 min interval (procedure C) of the sumatriptan intervention. A non-significant increase in the  $pCO_2$  level was observed at the 23 min sumatriptan intervention.



**Figure 7-2** R-values per slice (means; n = 6) for procedure A, (solid line) and procedure B (dashed line) with maximum and minimum SDs indicated, in (a) transaxial, (b) coronal and (c) sagittal views.



**Figure 7-3** R-values per slice (means; n = 6) for procedure C, (solid line) and procedure D (dashed line) with maximum and minimum SDs indicated, in (a) transaxial, (b) coronal and (c) sagittal views.

## 7.4 Discussion

The R-values for the ketamine-thiopentone control study (procedure A) are lower than the value 2, i.e. they relate to an augmented CBF during ketamine (from the equation) which corresponds to the increase in arterial BP and HR [16, 18, 19].

The prolonged maintenance of the animals under thiopentone in procedure D could account for the ratios R in this case being distinctly larger than 2. The barbiturates are known to accumulate in the muscle and subsequently in fatty tissue and an enhanced pCO<sub>2</sub> effect could result from their release during a prolonged study, leading to increased CBF from additional vasodilation (seen in the interventional phase of procedure D) in the absence of controlled ventilation [20].

The effect of sumatriptan after 10 min (procedure B) does not change the control values from procedure A significantly ( $P > 0.05$ ) although the values tend to be somewhat higher, pointing towards an increased CBF from the sumatriptan at 10 min. 23 min after the administration of sumatriptan, R-values (procedure C) are obtained which differ statistically significantly from the control value from procedure D. However, the R-values of procedure B and procedure C, i.e. 10 min and 23 min sumatriptan respectively are not significantly different. This means that the relationship:

$$\frac{\text{sumatriptan related count at 23 min}}{\text{sumatriptan related count at 10 min}}$$

introduces a factor into the R-values from procedure D which reduces these significantly. This factor has to be less than 1, and thus points to a higher CBF at 10 min than at 23 min. Also the effect of sumatriptan at 23 min is enhanced compared to the 10 min study. At 10 min after sumatriptan there was indeed no effect observed with relation to control R-values, (procedure A), whereas at 23 min a significant change (reduction) of 22% was measured (procedure D). This result suggests that sumatriptan returns the CBF to normality.

A transient slight increase in the peripheral blood pressure [21] and marginally lower heart rate, were observed at 10 min, which was not present at the 23 min interval. Slightly, but non-significant increases in pCO<sub>2</sub> and pO<sub>2</sub> levels were recorded for the procedures B and C under sumatriptan. No correlation is implicated in these slight changes in biochemical parameters.

This study shows that a substance with cerebrovasoconstrictor activities such as sumatriptan is beneficial in normalizing the effect, i.e. the increased CBF, of the anaesthetic regime used in this baboon model. It was recently demonstrated that the dilatation of the middle cerebral arteries, during a headache phase of a migraine attack, was reversed by sumatriptan [13, 22]. The present results may indicate that the serotonin, 5-HT<sub>1D</sub> receptors are likely to be involved in the normalization of the cerebral blood flow during our anaesthetic procedure with the sumatriptan intervention.

Investigation of other agents acting on the cerebrovascular serotonergic receptors can give further insight on the changes in the cerebral blood flow that occur during a migraine attack and the changes that were observed during the anaesthesia in our baboon model.

## 7.5 References

1. **KENYON, J.** (ed.). 1993. *Drugs and Therapy Perspectives*, Vol 1, pp 1-4 Aids International, Auckland, New Zealand.
2. **SKINHOJ E.** 1973. *Arch. Neurol* 29:95
3. **OLESEN J, LARSEN B, LAURITZEN M.** 1981. *Ann. Neurol* 9: 344
4. **LAURITZEN M, SKYHOJ, OLSEN T, LASSEN NA, PAULSON OB.** 1983. *Ann. Neurology* 13:633.
5. **SKYHOJ, OLSEN T, FRIBERG L, LASSEN NA.** 1987. *Arch. Neurol* 44:156.

6. LAURITZEN M, OLESON R, ROLAND PE, LASSEN NA. 1987. *Brain* 107:447.
7. OLESEN J, FRIBERG L, OLSEN TS. 1990. *Ann. Neurol* 28:791
8. FRIBERG L, SKYHOJ, OLSEN T, ROLAND PE, LASSEN NA. 1987. *Brain* 110:917
9. HUMPHREY PPA, FENIUK W, MARRIOT AS, TANNER RJ, JACKSON MR. 1991. *European Journal of Neurology* 31:282.
10. LANCE JW, LAMBERT GA, GOADSBY PJ, ZAGAMI AS. 1989. *Cephalalgia* 9:7.
11. OVERMEYER RH. 1990. *Tomorrow's Med. Today* 12:33.
12. DOENICKE A, BRAND J, PERRIN V. 1988. *Lancet* I 1309.
13. FRIBERG L, OLESEN J, IVERSEN H, SPERLING B. 1991. *Lancet* 338:13.
14. DIENER H-C, PETERS C, RUDIZIO M, NOE A, DICHGANS J, HAUX R, EHRIMANN R, TFELT, HANSEN PJ. 1991. *Neurology* 238:245.
15. HUMPHREY PPA, FENIUK W, PERREN MJ, OXFORD AW, BRITAIN RT. 1989. *Drugs Future* 14:35.
16. DORMEHL I, REDELINGHUYS F, HUGO N, OLIVER DW, PILLOY W. 1992. *Journal of Medical Primatology* 21:270.
17. PANTANO T, DU PIERO V, RICCI M, FIESCHI C, BOZZAO L, LIZENZI CNL. 1992. *European Journal of Nuclear Medicine* 19:939.
18. LASSEN NA, CHRISTENSEN MS. 1976. *British Journal of Anaesthesia* 48:719.
19. WOOD M, WOOD AJJJ. 1990. *Drugs Anaesthes.* 2:255.
20. KUSCHINSKY W. 1991. *Arzneimittel-Forschung* 41(I): 284.

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21. **FOWLER PA, LACEY LF, THOMAS M.** 1991. *European Journal of Neurology* 31:291.
  22. **MOSKOWITZ MA, HENRIKSON BM, MARKOWITZ S, SAITO K.** 1998. *In: Basic mechanisms of headache*, J. Olesen, L. Edvinsson (eds), pp. 429-437.

## Cerebral Blood Flow Effects of Sumatriptan in Drug Combinations in the Baboon Model\*

## Chapter 8

### Summary

*Sumatriptan (CAS 103628-46-2, Imigran®) has established itself as an important therapeutic agent in the treatment of migraine. Although considerable understanding of, in particular, the vascular pathophysiology of migraine has been gained during the past decade, the pathophysiology and mediators involved in the pain experience during migraine are not yet fully explained. The mechanisms behind the pharmacological effects of sumatriptan are still only partially understood. In the present study the effects of sumatriptan on drug induced cerebral blood flow increases in the baboon model were investigated using <sup>99m</sup>Tc-HMPAO (hexamethylpropylene amine oxime) and SPECT (single photon emission computed tomography). Sumatriptan selectively reduced drug induced cerebral blood flow increases. The effects of halothane anaesthesia and acetazolamide on cerebral blood flow were not reversed by sumatriptan, while the effect of nimodipine was attenuated by 47% (to the level of cerebral blood flow below the normal flow baseline). These results support multiple mechanisms for sumatriptan involving vascular neurotransmission and neurogenic inflammatory responses via serotonin receptor stimulation and Ca<sup>2+</sup> mobilization. Drug-drug interactions are further implicated through this study.*

**KEY WORDS:** Acetazolamide, Antimigraine drugs, CAS 103628-46-2, Cerebral blood flow, Halothane, Imigran®, Nimodipine, Sumatriptan, pharmacology.

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\* DORMEHL, I.C., OLIVER, D.W. & HUGO, N. 1995. Cerebral Blood Flow effects of Sumatriptan in Drug Combinations in the Baboon Model. *Arzneimittel Forschung/Drug Research*, 45 (9):952-956.

## 8.1 Introduction

Sumatriptan (CAS 103628-46-2) has effectively been used in the treatment of acute migraine and is furthermore a useful tool to investigate and elucidate drug mechanisms in migraine and related cerebrovascular headaches. We have recently successfully utilized a baboon model in the monitoring of pharmacological interventions [1-4], using  $^{99m}\text{Tc}$ -labelled hexamethylpropylene amine oxime (HMPAO). Increased cerebral blood flow (CBF) was observed after treatment with the carbonic anhydrase inhibitor, acetazolamide, and the calcium channel blocker, nimodipine [2, 3]. Sumatriptan reversed the increased cerebral blood flow induced by the prolonged anaesthesia [4]. It was also observed that a combination treatment of acetazolamide and nimodipine had no significant beneficial effect and showed a lower increase in the cerebral blood flow when compared with the individual drug responses. The current study focussed on the effects of combination drug treatment of sumatriptan with acetazolamide and sumatriptan with nimodipine in the baboon model, using single photon emission computed tomography (SPECT) techniques, specifically concentrating on the increases and reductions in CBF previously observed [2, 3, 4]. The objective of the present study was to determine and evaluate possible drug interactions of these combinations in the clinical application and from these results to further gain insight into the pharmacology of these drugs. In addition to the above-described procedures the effect of sumatriptan on the increased CBF, which has been observed during halothane anaesthesia [1], was also assessed in order to establish if sumatriptan is able to reverse this effect of halothane and to gain further information on specificity of the pharmacological action of sumatriptan and the interaction with specific anaesthesia regimes.

## 8.2 Materials and methods

Twelve adult male baboons (*Papio ursinus*, average weight 27 kg), divided into two groups of six each, were used for the investigation. The ages of the baboons are uncertain but they are mature adults of similar age, which have been in captivity for four years. They are housed, maintained and cared for

according to the guidelines laid down in the National Code for Animal Use in Research, Education, Diagnosis and Testing of Drugs and Related Substances in South Africa. These guidelines are in line with international standards.

Each animal was subjected to four different procedures (A to D for the one group and E to H for the second group) (Fig. 8-1), with at least a six-week interval between the consecutive procedures.

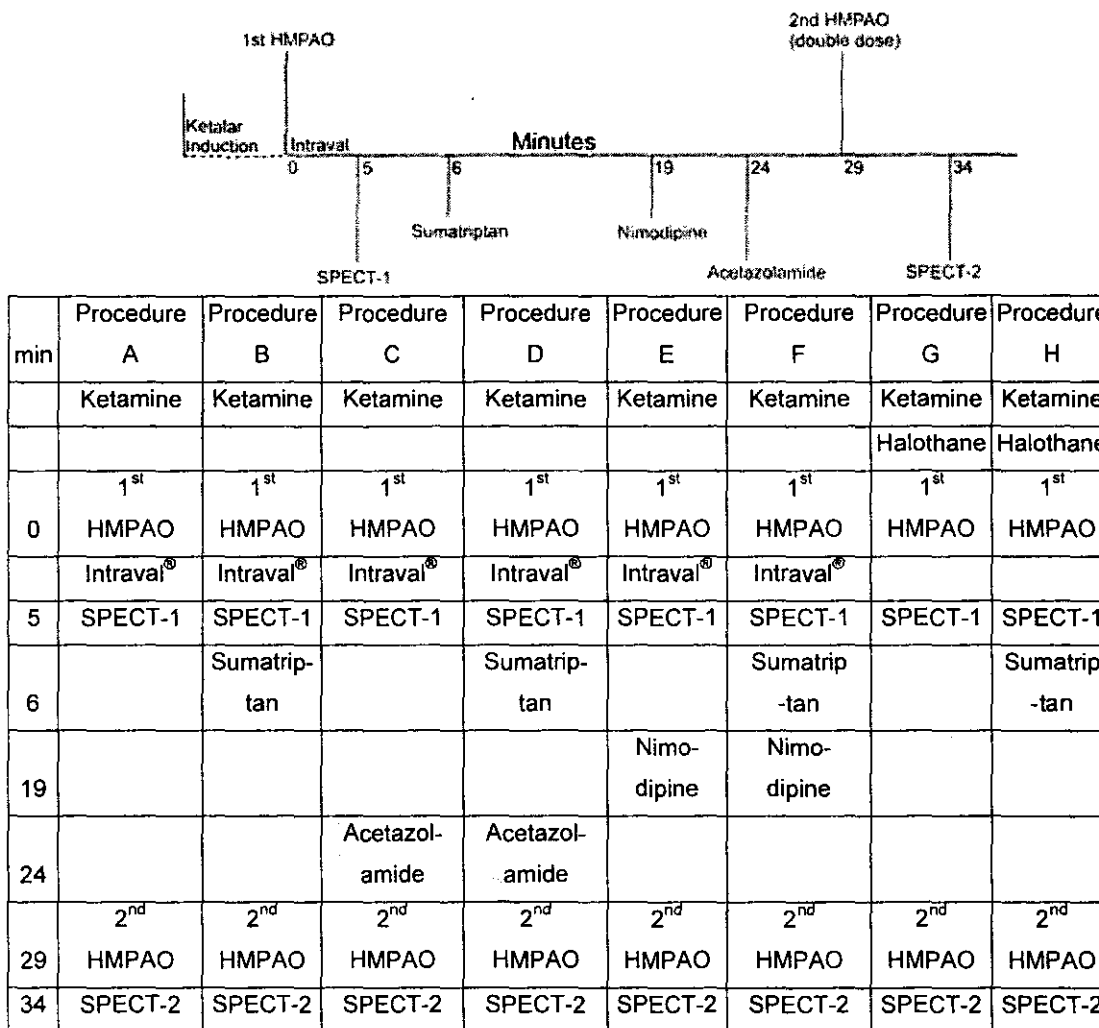


Figure 8-1 Time schedule for the various procedure protocols indicating time of each intervention

Procedure A was the control study under anaesthesia only [1] in which induction was obtained with ketamine hydrochloride (10 mg/kg<sup>-1</sup> i.v.) followed immediately by an i.v. injection of 148 MBq of <sup>99m</sup>Tc- HMPAO.

The animal was then maintained on an infusion of thiopentone sodium (70ml/h of a 0.05% solution), followed after 5 min by the first single photon

emission tomography acquisition (SPECT-1). The data of SPECT-1 represent the HMPAO distribution (uptake and retention) in the brain, resulting from CBF during ketamine sedation [5, 6]. The acquisition with a Siemens Orbiter gamma camera in 64 x 64 word mode, used 32 projections during a 360° rotation, allowing 15 per projection, i.e. about 12 min per rotation. 12 min after the end of tomography, a second i.v. administration <sup>99m</sup>Tc-HMPAO (296 MBq – double the first dose) followed, i.e. the split-dose method [7, 8], and 5 min later the second acquisition (SPECT-2) as before. These data represent a CBF pattern of HMPAO under thiopental anaesthesia, but with a background from the first HMPAO distribution under ketamine. After backprojection and reconstruction (Hamming/Hamm) the brain images consisted of two sets (SPECT1 and SPECT-2), each of four compacted transaxial, sagittal and coronal slices. The two sets represented CBF patterns respectively related to ketamine and to thiopental anaesthesia. Regions of interest (ROIs) were placed on the SPECT-1 and SPECT-2 data, in each view, and counts per pixel obtained from each slice. These values for each slice were inserted into the following equation.

$$R = \frac{[SPECT - 2](counts / pixel) - [SPECT - 1]^*(counts / pixel)}{SPECT - 1(counts / pixel)}$$

R represents the level changes of rCBF during the second anaesthesia, thiopentone with respect to rCBF during ketamine (the first anaesthesia) after subtraction of the background (\*), having been corrected for decay.

Procedure B followed the same protocol as above, except for an additional i.m. injection of sumatriptan (Imigran®, Glaxo S.A., Midrand, South Africa), 1 min into the SPECT-1 (ketamine related) acquisition. The double dose of HMPAO administered 12 min after the end of SPECT-1, will therefore represent the rCBF distribution due to sumatriptan (23 min p.i.), when compared to procedure A. Also for procedure C the protocol of the baseline procedure A was followed, but 7 min after completion of SPECT-1, i.e. 5 min before the second double dose of HMPAO, 500 mg of acetazolamide (100 mg/ml) was i.v. administered. Thus SPECT-2 reflected the rCBF 5 min after acetazolamide injection. In procedure E, the drug intervention was by nimodipine, which

started as an infusion (1  $\mu\text{g}/\text{kg}/\text{min}$ ) 10 min before the second HMPAO injection, and 2 min after the completion of SPECT-1, and continued for 5 min until SPECT-2 started. SPECT-2 would then reflect on rCBF after 10 min of nimodipine.

Procedures D and F concerned the two drug combinations, respectively acetazolamide plus sumatriptan and nimodipine plus sumatriptan as follows: For procedure D a combination protocol of procedure B and C is followed, i.e. an injection of sumatriptan 1 min into SPECT-1, and an injection of acetazolamide 7 min after SPECT-1, so that by the time of the second double dosage of HMPAO, 23 min will have passed after sumatriptan administration and 5 min after acetazolamide. Therefore SPECT-2 will reflect rCBF due to a drug combination along the lines of the protocols of the single drugs in procedure B and C.

Similarly procedure F (procedure B and E combined) will have SPECT-1 information on rCBF related to the ketamine anaesthesia as do all other procedures, but SPECT-2 will reflect on the effect of the 10 min nimodipine infusion which will start 2 min after SPECT-1 had ended, plus the effect of sumatriptan after 23 min, having been injected 1 min into SPECT-1.

The last two procedures G and H concerned the possible role of the halothane anaesthesia and the interaction with sumatriptan. The animals were placed on halothane (2% halothane/oxygen, Boyle's machine), immediately after having been immobilized by ketamine. The first injection of  $^{99\text{m}}\text{Tc}$ -HMPAO under halothane was followed five minutes later by SPECT-1, as above. 12 min after SPECT-1 another injection of HMPAO (double dose) was given and SPECT-2 followed 5 min later. This procedure was the baseline for procedure H, which followed the same course but with an injection of sumatriptan 1 min into SPECT-1 and 23 min before the second HMPAO injection.

During all procedures the blood pressure (BP), heart rates (HR) and blood gases were monitored before each drug intervention and before the second HMPAO injection.

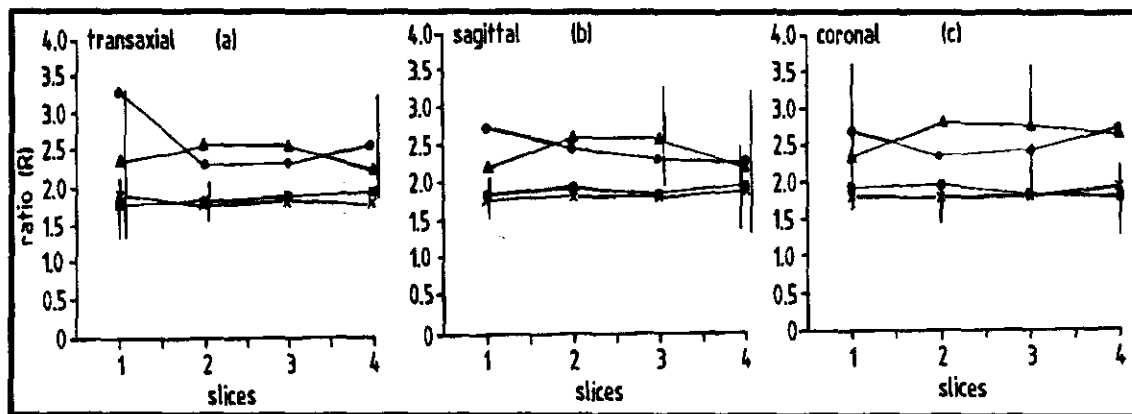
### 8.2.1 Statistical methods

Mean ratios  $R$  ( $n = 6$ ) and standard deviations were calculated per procedure for similar regions (slices). An average  $R$ -value was obtained for the total brain for a particular procedure by considering all slices of all views. These were compared for interprocedural effects and the comparisons assessed for significant differences, using Student's two-tailed  $t$ -test for paired variables on a 5% confidence level.

### 8.3 Results

The results are presented in Fig. 8-2 to 8-4, where the mean ratio ( $\pm$ SD) ( $n = 6$ ) for each slice is depicted in each procedure.

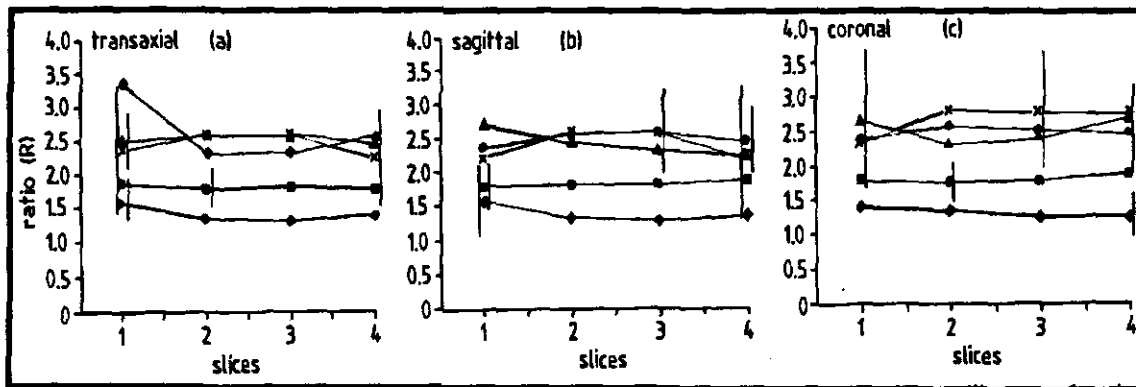
Fig. 8-2 displays procedure A (control), and procedures B, C and E, which are the single drug interventions respectively of sumatriptan, acetazolamide and nimodipine, in the transaxial (a), sagittal (b), and coronal (c) views. In Fig. 8-3 the single drug sumatriptan intervention (procedure B) is also compared to the combination drug intervention, procedures D and F.



**Figure 8-2** Curves of mean ratio ( $R$ ) ( $n = 6$ ) vs. slice number frontal to the occipital lobes transaxially, from right to left of the brain sagittally, and from the cerebellum to the dorsal slice of the cerebrum coronally. ■ Procedure A; x Procedure B; ▲ Procedure C; ● Procedure E. This figure compares single drug interventions with anaesthesia (ketamine-thiopental) baseline. The largest standard deviations for each procedure are indicated.

The possible role that the anaesthesia can play is illustrated in Fig. 8-4, where the two control studies without interventions viz the ketamine-thiopentone study (procedure A) and the halothane study (procedure G) are

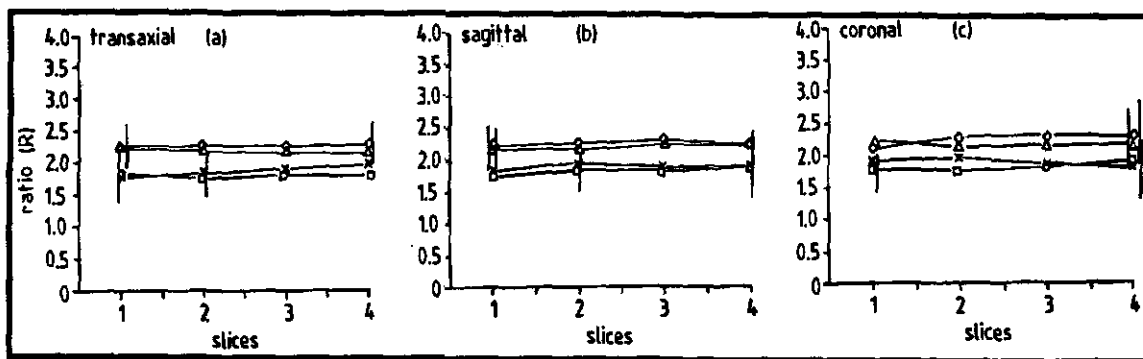
compared to corresponding studies with the sumatriptan interventions, i.e. procedures B and H.



**Figure 8-3** As in Fig 8-2. ■ Procedure B; ▲ Procedure D; ● Procedure F; x Procedure C; ● Procedure E. This figure illustrates drug interactions and comparative changes with respect to single drug effects.

In Table 8-1 means ( $n = 6$ )  $\pm$ SD of the ratio R are presented for the total brain (obtained by averaging R from all slices and all views in a procedure), with percentage changes between procedures, as well as (when applicable) significant changes in HR, BP,  $p\text{CO}_2$  and  $p\text{O}_2$ .

From Fig. 8-2 and Table 8-1 it is clear that the sumatriptan intervention did not change control R-values statistically significantly ( $1.87 \pm 0.36$  vs.  $1.82 \pm 0.55$ ,  $P > 0.05$ ); also not with halothane anaesthesia ( $2.18 \pm 0.32$  vs.  $2.23 \pm 0.28$ ,  $P > 0.05$ ); Fig. 8-4).



**Figure 8-4** As in Fig 8-2 and 8-3. x Procedure A; ■ Procedure B; ▲ Procedure G; ● Procedure H. The influence of the nature of anaesthesia is illustrated here.

Single drug interventions with acetazolamide and nimodipine increased CBF, as reflected in the R-values, by 32% and 35%, respectively, with respect to the

control values of procedure A. These increases are statistically significant ( $P < 0.05$ ). The drug combinations of sumatriptan plus acetazolamide (procedure D) and sumatriptan plus nimodipine (procedure F), influenced CBF in different ways. According to procedure D the sumatriptan had no statistically significant effect ( $P > 0.05$ ) on the acetazolamide-induced increases obvious from procedures C and A. However, the sumatriptan administered in procedure F reduced the nimodipine induced increases in CBF from procedure E statistically significantly ( $2.53 \pm 0.4$  vs.  $1.34 \pm 0.35$ ,  $P < 0.05$ ). These reduced R-values from procedure F are even significantly below the control values of procedure A ( $P < 0.05$ ).

**Table 8-1** Mean ( $\pm$  SD) ratios R for total brain as averaged from all slices and all views for all procedures, percentage changes of these R-values and parameter changes when statistically significant ( $P < 0.05$ )

	R $\pm$ SD	% Change in R	HR	BP	pCO <sub>2</sub>	PO <sub>2</sub>
<b>Procedure A</b>						
(Baseline)	1.87 $\pm$ 0.36		-	-	-	-
<b>Procedure B</b>						
(Sumatriptan)	1.82 $\pm$ 0.22		-	-	-	-
<b>Procedure C</b>						
(Acetazolamide)	2.49 $\pm$ 0.34		-	-7	-	+20
<b>Procedure D</b>						
(Acetazolamide + Sumatriptan)	2.54 $\pm$ 0.32		-	-	-	+7
<b>Procedure E</b>						
(Nimodipine)	2.53 $\pm$ 0.40		-	-18	-	-
<b>Procedure F</b>						
(Nimodipine + Sumatriptan)	1.34 $\pm$ 0.31		-	-55	-	-
<b>Procedure G</b>						
(Halothane Baseline)	2.18 $\pm$ 0.32		-	-	-	-
<b>Procedure H</b>						
(Halothane + Sumatriptan)	2.23 $\pm$ 0.28		-	-	-	-
Procedure A to C		+32				
Procedure A to D		+36				
Procedure A to E		+35				
Procedure A to F		-28				
Procedure B to D		+40				
Procedure B to F		-26				
Procedure E to F		-47				

No significant changes occurred for any of the interventional procedures in HR and  $p\text{CO}_2$  taken just before drug administration and just before the second HMPAO injection. Significant reductions ( $P < 0.05$ ) in BP occurred with the acetazolamide intervention (procedure C;  $\Delta\text{BP} = -7$ ), as well as with nimodipine (procedure E;  $\Delta\text{BP} = -18$ ), and the combination procedure F of sumatriptan and nimodipine (-55).  $p\text{O}_2$  increased significantly with acetazolamide (+20), and also when in combination with sumatriptan (+7).

## 8.4 Discussion

The results clearly indicate that sumatriptan had no effect (potentiation or antagonism) on the increased cerebral blood flow induced by acetazolamide within the current time scale and dose regimes. Similarly, sumatriptan did not influence the halothane anaesthesia, even though halothane pointed to increased CBF, when compared to the ketamine-thiopental alternative [1]. Conversely sumatriptan significantly influenced the increased cerebral blood flow induced by nimodipine. The increased blood flow effect of nimodipine was attenuated by 47% ( $P < 0.05$ ) by simultaneous treatment with sumatriptan. The reduced CBF-values noted in this procedure were also statistically significantly lower than the control values without drug treatment ( $P < 0.05$ ). These results clearly indicate that sumatriptan is a potent antagonist of nimodipine and that the pharmacological reversal of the induced increase in the CBF occurs not for all drug combinations. These results may further suggest that cerebral blood flow attenuation (vascular mechanism) is but one component of the pharmacology of sumatriptan as was previously outlined by Moskowitz [9]. The current data may give further insight into other mechanisms of action of sumatriptan and the pathological mechanisms in migraine.

The current research protocols for acetazolamide and halothane anaesthesia indicate no drug-drug interactions with simultaneous administration of sumatriptan. The increased CBF due to the action of acetazolamide and halothane was maintained. The blood pressure and oxygen ( $p\text{O}_2$ ) changes for the single drugs procedures are similar to those previously observed [2, 3]. Interesting is the marked drop in blood pressure in the sumatriptan-nimodipine

combination study, while a drop in cerebral flow is observed. Compensation responses could explain this occurrence.

Nimodipine, a dihydropyridine calcium channel antagonist has been shown to dilate cerebral arterioles and to increase cerebral blood flow (see review of nimodipine [10]). It has been suggested that nimodipine at a dose of 120 mg/day can effectively be used in the prophylactic treatment of migraine [11]. The potent antagonism by sumatriptan of the nimodipine-induced increase in CBF argues against simultaneous treatment of these two drugs. The combination could result in attenuation or diminishing of the effects of nimodipine. This result is however of importance to gain further knowledge on the actions of these drugs in the treatment of migraine.

Serotonin (5-HT) and some specific 5-HT-receptors are implicated in migraine (see reviews of 5-HT and the classification of receptors of 5-HT [12, 13]). Sumatriptan exhibits agonist activity at the 5-HT<sub>1D</sub>- and 5-HT<sub>1</sub> like-receptors [13]. 5-HT<sub>1D</sub>-receptors have been demonstrated in human cerebral arteries [14, 15] and to be of the type 5-HT<sub>1Dβ</sub>. Activation of these receptors results in the inhibition of forskolin-stimulated adenylyl cyclase activity [16]. However these receptors have been found to link positively and negatively to adenylyl cyclase depending on the cell line use for the expression of these receptors. Zgombick *et al.* have recently observed inhibition of adenylyl cyclase and stimulation of calcium mobilization [17]. The marked effect of sumatriptan to reduce the increased CBF due to nimodipine supports the involvement of Ca<sup>2+</sup> in the pharmacology of sumatriptan. This result suggests that sumatriptan is able to normalize the cerebrovascular bed via multiple mechanisms that include amongst others vasoconstriction and influencing Ca<sup>2+</sup> mobilization that might be important in the inflammatory and pain responses that occur through mediators such as nitric oxide (NO), histamine and other neurotransmitters and -peptides.

Moskowitz *et al.* have researched the neurogenic and vascular mechanism of sumatriptan in the migraine [18-25]. It was concluded that sumatriptan blocks the vascular headaches through blockade of neural transmission and neurogenic inflammatory responses and that post- and prejunctional 5-HT<sub>1D</sub>-

and 5-HT<sub>1</sub> like-receptors are implicated in the pharmacology of sumatriptan. It has been suggested that perivascular neurogenic inflammation around the dural and meningeal arteries could account for the migraine pain and that the process is associated with release of neuropeptide transmitters from perivascular trigeminal nerve endings [18]. Olesen *et al.* [26] have recently suggested that nitric oxide (NO) may play an important role in migraine and other vascular headaches. It was observed that glyceryl trinitrate (which can be regarded as a pro-drug for NO [27]) causes a reduction in the blood velocity in the middle cerebral artery [28]. No effect on regional cerebral blood flow was observed after glyceryl nitrate treatment, indicating an increased dilatation of the middle cerebral artery in migraine sufferers [29]. Furthermore, histamine-induced headache occurs most likely due to the formation of NO in the cranial blood vessels [30, 31]. NO through guanylate cyclase coupling has been postulated to trigger a chain of events that induce smooth muscle relaxation via decreases in cytosolic Ca<sup>2+</sup> concentrations [28, 29]. Sumatriptan apart from its vasoconstrictor effects might exert its therapeutic effects on the pain component of migraine via other mechanisms that might involve Ca<sup>2+</sup> mobilization. The findings that NO could be involved in migraine through vasodilatation and the induction of migraine-like headaches could be an important mechanism to explain pharmacology of sumatriptan. An inhibitory effect of sumatriptan on NO would result in the antagonism of both the vasodilatation and the possible effects on perivascular sensory nerves of NO. The effect on the calcium concentrations resulting in dilatation of cerebral blood arteries upon NO release could be antagonized by sumatriptan as was observed during the nimodipine combination procedure.

## 8.5 Conclusion

The current study strongly suggests that sumatriptan is able to reverse induced increases in cerebral blood flow due to pharmacological interventions. However, this reversal clearly does not occur in all the cases and is more specific than initially anticipated. The inability of sumatriptan to influence the acetazolamide and the halothane increases in CBF supports the specificity of sumatriptan, particularly in view of the marked effect on nimodipine. This study

was able to establish possible drug-drug interactions involving sumatriptan. The data clearly supports multiple mechanisms of action for sumatriptan during the treatment of migraine that involves both direct action on cerebral blood vessels via stimulation of amongst others 5-HT<sub>1D</sub>-receptors and effects of Ca<sup>2+</sup> mobilization. Sumatriptan's action could also involve the inhibition of endogenous substances, such as NO, histamine and other mediators, that are active in vascular neurotransmission and neurogenic inflammatory responses during migraine. The effect of sumatriptan on these substances is currently being investigated.

## 8.6 References

1. **DORMEHL IC, REDELINGHUYS IF, HUGO N, OLIVER D, PILLOY W.** 1992. *Journal of Medical Primatology* 21:270-274.
2. **OLIVER DW, DORMEHL IC, REDELINGHUYS IF, HUGO N, BEVERLY G.** 1993. *Nuklear Medizin* 32:292-298.
3. **DORMEHL IC, OLIVER DW, HUGO N.** 1995. *Nuclear Medicine Communications* 14:573-577.
4. **OLIVER DW, DORMEHL IC, HUGO N.** 1995. *Arzneimittel-Forschung* 44(2):925.
5. **BACCIOTTINI L, PUP A, FORMICONI AR, DE CRISTOFARO MTR, MELDOLESI U.** 1989. *Journal of Cerebral Blood Flow Metabolism* 9:S414.
6. **PUPI A, BISI G, SIAGRA R, SANTORO D, DE CRISTOFARO MTR, FORMICONI AR, MELDOLESI U.** 1989. *Journal of Cerebral Blood Flow Metabolism* 9:S411.
7. **WYPER DJ, HUNTER R, PATTERSON J, GOODWIN G, McCULLOCH J.** 1991. *Journal of Cerebral Blood Flow Metabolism (Suppl 2)* 11 :S449.
8. **HOLM S, MADSEN PL, RUBIN P, SPERLING B, FIRBERG L, LASSEN N.** 1991. *Journal of Cerebral Blood Flow Metabolism (Suppl 2)* 11 :S766.

9. **MOSKOWITZ MS.** 1992. *TIPS* 13:307.
10. **LANGLEY MS, SORKIN EM.** 1989. *Drugs* 37: 669-699.
11. **GAWEL MA.** 1987. *Cephalalgia* 7:453
12. **HUMPHREY PPA, APPERLEY E, FENIUK W, PERREN MJ,** in: Cardiovascular Pharmacology of 5-Hydroxytryptamine, pp. 417-431, Kluwer Academic, Dordrecht, The Netherlands. 1991.
13. **HOYER D, CLARKE DE, FOZARD JR, HARTIG PR, MARTIN GR, MYLECHARANE EJ, SAXENA PR, HUMPHREY PPA.** 1994. *Pharmacol. Rev. Pharmacol* 46:157.
14. **HAMEL E, GREGIOR L, LAY B.** 1993. *European Journal of Pharmacology* 242:75.
15. **HAMEL E, FAN E, LINVILLE D, YING V, VILLEMURE JG, CHIA LS.** 1993. *Molecular Pharmacology* 44:242.
16. **HOYER D, SCHOEFFTER P.** 1988. *European Journal of Pharmacology* 147:145.
17. **ZGOMBICK JM, BORDEN LA, COCHRAN TL, KUCHAREWICZ SA, WEINSHANK RL, BRANCHEK TA.** 1993. *Molecular Pharmacology* 44:575.
18. **MOSKOWITZ MA, MACFARLANE RC.** 1993. *Metab. Rev.* 3:1.
19. **MOSKOWITZ MA.** 1992. *Cephalalgia* 12:5.
20. **BUZZI MG, SAKAS DE, MOSKOWITZ MA.** 1989. *European Journal of Pharmacology* 165:251.
21. **BUZZI MG, MOSKOWITZ MA.** 1990. *British Journal of Pharmacology* 99:202.
22. **SAITO K, MARKOWITZ S, MOSKOWITZ MA.** 1988. *Ann Neurol* 224:732.

23. **MARKOWITZ S, SAITO K, MOSKOWITZ MA.** 1987. *Journal of Neuroscience* 7:4129.
24. **MATSUBARA T, MOSKOWITZ MA, BYUN BJ.** 1991. *British Journal of Pharmacology* 104:3.
25. **NOZAKI K, MOSKOWITZ MA, BOCCALINI P.** 1992. *British Journal of Pharmacology* 106:405.
26. **OLESON J, THOMSEN LL, IVERSEN HK.** 1994. *TIPS* 15:149.
27. **RAND M.** 1992. *Clinical and Experimental Pharmacology and Physiology* 19:147.
28. **THOMSEN LL, IVERSEN HK, BRINCK TA, OLESON J.** 1993. *Cephalalgia* 13:395.
29. **DAHL A, RUSSEL D, NYBERG-HANSEN R, ROOTWELL K.** 1989. *Stroke* 20:173.
30. **TODA N.** 1990. *American Journal of Physiology* 258:H311.
31. **OTTOSSON ALP, JANSEN I, LANGEMARK M, OLESEN J, EDVINSSON L.** 1991. *Cephalalgia* 11:183.

# A Comparative Cerebral Blood Flow Study in a Baboon Model with Acetazolamide Provocation: $^{99m}\text{Tc}$ -HMPAO vs. $^{123}\text{I}$ (IMP)\*

## Chapter 9

### Summary

*Pharmacological interactions are important when nuclear medical procedures are applied to patients under drug therapy, or drug provocation. This study compares in baboon models (regional) cerebral blood flow (rCBF) results from  $^{99m}\text{Tc}$ -HMPAO and  $^{123}\text{I}$ -iodoamphetamine, [ $^{123}\text{I}$ ]IMP, each with and without acetazolamide, the latter a suggested drug for testing cerebrovascular reserve. Expected differences in cerebral uptake were observed between the two radiotracers without acetazolamide. The increase in tracer uptake resulting from acetazolamide is significantly enhanced for [ $^{123}\text{I}$ ]IMP, which could have diagnostic implications.*

### 9.1 Introduction

Drug combinations when administered simultaneously have the potential to interact with each other on two main pharmacological levels, viz pharmacokinetically and/or pharmacodynamically (Stockley, 1991). Interactions are, therefore, also to be expected for radiopharmaceuticals when administered with drugs. This should be considered when nuclear medical procedures are applied to patients under drug therapy, when drugs are used provocatively to facilitate nuclear medical diagnosis (e.g. acetazolamide and cerebrovascular reserve, Devous *et al.*, 1992), and also when used to evaluate drugs in pharmaceutical development and for pharmacological responses.

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\* DORMEHL, I.C., OLIVER, D.W., HUGO, N. & ROSSOUW, D. 1995. A Comparative Cerebral Blood Flow Study in a Baboon Model with Acetazolamide Provocation:  $^{99m}\text{Tc}$ -HMPAO vs.  $^{123}\text{I}$ (IMP). *Nuclear medicine and biology*, 22(3):373-378.

Comparisons of the drug-induced behaviour of different radiopharmaceuticals during the above procedures are important in order to interpret differences in radiopharmaceutical responses, to understand and anticipate possible interactions and to acquire and quantify datasets obtained from different radiopharmaceuticals for practical diagnostic applications. With the above in mind a study was done to assess the results obtained from regional cerebral blood flow (rCBF) measurements using  $^{99m}\text{Tc}$ -hexamethylpropylene amine oxime (HMPAO) and *N*-isopropyl-*p*-(123)-iodoamphetamine,  $^{123}\text{I}$ (IMP), with and without acetazolamide (Diamox<sup>®</sup>, S.A. Cyanamid (Pty) Ltd) in a baboon model. Differences are explained in terms of induced metabolic acidosis, and alkaline urine, following from acetazolamide.

## 9.2 Materials and methods

Six adult male baboons (average weight 27 kg) were used for the investigation. The studies were performed after approval by the Ethics Committee of the University of Pretoria, according to the guidelines of the National Code for Animal Use in Research, Education Diagnosis and Testing of Drugs and Related Substances in South Africa. Each animal was subjected to four different procedures (A to D), with at least a six-week interval between consecutive procedures. Procedures A and C were control studies under anaesthesia only (Dormehl *et al.*, 1994) in which induction was performed with ketamine hydrochloride (10 mg/kg i.v.) (Ketalar<sup>®</sup>, Parke-Davis, Cape Town) and immediately followed by a maintained controlled infusion of thiopentone sodium (Intraval<sup>®</sup>, Sandoz S.A., Randburg) (70 ml/hr of a 0.5% solution) using an administration (drip) set. After a 10 min stabilization period under thiopentone, procedure A continued with an i.v. injection of 148 MBq of  $^{99m}\text{Tc}$ -HMPAO. Five minutes later the first SPECT acquisition (SPECT-1) followed with a Siemens Orbiter gamma camera, using 32 projections during a 360° rotation (10 sec per view). The baboons were viewed in a supine position with a special headrest to ensure a reproducible position to compare tomographic slices (Dormehl *et al.*, 1992). SPECT-1 was immediately followed by a second i.v. administration of  $^{99m}\text{Tc}$ -HMPAO (296 MBq, i.e. double the first dose), and 5 min later by the second similar SPECT acquisition (SPECT-2): the split-dose

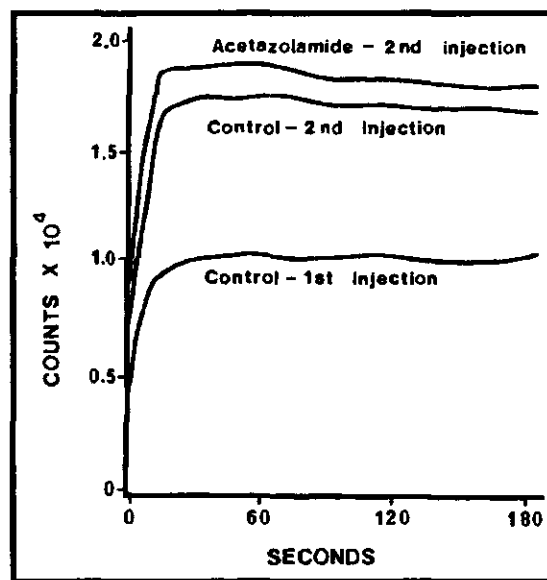
method (Holm *et al.*, 1991; Wyper *et al.*, 1991). The data from SPECT-1 and SPECT-2 represent HMPAO distributions (uptake and retention) in the brain resulting from CBF at two different times of prolonged thiopentone anaesthesia (Bacciottini *et al.*, 1989; Pup *et al.*, 1989). SPECT-2 data also contain background from the first HMPAO distribution.

Procedure C differed from procedure A by replacement of the two administrations of  $^{99m}\text{Tc}$ -HMPAO by, respectively, 148 and 296 MBq of [ $^{123}\text{I}$ ]IMP at corresponding times (as in procedure A) during the thiopentone anaesthesia.

Procedures B and D followed the same protocols, respectively, as procedures A and C until completion of the first SPECT acquisition (SPECT-1) but then this was immediately succeeded by an intravenous injection of 500 mg of acetazolamide (Oliver *et al.*, 1993; Dormehl *et al.*, 1993). Procedures B and D were continued 10 min later by, for procedure B, a second HMPAO injection (296 MBq), and similarly for procedure D, 296 MBq of [ $^{123}\text{I}$ ]IMP, to be followed after 5 min by SPECT-2 in both cases. Procedures B and D will, therefore, reflect on the effect of acetazolamide (10 min post administration) with respect to anaesthesia-only baselines using the two tracers HMPAO (procedure A) and amphetamine (procedure C) (Oliver *et al.*, 1994).

The split-dose method is based on the chemical properties of the tracer that crosses the blood-brain barrier and is trapped in brain cells. With HMPAO it becomes important to check radiochemical purity before its first application (within 5 min of preparation with a fresh eluate). For kit preparation each vial of HMPAO was reconstituted with 1110 MBq (30 mCi) of saline diluted  $^{99m}\text{Tc}$  in a 5 ml syringe. The lipophilic complex which determines the required lipophilic character and subsequent high brain retention (Ell *et al.*, 1985a, b; Homes *et al.*, 1985; Nowotnik *et al.*, 1985) was never found to be below 90%. This was checked in each case using chromatography on ITLC SG strips with methylethylketone (MEK) and saline as solvent.  $^{99m}\text{Tc}$ -HMPAO as a lipophilic complex runs with the solvent front in MEK but remains at the origin in saline. With time it could convert to a secondary complex which remains at the origin in both systems. The second injection of HMPAO following the first by 20-30 min

(depending on the procedure) was from the same  $^{99m}\text{Tc}$ -HMPAO preparation as the first and was accompanied on a count down by a dynamic data acquisition (15 sec per image for 4 min). Such a dynamic acquisition was also done with the first injection of HMPAO (Fig. 9-1). The shape of the time-activity curves thus obtained would reflect on the possibility of reduction of lipophilic  $^{99m}\text{Tc}$ -HMPAO. A tendency to wash out would prompt the termination of the study. Unchanged input functions with respect to the first HMPAO injection for SPECT-1 as seen in Fig. 9-1 for the second double dose injection (with and without Diamox<sup>®</sup>) permitted continuation with SPECT-2.



**Figure 9-1** Time-activity curves representing input functions from the dynamic acquisition on countdown of the first  $^{99m}\text{Tc}$ -HMPAO injection (control anaesthesia, first injection), of the second  $^{99m}\text{Tc}$ -HMPAO injection with acetazolamide, the latter two from double dosages of the tracer.

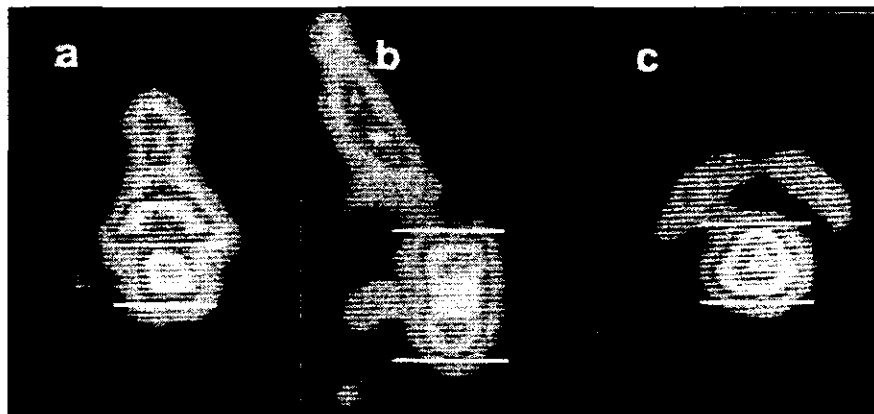
The efficacy of [ $^{123}\text{I}$ ]IMP is also a consequence of its lipophilicity, the initial uptake being a measure of perfusion while progressive brain accumulation is probably a combined sequence of intravascular/extravascular intracerebral pH gradients, favourable brain lipid/aqueous partition coefficients, and the affinity for high-capacity, relatively non-specific binding sites for amines located in the brain and/or brain capillary endothelium. High levels of brain activity are maintained for several hours (Winchell *et al.*, 1980a, b).

Following back projection and reconstruction, the brain images consisted of two sets of four compacted slices in the transaxial, sagittal and coronal views,

the two sets representing rCBF patterns from SPECT-1 and SPECT-2 for each procedure. Regions of interest (ROIs) were placed for the brain in the slices from SPECT-1 and -2 data in each view, for all the procedures, and counts per pixel obtained from each slice (Fig. 9-2). These values for each slice were inserted into the following equation, for each procedure:

$$R = \frac{[SPECT - 2](counts / pixel) - [SPECT - 1] * (counts / pixel)}{SPECT - 1(counts / pixel)}$$

R represents the level of change of rCBF during prolonged anaesthesia as a baseline, or during prolonged anaesthesia and acetazolamide intervention. [SPECT-1]\* decay is corrected (Dormehl *et al.*, 1992).



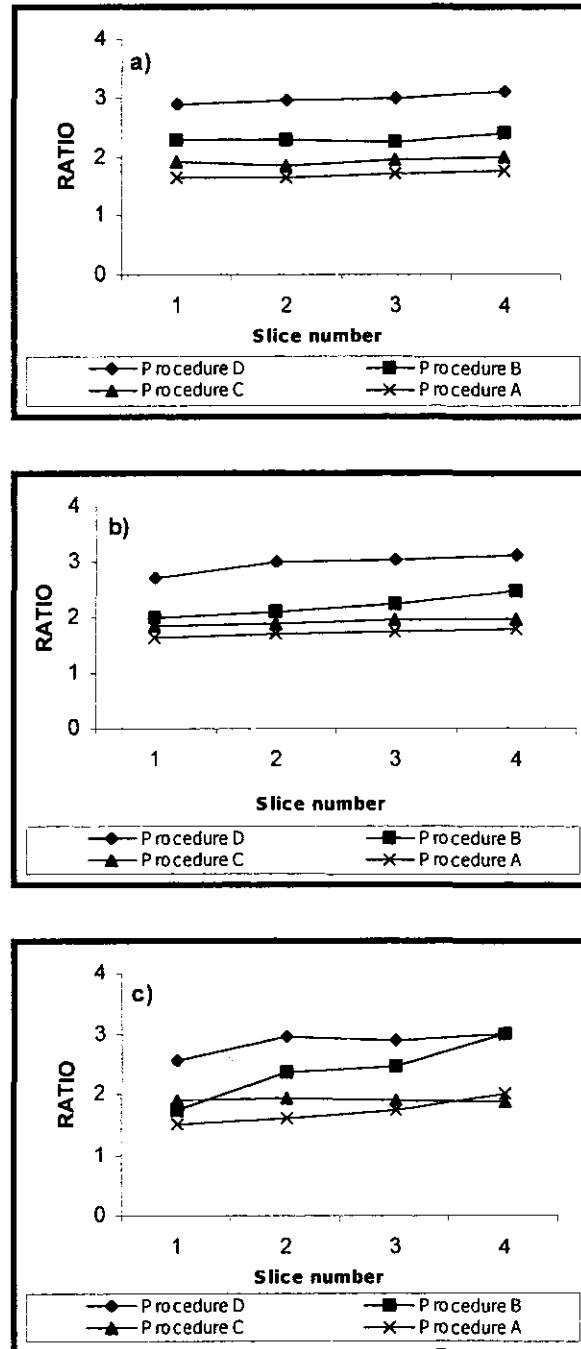
**Figure 9-2** Typical tomographic brain slices in the coronal (a), sagittal (b), and transaxial (c) views, indicating the regions of interest (ROI), i.e. the total brain.

From the individual baboon values mean regional and total brain ratios R were evaluated in each view for each of the procedures A-D, and compared for possible regional as well as procedural effects. The comparisons were assessed for significant differences using Student's two-tailed *t*-test at 1 and/or 5% level of confidence. The regional information refers to the tomographic slices rather than to anatomical structures.

### 9.3 Results

The results are summarized in Fig. 9-3a-c, where the mean ( $n = 6$ ) ratios R, indicating level changes in HMPAO and IMP uptake and retention during prolonged thiopentone anaesthesia, and because of the administration of

acetazolamide are shown for four compacted slices of the brain in three views: transaxial, sagittal, and coronal. The smallest and largest standard deviations are given for each procedure.



**Figure 9-3** Curves of the mean ratio ( $n = 6$ ) vs. slice number (region) starting at the frontal lobes to the occipital lobes transaxially (a), from right to left of the brain sagittally (b), and from the cerebellum to the dorsal slice of the cerebellum coronally (c). For each procedure the smallest and largest standard deviations are indicated. Ratio R represents the level of change of rCBF during prolonged anaesthesia as a baseline, or during prolonged anaesthesia and acetazolamide intervention.

Since no statistically significant regional differences ( $P > 0.05$ ) became apparent from the data (i.e. no statistically significant relative region-related changes in the curves), also not for procedure B in the dorsal region, Fig. 9-3c, the uptake and retention of the tracer in each procedure was equated to the average R-value from all the slices in all views, and referred to as the total brain value for that procedure. Changes in these average total brain R-values are summarized in Table 9-1 as percentage differences due to the various procedures.

It becomes apparent from Table 9-1 that the cerebral uptake of [ $^{123}\text{I}$ ]IMP is higher than of  $^{99m}\text{Tc}$ -HMPAO by about 13% ( $P > 0.05$ ), which agrees well with known data (Winchell *et al.*, 1980a, b; Dormehl *et al.*, 1994). No meaningful regional differences appear in the intraprocedural distribution of the two tracers, also not interprocedural.

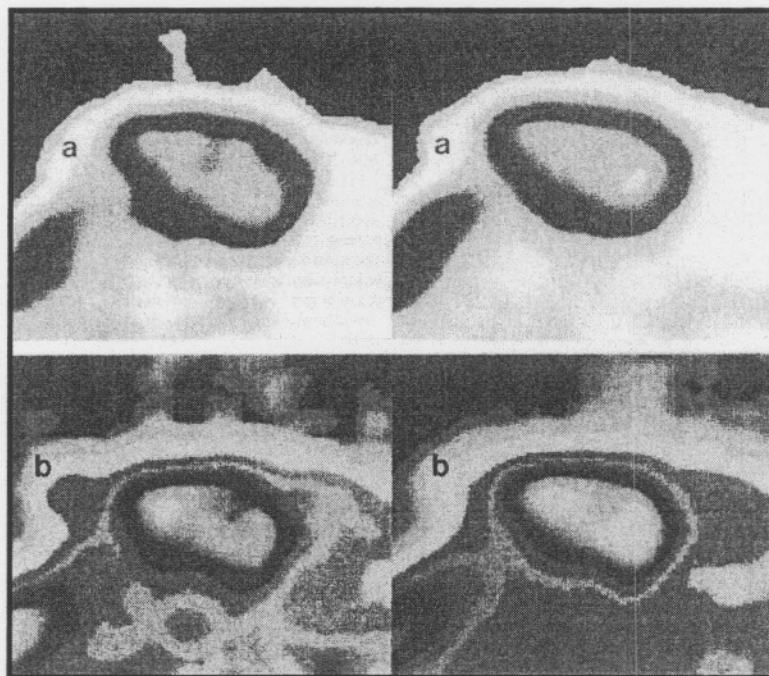
**Table 9-1** Percentage changes\* in total brain uptake of HMPAO and IMP because of the various procedures.

Procedures	Percentage change	Statistical significance P-values
A to C	+13 ± 1	$P > 0.05$
A to B	+37 ± 8	$P < 0.01$
C to D	+52 ± 3	$P < 0.01$
B to D	+26 ± 8	$P > 0.05$
A to D	+74 ± 4	$P < 0.01$
C to B	+21 ± 6	$P < 0.05, P > 0.01$

\* Percentage changes are expressed with respect to the procedure mentioned first

The influence of acetazolamide on rCBF when using  $^{99m}\text{Tc}$ -HMPAO as a tracer is one of a 37% increase (Table 9-1; Fig. 9-4a). This is significant ( $P < 0.01$ ) and in line with previous observations (Dormehl, 1993; Oliver *et al.*, 1993). Interesting is, however, the tendency of elevated rCBF (although not statistically significant,  $P > 0.05$ ) owing to acetazolamide in slice number 4 of the coronal view (Fig. 9-3c). This region is a dorsal representation of the brain and the observation has previously also been made (Dormehl, 1993), but remains unexplained, and maybe meaningless.

The increased CBF owing to acetazolamide, when using [ $^{123}\text{I}$ ]IMP is enhanced (52%;  $P < 0.01$ ), with respect to  $^{99m}\text{Tc}$ -HMPAO (Fig. 9-4b). The difference 37 vs. 52% is statistically significant ( $P < 0.01$ ). Regional patterns do not seem to be present.



**Figure 9-4** Sample images in the sagittal view for procedure B, with  $^{99m}\text{Tc}$ -HMPAO (a) and for procedure D, with [ $^{123}\text{I}$ ]IMP (b). The first image in each pair, from the split-dose method, represents the distribution in the brain from the first injection (SPECT-1). The second image represents the subtracted image (SPECT-2 - SPECT-1), to illustrate the effect of acetazolamide.

## 9.4 Discussion

Amphetamine-like drugs are all basic compounds that are able to penetrate the blood-brain barrier owing to their fairly high degree of lipophilicity. Drugs of this type are primarily excreted by the kidneys and re-absorption through the kidney tubules can occur which is dependent on the urinary pH. In acid urine these basic compounds are ionised (lipid insoluble) and are unable to diffuse back, and are subsequently excreted in the urine. Conversely alkaline urine would result in an increase of the serum levels of basic drugs owing to increase in the re-absorption with subsequent higher concentrations in the central nervous system compartment. Acetazolamide, a carbonic anhydrase inhibitor, is known to induce metabolic acidosis and alkaline urine (Gerhardt *et al.*, 1969).

It has previously been reported that the interaction between acetazolamide and quinidine may lead to quinidine intoxication due to increased serum levels because of the alkaline urine induced by acetazolamide (Goodman and Gilman, 1990). A similar mechanism could account for the higher values observed for IMP. IMP is, therefore, more susceptible to possible drug interactions (in particular those with marked influences on systemic and urine pH) than technetium-labelled HMPAO, and this could explain the enhanced CBF-effect with IMP and acetazolamide.

The mean R-values for the two acetazolamide procedures B and D respectively using HMPAO and IMP to measure rCBF, differ by about 26% ( $P > 0.05$ ). Procedure D yield the higher values and the percentage change exceeds that found between the baseline procedures A and C, i.e. 26 vs. 13%, which is statistically significant. This corresponds with the enhanced effect of acetazolamide on rCBF when measured with IMP.

In the light of these observations greatly over- and under-estimated changes in CBF can be expected, respectively, between procedures A and D, and C and B. This would logically argue against dual-isotope studies [ $^{99m}\text{Tc}$ -HMPAO and  $^{123}\text{I}$ (IMP)] in brain-stress testing after pharmacological intervention with acetazolamide for evaluating vasodilatory reserve.

Furthermore, the extent and reason for the acetazolamide-induced effect on CBF, which differ for  $^{99m}\text{Tc}$ -HMPAO and [ $^{123}\text{I}$ ]IMP, should also be considered when selecting the appropriate tracer for the diagnostic investigation or for experimental investigative purposes.

## 9.5 Conclusions

The current study clearly illustrates the importance of comparative nuclear medical studies in order to gain better insight into pharmacodynamical and pharmacokinetical interactions between drugs and radiopharmaceuticals. These data further emphasize that drugs that influence metabolic parameters systemically and urinary could indeed alter the bio distribution of

radiopharmaceuticals. Even when such changes are subtle they could still be meaningful and important.

## 9.6 References

**BACCIOTTINI L, PUP A, FORMICONI AR, DE CRISTOFARO MTR, MELDOLESI U.** 1989. Regional cerebral blood flow measurement in rat with HM-PAO and the indicator fractionation technique. *Journal of Cerebral Blood Flow and Metabolism* 9:S414

**BONTE FJ, DEVOUS MD, REISCH JS.** 1988. The effect of acetazolamide on regional cerebral blood flow in normal human subjects as measured by single photon emission computed tomography. *Investigative Radiology* 23:564-568.

**DEVOUS MD, PAYNE KJ, LOWE JL.** 1992. Dual-isotope brain SPECT imaging with technetium-99m and iodine-123: clinical validation using xenon-133 SPECT. *Journal of Nuclear Medicine* 33:1919-1924.

**DORMEHL IC, OLIVER DW, HUGO N.** 1995. Dose response form pharmacological interventions for CBF changes in a baboon model using  $^{99m}\text{Tc}$ -HMPAO and SPECT. *Nuclear Medicine Communications* 14:573-577.

**DORMEHL IC, PILLOY WP, HUGO N, ROSSOUW DT.** 1994. Regional cerebral blood flow patterns using [ $^{123}\text{I}$ ]IMP and  $^{99m}\text{Tc}$ -HMPAO with SPECT in the normal anaesthetised baboon. *European Journal of Nuclear Medicine* 21:848.

**DORMEHL IC, REDELINGHUYS IF, HUGO N, OLIVER D, PILLOY W.** 1992. The baboon model under anaesthesia for *in vivo* cerebral blood flow studies using SPECT techniques. *Journal of Medical Primatology* 21:270-274.

**ELL PJ, CULLUM I, COSTA DC, JARRITT PH, HOCKNELL JML, DIU D.** 1985a. Regional cerebral blood flow mapping with a new Tc-99m labelled compound. *The Lancet* 50-51.

**ELL PJ, HOCKNELL JML, JARRITT PA, PICKETT RD, CANNING LR, CULLUM I, LUI D, COMPAS COSTAS D, NOWOTNIK DP, NEIRINCKX RD.**

1985b. A Tc-99m-labelled radiotracer for the investigation of cerebral vascular disease. *Nuclear Medicine Communications* 6:437-441.

**GERHARDT RE, KNOUSS RF, THYRIUM PT, LUCHI RJ, MORRIS JJ.** 1969. Quinidine excretion in aciduria and alkaluria [Abstract]. *Ann Intern Med* 71:927.

**GOODMAN LS, GILMAN AG.** 1990. The pharmacological basis of therapeutics. (8<sup>th</sup> Edition. In: Gilman AG, Rall Tw, Nies AS, Talor P, eds. New York: Pergamon, 716-717).

**HOLM S, MADSEN PL, RUBIN P, SPERLING B, FRIBERG L, LASSEN N.** 1991. Tc-99m HMPAO activation studies: validation of the split-dose, image subtraction approach [Abstract]. *Journal of Cerebral Blood Flow and Metabolism* 11 (suppl) :S766.

**HOLMES RA, CHAPLIN SB, ROYSTON KG, HOFFMAN TJ, VOLKERT WA.** 1985. Cerebral uptake and retention of Tc-99m hexamethylpropylene amine oxime (Tc-99m HM-PAO). *Nuclear Medicine Communications* 6:443-447.

**NOWOTNIK DP, CANNING LR, CUMMING SA, HARRISON RC, HIGLEY B, NECHVATAL G, PIKETT RD, PIPER IM, BAYNE VJ, FORSTER AM, WEISNER PS, NEIRINCKS RD, VOLKERT WA, TRUTNER DE, HOLMES RA.** 1985. Development of a  $^{99m}\text{Tc}$ -labelled radiopharmaceutical for cerebral blood flow imaging. *Nuclear Medicine Communications* 6:499-506.

**OLIVER DW, DORMEHL IC, REDELINGHUYS IF, HUGO N, BEVERLY G.** 1993. Drug effects on cerebral blood flow in the baboon model. Acetazolamide and Nimodipine. *Nuklear Medizin* 32:292-298.

**PUPI A, BISI G, SIAGRA R, SANTORO D, DE CRISTOFARO MTR, FORMICONI AR, MELDOLESI U.** 1989. The comparison of brain distribution of HM-PAO and microspheres in humans. *Journal of Cerebral Blood Flow and Metabolism* 9:S411.

**STOCKLEY IH.** 1991. *Drug Interactions, A Sourcebook of Drug Interactions, Their mechanisms, Clinical Importance and Management*, 2<sup>nd</sup> Edn. Blackwell Scientific Publications, London.

## Technetium-99m-HMPAO, Technetium-99m-ECD and Iodine-123-IMP Cerebral Blood Flow Measurements with Pharmacological Interventions in Primates\*

## Chapter 10

### Summary

*Technetium-99m-bicisate ethyl cysteinate dimer (ECD) presents a different pattern from cerebral blood flow (CBF) in the sub acute phase of cerebral infarction, as measured by PET, perhaps due to lack of oxygen and enzyme activity; this pattern is contrary to that of hexamethylpropylene amine oxime (HMPAO) but similar to that of N-isopropyl-[<sup>123</sup>I] β-iodoamphetamine ([<sup>123</sup>I]IMP). This study explores possible CBF differences among HMPAO, ECD and IMP, with various relevant drug interventions. METHODS: Anaesthetized adult baboons were used in these SPECT studies. Four studies (n = 6 baboons for each study), one control study and three intervention studies involving intravenous acetazolamide, nimodipine infusion and intramuscular sumatriptan, were followed with <sup>99m</sup>Tc-HMPAO, <sup>99m</sup>Tc-ECD and [<sup>123</sup>I]IMP. The split-dose method was used as follows: For each tracer, intervention data from the second SPECT (SPECT-2) after the second tracer injection (444 MBq) reflected a change in CBF with respect to the baseline SPECT (SPECT-1) data from the initial injection (222 MBq). These changes as a ratio, R (R = SPECT-2/SPECT-1), for each study, and the R-values for each tracer were compared to R-values from the corresponding control studies, yielding a quantitative estimate of drug effects. RESULTS: There were no significant differences (P > 0.05) between HMPAO and ECD for the control, acetazolamide and sumatriptan studies, but*

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\* DORMEHL, I.C., OLIVER, D.W. HUGO, N., LANGEN, K-J. & CROFT, S. 1997. *Technetium-99m-HMPAO, Technetium-99m-ECD and Iodine-123-IMP Cerebral Blood Flow Measurements with Pharmacological Interventions in Primates. The journal of nuclear medicine, 38(12):1897 - 1901.*

*there was indeed a difference between the two for the nimodipine study, indicating a nimodipine-dependent underestimation of CBF with ECD (and also with IMP), with respect to HMPAO. A further significant difference was that larger CBF increases were observed with acetazolamide, as measured with [<sup>123</sup>I]IMP. CONCLUSION: This is a crucial observation for the clinical interpretation of CBF SPECT data and should direct the choice of tracer for a specific examination.*

**KEY WORDS:** *drug-tracer interaction, CBF SPECT, baboon model.*

## 10.1 Introduction

There is considerable interest in the development of tracers to measure cerebral blood flow (CBF) with SPECT. Such tracers should be trapped in the brain long enough so that their distribution can be quantitated and should demonstrate good spatial resolution.

Among the tracers that have been found useful are the iodine-labeled amines, e.g., N-isopropyl- [<sup>123</sup>I] β-iodoamphetamine ([<sup>123</sup>I] IMP) [1]. Its uptake linearly corresponds to a wide range of flow, as assessed by microspheres [2]. The brain retention of IMP will be a balance of washin and washout, which in turn will be influenced by blood flow, a retention mechanism that is stereo selective, and by metabolism of the tracer [3]. Despite its widespread use as a cerebral blood perfusion agent, IMP appears to redistribute in the brain with time [4]. Of several <sup>99m</sup>Tc-labelled compounds synthesized as cerebral perfusion agents, <sup>99m</sup>Tc-hexamethyl-propyleneamine oxime (<sup>99m</sup>Tc-HMPAO) has been used extensively in spite of its unfavourable stability after preparation. Its retention in the brain is limited to the enzymatic reactions with glutathione, of which there is a high prevalence [5, 6]. The CBF agent N,N' – 1,2-ethylene-di-yl-bis-L-cysteinate diethyl ester, labeled with <sup>99m</sup>Tc-bicisate ethyl cysteinate dimer (ECD), has a high initial brain extraction with a slow clearance [7]. The retention in the brain is associated with stereo specific deesterification to hydrophilic acid derivatives [8, 9]. As a CBF agent in healthy subjects, it corresponds well with <sup>133</sup>XE [10], although ECD underestimates higher flow rates, as HMPAO is also known to do. However, in cases of sub acute stroke,

ECD failed to show reflow hyperaemia in the infarct area, contrary to the action of HMPAO [11, 12] but similar to the known action (albeit to a lesser extent) of [<sup>123</sup>I]IMP [13].

It is important to know and understand quantitative and qualitative differences that are related to CBF, as measured by the various CBF agents. Such differences between the tracers may occur during various pathological conditions, as well as after relevant pharmacological interventions. Changes in the metabolic states of the brain appear to influence the kinetics and net accumulation of <sup>99m</sup>Tc-HMPAO at the cellular level by modifying the uptake, the backdiffusion or both [14]. Studies comparing these tracers under pharmacological intervention conditions have not yet been reported, and these comparisons were the aim of this study.

The drugs used for this purpose was chosen from previous studies reported in the literature. Acetazolamide has been used frequently in neuro-SPECT studies to evaluate cerebrovascular reserve. The recently developed lipophilic dihydropyridine calcium channel blocker, nimodipine, demonstrates the superiority in its influence on CBF compared to other calcium channel blockers and has been used for migraine and dementia [15]. The recent introduction of the 5-HT<sub>1D</sub> -agonist, sumatriptan, for the treatment of migraine has been a therapeutic breakthrough, with its undoubtable influence on abnormal CBF.

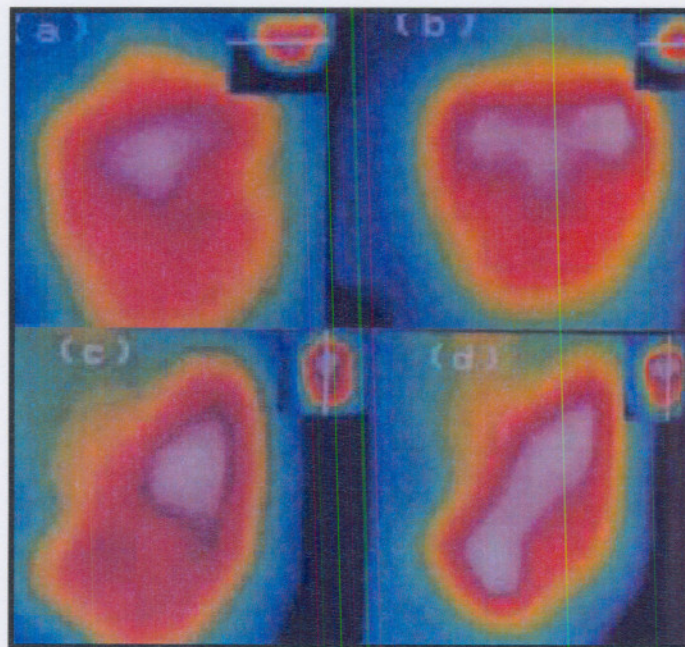
Drug intervention on CBF can ideally be investigated by the split-dose technique, whereby two doses of the tracer are administered within a one hour interval [16, 17], with the scan after the first administration acting as a control for the scan after the second administration, which is made with the subject at the appropriate response time of the drug to be evaluated. The baboon model, together with the split-dose method used in this study, has repeatedly proven to be ideally suited to the investigation of pharmacological interventions [18, 21].

This study reports the results of the above-mentioned pharmacological interventions on CBF as measured by the tracers <sup>99m</sup>Tc-HMPAO, <sup>99m</sup>Tc-ECD and [<sup>123</sup>I]IMP, using the baboon model and split-dose SPECT.

Procedure B was the same as procedure A until the completion of SPECT-1, which was then succeeded by an intravenous injection of 500 mg of acetazolamide (Diamox<sup>®</sup>, Cyanamid (Pty) Ltd, South Africa), at 24 min, i.e., 5 min before the second tracer administration. Thus, SPECT-2, which again followed 5 min after the second <sup>99m</sup>Tc-HMPAO/ECD injection, as in procedure A, reflected the influence of acetazolamide (5 min response time) on CBF.

Procedure C had the same protocol as procedure B, but the intervention was an infusion of nimodipine (Nimotop IV<sup>®</sup>, Bayer (Pty) Ltd), at 1 µg/kg/min, which started 10 min before the second tracer injection and continued as an infusion for a total of 15 min. SPECT-2 as before, began at this stage.

During procedure D, the effect of sumatriptan (Imigran<sup>®</sup>, Glaxo (Pty) Ltd), was investigated. A response time of 23 min was chosen to permit (because of stability limitations) the use of the same vial of HMPAO for the second injection at 29 min. The intramuscular administration of sumatriptan, therefore, took place at 6 min, i.e., 1 min after SPECT-1 had commenced. SPECT-2 thus reflected the effect of sumatriptan at the 23 min response time, which is close to the time that leads to optimal effect.



**Figure 10-1** Typical coronal (a and b) and sagittal (c and d) views, representing, in each view, baseline and changed post nimodipine CBF patterns obtained with <sup>99m</sup>Tc-HMPAO.

After backprojection and reconstruction of SPECT-1 and SPECT-2 data, the brain images in all procedures consisted of transaxial, sagittal and coronal slices, representing CBF- and regional CBF-related information. Eight slices of one pixel thickness represented the brain in all three views [Fig. 10-1, coronal (a and b) and sagittal views (c and d)].

Regions of interest were placed on the total brain, as viewed in each slice, and counting rate data (count/pixel) thus obtained were inserted into the following equation to obtain the ration R:

$$R = \frac{(SPECT - 2) - (SPECT - 1) *}{(SPECT - 1)}$$

where \* refers to decay-corrected data form SPECT-1 that is present during SPECT-2 and has to be subtracted from the SPECT-2 data as a background correction. R is an indication of the level change of regional CBF during extended anaesthesia or in addition, because of the drug interventions, as measured with <sup>99m</sup>Tc-HMPAO or <sup>99m</sup>Tc-ECD. A value of R = 2 indicates no change during the procedure. The arterial blood pressures (BPs) were recorded during all the procedures from a catheter in the femoral artery. Heart rates were also monitored, as were blood gases form an arterial line.

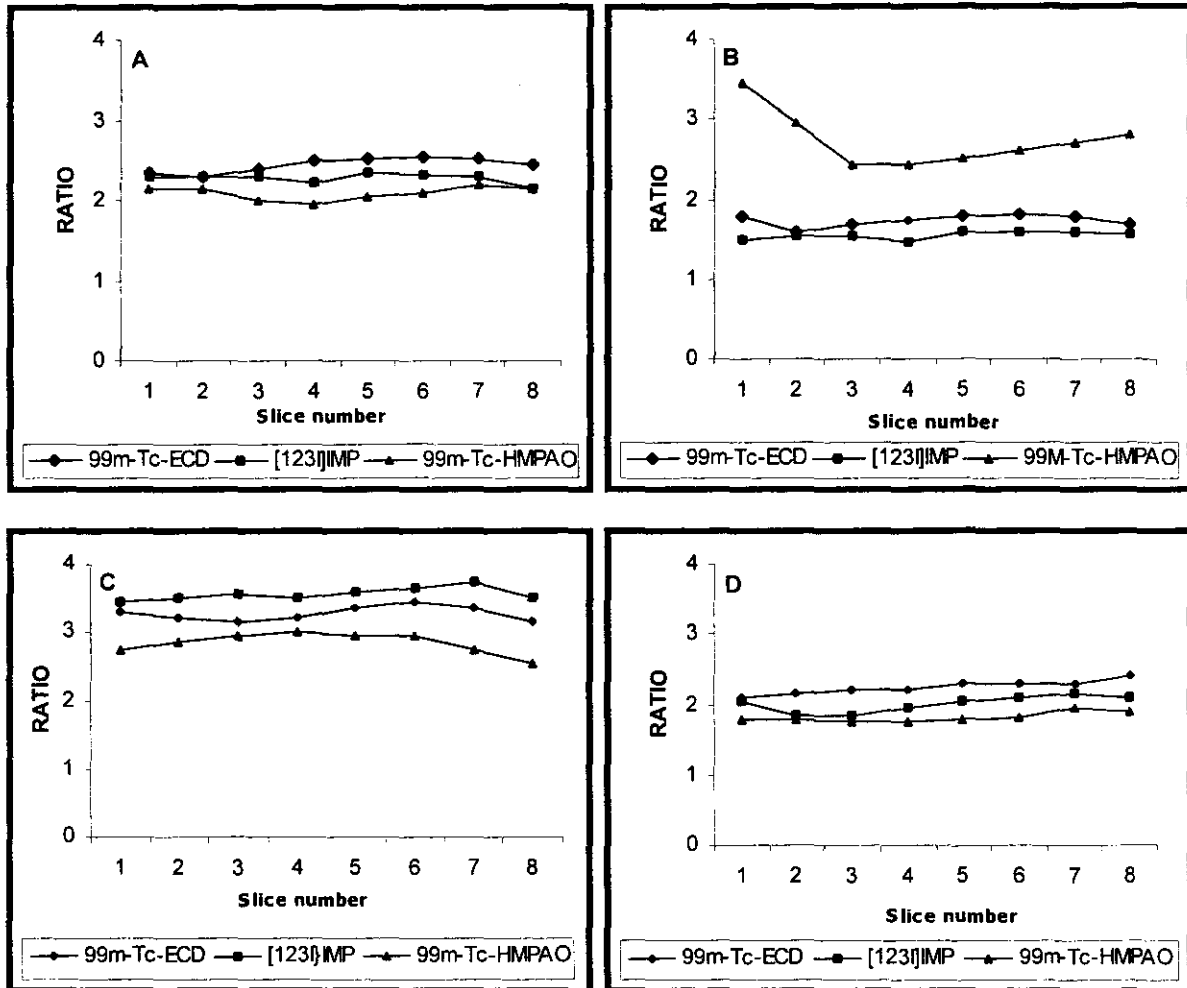
Procedures A – D were repeated using [<sup>123</sup>I]IMP (National Accelerator Center, Faure, South Africa) as tracer for both injections (i.e., 148-296 MBq) in the protocols described above.

The R-values for eight slices in transaxial, sagittal and coronal view could be compared between control and interventional studies for each tracer and also between tracers for similar procedures. A two-tailed Student's *t*-test for paired variables was used, with a 5% level of confidence.

### 10.3 Results

The results are summarized as curves of mean (n = 6) R-values vs. slice number in the transaxial view for all procedures and tracers (Fig. 10-2). Values from the sagittal and coronal views in all cases confirm the measured drug

effects, as represented by the transaxial view, and were, therefore, not included in the figure.



**Figure 10-2** (A-D) Mean ratio's R (n = 6) vs. tomographic slice number in the transaxial views (occipital to frontal) comparing <sup>99m</sup>Tc-HMPAO, <sup>99m</sup>Tc-ECD and [<sup>123</sup>I]IMP for the control (A) and each intervention, acetazolamide (B), nimodipine (C) and sumatriptan (D).

Regional effects are only crudely represented by the slice-related information of the method. No such regional effects could be established from any of the mean R vs. slice number curves as being meaningful interslice differences, except for nimodipine-induced changes, as measured by <sup>99m</sup>Tc-HMPAO. In this case, the increased CBF was significantly more pronounced in the cerebellum [Fig. 10-1, sagittal view (c and d)]. Average R-values were thus calculated using all slices to represent total brain R-values and are presented in Table 10-1.

**Table 10-1** Mean ratios from the total brain region as obtained from various interventions and percentage change from each with respect to its own control.

Intervention	Mean ratio (R) ± SD (n = 6)			% difference with respect to control		
	<sup>99m</sup> Tc-HMPAO	<sup>99m</sup> Tc-ECD	[ <sup>123</sup> I]IMP	<sup>99m</sup> Tc-HMPAO	<sup>99m</sup> Tc-ECD	[ <sup>123</sup> I]IMP
Control	1.79 ± 0.13	2.07 ± 0.24	1.95 ± 0.05			
Acetazolamide	2.53 ± 0.15	2.78 ± 0.13	3.02 ± 0.06	41.30%	34.30%	54.90%
Nimodipine	2.51 ± 0.14	1.67 ± 0.13	1.48 ± 0.34	40.20%	-19.3%	-24.1%
Sumatriptan	1.74 ± 0.10	2.09 ± 0.08	1.90 ± 0.06	-3%	1%	-2.5%

Percentage differences based on the R-values are presented for each procedure with respect to the control of that particular tracer (Table 10-1). For all three tracers, CBF increases were noted after acetazolamide intervention. Technetium-99m-HMPAO measured a CBF increase after nimodipine intervention, but <sup>99m</sup>Tc-ECD and [<sup>123</sup>I]IMP measurements showed decreases in CBF under similar conditions. Changes in the physiological parameters due to the drug interventions are presented in Table 10-2. The only statistically significant changes (*P* < 0.05) were seen in pO<sub>2</sub> and BP for acetazolamide (+20 and -8, respectively).

**Table 10-2** Effects for Procedures B (Acetazolamide), C (Nimodipine) and D (Sumatriptan) on heart rate, blood pressure, pCO<sub>2</sub> and pO<sub>2</sub> in arterial blood in baboons

Procedure	Heart rate	Blood pressure	pCO <sub>2</sub>	PO <sub>2</sub>
B1	116.25 ± 12.74	118.00 ± 2.58	41.10 ± 2.37	56.35 ± 4.91
B2	111.50 ± 16.84	110.25 ± 4.50*	40.08 ± 4.87	76.43 ± 7.47*
C1	115.33 ± 22.38	132.50 ± 29.93	42.85 ± 7.55	61.12 ± 4.39
C2	116.33 ± 24.58	112.89 ± 20.26	40.50 ± 1.27	68.50 ± 5.37
D1	115.31 ± 11.05	119.20 ± 9.90	41.91 ± 7.60	59.17 ± 8.39
D2	114.31 ± 14.13	112.13 ± 8.81	39.83 ± 5.33	66.55 ± 9.32

\* *P* < 0.05, for postintervention compared to corresponding preintervention values. Each value indicates the mean ± SD of six experiments. Subscript 1 and 2 refer to the measurements before the intervention and at the chosen response time, respectively.

## 10.4 Discussion

To date, various comparisons have been drawn between cerebral perfusion imaging agents for SPECT [13, 22-24], but the general conclusion remains that all currently available radiopharmaceuticals, including <sup>99m</sup>Tc-HMPAO, <sup>99m</sup>Tc-

ECD and [<sup>123</sup>I]IMP, are far from ideal. Although all three of the above tracers are neutral and lipophilic, their kinetic behaviour and trapping mechanisms differ. Consequently, there are certain conditions where they have been found to give different images; among these are cases of stroke and neurophysiological stimulation [11, 25]. From this study, it appears that interpretation of data from pharmacological interventions could also be a challenge and should be treated with caution.

R-values, as defined in this study, should ideally reach the value of 2 [18], to confirm the second double dose of tracer if no change in CBF or influencing metabolism had occurred before the second tracer injection. R-values from the interventional procedures can be compared to their corresponding controls to establish the effect of each intervention, which will be CBF- and/or metabolism-related, while controlling for common anaesthesia effects and tracer effects.

Of the mean control R-values obtained in this study, those obtained by <sup>99m</sup>Tc-ECD and [<sup>123</sup>I]IMP reach the value 2 more closely and are also not significantly statistically different ( $P > 0.05$ ) from each other. Neither do they differ statistically significantly from the mean control R-value obtained by the <sup>99m</sup>Tc-HMPAO studies ( $P > 0.05$ ), although the last appears to be lower. By now, this low R-value has been repeatedly reproduced with this experimental model and can be explained in terms of backdiffusion of the tracer [26] and the ketamine hydrochloride anaesthesia, which leads to increased BP (+30) and heart rate (+15) during the first <sup>99m</sup>Tc-HMPAO administration and is not maintained during the second barbiturate phase of the study [18].

The lower <sup>99m</sup>Tc-ECD back-flux from the brain leads to a higher retention of <sup>99m</sup>Tc-ECD than <sup>99m</sup>Tc-HMPAO, which may contribute to the higher control R-values obtained with <sup>99m</sup>Tc-ECD ( $2.07 \pm 0.24$  compared to  $1.79 \pm 0.13$ ) [26]. The time to reach the steady state is the same for these two tracers and will not contribute to the difference [26].

All three tracers measure the familiar increase of CBF by acetazolamide [12, 21, 27, 28). In comparison the highest increase (+54.9%) is measured by [<sup>123</sup>I]IMP, which has preciously been reported [19] and has been partly

attributed to a pH effect. Alkaline urine, which results from a carbonic anhydrase inhibitor such as acetazolamide ( $\Delta p\text{CO}_2 =$  );  $\Delta p\text{O}_2 = +20$ ;  $\Delta\text{BP} = -8$ ) [21], leads to reabsorption and an increase of the serum levels of basic drugs, with subsequent higher concentration in the central nervous system compartment [29, 30]. N-isopropyl- $\beta$ -iodoamphetamine as a basic compound is, therefore, more susceptible to possible drug interactions (in particular, those with marked influences on systemic and urine pH) than are  $^{99\text{m}}\text{Tc}$ -HMPAO and  $^{99\text{m}}\text{Tc}$ -ECD, explaining the significantly enhanced CBF effect with [ $^{123}\text{I}$ ]IMP and acetazolamide. N-isopropyl [ $^{123}\text{I}$ ]  $\beta$ -iodoamphetamine could, thus, be the more sensitive tracer to assess cerebrovascular reserve through acetazolamide intervention.

The underestimation of acetazolamide-induced CBF increases, which would normally have been expected with  $^{99\text{m}}\text{Tc}$ -HMPAO due to high flow, was not obvious in this study (41% compared to 35%) [26, 31] but was indicated with  $^{99\text{m}}\text{Tc}$ -ECD (34.3%) [32]. The first-pass extraction rate is flow-dependant for  $^{99\text{m}}\text{Tc}$ -ECD and  $^{99\text{m}}\text{Tc}$ -HMPAO, and back-flux affects  $^{99\text{m}}\text{Tc}$ -HMPAO retention more than it does  $^{99\text{m}}\text{Tc}$ -ECD retention. Therefore, the question arises of whether a certain degree of saturation of enzymatic reactions had occurred that might have accompanied the high rate of bioavailability of  $^{99\text{m}}\text{Tc}$ -ECD, as follows with a high CBF from acetazolamide. Protein binding plays an important role in the distribution of drugs, and the difference in binding between  $^{99\text{m}}\text{Tc}$ -HMPAO and  $^{99\text{m}}\text{Tc}$ -ECD could contribute to different concentrations of un-ionised hydrophobic metabolites so that a metabolic parameter, such as saturation, becomes a factor, particularly at higher flow rates. Saturation is not expected with  $^{99\text{m}}\text{Tc}$ -HMPAO [11].

The  $\text{Ca}^{2+}$ -blocker nimodipine was shown to increase CBF by 40.2% for  $^{99\text{m}}\text{Tc}$ -HMPAO and decrease it by 19.3% and 24.1% for  $^{99\text{m}}\text{Tc}$ -ECD and [ $^{123}\text{I}$ ]IMP, respectively; all changes were statistically significant ( $P < 0.05$ ) (Fig. 10-2). The larger effect measured by [ $^{123}\text{I}$ ]IMP as compared to that of  $^{99\text{m}}\text{Tc}$ -ECD was not significantly different ( $P > 0.05$ ). The difference in R-values among the tracers after the nimodipine intervention especially with  $^{99\text{m}}\text{Tc}$ -HMPAO, immediately warns that drug-tracer interaction must be considered

and allowed for when measuring CBF with SPECT. The role of cofactors in metabolism, such as magnesium cations, is well established, and drugs that influence such factors could indeed contribute to changed accumulation of compounds that are dependent on these factors for their metabolism. Nimodipine, as a calcium-channel blocker, may exert its effects through such a mechanism, resulting in unexpected blood flow patterns that manifest as being tracer-selective. It is unclear why nimodipine should influence the transport of  $^{99m}\text{Tc}$ -HMPAO across the blood brain barrier differently than it does the transport of  $^{99m}\text{Tc}$ -ECD and [ $^{123}\text{I}$ ]IMP.

It should, however, be noted that, for nimodipine, not only have CBF increases been measured [33] but also, at higher doses than those in the present experiment, CBF reductions have been accompanied by reduced BP [34], suggesting a loss of autoregulation at high doses. Studies indicate that even low doses of nimodipine can inhibit the autoregulatory adjustment to altered BP [33, 35], depending on the size of the BP stimulus. The large SD, which is more obvious for nimodipine, suggests a contribution in this study from biological variability of BP responses to the results.

The intervention with sumatriptan had no effect on normal CBF, as measured by all three tracers ( $P > 0.05$ ).

## 10.5 Conclusion

The most obvious observations noted in the CBF values, as measured by [ $^{123}\text{I}$ ]IMP, are the close approximation to  $R = 2$  in the control study and the high  $R$ -values with acetazolamide. Furthermore, [ $^{123}\text{I}$ ]IMP measures, after nimodipine intervention, a reduction in CBF, as does  $^{99m}\text{Tc}$ -ECD, contrary to  $^{99m}\text{Tc}$ -HMPAO. It is known that [ $^{123}\text{I}$ ]IMP follows a pattern linearity with flow over a wider flow range than do  $^{99m}\text{Tc}$ -HMPAO and  $^{99m}\text{Tc}$ -ECD, and it could, therefore, be regarded the truer CBF agent. On the other hand, as a basic drug, amphetamine uptake is influenced by urinary and intracellular pH, as is seen when it is used with acetazolamide (increased uptake) and in sub acute stroke (decreased uptake), respectively. Technetium-99m-ECD also demonstrates hypoactivity in sub acute stroke, which is linked to altered

esterase function in hypoxia, and its low CBF values, measured after the nimodipine intervention (which [ $^{123}\text{I}$ ]IMP also measures), could, therefore, be seen as relating to a drug-tracer interaction, in which metabolic processes play a role. Technetium-99m-HMPAO shows a focal area of hyperactivity during sub acute stroke. The inclination to caution due to drug-tracer interactions with CBF SPECT measurements is, therefore, not unfounded. The familiar differences of back-flux and, possibly, saturation between  $^{99\text{m}}\text{Tc}$ -HMPAO and  $^{99\text{m}}\text{Tc}$ -ECD were also observed, which could influence the diagnostic sensitivity of the particular tracer.

It is, in addition, an interesting finding that sumatriptan does not appear to change normal CBF.

This study confirms that the interpretation of CBF SPECT data after pharmacological interventions could be a challenge and should be viewed with cognisance of tracer and drug characteristics.

## 10.6 References

1. WINCHELL HS, BLADWIN RM, LIN TH. 1980. Development of I-123-labelled amines for brain studies: localization of I-123 iodo-phenylalkyl amines in rat brain. *Journal of Nuclear Medicine* 21:940-947.
2. KUHL DE, BARRIO JR, HUANG SC, *et al.* 1982. Quantifying cerebral blood flow by N-isopropyl-p- $^{123}\text{I}$ -iodoamphetamine (IMP) tomography. *Journal of Nuclear Medicine* 23: 196-203.
3. NISHIZAWA S, TANADA S, YONEKURA Y, *et al.* 1989. Regional dynamics of N-isopropyl-( $^{123}\text{I}$ )-iodo-amphetamine in human brain. *Journal of Cerebral Blood Flow and Metabolism* 30:150-156.
4. LASSEN NA, HENRIKSEN L, HOLM S, *et al.* 1983. Cerebral blood flow tomography: xenon-133 compared with isopropyl-amphetamine-iodine-123. *Journal of Nuclear Medicine* 27:17-21.

5. **LASSEN NA, ANDERSON AR, FRIBERG L, PAULSON OB.** 1988. The retention of [99mTc]-d,1-HMPAO in the human brain after intracarotid bolus injection: a kinetic analysis. *Journal of Cerebral Blood Flow and Metabolism* 8 (suppl 1): S13-S22.
6. **NIERINCKX RD, BURKE JF, HARRISON RC, FORSTER AM, ANDERSON AR, LASSEN NA.** 1988. The retention mechanism of technetium-99m-HM-PAO; Intracellular reaction with glutathione. *Journal of Cerebral Blood Flow and Metabolism* 8:S4-S12.
7. **WALOVITCH RC, HILL TC, GARRITY ST, et al.** 1989. Characterization of technetium-99m-L,L-ECD for brain perfusion imaging, part 1: pharmacology of technetium-99m-ECD in non-human primates. *Journal of Nuclear Medicine* 30:1892-1901.
8. **HOLMAN LB, HELLMAN RS, GOLDSMITH SJ, et al.** 1989. Biodistribution, dosimetry and clinical evaluation of technetium-99m ethyl cysteinate dimer in normal subjects and in patients with chronic cerebral infarction. *Journal of Nuclear Medicine* 30:1018-1024.
9. **VALLABHAJOSULA S, ZIMMERMANN RE, PICARD M, et al.** 1989. Technetium-99m-ECD: a new brain imaging agent. In vivo kinetics and bio distribution studies in normal human subjects. *Journal of Nuclear Medicine* 30:599-604.
10. **HUGLO D, ROUSSEAX M, LEYS D, FILADES P, STEINLING M.** 1994. Regional cerebral blood flow imaging: a quantitative comparison of 99m-Tc-bicisate with 133-Xe using single photon emission computed tomography. *Journal of Cerebral Blood Flow and Metabolism* 14 (suppl 1):S76-S83.
11. **LASSEN NA, SPERLING, B.** 1994. 99m-Tc-bicisate reliably images CBF in chronic brain diseases but fails to show reflow hyperaemia in sub acute stroke: report of a multicenter trail of 105 cases comparing 133-Xe and 99m-Tc-bicisate (ECD, Neurolite) measured by SPECT on the same day. *Journal of Cerebral Blood Flow and Metabolism* 14(suppl 1):S44-S48.

12. **NAKAGAWARA J, NAKAMURA J, TAKEDA R. et al.** 1994. Assessment of postischemic reperfusion and Diamox activation test in stroke using  $^{99m}\text{Tc}$ -ECD SPECT. *Journal of Cerebral Blood Flow and Metabolism* 14 (suppl 1):S49-S57.
13. **MORETTI J-L, DEFER G, TAMGAC F, WEINMANN CB, CESARO P.** 1994. Comparison of brain SPECT using  $^{99m}\text{Tc}$ -bicisate (L,L-ECD) and [ $^{123}\text{I}$ ]IMP in cortical and subcortical strokes. *Journal of Cerebral Blood Flow and Metabolism* 14 (suppl 1):S84-S90.
14. **AHN CS, TOW DE, YU CC, GREENE RW.** 1994. Effect of metabolic alterations on the accumulation of  $\text{Tc-}^{99m}$ -labelled D,L-HMPAO in slices of rat cerebral cortex. *Journal of Cerebral Blood Flow and Metabolism* 14:324-331.
15. **HÖLLERHAGE H-G, GAAB MR, ZUMKELLER M, WALTER GF.** 1988. Nimodipine treatment has been implicated in impaired CBF autoregulation. *Journal of Neurosurgery* 69:919-922.
16. **HOLM S, MADSEN PL, SPERLING B, LASSEN NA.** 1994. Use of  $^{99m}\text{Tc}$ -bicisate in activation studies by split-dose technique. *Journal of Cerebral Blood Flow and Metabolism* 14 (suppl 1):S115-S120.
17. **WYPER DJ, HUNTER R, PATTERSON J, GOODWIN G, McCULLOCH J.** 1991. A split-dose technique for measuring changes in cerebral blood flow patterns [Abstract]. *Journal of Cerebral Blood Flow and Metabolism* 11 (suppl 2) :S449.
18. **DORMEHL IC, REDELINGHUYS IF, HUGO N, OLIVER D, PILLOY W.** 1992. The baboon model under anaesthesia for *in vivo* cerebral blood flow studies using SPECT techniques. *Journal of Medical Primatology* 21:270-274.
19. **DORMEHL IC, OLIVER DW, HUGO N.** 1995. Dose response form pharmacological interventions for CBF changes in a baboon model using

- <sup>99m</sup>Tc-HMPAO and SPECT. *Nuclear Medicine Communications* 14:573-577.
20. **DORMEHL IC, OLIVER DW, HUGO N, ROSSOUW D.** A comparative cerebral blood flow study in a baboon model with acetazolamide provocation: <sup>99m</sup>Tc-HMPAO vs. [<sup>123</sup>I]IMP. *Nuclear Medicine and Biology* 22:373-379.
  21. **OLIVER DW, DORMEHL IC, REDELINGHUYS IF, HUGO N, BEVERLY G.** 1993. Drug effects on cerebral blood flow in the baboon model. Acetazolamide and Nimodipine. *Nuklear Medizin* 32:292-298.
  22. **MORETTI J-L, CAGLAR M, WEINMANN P.** 1995. Cerebral perfusion imaging tracers for SPECT: which one to choose? *Journal of Nuclear Medicine* 36:359-363.
  23. **SUZUKI S, SAKAI F, AKUTSU T, TAZAKI Y.** 1991. Tracer kinetics of [<sup>123</sup>I]IMP, <sup>99m</sup>Tc-HMPAO and <sup>99m</sup>Tc-Ecd: measurements of temporal changes in arterial and jugular venous radioactivity [Abstract]. *Journal of Cerebral Blood Flow and Metabolism* 11 (suppl 2) :S774.
  24. **YONEKURA Y, TSUCHIDA T, SADATO N. et al.** 1994. Brain perfusion SPECT with <sup>99m</sup>Tc-bicisate: comparison with PET measurement and linearization based on permeability-surface area product model. *Journal of Cerebral Blood Flow and Metabolism* 14 (suppl 1) :S58-S65.
  25. **HOLM S, MADSEN PL, RUBIN P, SPERLING B, FRIBERG L, LASSEN N.** 1991. Tc-99m HMPAO activation studies: validation of the split-dose, image subtraction approach [Abstract]. *Journal of Cerebral Blood Flow and Metabolism* 11 (suppl) :S766.
  26. **MURASE K, TANADA S, INOUE T. et al.** 1992. Kinetic behaviour of Tc-99m ECD in the human brain using compartment analysis and dynamic SPECT: comparison with Tc-99m HMPAO. *Journal of Nuclear Medicine* 33:135-143.

27. **BONTE FJ, DEVOUS MD, REISCH JS.** 1988. The effect of acetazolamide on regional cerebral blood flow in normal human subjects as measured by single photon emission computed tomography. *Investigative Radiology* 23:564-568.
28. **NAKAGAWARA J, TAKEDA R, TANAKA Y, NAKAMURA J, SUEMATSU K.** 1989. Assessment of haemodynamic reserve in candidates for carotid bypass surgery using SPECT and [<sup>123</sup>I]IMP activated with acetazolamide test. *Adv Funct Neuroimag* 2:8-15.
29. **GERHARDT RE, KNOUSS RF, THYRIUM PT, LUCHI RJ, MORRIS JJ.** 1969. Quinidine excretion in aciduria and alkaluria [Abstract]. *Ann Intern Med* 71:927.
30. **GOODMAN LS, GILMAN AG.** 1990. The pharmacological basis of therapeutics. (8<sup>th</sup> Edition. *In: Gilman AG, Rall Tw, Nies AS, Talor P, eds.* New York: Pergamon, 716-717).
31. **VORSTRUP S.** 1988. Tomographic cerebral blood flow measurements in patients with ischemic cerebrovascular disease and evaluation of the vasodilatory capacity by the acetazolamide test. *Acta Neurologica Scandinavica* 77(suppl 144): 1-48.
32. **FRIBERG L, ANDERSEN AR, LASSEN NA, HOLM S, DAM M.** 1994. Retention of 99mTc-bicisate in the human brain after intracarotid injection. *Journal of Cerebral Blood Flow and Metabolism* 14 (suppl) :S19-S27.
33. **McGALDEN TA, NATH RG.** 1989. Cerebrovascular autoregulation is resistant to calcium channel blockade with nimodipine. *Experientia* 45:305-306.
34. **HAWS CW, HEISTAD DD.** 1984. Effects of nimodipine on cerebral vasoconstrictor responses [Abstract]. *American Journal of Physiology* 247:H170.
35. **SAHLIN C, BRISMAR J, DELGADO T, OWMAN C, SALFORD LG, SVENGAARD NA.** 1987. Cerebrovascular and metabolic changes during

the delayed vasospasm following experimental subarachnoid haemorrhage in baboons, and treatment with a calcium antagonist. *Brain Research* 403-413.

## Cerebral Blood Flow Effects of Sodium Valproate in Drug Combinations in the Baboon Model\*

## Chapter 11

### Summary

*Sodium valproate (CAS 1069-66-5, Epilim®) has been used in the management of epilepsy during the last three decades. Although important information on the pharmacological actions and efficacy of sodium valproate has accrued to date, limited research has been conducted on its effects on cerebral blood flow. In recent years, with the aid of SPECT (single photon emission computed tomography) and PET (positron emission tomography) it has been shown that marked cerebral blood flow changes occur in epileptic patients. Furthermore it was established recently that sodium valproate influences the cerebral blood flow in children by decreasing the flow significantly. The present study investigated the effects of sodium valproate on the cerebral blood flow, using <sup>99m</sup>Tc-HMPAO (hexamethylpropylene amine oxime) and SPECT, in a primate model, as well as the effects of its drug interactions with therapeutic agents that influence cerebrovascular dynamics, e.g. sumatriptan, nimodipine and acetazolamide. The current study using single dose treatment with sodium valproate did not detect a decrease or increase of the cerebral blood flow when compared with control baseline results. Drug interaction between sodium valproate and nimodipine may occur as a reduction of 25% in cerebral blood flow from the baseline control was observed in this case. The effects observed for the combinations of sodium valproate respectively with sumatriptan and acetazolamide are attributed to the influences of the sumatriptan (decrease) and acetazolamide (increase) alone. The*

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*cerebral blood flow effects of these drugs and possible interactions during an acute epileptic seizure need to be investigated.*

**KEY WORDS:** Acetazolamide, CAS 1069-66-5, Epilim<sup>®</sup>, Nimodipine, Sodium valproate, cerebral blood flow effects, drug interactions, primate model, Sumatriptan.

## 11.1 Introduction

Sodium valproate (CAS 1069-66-5) has been introduced in Europe into the arsenal of anti-epileptic drugs about three decades ago and was approved by the Food and Drug Administration in the United States in 1978 [1]. Its effectiveness as an anti-epileptic drug is well established and it has been shown to inhibit seizures in a variety of models. Also its modes of action are well documented [1-5]. Recently an intravenous preparation of sodium valproate has been developed and is currently being marketed in South Africa as Epilim<sup>®</sup>, R&C Pharmaceuticals, Merebank, Republic of South Africa) and was obtained from the manufacturer for this study. Drug interactions of sodium valproate with significant clinical implications have been demonstrated: amongst others the plasma concentration of phenobarbital may rise by as much as 40% when valproate is administered concurrently, which may involve inhibition of the metabolism of phenobarbital [1]. Valproate also competes for plasma protein binding sites with subsequent increased plasma concentration of free (unbound) tolbutamide. The development of cerebral blood flow tracers such as technetium labelled <sup>99m</sup>Tc-HMPAO and <sup>99m</sup>Tc-ECD has made it possible to investigate the cerebral blood flow dynamics under various conditions, e.g. during pharmacological drug interventions, anaesthesia and in drug combination studies [6-11]. A non-human primate model developed by us [6], has proven to be adequately sensitive for measuring the effects of anaesthesia, pharmacological interventions, and drug combinations on CBF using single photo emission computed tomographic techniques and the above mentioned cerebral blood flow tracers.

It has recently been reported that there is a significant (although slight) reduction in cerebral blood flow in children upon treatment with sodium

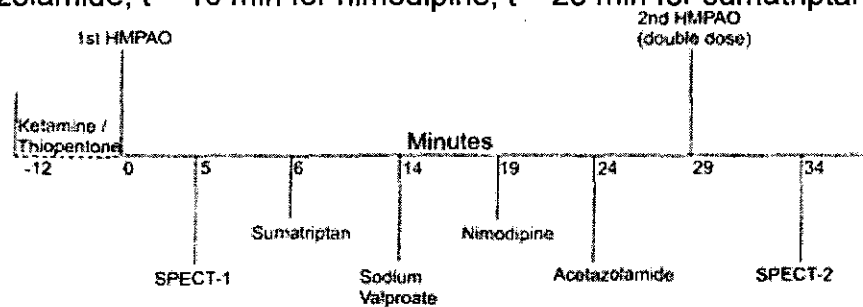
valproate and carbamazepine [12]. Several studies have indicated that cerebral blood flow abnormalities are present in epileptic patients [13-18]. The monitoring of cerebral blood flow of epileptic patients during therapy as well as during drug combination administrations is becoming more important for our understanding of the mechanisms involved in epilepsy. The objective of the current study was to investigate the possible drug interactions of sodium valproate with other cerebrovasoactive drugs, i.e. sumatriptan (CAS 103628-46-2) (used during the treatment of migraine), nimodipine (CAS 66085-59-4) (the calcium channel blocker) and acetazolamide (CAS 59-66-5) (the standard drug for investigating cerebrovascular reserve, a carbonic anhydrase inhibitor). Clinically important information on the influence of these combinations on cerebral blood flow could be gained from these studies. They could also add to the knowledge of the pharmacology of these drugs.

## 11.2 Materials and methods

Adult male baboons (*Papio ursinus*, average weight 27kg) were used for this study. The animals were obtained from Mr. E. Venter, Vaalwater, Northern Province, Republic of South Africa. The studies were performed after approval from the Ethics Committee of the University of Pretoria, according to the guidelines of the National Code for Animal Use in Research, Education and Testing of Drugs and Related Substances in South Africa. These guidelines are in line with international standards.

Five different procedures (A-E) with six animals per procedure were carried out (Fig. 9-1). Procedure A was a control study where changes in CBF that occurred during anaesthesia of 29 min were measured. This was also the duration of the anaesthesia used for procedures B-E when CBF changes were measured after sodium valproate (obtained from the manufacturer) only (B) and after various double drug interventions at appropriate response times. The drug interventions studied were: sodium valproate (100 mg/ml/min i.v. infused over 10 min; procedure B) at response time  $t = 15$ min; and then drug combination interventions which consisted of sodium valproate (100 mg/ml/min i.v. infused over 10 min) with one of either acetazolamide (100mg/ml i.v. [5 ml];

procedure C), nimodipine (1µg/kg/min infused over 10 min; procedure D), and sumatriptan (6mg i.m.; procedure E), each injected so as to allow measurement at the appropriate response time  $t = 15$  min for sodium valproate,  $t = 5$  min for acetazolamide,  $t = 10$  min for nimodipine;  $t = 23$  min for sumatriptan.



min	Procedure A	Procedure B	Procedure C	Procedure D	Procedure E
-12	Ketamine Thiopentone	Ketamine Thiopentone	Ketamine Thiopentone	Ketamine Thiopentone	Ketamine Thiopentone
0	1 <sup>st</sup> HMPAO	1 <sup>st</sup> HMPAO	1 <sup>st</sup> HMPAO	1 <sup>st</sup> HMPAO	1 <sup>st</sup> HMPAO
5	SPECT-1	SPECT-1	SPECT-1	SPECT-1	SPECT-1
6					Sumatriptan
14		Sodium Valproate	Sodium Valproate	Sodium Valproate	Sodium Valproate
19				Nimodipine	
24			Acetazolamide		
29	2 <sup>nd</sup> HMPAO	2 <sup>nd</sup> HMPAO	2 <sup>nd</sup> HMPAO	2 <sup>nd</sup> HMPAO	2 <sup>nd</sup> HMPAO
34	SPECT-2	SPECT-2	SPECT-2	SPECT-2	SPECT-2

**Figure 11-1** The time schedule for the various drug interventions procedure protocols indicating the time of each intervention

For each study the baboon was sedated with ketamine hydrochloride (10mg/kg i.m.) (Anaket-V<sup>®</sup>, Centaur Labs, Bryanston, Gauteng, SA). Was followed immediately by maintained and controlled infusion of thiopentone sodium (70 ml/hr of 0.5% solution) (Intraval<sup>®</sup>, Rhône-Poulenc, Rorer, S.A., Midrand, Gauteng, S.A.) using an administration (drip) set. After a 12-min stabilisation period under thiopentone, procedure A, the control study under anaesthesia, started with an i.v. injection of 222MBq of <sup>99m</sup>Tc - HMPAO at time  $t = 0$  (see Fig. 9-1). Five minutes later the first SPECT acquisition (SPECT-1) followed with a Siemens Orbiter gamma camera, using 32 projections of 20 seconds during a 360° rotation.

The baboons were always positioned in the supine position with a special headrest to ensure reproducible and comparable tomographic slices for all procedures. A second i.v. administration of  $^{99m}\text{Tc}$  - HMPAO (444 MBq, i.e. double the first dose) was administered at  $t = 29$  min taking care not to exceed the 30 minute time limit of use after reconstitution and allowing for pharmacologically appropriate response times as described above. Five minutes later a similar SPECT acquisition, SPECT 2 followed (the split dose method), which measures anaesthesia related changes in CBF taking place in between the two HMPAO administrations. Procedure B is similar to procedure A described above but includes an intervention with sodium valproate at  $t = 14$  minutes. Procedure C, D and E describe combination drug interventions with sodium valproate ( $t = 14$  min), vs. acetazolamide ( $t = 24$  min) (procedure C), nimodipine ( $t = 19$  min) (procedure D) and sumatriptan ( $t = 6$  min) (procedure E). The arterial blood pressures were recorded during all the procedures from a catheter in the femoral artery. Heart rates were monitored as well.

After back projection and reconstruction the brain images consisted of transaxial, sagittal and coronal slices representing CBF and rCBF information during conditions prevailing under the procedure of anaesthesia only, and under the various drug interventions. Eight transaxial slices represented the whole brain each of which was considered for count rate evaluation by the ROI (region of interest) feature. Similarly eight sagittal and eight coronal slices covering all of the brain were selected and analysed.

Count rate data for each slice in each view were then inserted into the following equation to obtain a corresponding ration R, for each procedure:

$$R = \frac{(SPECT - 2) - (SPECT - 1)^*}{(SPECT - 1)}$$

R is an indication of the level change of the CBF during the thiopentone anaesthesia (procedure A), or, in addition, due to the drug interventions (procedures B to E), measured by SPECT - 1 and SPECT - 2, at the selected response times. The equation allows for the subtraction of retained activity originating from the SPECT - 1 study after decay correction (\*). For all three

views graphs were plotted of R vs. slice number starting at the occipital lobes to the frontal lobes transaxially, from right to left sagittally and from the cerebellum to the dorsal slice of the cerebrum coronally (Table 11-1a-c).

### 11.2.1 Statistical methods

Mean ratios R ( $n = 6$ ) and standard deviations were calculated per procedure for similar regions (slices). An average R-value was obtained for the total brain for a particular procedure by considering all the slices of all the views. These were compared for interprocedural effects and the comparisons assessed for significant differences using Student's two-tailed *t*-test for paired variables on a 5% confidence limit.

## 11.3 Results

The curves of R-values (mean  $\pm$  SD) vs. slice number in all three views are plotted for each procedure in Table 11-1a-c for procedures A to E respectively. With no intraprocedural regional, slice dependent differences statistically significant, mean R-values were obtained and used to represent the total brain for each procedure (Table 11-2). Percentage changes with respect to the control anaesthesia only study (procedure A) are compared in Table 11-2.

**Table 11-1a** The mean R ratios ( $\pm$ SD) ( $n = 6$ ) from transaxial views of eight equal cerebral regions and total brain for the five different procedures with the slice number starting from the frontal to the occipital lobes

Procedure	Region 1	Region 2	Region 3	Region 4	Region 5
A	2.25 $\pm$ 0.32	2.12 $\pm$ 0.18	2.10 $\pm$ 0.13	2.14 $\pm$ 0.08	2.13 $\pm$ 0.06
B	2.19 $\pm$ 0.24	2.12 $\pm$ 0.17	2.05 $\pm$ 0.15	2.08 $\pm$ 0.13	2.09 $\pm$ 0.16
C	2.79 $\pm$ 0.48	2.76 $\pm$ 0.35	2.73 $\pm$ 0.30	2.81 $\pm$ 0.34	2.94 $\pm$ 0.39
D	1.67 $\pm$ 0.40	1.59 $\pm$ 0.32	1.53 $\pm$ 0.31	1.53 $\pm$ 0.29	1.55 $\pm$ 0.29
E	2.05 $\pm$ 0.17	1.93 $\pm$ 0.14	1.94 $\pm$ 0.15	1.96 $\pm$ 0.14	1.96 $\pm$ 0.14
	Region 6	Region 7	Region 8	Total brain	
A	2.09 $\pm$ 0.08	2.09 $\pm$ 0.08	2.00 $\pm$ 0.19	2.11 $\pm$ 0.08	
B	2.14 $\pm$ 0.18	2.14 $\pm$ 0.18	2.14 $\pm$ 0.12	2.13 $\pm$ 0.03	
C	2.88 $\pm$ 0.44	2.88 $\pm$ 0.44	2.85 $\pm$ 0.46	2.88 $\pm$ 0.11	
D	1.54 $\pm$ 0.34	1.54 $\pm$ 0.34	1.54 $\pm$ 0.32	1.56 $\pm$ 0.03	
E	2.01 $\pm$ 0.14	2.01 $\pm$ 0.14	1.99 $\pm$ 0.14	1.99 $\pm$ 0.04	

**Table 11-1b** The mean R ratios ( $\pm$ SD) (n = 6) from sagittal views of eight equal cerebral regions and total brain for the five different procedures with the slice number from left to right of the brain

Procedure	Region 1	Region 2	Region 3	Region 4	Region 5
A	2.25 $\pm$ 0.32	2.12 $\pm$ 0.18	2.10 $\pm$ 0.13	2.14 $\pm$ 0.08	2.13 $\pm$ 0.06
B	2.40 $\pm$ 0.44	2.26 $\pm$ 0.25	2.20 $\pm$ 0.17	2.13 $\pm$ 0.18	2.07 $\pm$ 0.12
C	3.15 $\pm$ 0.72	2.94 $\pm$ 0.50	2.93 $\pm$ 0.42	2.91 $\pm$ 0.43	2.89 $\pm$ 0.39
D	1.78 $\pm$ 0.32	1.63 $\pm$ 0.31	1.55 $\pm$ 0.30	1.52 $\pm$ 0.29	1.54 $\pm$ 0.29
E	1.93 $\pm$ 0.26	1.93 $\pm$ 0.18	1.95 $\pm$ 0.16	1.95 $\pm$ 0.14	1.96 $\pm$ 0.15
	Region 6	Region 7	Region 8	Total brain	
A	2.09 $\pm$ 0.11	2.09 $\pm$ 0.08	2.00 $\pm$ 0.19	2.11 $\pm$ 0.08	
B	2.04 $\pm$ 0.13	2.01 $\pm$ 0.19	1.97 $\pm$ 0.26	2.13 $\pm$ 0.09	
C	2.83 $\pm$ 0.40	2.76 $\pm$ 0.31	2.66 $\pm$ 0.42	2.88 $\pm$ 0.11	
D	1.56 $\pm$ 0.31	1.63 $\pm$ 0.34	1.65 $\pm$ 0.33	1.61 $\pm$ 0.02	
E	2.02 $\pm$ 0.17	2.04 $\pm$ 0.17	2.10 $\pm$ 0.23	1.99 $\pm$ 0.04	

**Table 11-1c** The mean R ratios ( $\pm$ SD) (n = 6) from coronal views of eight equal cerebral regions and total brain for the five different procedures with the slice number from the cerebellum to the dorsal slice of the cerebrum

Procedure	Region 1	Region 2	Region 3	Region 4	Region 5
A	2.06 $\pm$ 0.33	2.10 $\pm$ 0.19	2.12 $\pm$ 0.11	2.08 $\pm$ 0.14	2.14 $\pm$ 0.23
B	2.01 $\pm$ 0.28	2.05 $\pm$ 0.24	2.06 $\pm$ 0.17	2.09 $\pm$ 0.12	2.11 $\pm$ 0.13
C	2.65 $\pm$ 0.20	2.70 $\pm$ 0.28	2.72 $\pm$ 0.31	2.82 $\pm$ 0.37	2.86 $\pm$ 0.42
D	1.57 $\pm$ 0.44	1.52 $\pm$ 0.37	1.52 $\pm$ 0.33	1.51 $\pm$ 0.29	1.60 $\pm$ 0.27
E	1.88 $\pm$ 0.17	1.92 $\pm$ 0.14	1.96 $\pm$ 0.11	2.02 $\pm$ 0.16	2.00 $\pm$ 0.17
	Region 6	Region 7	Region 8	Total brain	
A	2.17 $\pm$ 0.24	2.19 $\pm$ 0.36	2.31 $\pm$ 0.76	2.14 $\pm$ 0.19	
B	2.11 $\pm$ 0.14	2.14 $\pm$ 0.17	2.23 $\pm$ 0.38	2.10 $\pm$ 0.08	
C	2.91 $\pm$ 0.47	2.89 $\pm$ 0.48	2.95 $\pm$ 0.70	2.81 $\pm$ 0.13	
D	1.60 $\pm$ 0.27	1.65 $\pm$ 0.30	1.71 $\pm$ 0.34	1.59 $\pm$ 0.05	
E	2.00 $\pm$ 0.17	2.03 $\pm$ 0.21	2.10 $\pm$ 0.36	1.99 $\pm$ 0.07	

Haemodynamic changes for procedures A - E appear in Table 11-3. The control R-value for cerebral blood flow under thiopentone anaesthesia obtained during the current study is similar to previous data [6] vs. R = 2.13 (Table 11-1a - c). From Table 11-1a - c and Table 11-2 it is clear that the sodium valproate

did not change the control R - values at all and therefore did not influence the cerebral blood flow.

**Table 11-2** The mean R-value  $\pm$  SD (n = 6) for total brain as averaged from all slices and all views for each procedure, percentage changes of these R-values with respect to control and significance.

Procedure	R-value	% Change	Significance <sup>a)</sup>
<b>Procedure A</b> (control)	2.13 $\pm$ 0.12		
<b>Procedure B</b> (Sodium valproate)	2.13 $\pm$ 0.12	0	$P > 0.05$
<b>Procedure C</b> (Sodium valproate + Acetazolamide)	2.84 $\pm$ 0.38	+34%	$P < 0.05$ $P < 0.01$
<b>Procedure D</b> (Sodium valproate + Nimodipine)	1.59 $\pm$ 0.31	-25%	$P < 0.05$ $P < 0.01$
<b>Procedure E</b> (Sodium valproate + Sumatriptan)	1.90 $\pm$ 0.14	-11%	$P > 0.05$ $P < 0.1$

<sup>a)</sup> With respect to control.

The drug combination interventions with sodium valproate and sumatriptan, or acetazolamide, or nimodipine reveal that the cerebral blood flow is differently influenced by these combinations. The combination (procedure C) of sodium valproate and acetazolamide (Table 11-1a - c and Table 11-2) yielded total brain R-values which were statistically significantly increased (34%) when compared to both the control and the single sodium valproate drug intervention (2.84  $\pm$  0.38 vs. 2.13  $\pm$  0.12,  $P < 0.01$ ). Conversely, the combination (procedure D) of sodium valproate and nimodipine (Table 11-1a - c and Table 11-2) yielded total brain R-values which were statistically significantly decreased (25%) when compared to both the control and the single sodium valproate drug intervention (1.59  $\pm$  0.31 vs. 2.13  $\pm$  0.12,  $P < 0.01$ ). From procedure E, i.e. the combination of sodium valproate and sumatriptan (Table 11-1a - c and Table 11-2) showed a slight decrease (11%) in cerebral blood flow approaching statistical significance difference when compared to both the control and the single sodium valproate intervention (1.90  $\pm$  0.14 vs. 2.13  $\pm$  0.12,  $P > 0.05$  to  $P < 0.1$ ).

**Table 11-3** The effect of all the procedures on peripheral blood pressure

Procedure	$\Delta$ BP% 0-29 min	$\Delta$ CBF%	$\Delta$ BP% 14-29 min (Sodium valproate) <sup>a</sup>
Procedure A (control)	+12	0	+7
Procedure B (Sodium valproate)	+19	0	+7
Procedure C (Sodium valproate + Acetazolamide)	+23	+34	+11
Procedure D (Sodium valproate + Nimodipine)	+9	-25	-3
Procedure E (Sodium valproate + Sumatriptan)	+19	-11	+7

<sup>a)</sup> With respect to control.

No significant changes occurred for any of the interventions in the heart rate taken just before drug administration versus just before the second HMPAO injection. Changes in blood pressure with respect to initial values (before drug intervention) (see Table 11-3) occurred with sodium valproate (increased 19%), combination of sodium valproate and acetazolamide (increased 23%), combination of sodium valproate and nimodipine combination (increased 9%) and combination of sodium valproate and sumatriptan (increased 19%). It was previously shown that drug interventions, such as acetazolamide, and combinations of sumatriptan may influence blood pressure [19]. Furthermore, anaesthesia is also known to influence the blood pressure [6,20,21]. The increase in blood pressure observed for the current anaesthetic regime (see Table 3), i.e. the control procedure A, was found to be approximately 12% with respect to the initial value at the beginning of the procedure. Based on the effect of the anaesthesia on blood pressure the following blood pressure effects can be deduced for the different procedures: procedure B (sodium valproate) increase of 12%; procedure C (sodium valproate and acetazolamide) increase of 11%; procedure D (sodium valproate and nimodipine) decrease of -3%; procedure E (sodium valproate and sumatriptan) increase 7%.

## 11.4 Discussion

The results clearly indicate that sodium valproate had no effect on the cerebral blood flow, both on the regional and total brain flow. This is in contrast to the significant decrease (although slight) in cerebral blood flow reported in

children upon treatment with sodium valproate [12]. The difference in the study procedures (Chronic vs. Acute) between the latter and the current study may explain the different outcomes. It was previously observed that acetazolamide increased the cerebral blood in the baboon model by approximately 35% [7,9]. This increase is nearly identical to the 34% observed for the drug combination of sodium valproate and acetazolamide in the current study, indicating the increase in cerebral blood flow is only due to the acetazolamide and supports the previous conclusion that sodium valproate does not influence the cerebral blood flow.

Nimodipine, the dihydropyridine calcium channel blocker, has recently been shown to diminish the increased cerebral blood flow induced by acetazolamide [9, 19] and that a combination of nimodipine and sumatriptan decreased the cerebral blood flow markedly [19]. The present study using nimodipine and sodium valproate is in line with the previous two findings and clearly shows that cerebral blood flow is decreased below the control baseline. Nimodipine has however previously been reported in a study with the same tracer to dilate the cerebral arterioles and thus to increase cerebral blood flow [19, 22]. The current study therefore points to an interaction of sodium valproate and nimodipine, and therefore calls for caution when simultaneously used.

Sumatriptan has been shown to reverse the increased cerebral blood flow due to prolonged anaesthesia [10]. The nearly significant ( $0.05 > P < 0.1$ ) decrease in cerebral blood flow upon simultaneous treatment with sodium valproate and sumatriptan suggests that the decrease may be due to the reversal of the anaesthesia induced increase [10] cerebral blood flow by sumatriptan. Drug interaction between sumatriptan and sodium valproate is therefore not implicated by this study. Although changes in the blood pressures (Table 3) were observed during these studies, the effect was only a slight increase that could be attributed to the action of sodium valproate. These were generally smaller than those observed during anaesthesia alone. No significant changes in blood pressure were noted for the combination studies with sodium valproate when compared to the anaesthesia control and no peripheral drug interaction is therefore envisaged.

## 11.5 Conclusion

The present study clearly indicated that sodium valproate exhibits no acute effects on cerebral blood flow. The current investigation did not address the effects and drug interactions upon chronic treatment with sodium valproate. The changes in the blood pressure observed during these studies were only insignificantly different than the increase during anaesthesia alone. No drug interactions with respect to cerebral blood flow dynamics are envisaged when sodium valproate and acetazolamide or sumatriptan is administered simultaneously in acute treatment. Possible drug interaction may occur between sodium valproate and nimodipine as shown in the current data upon single dose treatment. The current study could be followed up using halothane for anaesthesia, due to its marked induced increases in cerebral blood flow. The decrease in cerebral blood flow previously reported for sodium valproate might then be noticeable using halothane.

## 11.6 References

1. **McNAMARA JO**, in: Goodman and Gilman's The Pharmacological Basis of Therapeutics 9<sup>th</sup> Edition, JG Hardman, LE Limbird, PB Molinoff *et al.*, (eds.), pp. 476, McGraw-Hill, New York (1996).
2. **CHAPMAN A, KEANE PE, MELDRUN BS, et al.** 1982. *Prog. Neurobiol* 19:315.
3. **PHILLIPS NI, FOWLER LJ.** 1982. *Pharmacol* 31: 2257
4. **MCLEAN MJ, MACDONALD RL.** 1986. *Pharmacol Exp Ther* 238:727.
5. **COULTER DA, HUGUENARD JR, PRINCE DA.** 1990. *British Journal of Pharmacology* 100:800.
6. **DORMEHL IC, REDELINGHUYS F, HUGO N, et al.** 1992. *Medical Primatology* 21:270.

7. **DORMEHL IC, OLIVER DW, HUGO N.** 1993. *Nuclear Medicine Communications* 14:573.
8. **DORMEHL IC, LIPP MDW, HUGO N, et al.** 1993. *Journal of Nuclear Medicine* 20:11.
9. **OLIVER DW, DORMEHL IC, REDELINGHUYS F, et al.** 1993. *Nuklear Medizin* 32:292.
10. **OLIVER DW, DORMEHL IC, HUGO N.** 1994. *Arzneimittel Forschung / Drug Research* 44(2):925.
11. **JORDAAN B, OLIVER DW, DORMEHL IC, et al.** 1996. *Arzneimittel Forschung / Drug Research* 46(2):844.
12. **FUTAGI Y, OTANI K, IMAI K.** 1994. *Epilepsia* 35:827.
13. **FEGGI L, SCARPA P, CALZOLARI F.** 1995. *Clinical Nuclear Medicine* 20:42.
14. **SPENCER SS.** 1994. *Epilepsia* 35:72.
15. **NEHLIG A, ERGNES M, WAYDEDLICH R, et al.** 1996. *Journal of Cerebral Blood Flow and Metabolism* 16:147.
16. **NEWTON MR, BERKOVIC SF, AUSTIN MC, et al.** 1994. *European Journal of Nuclear Medicine* 21:1067.
17. **WIESER HG.** 1994. *European Neurology* 34:58.
18. **OGASAWARA K, FUJIWARA S, YOSHIMOTO T.** 1995. *Kaku Igaku (Japan)* 32:1241.
19. **DORMEHL IC, OLIVER DW, HUGO N.** 1995. *Arzneimittel Forschung / Drug Research* 45(2):952.
20. **LASSEN NA, CHRISTENSEN MS.** 1976. *British Journal of Anaesthesia* 48:719.

21. **WOOD M, WOOD AJJ.** 1990. *Drugs Anaesthes.* 2:179.
22. **LANGLEY MS, SORKIN EM.** 1989. *Drugs* 27:669.

## Cerebral Blood Flow Effects of the Nitric Oxide Donor, Nitroglycerin and its Drug Combinations in the Non-human Primate Model\*

## Chapter 12

### Summary

*Nitroglycerin (CAS 55-63-0, Nitrocene®) has successfully been used in the management of angina during the last several decades. Although important information on the pharmacological actions and efficacy of nitroglycerin has been extracted, to date, limited research has been conducted on its effects on cerebral blood flow. In recent years, with the aid of SPECT (single photon emission computed tomography) and PET (positron emission tomography) it has been shown that marked cerebral blood flow changes occur under treatment of a wide variety of drugs. Illucidation of the pharmacological mode of action of nitroglycerin has gained momentum with the discovery of nitric oxide (NO) as an endogenous mediator and with the knowledge that nitroglycerin acts as a NO donor. The present study investigated the effects of nitroglycerin (0.25 µg/kg/min over 10 min) on the cerebral blood flow, using <sup>99m</sup>Tc-HMPAO (hexamethylpropylene amine oxime) and SPECT, in an anaesthetised primate model, as well as the effects of its drug interactions with therapeutic agents that influence cerebrovascular dynamics, e.g. sumatriptan, nimodipine and acetazolamide. The present study with nitroglycerin indicates that the response time to measure cerebral blood flow effects seems to be present and an important factor as the transient is relatively short. The current treatment regime with nitroglycerin indicates a slight increase, when compared with control results, although not significant, except for regional significant increases in particular the occipital regions of the brain. Drug interaction between*

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\* OLIVER, D.W. & DORMEHL, I.C. 1999. Cerebral Blood Flow Effects of the Nitric Oxide Donor, Nitroglycerin and its Drug Combinations in the Non-Human Primate Model. *Arzneimittel Forschung/Drug Research*, 49:732-739.

*nitroglycerin and nimodipine may occur as a reduction of 20% in cerebral blood flow from the control was observed in this case. The results for the combination of nitroglycerin with sumatriptan showed a further increase of the cerebral blood flow to near significance, when compared with the control results and is significantly increased (+27%) when compared with sumatriptan treatment alone. Effective treatment with sumatriptan may therefore be compromised with simultaneous administration of nitroglycerin or NO donor drugs. The combination of nitroglycerin and acetazolamide suggested that the increase in cerebral blood flow is primarily attributed to the influence of acetazolamide. The cerebral blood flow effects of these drugs and possible interactions during an angina attack need to be investigated.*

**KEY WORDS:** *Acetazolamide, CAS 55-63-0, Nimodipine, Nitrocene ®, Nitroglycerine, cerebral blood flow effects, drug interactions, primate model, Sumatriptan.*

## **12.1 Introduction**

Since the discovery, identification and subsequent characterisation of the nitric oxide, NO (CAS 10102-43-9) as an important endogenous vascular and inflammatory mediator in living organisms, NO has received ever increasing attention from researchers investigating its wide range of biological effects and its biochemistry [1, 2]. NO is synthesised from L-arginine and molecular oxygen by nitric oxide synthase (NOS) through enzymatic deamination of L-arginine to L-citrulline. Several isoforms of NOS have been sought and discovered [3, 4]. Subsequently novel structures have been searched for and have been discovered, such as the nitric oxide synthase inhibitors, such as methyl arginine and nitroarginine, that influence the vast array of responses observed for NO [1]. The effect of NO or NO donors on cerebral blood flow has recently been the focus of various studies using a variety of experimental models [5, 6, 7, 8]. Part of the pharmacological mode of action of the classical cardiovascular drugs, such as nitroglycerine and sodium nitroprusside is now attributed to their decomposition and subsequent action as NO donors [1, 9, 10,

11]. They therefore are able to mimic some of the effects of endogenously released nitric oxide.

The success of the non-human primate model developed by the present authors [12-21], to investigate drug interventions prompted the study of the effects of the NO donor, nitroglycerine (CAS 55-63-0) on the cerebral blood flow. The baboon (*Papio ursinus*) model has previously been used, for single photon emission computed tomography (SPECT) brain imaging utilising the radiopharmaceutical, Tc-99m hexamethylpropylene amine oxime ( $^{99m}\text{Tc}$ -HMPAO), to measure cerebral blood flow during pharmacological interventions with drugs used in different pharmacological fields, i.e. migraine (serotonin receptors), cardiovascular disease (calcium channel blockers), epilepsy, cognitive disorders and glaucoma (carbonic anhydrase inhibition) [12-21]. The current study reports the cerebrovascular effects of the NO donor, nitroglycerine, also in its drug combinations with other known cerebrovascular drugs, i.e. sumatriptan (CAS 103628-46-2), nimodipine (CAS 66085-59-4) and acetazolamide (CAS 59-66-5) in the primate model, using the split-dose [22, 23] method.

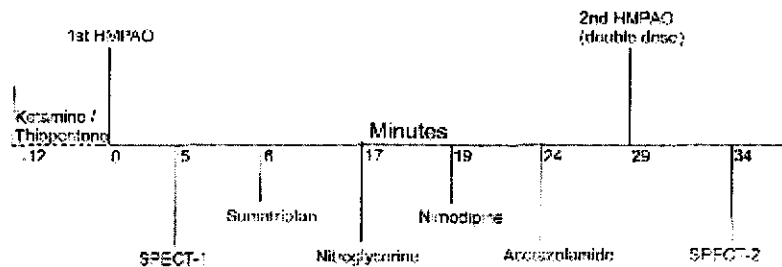
## **12.2 Materials and methods**

Six adult male baboons (*Papio ursinus*, average weight 27 kg) were used for this investigation. The animals were obtained from Mr. E. Venter, Vaalwater, Northern Province (Republic of South Africa). The baboons were housed, maintained and cared for according to the guidelines laid down in the National Code for Animal Use in Research, Education, Diagnosis and Testing of Drugs and related substances in South Africa. These guidelines comply with international standards. The studies were performed after the approval of the Ethics Committee of the University of Pretoria. Each baboon was subjected to 5 different procedures (A-E) at least six weeks apart (Table 10-1).

As limited dose-response data have been reported using nitroglycerine with respect to the current baboon model, a preliminary study was conducted to investigate the effect of dose-time responses upon treatment with nitroglycerine in order to select a treatment regime which could be followed during the current

study. The following dose-time intervals were investigated for cerebral blood flow effects: 0.03 µg/kg/min at 12 min; 0.166 µg/kg/min at 5 min; 0.166 µg/kg/min at 10 min; 0.25 µg /kg/min at 2 min; 0.25 µg/kg/min at 5 min; 0.25 µg/kg/min at min; 1.0 µg/kg/min, at 5 min. For all the above-described regimes the nitroglycerine was initially administered over 10 minute before the time intervals as indicated were started. Based on the results presented in Table 1, a dose-time regime of 0.25 µg/kg/min at 2 min was selected for the present study, .i.e. procedures B-E.

**Table 12-1** The time schedule for the various drug intervention protocols indicating the time of each intervention



min	Procedure A	Procedure B	Procedure C	Procedure D	Procedure E
-12	Ketamine Thiopentone	Ketamine Thiopentone	Ketamine Thiopentone	Ketamine Thiopentone	Ketamine Thiopentone
0	1 <sup>st</sup> HMPAO	1 <sup>st</sup> HMPAO	1 <sup>st</sup> HMPAO	1 <sup>st</sup> HMPAO	1 <sup>st</sup> HMPAO
5	SPECT-1	SPECT-1	SPECT-1	SPECT-1	SPECT-1
6					Sumatriptan
17		Nitroglycerine	Nitroglycerine	Nitroglycerine	Nitroglycerine
19				Nimodipine	
24			Acetazolamide		
29	2 <sup>nd</sup> HMPAO	2 <sup>nd</sup> HMPAO	2 <sup>nd</sup> HMPAO	2 <sup>nd</sup> HMPAO	2 <sup>nd</sup> HMPAO
34	SPECT-2	SPECT-2	SPECT-2	SPECT-2	SPECT-2

Procedure A concerned the control cerebral blood flow (CBF) study using the <sup>99m</sup>Tc-HMPAO split-dose method [22, 23] with two respective SPECT acquisitions after two consecutive <sup>99m</sup>Tc-HMPAO administrations at chosen times (see Fig.10-1), under standard anaesthesia conditions. This entails

induction with ketamine hydrochloride (Anaket-V<sup>®</sup>, Centaur Labs, Bryanston, Gauteng, S.A.; 10 mg/kg), followed by maintenance on thiopentone sodium (Sandothal<sup>®</sup>, Sandoz Products, Randburg, Gauteng, S.A.; 70 ml/h of a 0.5 % solution, procedure A). A period of 12-minute stabilisation under anaesthesia followed before the start of the each study.

Procedures B, C, D and E investigated the CBF changes at 2 minutes post the end of alternatively administration of nitroglycerine (Nitrocene<sup>®</sup>, Schwarz Pharma AG, Randburg, S.A.; 0.25 µg/kg/min for 10 minutes, procedure B), a combination of nitroglycerine (Nitrocene<sup>®</sup>, Schwarz Pharma AG.; 0.25 µg/kg/min for 10 minutes) and sumatriptan (Imigran<sup>®</sup>, Glaxo-Wellcome, Midrand, S.A.; 6 mg, procedure C), a combination of nitroglycerine (Nitrocene<sup>®</sup>, Schwarz Pharma AG; 0.25 µg/kg/min over 10 minutes) and nimodipine (Nimotop IV<sup>®</sup>, Bayer SA, Isando, S.A.; 1 µg/kg/min over 10 minutes, procedure D), and a combination of nitroglycerine (Nitrocene<sup>®</sup>, Schwarz Pharma AG; 0.25 µg/kg/min over 10 minutes) and acetazolamide at 24 min (Diamox<sup>®</sup>, Cyanamid S.A. Isando, S.A.; 500 mg/5ml, Procedure E). The test substances were obtained from the respective manufacturers..

### 12.2.1 Procedure A (control study)

Anaesthesia was induced in each animal by darting with ketamine hydrochloride, and maintained with thiopentone sodium, using an administration (drip) set. The baboon was placed in the supine position, positioned with a special headrest to ensure a reproducible position to compare tomographic slices. After a period of 12 minute stabilisation, the control study under anaesthesia (thiopentone - thiopentone), started at time  $t = 0$  with an i.v. injection of 222 MBq <sup>99m</sup>Tc-HMPAO. The baboon was maintained and controlled with thiopentone for the duration of the study (Fig 10-1). The first acquisition, SPECT-1, was performed at 5 minutes with a Siemens Orbiter gamma camera coupled to a Sophy 256G computer using 32, 20 second views and a 360° rotation, in 64 x 64 word mode with the baboon in a supine position. At 29 minutes, the baboon was reinjected with <sup>99m</sup>Tc-HMPAO (444 MBq, i.e. double the first dosage) and SPECT-2 performed at 34 minutes [22, 23]. These

data represented the CBF pattern of  $^{99m}\text{Tc}$ -HMPAO under thiopental anaesthesia to be compared with the patterns after the drug interventions.

### **12.2.2 Procedure B (Nitroglycerine)**

Procedure B (nitroglycerine) followed the same protocol as described for the control study except that the nitroglycerine (0.25  $\mu\text{g}/\text{kg}/\text{min}$  over 10 min) was i.v. administered at 17 minutes post administration of the first  $^{99m}\text{Tc}$ -HMPAO injection (Fig 10-1). The 2<sup>nd</sup>  $^{99m}\text{Tc}$ -HMPAO administration and 2<sup>nd</sup> scan followed the same protocol as the control study. Results for procedure B would depict cerebral blood flow changes due to the nitroglycerine intervention, 2 min after completion of the drug intervention.

### **12.2.3 Procedures C (nitroglycerine and sumatriptan), Procedure D (nitroglycerine and nimodipine), Procedure E (nitroglycerine and acetazolamide)**

The drug combination procedures C, D, and E followed the same sequence as procedure B, the nitroglycerine study (administered at 17 minutes over 10 minutes), but with the additional administration of an i.m. injection of sumatriptan, 6 mg at 6 minutes of the study, i.e. 23 min drug response time (procedure C), administration of nimodipine, 1  $\mu\text{g}/\text{kg}/\text{min}$  over 10 minutes starting at 19 minutes of the study (procedure D), and administration of acetazolamide, 500 mg i.v. at 24 minutes of the study, i.e. 5 min drug response time (procedure E). The 2<sup>nd</sup>  $^{99m}\text{Tc}$ -HMPAO administration (at 29 min) and 2<sup>nd</sup> scan, SPECT-2 (at 34 min) followed again the same protocol as the control study (Fig 1). The results from these procedures C to E, would represent the effects of these drug combinations on the cerebral blood flow in the baboon.

The arterial blood pressures were recorded during all the procedures via a catheter in the femoral artery. Heart rates were monitored together with a 12 lead ECG.

After backprojection and reconstruction (filter: Butterworth 4.25), the brain images in all procedures consisted of transaxial, sagittal and coronal slices

representing rCBF related information due to the above mentioned drug interventions. Eight slices of one pixel thickness each, represented the brain area in each slice, and in all three views. Regions of interest (ROIs) were placed on the total brain area and count rate data (counts/pixel) thus obtained were inserted into the following equation to obtain the ratio R :

$$R = \frac{(SPECT - 2) - (SPECT - 1)^*}{(SPECT - 1)}$$

where \* refers to decay corrected data from SPECT-1, present during SPECT-2, which has to be subtracted from the SPECT-2 data, as background; R is an indication of the level change of rCBF due to the changed conditions prevailing during the second HMPAO injection with respect to that of the first injection, i.e. due to prolonged anaesthesia alone (procedure A), or additionally with drug interventions.

A value of  $R = 2$  (due to the double second dose of  $^{99m}\text{Tc}$ -HMPAO) will indicate no rCBF change during procedure A due to prolonged anaesthesia. R for procedure B, C, D and E will additionally reflect on changes due to the nitroglycerine (B), the combination of nitroglycerine and sumatriptan (C), of nitroglycerine and nimodipine (D) and the nitroglycerine and acetazolamide (E), and was compared to R (procedure A as control) to assess the effects of these drugs.

#### 12.2.4 Statistical Analysis

Mean ratios ( $n = 6$ ) and standard deviations (SD) were calculated per procedure for similar regions (slices) and for the total brain in the various projections. These were compared for interprocedural effects as well as regional effects for a particular procedure. The comparisons were assessed for significant differences using Student's two-tailed *t*-test on a 1% and 5 % level of confidence.

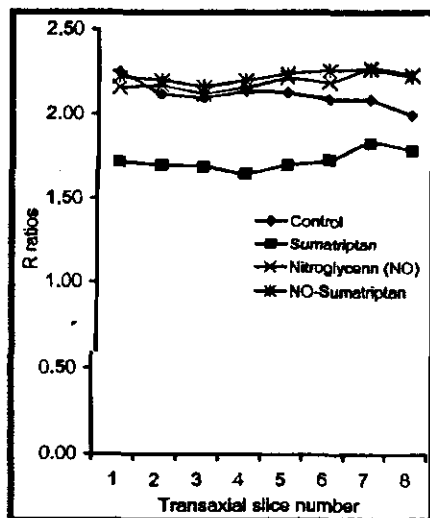
### 12.3 Results

Table 12-2 presents the data reflecting the time-dose cerebral blood flow responses upon treatment with nitroglycerine.

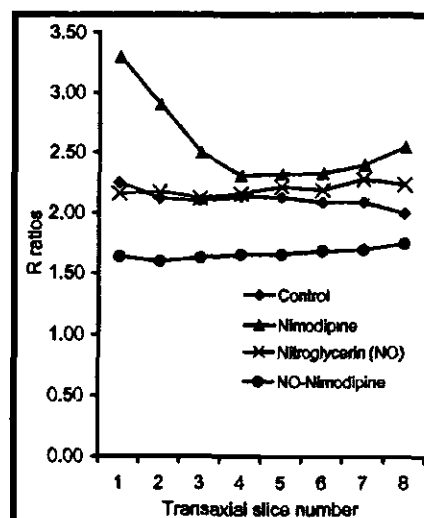
**Table 12-2** The mean ratios for the time-dose cerebral blood flow responses at time (min) post the administration for nitroglycerine ( $\mu\text{g}/\text{kg}/\text{min}$  over 10 min) interventions compared to the control

Dose	Time (min)	R ratio
Control	-	2.13 $\pm$ 0.11
0.03	12	2.11 $\pm$ 0.16
0.166	10	2.09 $\pm$ 0.04
0.166	20	2.03 $\pm$ 0.00
0.25	2	2.23 $\pm$ 0.12
0.25	5	2.06 $\pm$ 0.18
0.25	10	1.74 $\pm$ 0.25
1.00	5	2.05 $\pm$ 0.02

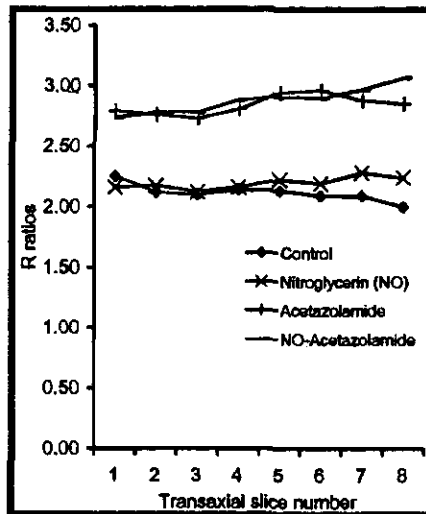
Based on these preliminary results the treatment regime for the current study was determined (i.e. 0.25  $\mu\text{g}/\text{kg}/\text{min}$  administered over 10 minutes with 2 minutes response time, after the end of the nitroglycerine administration, before the 2<sup>nd</sup> HMPAO injection at 29 min). The data are presented in Tables 12-3, 12-4 and 12-5 and Figures 12-1 and 12-2.



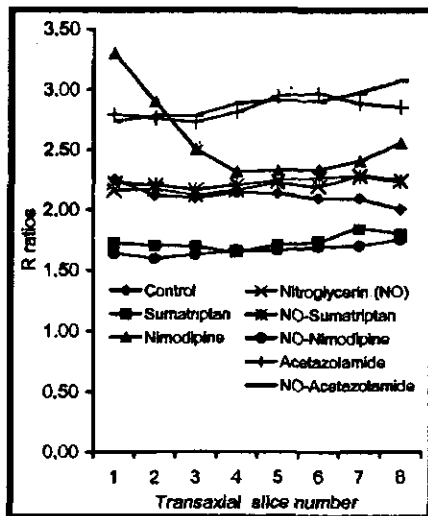
**Figure 12-1a** Transaxial curves of mean ratio (R) ( n = 6) vs. slice number starting at the frontal to the occipital lobes for the control, nitroglycerine, sumatriptan and combination of sumatriptan and nitroglycerine.



**Figure 12-1b** Transaxial curves of mean ratio (R) ( n = 6) vs. slice number starting at the frontal to the occipital lobes for the control, nimodipine and combination of nimodipine and nitroglycerine.



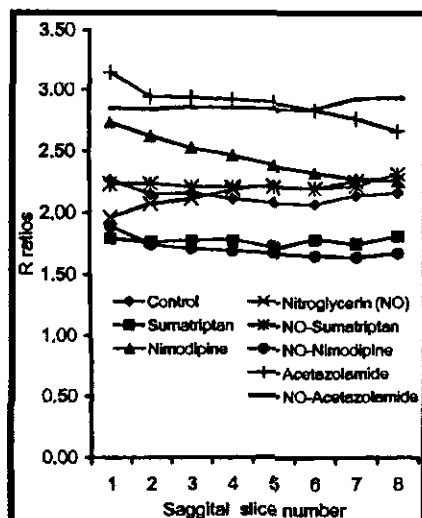
**Figure 12-1c** Transaxial curves of mean ratio (R) (n = 6) vs. slice number starting at the frontal to the occipital lobes for the control, acetazolamide and combination of acetazolamide and nitroglycerine.



**Figure 12-2a** Curves of mean ratio (R) (n = 6) vs. slice number of all procedures and single drug interventions in the transaxial view.



**Figure 12-2b** Curves of mean ratio (R) (n = 6) vs. slice number of all procedures and single drug interventions in the coronal view.



**Figure 12-2c** Curves of mean ratio (R) (n = 6) vs. slice number of all procedures and single drug interventions in the sagittal view.

Tables 12-3 and 12-4 present the mean ratios ( $R \pm SD$ ) ( $n = 6$ ) for the total brain under anaesthesia only (procedure A: control), and after the various drug interventions (procedure B: nitroglycerine (NO), procedure C: nitroglycerine and sumatriptan, procedure D: nitroglycerine and nimodipine, procedure E: nitroglycerine and acetazolamide). Mean percentage (%) changes of ratios from the total brain, comparing the different procedures with each other, are also reflected in Tables 12-3 and 12-4. Table 12-3 presents the mean percentage (%) changes of the ratios, comparing with the drugs previously investigated as single drug administrations, i.e. sumatriptan, nimodipine and acetazolamide.

**Table 12-3** The mean R-value ( $\pm SD$ ) ( $n = 6$ ) for total brain as averaged from all slices and all views for each procedure, percentage changes of these R-values with respect to the different procedures, and the statistical significance

Procedure	R-value	% Change with respect to control	% Change with respect to nitroglycerine	% Change with respect to nitroglycerine and sumatriptan	% Change with respect to nitroglycerine and nimodipine
Procedure A (Control)	2.13 $\pm$ 0.11				
Procedure B (Nitroglycerine)	2.19 $\pm$ 0.08	+2.8 (P > 0.05)			
Procedure C (Nitroglycerine + Sumatriptan)	2.22 $\pm$ 0.07	+4.2 (P > 0.05 P < 0.10)	+1.4 (P > 0.05)		
Procedure D (Nitroglycerine + Nimodipine)	1.69 $\pm$ 0.18	-20.7 (P < 0.10)	-22.8 (P < 0.01)	-23.9 (P < 0.01)	
Procedure E (Nitroglycerine + Acetazolamide)	2.89 $\pm$ 0.14	+35.7 (P < 0.01)	+32.0 (P < 0.01)	+30.2 (P < 0.01)	+71 (P < 0.01)

**Table 12-4** The mean R-value ( $\pm$ SD) ( $n = 6$ ) for total brain as averaged from all slices and all views for each procedure, percentage changes of these R-values with respect to sumatriptan, nimodipine and acetazolamide only interventions, and statistical significance.

Procedure	R-value	% Change with respect to control (significance)	% Change with respect to nitroglycerine (significance)	% Change with respect to sumatriptan (significance)	% Change with respect to nimodipine (significance)
Procedure A (Control)	2.13 $\pm$ 0.11				
Procedure B (Nitroglycerine)	2.19 $\pm$ 0.08	+2.8 ( $P > 0.05$ )			
Procedure C (Nitroglycerine + Sumatriptan)	2.22 $\pm$ 0.07	+4.2 ( $P > 0.05$ $P < 0.10$ )	+1.4 ( $P > 0.05$ )	+27.6 ( $P < 0.01$ )	-11.6 ( $P < 0.02$ )
Procedure D (Nitroglycerine + Nimodipine)	1.69 $\pm$ 0.18	-20.7 ( $P < 0.10$ )	-22.8 ( $P < 0.01$ )	-2.9 ( $P > 0.05$ )	-32.6 ( $P < 0.01$ )
Procedure E (Nitroglycerine + Acetazolamide)	2.89 $\pm$ 0.14	+35.7 ( $P < 0.01$ )	+30.2 ( $P < 0.01$ )	+66.1 ( $P < 0.01$ )	+13.1 ( $P < 0.05$ )
Sumatriptan [16]	1.74 $\pm$ 0.10	-18.3 ( $P < 0.05$ )	-20.5 ( $P < 0.01$ )		
Nimodipine [15]	2.51 $\pm$ 0.14	+17.8 ( $P < 0.01$ )	+14.6 ( $P < 0.02$ )	+44.3 ( $P < 0.01$ )	
Acetazolamide [19]	2.84 $\pm$ 0.38	+33.3 ( $P < 0.01$ )	+29.7 ( $P < 0.01$ )	+63.2 ( $P < 0.01$ )	+13.1 ( $P < 0.05$ )

Table 12-5 presents the effects of these drug interventions during the different procedures A to E and sumatriptan, nimodipine and acetazolamide, on the cardiovascular parameters, i.e. heart rate and blood pressure. The data has been transformed to difference data and compared with the control data. Figures 1a,b, c present R-values vs. slice number for the transaxial views for the different procedures. Figures 12-2a,b, c present R-values vs. slice number for the transaxial, coronal and sagittal views for the combined data for all the procedures. Only the transaxial views (Fig. 12-1a,b,c) are presented here as the sagittal and coronal views presented similar ratios.

**Table 12-5** The effects of all the procedures on heart rate and blood pressure with respect to the control

Procedure	$\Delta$ HR	$\Delta$ HR%	$\Delta$ BP	$\Delta$ BP%
Procedure B (Nitroglycerine)	0	0	+13 $\pm$ 4	+16
Procedure C (Nitroglycerine + sumatriptan)	+0.2 $\pm$ 5.8	+0.2	+4.2 $\pm$ 5.4	+5
Procedure D (Nitroglycerine + nimodipine)	+9.2 $\pm$ 21	+8	-134 $\pm$ 9.8	-7
Procedure E (Nitroglycerine + Acetazolamide)	-1.6 $\pm$ 3.6	-1.4	+10.8 $\pm$ 11.6	+9
Sumatriptan [16]	-1.5 $\pm$ 5	-1.4	+5.3 $\pm$ 3.8	+5
Nimodipine [15]	+4.5 $\pm$ 7.9	+4	-13 $\pm$ 7.9	-7
Acetazolamide [19]	-5.0 $\pm$ 14.0	-4	-7.8 $\pm$ 3.5	-7

It is clear from Table 12-2 and Fig. 12-1 and 12-2 that the nitroglycerine (R value  $2.19 \pm 0.08$ ) did not significantly alter the cerebral blood flow when compared with the control R value of  $2.13 \pm 0.12$ , although an increasing trend was noted. The drug combination interventions with nitroglycerine and sumatriptan (procedure C), or nimodipine (procedure D), or acetazolamide (procedure E) reveal distinct different influences on the cerebral blood flow by these combinations (See Table 12-3 for percentage changes with respect interprocedural differences). The combination of nitroglycerine and acetazolamide (procedure E, Table 12-3) significantly increased the cerebral blood flow (total brain R values increased by 35%) when compared with both the control ( $2.89 \pm 0.14$  vs.  $2.13 \pm 0.12$ ,  $P < 0.01$ ) and the nitroglycerine response alone ( $2.89 \pm 0.14$  vs.  $2.19 \pm 0.08$ ,  $P < 0.01$ ). Conversely, the combination of nitroglycerine and nimodipine (procedure D, Table 3) yielded total brain R values which were statistically significantly decreased when compared with both the control (-20%;  $1.69 \pm 0.18$  vs.  $2.13 \pm 0.11$ ,  $P < 0.01$ ) and the single nitroglycerine drug intervention (-22%;  $1.69 \pm 0.18$  vs.  $2.19 \pm 0.08$ ,  $P < 0.01$ ). The combination of nitroglycerine and sumatriptan (procedure C, Table 12-3) did not significantly alter the R-values when compared with both the control ( $2.22 \pm 0.07$  vs.  $2.13 \pm 0.11$ ,  $P > 0.05$ ) and the nitroglycerine alone ( $2.22 \pm 0.08$  vs.  $2.19 \pm 0.08$ ,  $P > 0.05$ ).

Table 12-4 presents the total brain R-values for all the procedures in the current study (A to E), compared to single drug interventions with sumatriptan, nimodipine and acetazolamide [15,16,19]. Comparative % changes of all these data sets will follow in the Discussion.

No significant changes occurred for any of the interventions in the heart rate for the procedures A to E (Table 12-5). The largest increase in heart rate (8%) was noted for the combination of nitroglycerine and nimodipine but was statistically insignificant. Changes of blood pressure of more than 10% with respect to initial values (before drug intervention) (Table 12-5) occurred with nitroglycerine (procedure B) (increase 16%) alone. It is known that anaesthesia also influences the blood pressure and it was previously shown that the current anaesthetic regime for control procedure A increased the blood pressure with approximately 12% with respect to the initial value at the beginning of the procedure [19]. The increase (16%) observed after the single nitroglycerine intervention is therefore not meaningful.

## 12.4 Discussion

It was previously demonstrated that various forms of anaesthesia [13, 24, 25] influence cerebral blood flow. Sumatriptan [16], nimodipine [15] and acetazolamide [14, 15] also caused statistically significant changes in the cerebral blood flow with respect to control (see Table 10-3). However, contrary to a literary report [26], sodium valproate did not influence the cerebral blood flow in the primate model [19]. It was suggested that chronic treatment used in the literature report [26] compared to the acute single dose approach used in the primate study [19] might explain the inconsistencies in cerebral blood flow effects.

Although in the current study only a slight cerebral blood flow increase of the total brain was observed for nitroglycerine, when compared with the control study, it was not statistically significant with respect to the control ( $R = 2.19$  vs.  $2.13$ ). However, statistically significant regional increases in cerebral blood flow was observed for the occipital areas of the brain, i.e. slice 7 for the transaxial view, with an increase of approximately to 10% ( $R = 2.24$  vs.  $2.0$ ;  $P <$

0.05). Similar increases were observed in the occipital regions for the combination of sumatriptan and nitroglycerin ( $R = 2.23$  vs.  $2.0$ ). Diverse cerebral blood flow responses have been reported after nitroglycerine administration. A study in cynomolgus monkeys suggests that tonic production of NO contributes to the control of cerebral blood flow during isoflurane anaesthesia, which is decreased by nitric oxide synthase inhibition, i.e. with N omega-nitro-L-arginine methyl ester (L-NAME) [6]. This study was performed by firstly NOS inhibition by L-NAME, and secondly followed by L-arginine administration which reversed the initial decreased cerebral blood flow [6]. This reversal was however not above the control values. Studies have also showed decreases in cerebral blood flow after inhibition of nitric oxide synthase for different anaesthetic regimes [5, 8]. It was also shown that nitroglycerine increases local cerebral blood flow in man [27]. Furthermore, it was shown that cerebral blood flow was unchanged in Beagle dogs after nitroglycerine treatment [28]. From the above and our preliminary investigation it is clear that nitroglycerine treatment regime can be of importance in the clinical situation; furthermore that nitroglycerine related increases in cerebral blood flow may be more evident through the reversal of decreased cerebral blood flow after NOS inhibition.

For the drug combinations it was found that nitroglycerine reversed the nimodipine-induced increase in cerebral blood flow to below the control control value ( $R = 2.51$  vs.  $1.69$ ). An increase in cerebral blood flow after nimodipine administration has also been reported previously [29]. A similar reversal was recently observed upon the combination treatment with sodium valproate and nimodipine [19]. This marked influence supports the importance of calcium and the action of nitroglycerine through the NO process. Drug interaction from simultaneous administration of these two drugs is therefore implicated by these results.

The sumatriptan-induced decreased cerebral blood flow [16] was reversed by nitroglycerine to values that are slightly higher than the control but not statistically significantly. This result indicates that sumatriptan did not influence the cerebral blood flow due to nitroglycerine treatment which may suggest that

sumatriptan would not be effective in treating cerebral blood flow increases due to nitroglycerine therapy, i.e. the nitroglycerine induced headache [9, 10]. These results may also suggest that in so far as the action of sumatriptan is concerned in the treatment of migraine, NO seems to play a lesser role in the cerebrovascular pharmacological mode of action of sumatriptan. However the effective treatment of migraine with sumatriptan may be compromised from simultaneous treatment with nitroglycerine as was indicated by reversal of the effect of sumatriptan.

No significant interaction between nitroglycerine and acetazolamide is envisaged and the effects observed are primarily attributed to acetazolamide for the drug combination. A similar observation was reported in the drug combination of acetazolamide with the anti-epileptic drug, sodium valproate [19] with no drug interactions envisaged.

Nitroglycerine treatment alone exhibited the largest influence on the arterial blood pressure, i.e. an increase of 16% with respect to the control. The increase is insignificant when the increase (12%) due to the anaesthesia alone [19] is taken into consideration. The increase in blood pressure was lower for all the combination studies with nitroglycerine and a decrease was even observed for the combination of nitroglycerine and nimodipine. The heart rates were only marginally influenced with the largest effect being an increase of 8% for the combination of nitroglycerine and nimodipine. The dosage regimes used in the present study for nitroglycerine and the other drugs therefore exhibit insignificant cardiovascular effects when compared with the control.

## 12.5 Conclusion

The current study has emphasised the clinical importance of a NO donor drug in drug combinations with respect to cerebrovascular effects. The study has shown that, although only marginal total brain cerebral blood flow increases were observed on nitroglycerine treatment, significant regional increases in the occipital areas were noted. Nitroglycerine influences the effects of various cerebrovascular drugs administered simultaneously, in different ways: drug interactions seem possible between nitroglycerine and nimodipine;

nitroglycerine treatment may compromise migraine treatment with sumatriptan; the combination of acetazolamide and nitroglycerine was found to be acceptable with respect to cerebral blood flow responses. The influences of the drug combinations of nitroglycerine on the haemodynamic parameters (blood pressure and heart rate) were found to be insignificant with respect to anaesthesia control data. The cerebral blood flow effects of NO synthase inhibitors, such as L-NAME are currently being under investigation.

## 12.6 References

1. **McNAMARA JO.** *In: Goodman and Gilman's The Pharmacological Basis of Therapeutics, 9<sup>th</sup> ed., JG Hardman, LE Limbird, PB Molinoff et al. (eds), pp. 137, 254, 286, 827, 830, McGraw-Hill, New York. 1996.*
2. **MONCADA S, PALMER RMJ, HIGGS EA.** 1991. *Pharmacol Rev* 43:109.
3. **BREDT DS, SNYDER SH.** 1994. *Annual Review of Biochemistry* 63:174.
4. **MARLETTA MA.** 1993. *J. Biol. Chem* 268:12231.
5. **TODD MM, WU B, WARNER DS, MAKTABI M.** 1994. *Anaesthesiology* 80:1128.
6. **MCPERSON RW, KIRCH JR, TOBIN JR, et al.** 1994. *Anaesthesiology* 80:1320.
7. **TRUCCO M, CANANZI C, SLAVADORI PR, et al.** 1994. *Funct. Neurology* 9:247.
8. **HYLLAND P, NILSSON GE, LUTZ PL.** 1996. *Journal of Cerebral Blood Flow and Metabolism* 16:290.
9. **THOMSEN LL, IVERSEN HK, BRINCK TA, et al.** 1993. *Cephalalgia* 13:396.
10. **IVERSEN HK.** 1995. *Cephalalgia* 15:281.
11. **OLESEN J, IVERSEN HK, THOMSEN LL.** 1993. *Neuroreport* 4:1027.

12. **DORMEHL IC, OLIVER DW, HUGO N**, *in* : SPECT in Neurology and Psychiatry, PP de Deyn, RA Driecks, A Alavie *et al.*), Chapter 62, pp. 521-535, John Libbey and Company, Amsterdam (1997).
13. **DORMEHL IC, REDELINGHUYS F, HUGO N**, *et al.* 1992. *Medical Primatology* 21:270.
14. **OLIVER DW, DORMEHL IC, REDELINGHUYS F**, *et al.* 1993. *Nuklear Medizin* 32:292.
15. **DORMEHL IC, OLIVER DW, HUGO N**. 1993. *Nuclear Medicine Communications* 14:573.
16. **OLIVER DW, DORMEHL IC, HUGO N**. 1994. *Arzneimittel Forschung / Drug Research* 44(2):925.
17. **DORMEHL IC, OLIVER DW, HUGO N**. 1995. *Arzneimittel Forschung / Drug Research* 45(2):952.
18. **JORDAAN B, OLIVER DW, DORMEHL IC**, *et al.* 1996. *Arzneimittel Forschung / Drug Research* 46(2):844.
19. **OLIVER DW, DORMEHL IC**. 1998. *Arzneimittel Forschung / Drug Research* 48(2):1058.
20. **DORMEHL IC, OLIVER DW, HUGO N**, *et al.* 1995. *Nuclear Medicine and Biology* 22:373.
21. **DORMEHL IC, OLIVER DW, LANGEN K-J**, *et al.* 1997. *Journal of Nuclear Medicine* 38:1897.
22. **WYPER DJ, HUNTER R, PATTERSON J**, *et al.* 1991. *Journal of Cerebral Blood Flow and Metabolism* 11:S449
23. **HOLM S, MADSEN PL, RUBIN P**, *et al.* 1991. *Journal of Cerebral Blood Flow and Metabolism* 11:S766.

24. **LASSEN NA, CHRISTENSEN MS.** 1976. *British Journal of Anaesthesia* 48:719.
25. **WOOD M, WOOD AJJ.** 1990. *Drugs Anaesthes.* 2:179.
26. **FUTAGI Y, OTANI K, IMAI K.** 1994. *Epilepsia* 35:827.
27. **ABE K.** 1993. *Canadian Journal of Anaesthesia* 40:775.
28. **HAMAGUCHI M, ISHIBASHI T, KATSUMATA N, et al.** 1992. *Cardiovascular Drugs Ther* 6:611.
29. **LANGLEY MS, SORKIN EM.** 1989. *Drugs* 27:669.

**Effect of Cyclodextrin  
Complexation on the in vivo  
Disposition of the Brain Imaging  
Radiopharmaceutical  
<sup>99m</sup>Techneium Ethyl Cysteinate  
Dimer (<sup>99m</sup>Tc-ECD)\***

**Chapter  
13**

### Summary

The brain imaging radiopharmaceutical, <sup>99m</sup>Techneium ethyl cysteinate dimer (<sup>99m</sup>Tc-ECD, <sup>99m</sup>Tc-bicisate) is the most recent addition to the available set of radiopharmaceuticals for measuring cerebral blood flow. Ideally radiotracers should be trapped in the brain long enough so that their distribution can be quantitated and should demonstrate good spatial resolution. Furthermore, the stability (chemical and metabolic) and bioavailability of radiopharmaceuticals have in general proved to be a challenge during development and clinical administration. In view of these challenges and background, this study with <sup>99m</sup>Tc-ECD is presented. The aims of this research program were to develop novel approaches to improve the chemical and metabolic stability and the bioavailability of <sup>99m</sup>Tc-ECD across the blood brain barrier for cerebral blood flow determinations, using the well known non-human primate in vivo baboon model. These aims were addressed by investigating the influence of cyclodextrin-<sup>99m</sup>Tc-ECD complexation on normal cerebral blood flow patterns, using two different cyclodextrins, i.e.,  $\gamma$ -cyclodextrin (CAS 17465-86-0) and  $\beta$ -trimethylcyclodextrin (CAS 55216-11-0). The effect of incubation of <sup>99m</sup>Tc-ECD (with or without cyclodextrin complexation) in plasma, on metabolic esterase action, was also investigated. Possible protection against plasma esterase by acetylcholine (CAS 51-84-3) of <sup>99m</sup>Tc-ECD was further determined.

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\* OLIVER, D.W. & DORMEHL, I.C., LOUW, W., MORETTI, J-L. & KILLIAN, E. 2000. Effect of Cyclodextrin Complexation on the in vivo Disposition of the Brain Imaging Radiopharmaceutical, <sup>99m</sup>Techneium Ethyl Cysteinate Dimer (<sup>99m</sup>Tc-ECD). *Arzneimittel Forschung/Drug Research*, 50:75-81.

*The current study has shown that cyclodextrin complexation of  $^{99m}\text{Tc}$ -ECD indeed offers a useful approach to improve the stability of the radiopharmaceutical against peripheral metabolism. The acetylcholine shows also potential to protect  $^{99m}\text{Tc}$ -ECD. However, it is clear from the current data that the choice of cyclodextrin is of utmost importance, as has been observed from significantly reduced the bioavailability of  $^{99m}\text{Tc}$ -ECD when complexed with  $\beta$ -trimethylcyclodextrin. The plasma incubation procedures showed that  $\gamma$ -cyclodextrin offers protection with only slightly reduced bioavailability. This study has indicated that novel approaches, such as cyclodextrin technologies, indeed show potential to modify the performance in its currently available  $^{99m}\text{Tc}$ -ECD form.*

**KEY WORDS:** Brain imaging, Cyclodextrin - complexation with technetium- $^{99m}$  ethyl cysteinate dimer, Radiopharmaceuticals, Technetium- $^{99m}$  ethyl cysteinate dimer - complexation with cyclodextrin.

## **13.1 Introduction**

Several radiopharmaceutical agents, e.g., N-isopropyl- $^{123}\text{I}$ B-iodoamphetamine ( $^{123}\text{I}$ IMP),  $^{99m}\text{Tc}$  hexamethylpropyleneamine oxime ( $^{99m}\text{Tc}$ -HMPAO) and  $^{99m}\text{Tc}$  ethyl cysteinate dimer ( $^{99m}\text{Tc}$ -ECD) have found useful application as tracers to measure cerebral blood flow (CBF) [1,2]. Ideally these tracers should be trapped in the brain long enough so that their distribution can be quantitated, and should demonstrate good spatial resolution. The abovementioned agents each show unique properties that, although they are far from the optimal, warrant their clinical application in nuclear medicine. Despite its widespread use, the iodine labelled amphetamine, IMP appears to redistribute in the brain with time [3], and its retention mechanism is stereo selective that depends on its metabolism [4]. Furthermore, the stability (chemical and metabolic) and bioavailability of radiopharmaceuticals have in general proved to be a challenge during development and clinical administration. The retention of  $^{99m}\text{Tc}$ -HMPAO in the brain is further limited to the enzymatic reactions with glutathione [5,6].  $^{99m}\text{Tc}$ -

ECD exhibits a high initial brain extraction with a slow clearance [7] with brain metabolism yielding hydrophylic monoacid esters trapped in the primate brain [8]. Peripheral systemic enzymatic esterase metabolism [9,10] of  $^{99m}\text{Tc}$ -ECD, further negatively impacts on the amount of brain extraction for the tracer, due the formation of hydrophylic acid derivatives, unable to cross the hydrophobic blood brain barrier. Recently, improved stability was demonstrated with  $^{99m}\text{Tc}$ -labeled liposomes through formation of a hydrazino nicotyl derivative [11]. Moretti's group recently investigated the uptake of liposome-encapsulated  $^{99m}\text{Tc}$ -MIBI by both sensitive and multidrug-resistant tumour cell lines [12]. Cyclodextrin technologies have frequently been employed during the last decade to improve the bioavailability of poor water-soluble drugs, i.e. miconazole, lorazepam, cyclosporin A and others [13 -15]. In view of these challenges and background, the current study with  $^{99m}\text{Tc}$ -ECD is presented. The aims of this research program were to develop novel approaches to improved the metabolic stability and the bioavailability of  $^{99m}\text{Tc}$ -ECD across the blood brain barrier for cerebral blood flow determinations, using the well known non-human primate *in vivo* baboon model [1]. These aims were addressed using cyclodextrin complexation technologies (using two different cyclodextrins, i.e.,  $\gamma$ -cyclodextrin (CAS 17465-86-0) and  $\beta$ -trimethylcyclodextrin, i.e. heptakis 2,3,6-tri-O-methyl- $\beta$ -cyclodextrin (CAS 55216-11-0)) and incubating  $^{99m}\text{Tc}$ -ECD in plasma with or without cyclodextrin complexation prior to *in vivo* administration. It was further assessed if acetylcholine (CAS 51-84-3) was able to protect  $^{99m}\text{Tc}$ -ECD from peripheral esterase metabolism.

## 13.2 Materials and methods

Six adult male baboons (*Papio ursinus*, average weight 25 kg) were used for this study. The animals were obtained from Mr. E. Venter, Northern Province (Republic of South Africa). The studies were performed after approval by the Ethics Committee of the University of Pretoria, according to the guidelines of the National Code for Animal Use in Research, Education and Testing of Drugs and Related Substances in South Africa. These guidelines are in line with international standards. Previously conducted studies used the sensitive

familiar baboon model developed for cerebral blood flow determinations with single photon emission computed tomography [16 - 23]. An identical approach was followed for the current investigation.

Six different procedures (A -F) were carried out on each of the six baboons with three-week intervals between. These procedures were: five modifications in the use of  $^{99m}\text{Tc}$ -ECD as CBF tracer with hopefully higher bioavailability to the brain than is the case of unmodified, conventional  $^{99m}\text{Tc}$  labelled ECD which made out the sixth study (see procedure A in Table 13-1).

For the control study (procedure A), each baboon was sedated with ketamine hydrochloride (10 mg/kg i.m)(Anaket-V<sup>®</sup>, Centaur Labs, Bryanston, Gauteng, SA). This was followed immediately by maintained and controlled infusion of thiopentone (thiopental) sodium (70 ml/h of 0,5% solution) (Intraval<sup>®</sup>, Rhône-Poulenc, Rorer, S.A., Midrand, Gauteng, SA). After a 12-min-stabilisation period under thiopentone, procedure A, the control study started at  $t = 0$  with an i.v. injection of 222 MBq of  $^{99m}\text{Tc}$ -Ethyl Cysteinate Dimer (ECD), Neurolite<sup>®</sup>, Du Pont Pharma). The ECD was labelled according to the manufacturer's directions. Five min after the tracer injection for procedure A, the first SPECT acquisition (SPECT-1) followed with a Siemens Orbiter gamma camera, using 32 projections of 20 sec per view during a 360° rotation. The baboons were always positioned in the supine position with a special headrest to ensure reproducible and comparable tomographic slices for all procedures. SPECT-1 was followed by a second intravenous administration of tracer  $^{99m}\text{Tc}$ -ECD of double the radioactivity dose (i.e. 444 MBq) at  $t = 20$  min. After another 5 min a similar SPECT acquisition, SPECT-2 followed (the split dose method), which, for procedure A, measures the anaesthesia related CBF changes taking place in between the two ECD administrations. procedure B, was the same as procedure A, except that the second tracer application was of  $^{99m}\text{Tc}$ -ECD incubated in 2 ml plasma, which was obtained from 10 ml of the baboon's blood which was previously drawn and centrifuged.

**Table 13-1** The time schedule for the various tracer procedure protocols indicating the time of each procedure.

Min	Procedure A	Procedure B	Procedure C	Procedure D	Procedure E	Procedure F
	Ketamine	Ketamine	Ketamine	Ketamine	Ketamine	Ketamine
-12	Thiopental	Thiopental	Thiopental	Pentobarbital	Pentobarbital	Pentobarbital
0	1 <sup>st</sup> $^{99m}\text{Tc}$ -ECD	1 <sup>st</sup> $^{99m}\text{Tc}$ -ECD	1 <sup>st</sup> $^{99m}\text{Tc}$ -ECD	1 <sup>st</sup> $^{99m}\text{Tc}$ -ECD	1 <sup>st</sup> $^{99m}\text{Tc}$ -ECD	1 <sup>st</sup> $^{99m}\text{Tc}$ -ECD
5	SPECT-1	SPECT-1	SPECT-1			
6		Mix ECD + plasma				
7			Mix + plasma + acetylcholine			
8		Incubation (37°, 10min)	Incubation (37°, 10min)			
18		Mix $^{99m}\text{Tc}$ + ECD + plasma	Mix $^{99m}\text{Tc}$ + ECD + acetylcholine + plasma			
20	Injection 2 <sup>nd</sup> $^{99m}\text{Tc}$ -ECD	Injection 2 <sup>nd</sup> $^{99m}\text{Tc}$ -ECD + plasma	Injection 2 <sup>nd</sup> $^{99m}\text{Tc}$ -ECD + acetylcholine + plasma			
25	SPECT-2	SPECT-2	SPECT-2			
30				Complexation 2 <sup>nd</sup> $^{99m}\text{Tc}$ -ECD + $\gamma$ -cyclodextrin	Complexation 2 <sup>nd</sup> $^{99m}\text{Tc}$ -ECD + $\beta$ -trimethyl- cyclodextrin	Complexation 2 <sup>nd</sup> $^{99m}\text{Tc}$ -ECD + $\gamma$ -cyclodextrin
45				SPECT-1	SPECT-1	SPECT-1 and mix $^{99m}\text{Tc}$ -ECD + $\gamma$ -cyclodextrin + plasma
47						incubation (37°C)
60				Injection 2 <sup>nd</sup> $^{99m}\text{Tc}$ -ECD + $\gamma$ -cyclodextrin	Injection 2 <sup>nd</sup> $^{99m}\text{Tc}$ -ECD + $\beta$ -trimethyl - cyclodextrin	Injection 2 <sup>nd</sup> $^{99m}\text{Tc}$ -ECD + $\gamma$ -cyclodextrin + plasma
105				SPECT-2	SPECT-2	SPECT-2

The ECD and plasma were mixed at 6 min and at t = 8 min the incubation period for 10 min (37 °C) started. At 18 min, double the first activity dose of  $^{99m}\text{Tc}$  (444 MBq) was mixed with ECD in plasma and the second i.v. administration of  $^{99m}\text{Tc}$ -ECD in plasma took place at t = 20 min. Thus, SPECT-2, which again followed 5 min after the second injection at t = 25 min

reflected the influence of the plasma incubation of ECD in relation to its bioavailability to the brain.

Procedure C had the same protocol as procedure B, but the second application was an infusion of Tc-labelled ECD, plasma and acetylcholine (CAS 51-84-3; 1% solution), the latter to act as an esterase substrate in the plasma. This tracer modification was achieved as follows: at  $t = 7$  min the plasma and acetylcholine were mixed and incubation ( $37\text{ }^{\circ}\text{C}$ ) started at  $t = 8$  min, for 10 min. At 18 min double the first dose of  $^{99m}\text{Tc}$ -ECD (444 MBq) was mixed with the plasma and acetylcholine. This was injected i.v. as a second tracer at  $t = 20$  min. SPECT 2 followed 5 min later ( $t = 25$  min). SPECT-2 should reflect improved brain bioavailability with respect to SPECT-1, if the acetylcholine protected the ECD from degradation by plasma esterases.

Procedure D and E concerned complexation of the ECD into two different cyclodextrin formulations,  $\gamma$ -cyclodextrin (CAS 17465-86-0) and  $\beta$ -trimethylcyclodextrin, i.e. heptakis 2,3,6-tri-O-methyl- $\beta$ -cyclodextrin (CAS 55216-11-0). For both these procedures the maintaining anaesthesia was changed to the long acting sodium pentobarbitone (30ml/hr of a 0.8% solution)(Sagatal<sup>®</sup>, Kyron Laboratories (Pty) Ltd, Benrose, Gauteng, SA), due to the longer time involved, to allow for release of the ECD from the complex to cross the blood brain barrier (see Table 13-1).  $^{99m}\text{Tc}$ -ECD was complexed with  $\gamma$ -cyclodextrin by incubating the  $^{99m}\text{Tc}$ -ECD with a 10 – 30 molar excess ( $\gamma$ - CD : ECD) for 30 min at room temperature in physiological normal saline solution. In procedure E, the  $\gamma$ -cyclodextrin was replaced by  $\beta$ -trimethylcyclodextrin and the complexation performed as before. Split dose SPECT was performed as in procedures A – C, except that SPECT-1 followed 45 min after the first i.v. administration of  $^{99m}\text{Tc}$ -ECD, and the second SPECT-2, likewise 45 min after either ECD +  $\gamma$ -cyclodextrin, or ECD +  $\beta$ -trimethylcyclodextrin, labelled with the double dose of  $^{99m}\text{Tc}$ -ECD, was administered.

Additionally, procedure F was eventually performed, similarly to procedure D, but the second injection was of  $^{99m}\text{Tc}$ -ECD +  $\gamma$ -cyclodextrin with additional

incubation done in 2 ml plasma for 10 min, starting at 47 min, injected at 60 min and scanning started at 105 min.

During all the above procedures arterial blood pressures were recorded from a catheter in the femoral artery and heart rates were monitored.

After backprojection and reconstruction of SPECT-1 and SPECT-2 data, the brain images in all procedures consisted of transaxial, sagittal and coronal slices, representing total brain CBF and some regional related CBF information. Eight slices of one pixel thickness each represented the brain in all three views, as mentioned above.

Regions of interest were placed on the total brain, as viewed in each slice and count rate data (counts/pixel) thus obtained were inserted into the following equation to obtain the ratio R:

$$R = \frac{(SPECT - 2) - (SPECT - 1)^*}{(SPECT - 1)}$$

where \* refers to decay-corrected data from SPECT-1 which is present during SPECT-2 and has to be subtracted from the SPECT-2 data as a background correction. R is an indication of the level change of (r) CBF during extended anaesthesia using  $^{99m}\text{Tc}$ -ECD, or in addition, because of modifications of the second tracer in each study.

### 13.2.1 Statistical methods

The R-values for eight slices in transaxial, sagittal and coronal view could be compared between control and modified studies and between modified tracers studies. A two-tailed Student's *t*-test for paired variables was used, to express significance with a 5% level of confidence.

## 13.3 Results

R-values (mean  $\pm$  SD) are presented in Tables 13-2 to 13-4 (transaxial, sagittal and coronal), for each of the eight slices, and each Procedure A – F. It is clear from Tables 13-2 to 13-4 that the biodisposition of the Tc-ECD

radiopharmaceutical is influenced differently. With no intra-procedural regional, slice dependent differences showing statistical significance (see Fig. 13-1 – transaxial), mean R-values including all slices and all views were obtained, and used to represent the total brain for each procedure, as shown in Table 13-4. Percentage changes due to the tracer modification ( $\Delta R$ ) are given between the different procedures in Table 13-5.

**Table 13-2** The mean R-ratios ( $\pm$  SD) ( $n = 6$ ) from transaxial views of eight equal cerebral slices for the six different procedures with the slice number starting from the frontal to the occipital lobes

Procedure	Slice 1	Slice 2	Slice 3	Slice 4
A	2.03 $\pm$ 0.15	1.99 $\pm$ 0.21	2.05 $\pm$ 0.26	2.10 $\pm$ 0.28
B	1.68 $\pm$ 0.13	1.73 $\pm$ 0.10	1.75 $\pm$ 0.14	1.76 $\pm$ 0.12
C	1.77 $\pm$ 0.04	1.78 $\pm$ 0.11	1.81 $\pm$ 0.16	1.80 $\pm$ 0.22
D	2.00 $\pm$ 0.46	1.98 $\pm$ 0.34	2.02 $\pm$ 0.31	2.03 $\pm$ 0.32
E	1.66 $\pm$ 0.08	1.65 $\pm$ 0.10	1.66 $\pm$ 0.12	1.66 $\pm$ 0.12
F	1.92 $\pm$ 0.23	1.95 $\pm$ 0.28	1.96 $\pm$ 0.15	1.99 $\pm$ 0.30
Procedure	Slice 5	Slice 6	Slice 7	Slice 8
A	2.13 $\pm$ 0.30	2.19 $\pm$ 0.28	2.14 $\pm$ 0.28	2.07 $\pm$ 0.36
B	1.78 $\pm$ 0.10	1.73 $\pm$ 0.12	1.72 $\pm$ 0.10	1.77 $\pm$ 0.15
C	1.75 $\pm$ 0.01	1.76 $\pm$ 0.15	1.89 $\pm$ 0.08	1.96 $\pm$ 0.01
D	2.00 $\pm$ 0.30	2.02 $\pm$ 0.31	2.04 $\pm$ 0.18	2.04 $\pm$ 0.15
E	1.68 $\pm$ 0.10	1.69 $\pm$ 0.09	1.69 $\pm$ 0.10	1.68 $\pm$ 0.02
F	2.00 $\pm$ 0.30	2.03 $\pm$ 0.30	1.98 $\pm$ 0.23	1.83 $\pm$ 0.26

From Table 13-5 it is clear that incubating the ECD with the animal's plasma for 10 min before injection for the 2<sup>nd</sup> scan (procedure B) significantly reduced ( $R = 1.75 \pm 0.11$ ) the bioavailability to the brain by 15.5 % ( $P < 0.05$ ), and that this attenuation was only slightly altered (- 13%) with the addition of acetylcholine (Procedure C) to the plasma ( $R = 1.80 \pm 0.20$ , % change -13 %,  $P > 0.05$ ,  $P < 0.10$  with respect to Control).

**Table 13-3** The mean R-ratios ( $\pm$  SD) (n = 6) from sagittal views of eight equal cerebral slices for the six different procedures with the slice number starting from the left to the right

Procedure	Slice 1	Slice 2	Slice 3	Slice 4
A	2.18 $\pm$ 0.40	2.12 $\pm$ 0.30	2.12 $\pm$ 0.27	2.09 $\pm$ 0.26
B	1.75 $\pm$ 0.07	1.74 $\pm$ 0.07	1.74 $\pm$ 0.11	1.81 $\pm$ 0.13
C	1.59 $\pm$ 0.37	1.70 $\pm$ 0.25	1.75 $\pm$ 0.13	1.79 $\pm$ 0.05
D	1.73 $\pm$ 0.85	2.05 $\pm$ 0.25	2.02 $\pm$ 0.27	2.00 $\pm$ 0.28
E	1.83 $\pm$ 0.20	1.71 $\pm$ 0.13	1.68 $\pm$ 0.12	1.66 $\pm$ 0.11
F	2.08 $\pm$ 0.63	1.87 $\pm$ 0.27	1.83 $\pm$ 0.27	1.92 $\pm$ 0.27
Procedure	Slice 5	Slice 6	Slice 7	Slice 8
A	2.10 $\pm$ 0.15	2.07 $\pm$ 0.30	2.06 $\pm$ 0.30	2.03 $\pm$ 0.15
B	1.78 $\pm$ 0.16	1.74 $\pm$ 0.11	1.75 $\pm$ 0.11	1.74 $\pm$ 0.12
C	1.86 $\pm$ 0.01	1.81 $\pm$ 0.01	1.94 $\pm$ 0.16	2.04 $\pm$ 0.35
D	2.00 $\pm$ 0.27	2.01 $\pm$ 0.25	1.98 $\pm$ 0.24	1.96 $\pm$ 0.27
E	1.66 $\pm$ 0.12	1.67 $\pm$ 0.11	1.67 $\pm$ 0.09	1.68 $\pm$ 0.07
F	1.95 $\pm$ 0.28	1.96 $\pm$ 0.28	1.92 $\pm$ 0.27	1.84 $\pm$ 0.28

**Table 13-4** The mean R-ratios ( $\pm$  SD) (n = 6) from coronal views of eight equal cerebral slices for the six different procedures with the slice number starting from the cerebellum to the dorsal slice of the cerebrum

Procedure	Slice 1	Slice 2	Slice 3	Slice 4
A	2.12 $\pm$ 0.45	2.15 $\pm$ 0.41	2.11 $\pm$ 0.33	2.11 $\pm$ 0.26
B	1.77 $\pm$ 0.12	1.75 $\pm$ 0.08	1.76 $\pm$ 0.11	1.75 $\pm$ 0.08
C	1.45 $\pm$ 0.78	1.38 $\pm$ 0.51	1.57 $\pm$ 0.30	1.71 $\pm$ 0.08
D	1.94 $\pm$ 0.28	1.99 $\pm$ 0.26	2.04 $\pm$ 0.28	2.01 $\pm$ 0.24
E	1.63 $\pm$ 0.09	1.65 $\pm$ 0.10	1.66 $\pm$ 0.10	1.67 $\pm$ 0.10
F	2.01 $\pm$ 0.37	2.09 $\pm$ 0.34	2.07 $\pm$ 0.31	1.96 $\pm$ 0.25
Procedure	Slice 5	Slice 6	Slice 7	Slice 8
A	2.06 $\pm$ 0.26	2.03 $\pm$ 0.24	1.92 $\pm$ 0.27	1.71 $\pm$ 0.68
B	1.75 $\pm$ 0.13	1.73 $\pm$ 0.10	1.73 $\pm$ 0.10	1.72 $\pm$ 0.10
C	1.85 $\pm$ 0.04	1.95 $\pm$ 0.07	2.08 $\pm$ 0.30	2.32 $\pm$ 0.60
D	2.04 $\pm$ 0.24	2.01 $\pm$ 0.26	2.04 $\pm$ 0.24	2.02 $\pm$ 0.24
E	1.69 $\pm$ 0.11	1.70 $\pm$ 0.11	1.71 $\pm$ 0.12	1.71 $\pm$ 0.15
F	1.82 $\pm$ 0.25	1.74 $\pm$ 0.25	1.63 $\pm$ 0.26	1.51 $\pm$ 0.46

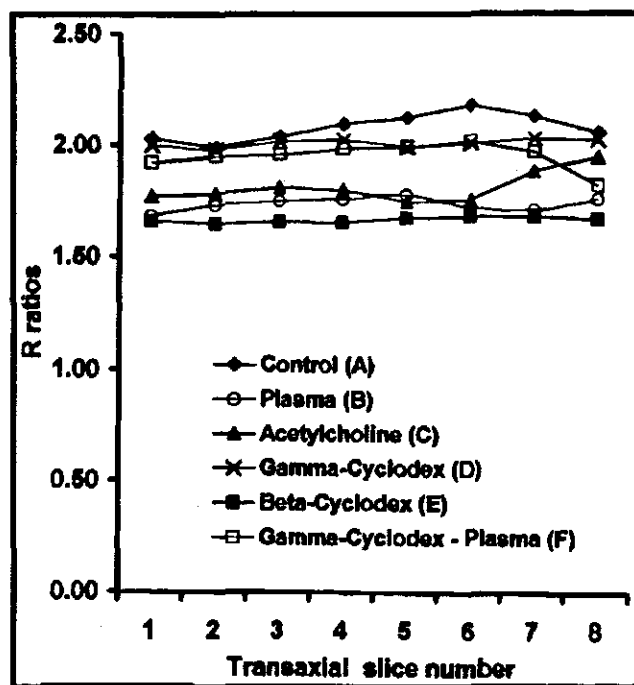


Figure 13-1 Curves of the mean ratio (R) (n = 6) vs. slice number of all the procedures (A-F) in the transaxial view starting from the frontal to the occipital lobes.

The effect of cyclodextrin complexation with the radiotracer is clearly evident in the results obtained from procedures D and E. Complexing ECD with  $\gamma$ -cyclodextrin for SPECT-2 as described in procedure D, yielded an R-value of  $2.00 \pm 0.30$ , which although 3.4 % below the control of Procedure A, was not statistically significantly different from the control value ( $P > 0.05$ ). On the other hand, the complex between ECD and  $\beta$ -trimethylcyclodextrin resulted in significantly decreased R-value when compared to both the control, procedure A ( $R = 1.68 \pm 0.11$  vs.  $2.07 \pm 0.15$ ,  $P < 0.02$ ) and the  $\gamma$ -cyclodextrin complex, procedure D ( $R = 1.68 \pm 0.11$  vs.  $2.00 \pm 0.30$ ,  $P < 0.05$ ). The changes are -18.8% and -16% between procedures A and D and procedures B and D respectively. The result from procedure F, where the complex of ECD and  $\gamma$ -cyclodextrin was incubated in plasma shows R-value of  $R = 1.91 \pm 0.12$ . This result was significantly higher (+9%) than the  $R = 1.80 \pm 0.20$  observed in procedure B, for uncomplexed ECD that was incubated in plasma. Conversely, the result from procedure F is a slightly but not significantly lower (-7.7%) R-

value than the control result ( $R = 2.07 \pm 0.15$ ). The results from procedures D ( $2.00 \pm 0.30$ ) and F ( $1.91 \pm 0.12$ ) are similar with no significant difference.

**Table 13-5** The mean R-values ( $\pm$  SD) ( $n = 6$ ) for total brain as averaged from all slices and all views for each procedure, percentage changes ( $\Delta R$ ) of these R-values with respect to the procedure indicated in brackets

Procedure	R-value	Change ( $\Delta R$ )	Significance
<b>Procedure A</b> (Control)	$2.07 \pm 0.15$		
<b>Procedure B</b> (ECD in plasma incubated)	$1.75 \pm 0.11$	-15.5% (A)	$P < 0.05$
<b>Procedure C</b> (ECD + plasma + acetylcholine incubated)	$1.80 \pm 0.20$	-13.0% (A) +3.0% (B)	$P < 0.10, P > 0.05$ $P > 0.05$
<b>Procedure D</b> (ECD + $\gamma$ -cyclodextrin)	$2.00 \pm 0.30$	-3.4% (A) +8.6% (B)	$P > 0.05$ $P > 0.05, P < 0.10$
<b>Procedure E</b> (ECD + $\beta$ -trimethylcyclodextrin)	$1.68 \pm 0.11$	-18.8% (A) -16.0% (D)	$P < 0.02$ $P < 0.05$
<b>Procedure F</b> (ECD + $\gamma$ -cyclodextrin in plasma incubated)	$1.91 \pm 0.12$	-7.7% (A) +9.0% (B) -4.5% (D)	$P > 0.05$ $P < 0.05$ $P > 0.05$

## 13.4 Discussion

The current study indicated that the metabolic stability and central nervous system bioavailability across the blood brain barrier are important components for cerebral blood flow radioactive tracers, such as technetium labelled ECD. Furthermore, these aspects can be influenced, e.g. to improve the brain imaging properties of the radiopharmaceutical. The present study focussed on the effect of complexation of the ECD with two different cyclodextrins during cerebral blood flow determinations. The instability of ECD due to metabolism by plasma esterases was further pointed out by incubating ECD *in vitro* in plasma with or without being complexed with the cyclodextrin prior *in vivo* SPECT investigation.

The marked reduction (-15.5%) in the R-value for Procedure B (plasma incubation) when compared with the control (Procedure A) clearly indicate that the incubation of ECD in plasma adversely affected the ECD metabolic stability. This additional exposure of ECD to the plasma esterases *ex vivo* before the *in vivo* administration clearly resulted in significant additional esterase metabolism of ECD. Inoue and co-workers [24] earlier reported the *in vitro* metabolism of ECD in blood. They concluded that the majority of the enzyme that is involved in the metabolism of ECD exists in the red blood cells. An approach to protect ECD from this esterase degradation was attempted with Procedures C (adding acetylcholine) and Procedures D and E (using different cyclodextrins complexes). The improvement of 3 % in the case of Procedure C (adding acetylcholine) above Procedure B is measurable and maybe indicative of the slight protection of acetylcholine towards the esterase degradation of Tc-ECD, but insignificant ( $P > 0.05$ ) under the current dosage regimes.

On the other hand, complexing ECD with  $\gamma$ -cyclodextrin for SPECT-2 as described in procedure D, yielded an R-value of  $2.00 \pm 0.30$ , which although 3.4 % below the control of procedure A, was not statistically significantly different from it ( $P > 0.05$ ). The cyclodextrin complexation showed protection of ECD when compared with procedure B. An improvement of 8.6% was observed for the complexed ECD over the ECD not being complexed with the cyclodextrin and incubated in plasma. This finding is further supported by the result obtained from procedure F, i.e. that the  $\gamma$ -cyclodextrin offered some protection to the ECD against degradation. This can be seen from the R-value,  $R = 1.91 \pm 0.12$  for procedure F, where the incubation of  $\gamma$ -cyclodextrin complexed ECD in plasma did not show the same degree of degradation as in procedure B ( $\Delta R = +9 \%$ ,  $p < 0.05$ ), while not significantly differing from procedure A ( $\Delta R = -7.70 \%$ ,  $p > 0.05$ ) or from procedure D ( $\Delta R = -4.5 \%$ ,  $p > 0.05$ ) where no plasma incubation was involved.

The somewhat lower bioavailability of the  $\gamma$ -cyclodextrin complexed ECD to the brain (procedure D vs. procedure A,  $\Delta R = -3.4 \%$ ) although not significant, could point to a slightly decreased release of the  $^{99m}\text{Tc}$ -ECD from the complex, to cross the blood brain barrier. This seems to be confirmed by the results from

procedure E, where complexation of  $^{99m}\text{Tc}$ -ECD with the other cyclodextrin, i.e.  $\beta$ -trimethylcyclodextrin for the SPECT-2 injection rendered R-values significantly lower than in the control procedure A ( $R = 1.68 \pm 0.11$ ,  $\Delta R = -18.80\%$ ,  $P < 0.02$ ). The  $\beta$ -trimethylcyclodextrin was shown to display a binding by a factor of approximately 66 % stronger than  $\gamma$ -cyclodextrin ( $\Delta R = -16\%$  between procedure D and E,  $P < 0.05$ )(J.-L. Moretti, Personal Communication). A marked reduction in the release of the  $^{99m}\text{Tc}$ -ECD from the  $\beta$ -trimethylcyclodextrin complex or a too slow release from the complex could account for the significantly lower R-value for procedure E. The complex of ECD with  $\beta$ -trimethylcyclodextrin therefore reduces the availability of ECD for crossing the blood brain barrier during the course of the experiment, while protection against the esterase degradation is observed through the complex formation.

### 13.5 Conclusion

The complex of ECD with  $\gamma$ -cyclodextrin showed similar R-ratios as both the control and when the complex was incubated in plasma. In support of this finding, incubation of ECD alone showed significantly lower R-ratios. This study therefore revealed that biodegradation could indeed be reduced with the complexation approach using cyclodextrin technologies. The  $\beta$ -trimethylcyclodextrin however, reduced the availability of the ECD significantly, resulting in low R-ratio values. Therefore, this study has also shown that the availability of the radiotracer from the complex is of significant importance with respect to the timely release of the radiopharmaceutical, which is critical as not to negatively influence its availability and clinical application. The choice of cyclodextrin for the complexation with ECD must therefore be directed to protect the ECD against metabolic degradation as well as to ensure timely release for successful imaging characteristics.

## 13.6 References

1. **DORMEHL IC, OLIVER DW, HUGO N.** 1997. *SPECT in Neurology and Psychiatry*, P.P. de Deyn, R.A. Driecks, A. Alavi *et al.* (eds), Chapter 62, pp. 521-535, John Libbey and Company, Amsterdam.
2. **DORMEHL IC, OLIVER DW, LANGEN K-J, et al.** 1997. Technetium-99Tc-HMPAO, Technetium-99Tc-ECD and Iodine-123-IMP Cerebral blood flow measurements with pharmacological interventions in primates. *Journal of Nuclear Medicine* 38:1897.
3. **NISHIZAWA S, TANADA S, YONEKURA Y, et al.** 1989. Regional dynamics of N-isopropyl-( $^{123}\text{I}$ )-iodo-amphetamine in human brain. *Journal of Cerebral Blood Flow and Metabolism* 30:150-156.
4. **LASSEN NA, HENRIKSEN L, HOLM, S, et al.** 1983. Cerebral blood flow tomography xenon-133 compared with isopropyl-amphetamine-iodine-123. *Journal of Nuclear Medicine* 27.
5. **LASSEN NA, ANDERSON AR, FRIBERG L, PAULSON OB.** 1988. The retention of [99mTc]-d,1-HMPAO in the human brain after intracarotid bolus injection: a kinetic analysis. *Journal of Cerebral Blood Flow and Metabolism* 8 (suppl 1): S13-S22.
6. **NIERINCKX RD, BURKE JF, HARRISON RC, FORSTER AM, ANDERSON AR, LASSEN NA.** 1988. The retention mechanism of technetium-99m-HM-PAO; Intracellular reaction with glutathione. *Journal of Cerebral Blood Flow and Metabolism* 8:S4-S12.
7. **WALOVITCH RC, HILL TC, GARRITY ST, et al.** 1989. Characterization of technetium-99m-L,L-ECD for brain perfusion imaging, part 1: pharmacology of technetium-99m-ECD in non-human primates. *Journal of Nuclear Medicine* 30:1892-1901.
8. **WALOVITCH RC, CHEESMAN EH, MAHEU LJ, et al.** 1994. Studies of the retention mechanism of the brain perfusion imaging agent  $^{99m}\text{Tc}$ -

- bicisate ( $^{99m}\text{Tc}$ -ECD). *Journal of Cerebral Blood Flow and Metabolism* 14:S4.
9. **HOLMAN LB, HELLMAN RS, GOLDSMITH SJ, et al.** 1989. Biodistribution, dosimetry and clinical evaluation of technetium-99m ethyl cysteinate dimer in normal subjects and in patients with chronic cerebral infarction. *Journal of Nuclear Medicine* 30:1018-1024.
  10. **VALLABHAJOSULA S, ZIMMERMANN RE, PICARD M, et al.** 1989. Technetium-99m-ECD: a new brain imaging agent. In vivo kinetics and bio distribution studies in normal human subjects. *Journal of Nuclear Medicine* 30:599-604.
  11. **LAVERMAN P, DAMS ET, OYEN WJ, et al.** 1999. A novel method to label liposomes with  $^{99m}\text{Tc}$  by the hydrazino nicotinyl derivative. *Journal of Nuclear Medicine* 40:192.
  12. **DURAN CORDOBES M, MORETTI J-L, DE BECO V, et al.** 1999. Uptake of liposome-encapsulated  $^{99m}\text{Tc}^{\text{ni}}$ -MIBI by sensitive and multidrug-resistant tumour cell lines. *Nuclear Medical Communications* 20:433.
  13. **JENJARLA S, PURANJOTI P, KASINA R, et al.** 1998. Preparation, characterization and evaluation of miconazole cyclodextrin complexes for improved oral and topical delivery. *Journal of Pharmaceutical Sciences* 87:425.
  14. **MIYAKE K, ARIMA H, IRIE T, et al.** 1999. Enhanced absorption of cyclosporin A by complexation with dimethyl-beta cyclodextrin in bile duct cannulated and -noncannulated rats. *Biological and Pharmaceutical Bulletin* 22:66.
  15. **MAHROUK GM, AL-ANGARY AA, AL-MESHAL MA.** 1994. Modified beta-cyclodextrin lorazepam inclusion complexation. *Pharm Ind.* 56:1080.
  16. **DORMEHL IC, REDELINGHUYS IF, HUGO N, OLIVER D, PILLOY W.** 1992. The baboon model under anaesthesia for *in vivo* cerebral blood

- flow studies using SPECT techniques. *Journal of Medical Primatology* 21:270-274.
17. **OLIVER DW, DORMEHL IC, REDELINGHUYS IF, HUGO N, BEVERLY G.** 1993. Drug effects on cerebral blood flow in the baboon model. Acetazolamide and Nimodipine. *Nuklear Medizin* 32:292-298.
  18. **DORMEHL IC, OLIVER DW, HUGO N.** 1995. Dose response form pharmacological interventions for CBF changes in a baboon model using  $^{99m}\text{Tc}$ -HMPAO and SPECT. *Nuclear Medicine Communications* 14:573-577.
  19. **OLIVER DW, DORMEHL IC, HUGO N.** 1995. The effect of sumatriptan on cerebral blood flow in the baboon model. *Arzneimittel-Forschung* 44(2):952.
  20. **DORMEHL IC, OLIVER DW, HUGO N.** 1995. Cerebral blood flow effects of sumatriptan in drug combinations in the baboon model. *Arzneimittel-Forschung* 45(2):952.
  21. **JORDAAN B, OLIVER DW, DORMEHL IC, et al.** 1996. Cerebral blood flow effects of piracetam, pentifylline and nicotinic acid in the baboon model compared with the known effect of acetazolamide. *Arzneimittel-Forschung* 45(2):844.
  22. **OLIVER DW, DORMEHL IC.** 1998. Cerebral blood flow effects of Sodium Valproate and its drug combinations in the baboon model. *Arzneimittel-Forschung* 48(1):692.
  23. **OLIVER DW, DORMEHL IC.** 1999. Cerebral blood flow of the nitric oxide donor, nitroglycerine and its drug combinations in the non-human primate model. *Arzneimittel-Forschung* 49(2):732.
  24. **INOUE Y, MOMOSE T, AMO K, et al.** Metabolism of  $^{99m}\text{Tc}$ -ethyl cysteinate dimer ( $^{99m}\text{Tc}$ -ECD) in blood – mainly in vitro. *Kaku Igaku* (Japan).

## Summary Discussion and Conclusions

## Chapter 14

### 14.1 Introduction

This thesis describes the scientific and experimental development of a baboon *Papio ursinus* model for *in vivo* cerebral blood flow determinations. The model consists of the non-human primate in conjunction with very specific radiotracer measurement techniques. In this thesis the validation and successful application of the model in various drug studies for the evaluation of pharmacological interventions, are described. The specific objectives of this study were:

- The development of a cerebral perfusion baboon model under anaesthesia, for cerebral perfusion measurements;
- The validation with acetazolamide;
- The measurement of cerebral blood flow effects of selected drugs;
- The determination of cerebral perfusion effects after various pharmacological interventions;
- The determination of cerebral blood flow effects following drug interventions using different brain perfusion agents to evaluate their performance;
- The possible improvement of the tracer performance by investigation of cyclodextrine technology with the brain imaging agents.

A discussion of the contributions of the ten studies included in the thesis, towards the achievement of this aim will follow:

### 14.2 Discussion

The success of the split-dose method in humans suggested that such an approach could be followed in the baboon. The main difference between the human and the baboon study would be the need for the studies to be

conducted with the baboon under anaesthesia. The successful initial development of such a model under anaesthesia for cerebral perfusion measurement was essential for the subsequent studies in this thesis. The ability to understand and explain the effects of different anaesthesia regimes on perfusion was important, as was reproducibility of results and sufficient sensitivity under anaesthesia to measure moderate changes in perfusion. As such, the model under anaesthesia could serve as a control for comparisons during pharmacological interventions.

It is clear from the initial developmental study (Chapter 4) that the split-dose method is able to show changes in CBF due to the anaesthesia. These CBF changes expressed as R-values as calculated for the split-dose method, are compared to the intervention in subsequent studies. Furthermore, the various anaesthesia regimes induced different effects on cerebral perfusion. Halothane is known for its marked increase of CBF in humans and the split-dose baboon model was able to reproduce this increase. The duration of the anaesthesia, i.e. short acting vs. long acting barbiturate for the anaesthesia maintenance, induced differences in the cerebral perfusion, which could be illustrated. The increases found for longer anaesthesia studies, are of importance not only as control for longer intervention studies, but also in the clinical situation for comparing perfusion data of patients under anaesthesia and awake patients. This developmental study also showed that the split-dose method could be used with confidence in the *Papio ursinus* baboon using a radiotracer frequently applied in humans, viz.  $^{99m}\text{Tc}$ -hexamethylpropylene amine oxime ( $^{99m}\text{Tc}$ -HMPAO). Two other brain perfusion tracers, e.g.  $^{99m}\text{Tc}$ -ethylcysteinatedimer ( $^{99m}\text{Tc}$ -ECD) and  $^{123}\text{I}$ -Iodoamphetamine were also successfully applied and their results explained in the studies from Chapters 8,10 and 13, as such enhancing the successes of the baboon model.

Additional evaluation and confirmation of the split-dose method in the baboon model under anaesthesia was continually performed in those studies where acetazolamide was used. Acetazolamide, a drug that is well established in the determination of cerebral perfusion reserve, is known for its marked increase of perfusion ranging from 30% upwards in humans. Several of the

present baboon studies showed perfusion increases in the order of 35% upon acetazolamide treatment, which is similar to the increases reported in humans (Chapters 5,6,7,8,10). From these acetazolamide perfusion studies it can be confidently concluded that the model is sufficiently sensitive to serve in the evaluation of other cerebrovasoactive drugs for perfusion induced changes. This model is furthermore non-invasive in nature and the study is of short duration, i.e. it takes less than 150 minutes starting from the induction of the anaesthesia to the total recovery of the baboon from anaesthesia. In fact swift completion of the studies is called for by the radiopharmaceutical properties of  $^{99m}\text{Tc}$ -HMPAO tracer. The *in vivo* character of this model is naturally an advantage with respect to *in vitro* studies, as all the pharmacodynamic and – kinetic properties of the drugs are then taken into consideration and the results would be applicable in the clinical setting.

Following the evaluation and confirmation of the baboon model for CBF determinations, several drug intervention studies were conducted (Chapters 5-11). These studies ranged from single drug only, to drug combinations with variations in pharmacological response times and dosages.

Studies with nimodipine (Chapter 5) showed similar increases (+35%) in CBF as were found for acetazolamide compared to control values. A combination of nimodipine and acetazolamide, did not lead to a further increase in the CBF, to the contrary an attenuation of the effect of acetazolamide was observed. This attenuation of CBF effects was also observed when nimodipine was combined with sumatriptan (Chapter 9), sodium valproate (Chapter 11), and nitroglycerin (Chapter 12). These combination studies clearly suggest that drug-drug interactions are implicated for these combinations and that failing such considerations, therapies might significantly be compromised. Furthermore, decreases in the CBF patterns were observed for nimodipine when either ECD or IMP (Chapters 8, 10) was used as radiotracer, indicating that the choice of tracer is of utmost importance for clinical interpretation of CBF SPECT data. With acetazolamide treatment IMP indicated an increase of 52% in CBF, however HMPAO showed a significantly lower R-value of 37% (Chapter 8). The differences between the tracers with respect to their trapping

mechanisms in the brain probably contribute to these results. These studies also accentuated the use of the same tracer during a series of CBF investigations in the same patient. Sumatriptan showed a selective decrease in CBF when used in combination with some agents which induce increases in CBF. In this respect, the increases in CBF due to acetazolamide or halothane were not influenced (Chapter 7), whereas the increase due to barbiturate anaesthesia (long duration) was reversed to approximate control values. Sumatriptan did not influence the CBF pattern during short duration barbiturate anaesthesia (Chapters 7, 9). Although nitroglycerin (nitric oxide donor) presented no significant effect on CBF (Chapter 12), a significant decrease in CBF (-21%) was observed in combination with nimodipine. A combination of nitroglycerin with acetazolamide resulted in a CBF increase similar to the increase of acetazolamide alone (+35%). These various effects on CBF from various drug combinations are extensively discussed in the respective chapters and explanations offered.

The studies here reported, support the notion that the split-dose method is suitable to investigate pharmacological interventions and that it has been applied successfully in a variety of drug intervention studies as described in this thesis. The non-human primate model allows measurement of cerebral perfusion effects of drugs and yields information assisting the prediction of potential drug interactions in the clinical human setting, as well as provides information on *in vivo* pharmacological dynamics. The model is further validated by other studies than those discussed in this thesis, where it was used effectively (See Appendix 1, list of publications using this model).

Since current brain perfusion tracers still lack ideal properties, studies (Chapters 8, 10, 13) on the performance of  $^{99m}\text{Tc}$ -hexamethylpropylene amine oxime ( $^{99m}\text{Tc}$ -HMPAO),  $^{99m}\text{Tc}$ - ethylcysteinatedimer ( $^{99m}\text{Tc}$ -ECD), and  $^{123}\text{I}$ -lodoamphetamine were undertaken and cyclodextrin technology (Chapter 13) applied to attempt to improve the performance. The results of cyclodextrin complexation on the *in vivo* disposition of the tracer ECD (Chapter 13) emphasized the plasma instability of ECD indicated by a lower R-value (-15%) when compared to control R-values. The two types of cyclodextrins used, i.e.

beta and gamma cyclodextrin behaved differently with respect to the calculated R-values. Beta-cyclodextrin gave a R-value (-19%) that is significantly lower than the control, whereas gamma-cyclodextrin showed an insignificant difference, even when the complex was incubated in plasma. These studies clearly showed that gamma-cyclodextrin is able to protect ECD against plasma esterase degradation in plasma and that this technology is a promising approach to improve the stability of radiopharmaceuticals. These results further point towards investigating other types of cyclodextrins, which exhibit different complexing properties, in order to search for improvement in tracer performance.

In summary all these studies clearly showed differences between the tracers used, emphasising that the selection of a specific tracer is very important in order to decide accurately on patient deviations in perfusion from the normal in the clinical setting. These differences, if not taken into consideration, may lead to inappropriate clinical conclusions and subsequent ineffective or even contradictory therapeutic decisions. Although cyclodextrine technology did not markedly improve the brain disposition of the tracer, the study still showed potential for further exploration of this technique to afford protection to the tracer against chemical and metabolic degradation. The wide variety of cyclodextrines with diverse properties currently available, indeed opens the potential use of this technology in the development of brain perfusion tracers.

### **14.3 Future Studies and Applications**

It follows from the studies presented in this thesis as well as from several other studies using this model, that the non-human primate model with split-dose methodology to measure cerebral blood flow, could effectively be used in future pharmacological intervention studies. The model, although exclusively applied in the current studies for acute drug studies to assess acute effects, could equally be applied to determine perfusion effects from chronic drug treatments. The non-invasiveness of the model may permit it to be used to induce transient agent (drug) pathological simulations in order to investigate treatments. The model may also be combined with other established non-

human primate pathological models (Chapter 2) to investigate CBF in such pathologies and subsequent treatment. Apart from extending the studies further to the field of cyclodextrines, other approaches for modifying the performance of current tracers, such as protection by liposomes, should be investigated. This model may well be effectively applied for investigating CBF effects in novel drug development.

From the above, it is clear that the versatility of the perfusion model allows for a series of future studies to be conducted, ranging from pathology to therapy.

## 14.4 Conclusion

The current collective study set out to achieve the development and effective application of a non-human primate model, for cerebral blood flow determinations, for use in the field of pharmacological interventions.

Split-dose radiotracer methodology allowed the successful development of such a baboon model under anaesthesia. The model was justified with the configuration of acceptable cerebrovascular reserve determinations, indicating sensitivity to CBF changes. In subsequent studies, the model was effectively applied in several pharmacological intervention studies, whereby cerebropharmacodynamics of selected drugs were investigated and established. These studies not only yielded the perfusion effects of these drugs but also assisted in identification of clinically important drug interactions. The studies on the performances of the various tracers showed differences in the various CBF data sets, with important implications for considerations in the selection of a particular tracer for specific conditions in the clinical setting. Finally, cyclodextrine technology holds potential in modifying the performance of tracers amongst others through protection of the tracer against degradation.

In conclusion, the non-human primate *Papio ursinus* baboon cerebral blood flow model can be claimed to have been successfully developed and to have proved very useful and sensitive to investigate a wide variety of pharmacological interventions. This model has served many studies

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successfully during the past 12 years and could do so in the future and could indeed expand its investigative abilities with improved technologies.

## Glossary of abbreviations and terms

## Appendix 1

AD	Alzheimer's Disease
ADC	AIDS Dementia Complex
AIDS	Acquired Immune Deficiency Syndrome
ATP	Adenosine Triphosphate
BBB	Blood Brain Barrier
CNS	Central Nervous System
CT	Computed Tomography
CBF	Cerebral Blood Flow
CBV	Cerebral Blood Volume
CVR	Cerebrovascular Reserve
DA	Dopamine
ECD	Ethyl Cysteinate Dimer
EEG	Electroencephalography
GMP	Good Manufacturing Practise
5-HT	Serotonin
HIV	Human Immunodeficiency Virus
HMPAO	Hexamethylpropylene Amine Oxime
IMP	Iodoamphetamine
$K_{pat}$	Patlak value (graphic)
MCA	Middle Cerebral Artery
MELAS	Mitochondrial Myopathy, Encephalopathy, Lactic Acidosis
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MRI	Magnetic Resonance Imaging
NaI	Sodium Iodide
$^{18}\text{F}$ -FDG	Fluorine-18-labeled fluorodeoxyglucose

$^{18}\text{F}$	Fluorine-18
$^{123}\text{I}$	Iodine-123
$^{99}\text{Mo}$	Molybdenum-99
$^{99\text{m}}\text{Tc}$	Technetium-99 metastable
OCD	Obsessive Compulsive Disorder
6-OHDA	6-Hydroxydopamine
PET	Positron Emission Tomography
ROI	Region of interest
rCBF	Regional cerebral blood flow
rCBV	Regional cerebral blood volume
rCMRGlc	Regional cerebral metabolic rate of glucose
rCMRO <sub>2</sub>	Regional cerebral metabolic rate of oxygen
Sn	Zink
SD	Standard deviation
SIV	Simian Immunodeficiency Virus
SPECT	Single Photon Emission Computed Tomography
TAC	Time activity curve
Tc	Technetium
VD	Vascular Dementia
WCST	Wisconsin Card Sorting Test

## Publications and Presentations applying the Cerebral Perfusion Baboon Model

## Appendix 2

### 2.1 Publications and Congress Proceedings

1. DORMEHL, I.C., REDELINGHUYS, F., HUGO, N., OLIVER, D.W. & PILLOY, W. 1992. The Baboon model under anaesthesia for in vivo cerebral blood flow studies using single photon emission computed tomographic (SPECT) techniques. *Journal of medical primatology*, 21: 270-274.
2. DORMEHL, I.C., HUGO, N. & BEVERLEY, G. 1992. The baboon -an ideal model in biomedical research. *Anaesth Pain Contr Dent*, 1:109-115.
3. DORMEHL, I.C., LIPP, M.D.W., HUGO, N., DAUBLÄNDER, M. & PICARD, J.A. 1993. Influence of i.v. administered Lidocaine on cerebral blood flow in a baboon model standardized under controlled general anaesthesia using single photon emission computed tomography (SPECT) and <sup>99m</sup>Tc-HMPAO. *European journal of nuclear medicine*, 20(11):1095-1098.
4. DORMEHL, I.C., OLIVER, D.W. & HUGO, N. 1993. Dose response from pharmacological interventions for CBF changes in a baboon model using <sup>99</sup>Tcm-HMPAO and SPECT. *Nuclear medicine communications*, 14:573-577.
5. OLIVER, D.W., DORMEHL, I.C., REDELINGHUYS, F., HUGO, N. & PILLOY, W. 1993. Drug effects of cerebral blood flow in the baboon model. Acetazolamide and nimodipine. *Nuklear Medizin/Nuclear Medicine*, 32:292-298.
6. OLIVER, D.W., DORMEHL, I.C. & HUGO, N. 1994. The effect of sumatriptan on cerebral blood flow in the baboon model. *Arzneimittel Forschung/Drug Research*, 44: 925-928.

7. **DORMEHL, I.C., OLIVER, D.W., HUGO, N. & ROSSOUW, D.** 1995. A Comparative Cerebral Blood Flow Study in a Baboon Model with Acetazolamide Provocation: 99mTc-HMPAO vs 123I(IMP), *Nuclear medicine and biology*, 22(3):373-378.
8. **DORMEHL, I.C., OLIVER, D.W & HUGO, N.** 1995. Cerebral Blood Flow effects of Sumatriptan in Drug Combinations in the Baboon Model. *Arzneimittel Forschung/Drug Research*, 45 (9): 952-956.
9. **OLIVER, D.W., DORMEHL, I.C. & HUGO, N.** 1995. Sumatriptan in drug combinations in the cerebral blood flow baboon model. *Pharmacological Research*, First European Congress of Pharmacology, 16-19 June 1995, Milan, ITALY 31(suppl), 348.
10. **VD SCHYF, C.J., DORMEHL, I.C., OLIVER, D.W., HUGO, N., KEEVE, R., MULLER-GARTNER, H.-W., POND, S.M. & CASTAGNOLI, N JR.** 1996. Long-term treatment with the Tetrahydropyridine Analog (HPTP) of Haloperidol, on Dopamine influences Dopamine Ligand Binding in Baboon Brain. An 123I-Iodobenzamide (IBZM) SPECT Study. *Molecular brain research*, 43:251-258.
11. **BRAND, L., OLIVER, D.W., VD SCHYF, C.J., POND, S.M. & CASTAGNOLI, N JR.** 1996. Dopamine Receptor Binding of 4-(4-chlorophenyl)-1-[4-(4-fluorophenyl) 4-oxobutyl]- 1,2,3,6-tetrahydropyridine (HPTP), an intermediate metabolite of Haloperidol. *Life Sciences*, 59 (10):815-820.
12. **JORDAAN, B., OLIVER, D.W., DORMEHL, I.C. & HUGO, N.** 1996. Cerebral Blood Flow Effects of Piracetam, Pentifylline and Nicotinic in the Baboon Model compared with the known effect of Acetazolamide. *Arzneimittel Forschung/Drug Research*, 46(9): 844-847.
13. **VD SCHYF, C.J., DORMEHL, I.C., OLIVER, D.W., HUGO, N., KEEVE, R., MULLER-GARTNER, H.-W., POND, S.M. & CASTAGNOLI, N JR.** 1996. Long-term treatment with the Tetrahydropyridine Analog (HPTP) of Haloperidol, on Dopamine influences Dopamine Ligand Binding in Baboon Brain. An 123I-

- Iodobenzamide (IBZM) SPECT Study. *Molecular brain research*, 43:251-258.
14. **DORMEHL, I.C., OLIVER, D.W & HUGO, N.** 1997. The Primate Model in Neuropharmacology for Cerebral Blood Flow Determinations with HMPAO SPECT. *In: Neurospect in Neurology and Psychiatry*, eds P.P. de Deyn, R.A. Drieckx, A Alavi, B.A. Pickut, John Libbey and Company, Amsterdam, the Netherlands, Chapter 62, pp 521-535.
  15. **OLIVER, D.W., DORMEHL, I.C., VD SCHYF, C.J., HUGO, N., KEEVE, R., MULLER-GARTNER, H.-W. & CASTAGNOLI, N. JR.** 1997. Effect of the haloperidol tetrahydropyridine metabolite, 4-(4-chlorophenyl)-1-[4-(4-fluorophenyl) 4-oxobutyl]- 1,2,3,6-tetrahydropyridine on dopamine receptor and transporter binding. A nonhuman primate 123I-Iodobenzamide and 28-carbomethoxy-38(4-iodophenyl)tropane single photon emission computed tomographic study. *Arzneimittel Forschung/Drug Research*, 47(6): 692- 699.
  16. **DORMEHL, I.C., OLIVER, D.W., HUGO, N., LANGEN, K-J. & CROFT, S.** 1997. Technetium-99m-HMPAO, Technetium-99m-ECD and Iodine-123-IMP Cerebral Blood Flow Measurements with Pharmacological Interventions in Primates. *The journal nuclear of medicine*, 38 (12):1897 – 1901.
  17. **OLIVER, D.W. & DORMEHL, I.C.** 1998. Cerebral blood flow effects of Sodium Valproate and its drug combinations in the baboon model. *Arzneimittel Forschung/Drug Research*, 48(11): 1058-1063.
  18. **DORMEHL, I.C., JORDAAN, B., OLIVER, D.W & CROFT, S.** 1999. SPECT Cerebral blood flow improvement of elderly patients on long-term treatment with nootropic drugs. *Clinical nuclear medicine*, 24:29-34.
  19. **OLIVER, D.W. & DORMEHL, I.C.** 1999. Cerebral blood flow of the nitric oxide donor, nitroglycerin and its drug combinations in the non-human primate model. *Arzneimittel Forschung/Drug Research*, 49:732-739.

20. **DORMEHL, I.C., JORDAAN, B., OLIVER, D.W. & KILIAN, E.** 1999. SPECT Cerebral blood flow improvement of elderly patients on long-term treatment with nootropic drugs Proceedings Chapter, First International Congress on Vascular Dementia, Geneva, SWITZERLAND 3-6 October 1999, Editor AD Korczyn, Monduzzi Editore, Bologna, Italy September pp 119-122.
21. **OLIVER, D.W., DORMEHL, I.C., LOUW, W.K.A., MORETTI, J-L. & KILIAN, E.** 2000. Effect of Cyclodextrin Complexation on the *in vivo* Disposition of the Brain Imaging Radiopharmaceutical, <sup>99m</sup>Tc-ethyl Cysteinate Dimer (<sup>99m</sup>Tc-ECD). *Arzneimittel Forschung/Drug Research*, 50:758-764.
22. **OLIVER, D.W., DORMEHL, I.C., LOUW, W.K.A., MORETTI, J-L. & KILIAN, E.** 2000. Effect of cyclodextrin complexation of the *in vivo* disposition of <sup>99m</sup>Tc-ethyl Cysteinate. *European Journal of Nuclear Medicine, Proceedings abstract: European Association of Nuclear Medicine*, 27(8):1116.
23. **OLIVER, D.W., DORMEHL, I.C. & KILIAN, E.** 2000. Cerebral blood flow determinations in drug intervention studies in an *in vivo* primate SPECT model. *British Journal of Pharmacology, Proceedings abstract: VII World Conference on Clinical Pharmacology and Therapeutics IUPHAR, CPT2000*, July, Florence, Italy, page 185-186.
24. **CLAUSS, R.P., DORMEHL, I.C., OLIVER, D.W., NEL, H.W., KILIAN, E. & LOUW, W.K.A.** 2001. Measurement of cerebral perfusion after zolpidem administration in the baboon model. *Arzneimittel Forschung/Drug Research*, 51 (8):619-622.
25. **OLIVER, D.W., CLAUSS, R.P., DORMEHL, I.C., NEL, H.W., KILIAN, E. & LOUW, W.K.A.** 2002. Cerebral Perfusion changes measure with <sup>99m</sup>Tc-HMPAO SPECT after zolpidem and flumazenil intervention, Proceedings Chapter, 2<sup>nd</sup> International Congress on Vascular Dementia, ed AD Korczyn, Monduzzi Editori, 81-88.
26. **CLAUSS, R.P., DORMEHL, I.C., OLIVER, D.W., NEL, H.W., KILIAN, E. & LOUW, W.K.A.** 2002. Cerebral blood perfusion after

- treatment with Zolpidem and Flumazenil in the baboon. *Arzneimittel Forschung/ Drug Research*, 52:740-744.
27. **OLIVER, D.W., CLAUSS, R.P., DORMEHL, I.C., NEL, H.W., KILIAN, E. & LOUW, W.K.A.** 2002. Cerebral Perfusion changes measure with 99m-Techneium HMPAO SPECT after zolpidem and flumazenil intervention. *Journal of the Neurological Sciences, Proceedings abstract: 2<sup>nd</sup> International Congress on Vascular Dementia*, ed AD Korczyn, Monduzzi Editori, Vol 203-204, p317.
  28. **OLIVER, D.W & DORMEHL, I.C.** 2002. *In vivo* pharmacological models for drug interventions using non-human primates: The Cerebral Blood Flow Model. *The Pharmacologist. Proceedings abstract: The 15<sup>th</sup> World Congress of Pharmacology, The new century of Pharmacology, IUPHAR, 7-11 July 2002, San Francisco, USA Vol 44, No 2. Suppl. 1. pA47.*

## **2.2 Presentations and Guest lectures International**

1. **DORMEHL, I.C., REDELINGHUYS, F., OLIVER, D.W., HUGO, N. & PILLOY, W.** Anaesthesia and brain SPECT investigations using 99mTc-HMPAO in the baboon model. European Association of Nuclear Medicine. 22-27 August 1992, Lissabon, PORTUGAL.
2. **DORMEHL, I.C., REDELINGHUYS, F., OLIVER, D.W., HUGO, N. & PILLOY, W.** Anaesthesia and brain spect investigations using 99mTc-HMPAO in the baboon model. Polish Society of Nuclear Medicine. 21-23 September 1992, Cieszyn, POLAND
3. **DORMEHL, I.C., OLIVER, D.W., HUGO, N. & ROSSOUW, N.** A comparative cerebral flow study in a baboon model with acetazolamide provocation: 99mTc-HMPAO vs 123I(IPM). Nuklear Medizine Conference, August 1994, Dusseldorf, GERMANY
4. **LIPP, M., DORMEHL, I.C. & DAUBLÄNDER, M.** Influence of i.v. Articaine and Lidocaine on cerebral blood flow in a baboon model standardized under general anaesthesia using single photon

- tomography. Euroanaesthesia 94 European Academy of Anaesthesiology, Mannheim, Germany, March 1994. GERMANY
5. **LIPP, M., DORMEHL, I.C. & DAUBLÄNDER, M.** Influence of i.v. Articaine and Lidocaine on cardiovascular parameters and cerebral blood flow in a standardised baboon model. Influence of i.v. Articaine and Lidocaine on cardiovascular parameters and cerebral blood flow in a standardised baboon model. European Congress of Anaesthesiology, Jerusalem, October 5-9, 1994 ISRAEL.
  6. **OLIVER, D.W., DORMEHL, I.C. & HUGO, N.** Sumatriptan in drug combinations in the cerebral blood flow baboon model. , First European Congress of Pharmacology, 16-19 June 1995, Milan, ITALY.
  7. **DORMEHL, I.C., OLIVER, D.W. & HUGO, N.** Effect of sumatriptan on drug induced increased cerebral blood flow in the baboon mode. , NEUROSPECT, International Symposium on SPECT in Clinical Neurology and Psychiatry, 31 Aug-2 Sept 1995, Antwerp, BELGIUM
  8. **DORMEHL, I.C., OLIVER, D.W. & HUGO, N.** Cerebral Blood Flow Studies in the Baboon. The Split-Dose Method, Research Lecture: Department of Nuclear Medicine, 24-26 May 1995, University Hospital Michigan, Ann Arbor, USA.
  9. **JORDAAN, B., OLIVER, D.W., DORMEHL, I.C. & HUGO, N.** Effect of a combination of pentifylline and nicotinic acid vs piracetam on cerebral blood flow in the baboon model as compared with the known effect of acetazolamide., Hayman Island Neuropsychology Symposium, 30 June-3 July 1995, Melbourne, AUSTRALIA.
  10. **DORMEHL, I.C., OLIVER, D.W. & HUGO, N.** The potential of the primate model for cerebral blood flow determinations with SPECT. NEUROSPECT, International Symposium on SPECT in Clinical Neurology and Psychiatry, 31 Aug-2 Sept 1995, Antwerp, BELGIUM.

11. **DORMEHL, I.C., OLIVER, D.W., HUGO, N., LANGEN, K-J. & CROFT, S.** 99m-Tc-HMPAO and ECD compared for cerebral blood flow (CBF) measurements with various Pharmacological Interventions in the primate model. , European Annual Nuclear Medicine Conference, September 16-20, 1996, Copenhagen, DENMARK.
12. **OLIVER, D.W., DORMEHL, I.C., HUGO, N., LANGEN, K-J. & CROFT, S.** 99m-Tc-HMPAO and ECD compared for cerebral blood flow (CBF) measurements with various Pharmacological Interventions in the primate model. First International Conference on Pharmaceutical and Pharmacological Sciences (SA) ICPPS, September 22-25, 1996, Midrand, SOUTH AFRICA.
13. **BOY, A., KLIMKE, M., HOLSCHBACH, H., HERZOG, E., ROTA-KOPS, F., SONNENBERG, H., MÜHLENSIEPEN, W., GAEBEL, E., MARKSTEIN, P., SEEMAN, H., VAN TOL, DORMEHL, I.C., STÖCKLIN, I. & MÜLLER-GÄRTNER, H-W.** Darstellung cerebraler dopamin- D<sub>4</sub>-ähnlicher bindungs-stellen bei primaten mit dem pet-liganden [<sup>11</sup>C] SDZ GLC C. 756. Deutsche Gesellschaft für Nuklearmedizin e.v. Jahrestagung, 17-20 April 1996, Münster, Westfalen, GERMANY.
14. **OLIVER, D.W., DORMEHL, I.C., VD SCHYF, C.J., HUGO, N., KEEVE, R., MULLER-GARTNER, H-W. & CASTAGNOLI, N. JR.** A nonhuman primate study on the effects of haloperidol tetrahydropyridine metabolite, HPTP on the dopamine receptor and transporter binding. 5th International Symposium on Pharmaceutical Sciences (ISOPS-5), June 24-27, 1997, Ankara, TURKEY.
15. **OLIVER, D.W.** Cerebral Blood Flow Studies and Drugs Interventions. The Baboon Model. Visiting Professor, School of Pharmacy and Drug Design and Development Centre, University of Queensland, Brisbane, St Lucia, Proff Sue Tett and Peter Andrews, 3 September 1998, Queensland, AUSTRALIA.
16. **OLIVER, D.W. & DORMEHL, I.C.** Effect of the Nitric Oxide Donor, Nitroglycerin on Cerebral Blood Flow in the Baboon. 2ND

- EPHAR (European Congress of Pharmacology, Drugs against disease to improve quality of life: gateway to the 21st Century through EPHAR Symposia, Budapest, 3-7 July 1999 HUNGARY.
17. **DORMEHL, I.C., JORDAAN, B., OLIVER, D.W. & KILIAN, E.** SPECT Cerebral blood flow improvement of elderly patients on long-term treatment with nootropic drugs. First International Congress on Vascular Dementia, Geneva, 3-6 October 1999, SWITZERLAND.
  18. **OLIVER, D.W., DORMEHL, I.C. & KILIAN, E** Cerebral Blood Flow Drug Intervention Studies. In Vivo non-human Primate model. VII World Conference on Clinical Pharmacology and Therapeutics July 2000, Florence, ITALY.
  19. **OLIVER, D.W. & DORMEHL, I.C.** Effect of cyclodextrin complexation on the in vivo disposition of <sup>99m</sup>Techneium ethyl cysteinate European Association of Nuclear Medicine Congress September 2000 Paris FRANCE.
  20. **OLIVER, D.W. & DORMEHL, I.C.** In Vivo models for drug Studies using non-human primates. The cerebral blood flow model. International Symposium on Current Trends in Drug Research (CTDDR 2001), 11-15 February 2001, Lucknow, INDIA.
  21. **OLIVER, D.W. & DORMEHL, I.C.** The Effect of Sodium Valproate on the Cerebral Blood Flow in the Non-human Primate Baboon Model, 3rd EPHAR Federation of the European Pharmacological Societies Congress, 6-9 July 2001 Lyon, FRANCE.
  22. **OLIVER, D.W., CLAUSS, R.P., DORMEHL, I.C., NEL, H.S.W., KILIAN, E. & LOUW, W.K.A.** Cerebral perfusion changes measured with <sup>99m</sup>- Technetium HMPAO SPECT after Zolpidem and Flumazenil intervention, 2nd International congress on vascular Dementia.. Vascular Dementia, 24-29 January 2002, Salzburg, AUSTRIA.
  23. **OLIVER, D.W. & DORMEHL, I.C.** In Vivo pharmacological models for drug interventions using non-human primates: The Cerebral Blood Flow Model. The 15th World Congress of

Pharmacology, The new century of Pharmacology, IUPHAR, 7-11 July 2002, San Francisco, USA.

24. **OLIVER, D.W.** Non-Human Primate models in Medicine. Measuring Cerebral Blood Flow Changes, IBILI, University of Coimbra, Guest Prof Pedroso de Lima, October 2002, PORTUGAL.
25. **OLIVER, D.W. & DORMEHL, I.C.** Non-human Primate study of PR9. Cerebral Blood Flow and Cardiovascular Effects. University of Uppsala, Guest of Prof Jarl Wikberg, Department Pharmacology, October 2002 Uppsala, SWEDEN.
26. **OLIVER, D.W. & DORMEHL, I.C.** Non-human Primate study of PR9. Cerebral Blood Flow and Cardiovascular Effects. Melacure Pharmaceutical Company, October 2002 Uppsala, SWEDEN.
27. **OLIVER, D.W., DORMEHL, I.C.** Non-human primate SPECT model for determining cerebral perfusion effects and drug interactions of cerebrovasoactive drugs acting via multiple modes of pharmacological action, 3<sup>rd</sup> International Congress on Vascular Dementia, 23-26 October 2003, Prague, CZECH REPUBLIC.

## Reprints

## Appendix

## 3

Short Papers

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## The baboon model under anesthesia for in vivo cerebral blood flow studies using single photon emission computed tomographic (SPECT) techniques

Dormehl I, Redelinghuys F, Hugo N, Oliver D, Pillay W. The baboon model under anesthesia for in vivo cerebral blood flow studies using single photon emission computed tomographic (SPECT) techniques. *J Med Primatol* 1992;21:270-274.

Single photon emission computed tomography of the brain can be useful in animal experimentation directed toward cerebral conditions. A well established and understood baboon model, necessarily under anesthesia, could be especially valuable in such investigations. Six normal baboons were studied under various anesthetic agents and their combinations: ketamine, thiopentone, pentobarbitone, and halothane. Cerebral blood flow (CBF) studies were performed with  $^{99m}\text{Tc}$ -HMPAO. CBF effects from various anesthesia were detected, requiring careful choice of the anesthesia for cerebral investigations.

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Key words: primate model - CBF - SPECT imaging.

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### Introduction

Single photon emission computed tomographic (SPECT) imaging of the brain to establish cerebral blood flow (CBF) patterns could provide valuable adjunctive information in those neurological diseases where blood flow imaging has diagnostic and prognostic value [3,7]. Patients with ischemic brain lesions, dementia, psychiatric disorders, or other neurological signs or symptoms may be followed up with SPECT for disease progression and for monitoring the efficiency of pharmacological interventions [10]. Additionally, results of cerebral blood flow studies and metabolic studies can be matched [4].

The radiopharmaceutical hexamethylpropylene amine oxime ( $^{99m}\text{Tc}$ -HMPAO) is taken up rapidly in the brain tissue. It exhibits prolonged retention in the brain because of intracellular conversion to a hydrophilic compound that diffuses poorly across cell membranes [8,9]. It has been validated as a marker of regional CBF (rCBF) although it is not linearly so dependent, and provides high resolution static imaging of brain perfusion [1,6,13]. The pattern of its distribution is representative of the blood flow conditions during its injection, with no redistribution taking place.

The purpose of this study was the standardization

of a baboon model for in vivo CBF studies using SPECT and  $^{99m}\text{Tc}$ -HMPAO. The baboon model necessitates the use of anesthesia for the duration of the investigation. First and foremost then would be to establish the influence of various forms of anesthesia on CBF patterns, bearing in mind the essential procedure of initial darting with ketamine hydrochloride and subsequent maintenance of the animal on long- or short-acting anesthesia depending on the duration of the study. Ketamine, producing so-called dissociative anesthesia, does not serve the last purpose well because of ensuing hallucinations, which result in undesirable involuntary movement. An established baboon model with the effects of anesthesia determined and understood can be used to assess surgical and pharmacological interventions by SPECT imaging.

### Materials and methods

Six adult male baboons (*Papio ursinus*, average weight 27 kg) were selected for this study. Anesthesia was induced in each by darting with ketamine hydrochloride (Ketalar, Parke-Davis, S.A.; 10 mg/kg) and was followed immediately by an intravenous injection of  $^{99m}\text{Tc}$ -HMPAO (148 MBq), the

## Baboon model for cerebral blood flow of SPECT

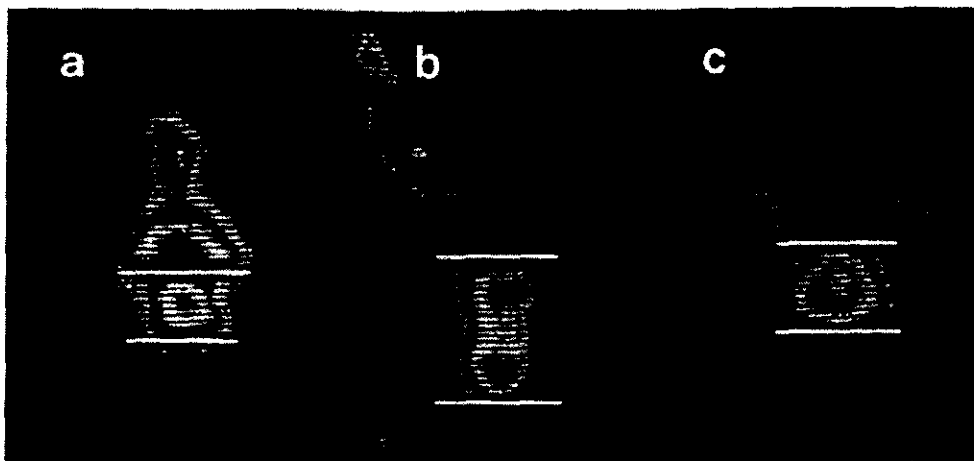


Fig. 1. Typical tomographic brain slices in the coronal (a), sagittal (b), and transaxial (c) view, indicating the position of the regions of interest (ROI), i.e., the total brain between solid lines.

distribution of which would then represent the effect of ketamine. Five minutes later the baboon was intubated, maintained, and controlled for the duration of the study under a second anesthetic agent, which was alternatively thiopentone sodium (Intraval, Maybaker, S.A.), pentobarbitone sodium (Sagatal, Maybaker, S.A.), or halothane (Fluothane, Maybaker, S.A.).

The subsequent SPECT acquisition to obtain the HMPAO distribution in the brain during ketamine was done with a Siemens Orbiter gamma camera coupled to an A<sup>3</sup> MDS computer, using 32 views and 360° (10 sec/view). Following the first acquisition, the baboon was reinjected with <sup>99m</sup>Tc-HMPAO (296 MBq) and tomographed to detect the radionuclide distribution during the anesthesia of the second agent. Care was taken to administer the two HMPAO injections at its latest within 30 minutes after reconstitution with <sup>99m</sup>Tc. Baboons were viewed in a supine position with a special head rest to ensure a reproducible position for comparable tomographic slices.

Tomographic procedures took place for all six baboons, with the following combinations of anesthesia. A) thiopentone-thiopentone, B) ketamine-thiopentone, C) ketamine-pentobarbitone, and D) ketamine-halothane, with thiopentone (in A) and ketamine (in B, C, and D) being designed as baseline studies and thiopentone (in A and B), pentobarbitone (C) and halothane (D) as interventions. In the case of thiopentone-thiopentone the anesthesia was first induced by darting with ketamine and was then immediately followed by, and maintained by a controlled i.v. infusion of thiopentone (70 ml/hr of a 0.5% solution) using an administration (drip) set. After 30 minutes when the thiopentone blood

levels predominated, <sup>99m</sup>Tc-HMPAO was injected to obtain, through SPECT, a distribution in the brain under the influence of thiopentone. The baboon remained on thiopentone anesthesia for the second HMPAO administration and subsequent tomography. This study (A) formed a baseline reference to evaluate the effect of ketamine as would occur in procedures B, C, and D.

For procedure B thiopentone was maintained as described above. The pentobarbitone was maintained during procedure C with an infusion pump (30 ml/hr of a 9 mg/ml solution). For the halothane procedure (D) 2% halothane/oxygen (Boyle's machine) was used for maintenance of anesthesia.

During all procedures the blood pressures (BP), heart rates (HR) and blood gases were monitored.

After backprojection and reconstruction the brain images consisted of transaxial, sagittal, and coronal slices representing CBF information during conditions prevailing under the various forms of anesthesia. Sixteen transaxial slices represented the whole brain, of which every second slice was considered for count rate evaluation by the region of interest (ROI) feature. Similarly six sagittal slices and five coronal slices were selected to cover all of the brain. In subsequently placing the respective ROIs (Fig. 1, a,b,c) for count/pixel values of the brain slices care had to be taken to avoid the baboon sinus cavities and salivary glands. Count rate data were then inserted into the following equation to obtain the ratio R

$$R = \frac{\left( \begin{array}{c} \text{Intervention} \\ \text{2nd anesthesia} \end{array} \right) - \left( \begin{array}{c} \text{Baseline} \\ \text{1st anesthesia} \end{array} \right)}{\text{Baseline (1st anesthesia)}}$$

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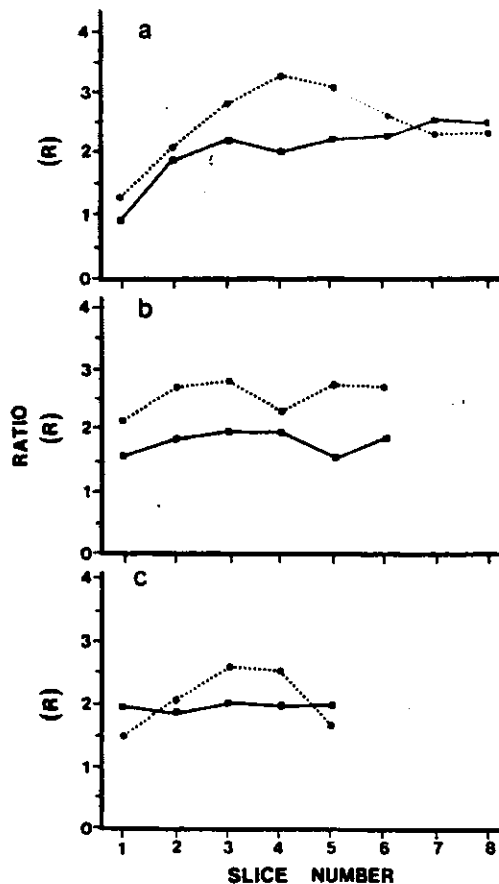


Fig. 2. A set of typical curves of ratio (R) versus slice number starting at the occipital lobes to the frontal lobes transaxially (a), from right to left of the brain sagittally (b), and from the cerebellum to the dorsal slice of the cerebrum coronally (c). Dotted lines represent ketalar-halothane data; solid lines ketalar-intraval data.

which is an indication of the level change of the rCBF during the second anesthesia ("intervention") with respect to that during the first anesthesia ("baseline") allowing for subtraction of retained activity from the first anesthesia. For all three views graphs were plotted of R versus slice numbers starting at the occipital to frontal lobes transaxially, from right to left sagittally, and from the cerebellum to the dorsal slice of the cerebrum coronally (see Fig. 2, a,b,c). The coronal slices were not aligned to the axis of the brain (Fig. 3), and they largely resemble transaxial slices of the human brain. The brain was then divided into four equal, not anatomically specific, regions or segments using the curves described in Figure 2 (divided into four segments along the abscissa) as guidelines. Relating to these representative regional blood flow values were obtained. Radioactive decay corrections were made throughout.

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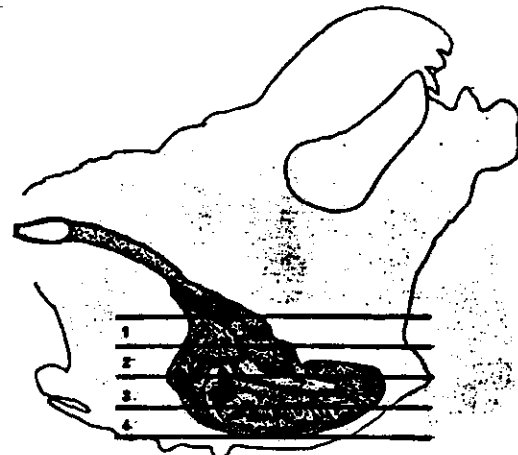


Fig. 3. The position of the four coronal slices of the baboon brain, in this study not aligned to brain axis, and resembling transaxial slices of the human brain.

#### Statistical methods

Mean ratios and standard deviations (SD) were evaluated for similar regions and for the total brain in the various projections as obtained from the baboons for the different procedures, and these were compared for procedural as well as regional effects. The comparisons were assessed for significant differences using Student's two-tailed t-test for paired observations.

#### Results

Tables I, II, and III present the mean ( $n = 6$ ) ratios (R) and SD obtained from the four brain regions as from Figure 2, and the total brain viewed respectively transaxially, sagittally, and coronally under the various conditions of anesthesia.

The total brain ratios for the anesthesia procedures A, B, and C to a large degree tend to approach, but not reach, the value 2, which would correspond with the second double dosage of  $^{99m}\text{Tc}$ -HMPAO and also agree to small CBF changes due to intraprocedural anesthesia variations. For these three procedures there are, furthermore, no statistically significant differences between the intraprocedural regional ratios ( $P > 0.05$ ), thus indicating no influence from A, B, and C on rCBF.

The values from ketamine-thiopentone and ketamine-pentobarbitone are significantly similar ( $P > 0.05$ ) as is to be expected since only the pharmacokinetic properties of thiopentone and pentobarbitone differ, with the latter the long acting barbiturate.

Procedure A ratios tend to be larger regionally and also for the total brain than those from proce-

## Dose response from pharmacological interventions for CBF changes in a baboon model using $^{99}\text{Tc}^{\text{m}}$ -HMPAO and SPECT

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### Summary

This study assesses the sensitivity of the baboon model under anaesthesia to determine by single photon emission computed tomography (SPECT) and  $^{99}\text{Tc}^{\text{m}}$ -hexamethylpropyleneamine oxime (HMPAO) dose responses from drugs (acetazolamide) with known regional cerebral blood flow (rCBF) effects on humans. Three dosages of acetazolamide were chosen: 250, 500 and 750 mg. The effects of these were studied by conventional SPECT 5 min after intravenous (i.v.) administration and compared to previous studies of rCBF with the baboons under anaesthesia only. An additional study concerned the effect of 500 mg acetazolamide at 15 min after administration. Haemodynamic parameters and blood gases were also monitored. No statistically significant regional effects were noted ( $P > 0.05$ ). The largest increase in CBF (39%) was observed from 500 mg acetazolamide after 5 min. This was statistically significantly different from control values only at a 10% level of confidence; then followed a 27% increase above control values after 750 mg (5 min). At 15 min 500 mg yielded values lower by 10% than the high dose. No effects were observed from 250 mg acetazolamide; only  $p\text{O}_2$  showed changes which largely confirm the CBF findings. The model did not give significant results at a 5% level of confidence but large fluctuations were observed, also in the haemodynamic and blood gas values. At a 10% level a significant dose response was confirmed for acetazolamide.

### Introduction

A baboon (*Papio ursinus*) model has been developed to assess *in vivo* the effect of drugs on cerebral blood flow (CBF) and to an extent on regional CBF (rCBF) using  $^{99}\text{Tc}^{\text{m}}$ -hexamethylpropyleneamine oxime (HMPAO). This model [1], of necessity under anaesthesia, confirmed the influence of anaesthetic drugs on CBF, and prompted the use of anaesthesia by induction with ketamine hydrochloride and subsequent maintenance on a long- or short-acting barbiturate in a prerequisite control study for each experimental brain blood flow investigation. The success of the model to detect, over and above the anaesthesia, the effects of drugs with known CBF influences in humans, (acetazolamide, nimodipine) has likewise been established [2]. For pharmacological investigations the sensitivity of the

model for allowing dose response determinations becomes important. This study therefore concerns the efficacy of the model for yielding a sensitive dose response to the administration of acetazolamide (Diamox, S.A. Cyanamid (Pty) Ltd), a drug chosen for its present day wide application as a provocative intervention in nuclear medicine to facilitate diagnosis of stroke in patients and for its therapeutic use in patients with glaucoma and to a limited extent epilepsy [3]. In cases of glaucoma acetazolamide inhibits the carbonic anhydrase to reduce the intraocular pressure [4]. Three dosages of acetazolamide were chosen for the investigation: 250, 500 and 750 mg. The effects of these were studied 5 min after intravenous (i.v.) administration. An additional study concerned the effect of 500 mg acetazolamide at a time interval of 15 min after administration [5].

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#### Materials and methods

Six adult male baboons (average weight 27 kg) were used for the investigation. Each animal was subjected to five different procedures (A to E) with at least a 6-week interval between the consecutive procedures. Procedure A was the control study under anaesthesia only [1] in which induction was with ketamine hydrochloride (10 mg kg<sup>-1</sup> i.v.) (Ketalar, Parke Davis, Cape Town) followed immediately by an i.v. injection of 148 MBq <sup>99</sup>Tc<sup>m</sup>-HMPAO. Five minutes later the animal was put on an infusion of thiopentone sodium (70 ml h<sup>-1</sup> of a 0.05% solution) (Intraval, Sandos S.A., Randburg) with the first single photon emission computed tomography (SPECT) acquisition (SPECT-1) following after five more minutes. The data of SPECT-1 represent the HMPAO distribution (uptake and retention) in the brain resulting from CBF during ketamine sedation [6, 7]. This acquisition, using a Siemens Orbiter gamma camera with 32 projections during a 360° rotation and allowing 10 s per view, was immediately followed by a second i.v. administration of <sup>99</sup>Tc<sup>m</sup>-HMPAO (296 MBq, i.e. double the first dosage), the split-dose method [8, 9], and 5 min later by the second SPECT acquisition (SPECT-2) as before. These data represent a CBF pattern of HMPAO under thiopentone anaesthesia, but with a background from the first HMPAO distribution under ketamine.

Procedures B to D followed the same protocol until the completion of the first SPECT acquisition (SPECT-1), but this was immediately followed by an i.v. injection of acetazolamide. For procedure B, 250 mg acetazolamide was administered, for procedures C and D, 500 and 750 mg, respectively. The protocol was continued for all three procedures 5 min later by a second HMPAO injection (296 MBq), to be followed after another 5 min

by SPECT-2, of which the acquired data now also represent the effect on CBF patterns of the various doses of acetazolamide at a time 5 min after their administration [6-9]. Procedure E differed from the above acetazolamide procedures in that the second HMPAO injection (296 MBq) was administered 15 min after the acetazolamide injection (500 mg). The SPECT-2 data here represent the CBF pattern of HMPAO 15 min after the administration of 500 mg acetazolamide.

The split-dose method is based on the chemical properties of the tracer that crosses the blood-brain barrier and is trapped in brain cells. Radiochemical purity of HMPAO was consequently checked before its first application (within 5 min of preparation with a fresh eluate) for each procedure, and the lipophilic complex was never found to be below 90%. The second injection of HMPAO following the first by 15 to 30 min (depending on the procedure) was accompanied on a count down by a dynamic data acquisition (15 s per image for 4 min). The shape of the time-activity curve reflected the reduction of lipophilic <sup>99</sup>Tc<sup>m</sup>-HMPAO, and was a determining factor for proceeding with SPECT-2.

Reproducible positioning of the animal for SPECT procedures allowing eventual comparisons was ensured by a special headrest with the baboon in the supine position.

Arterial blood pressure (BP), heart rates (HR) and blood gases (pCO<sub>2</sub> and pO<sub>2</sub>) were also measured during each procedure (i.e. immediately before the administration of the acetazolamide and then again 5 min thereafter for procedures B, C and D, and after 15 min for E).

Following backprojection and reconstruction the brain images consisted of two sets each of the following compacted slices, eight transaxial slices, six sagittal slices and five coronal slices, the two sets representing

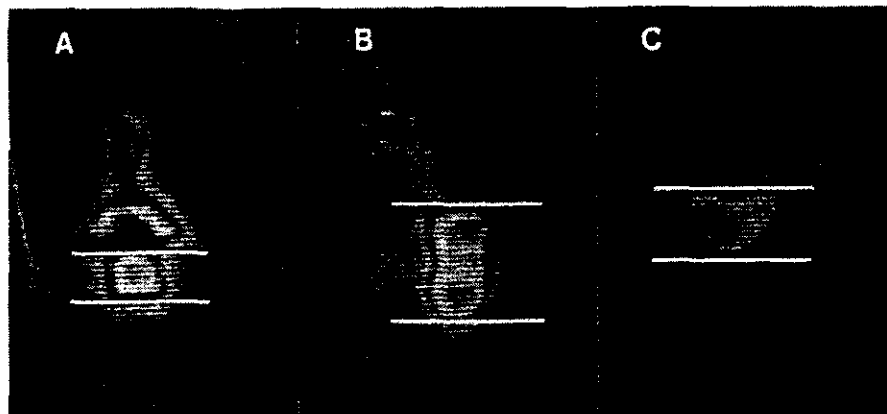


Fig. 1. Typical tomographic brain slices in the (A) coronal, (B) sagittal and (C) transaxial views indicating the position of the regions of interest, i.e. the total brain between solid lines.

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rCBF patterns, respectively, from SPECT-1 (ketamine related) and SPECT-2 (related to thiopentone without or with the various dosages of acetazolamide). Regions of interest (ROIs) were placed on the brain slices from SPECT-1 and SPECT-2 data, in each view, for all the procedures, and counts per pixel obtained from each slice (Fig. 1). These values for each slice were inserted into the following equation.

$$R = \frac{[\text{SPECT-2}] (\text{counts pixel}^{-1}) - [\text{SPECT-1}]^* (\text{counts per pixel}^{-1})}{\text{SPECT-1} (\text{counts pixel}^{-1})} \quad (1)$$

Where  $R$  represents the level of change of rCBF during

the second anaesthesia, thiopentone (without or with acetazolamide), with respect to that during ketamine (the first anaesthesia) after subtraction of the background (indicated by \*), having corrected for decay.

Not much anatomical structure was noted from the various slices. Further compacting of the slices led to their consolidation into four equal brain regions, 1 to 4, in each view, and to subsequent  $R$  values relating to the regions. From the individual baboon values mean regional and total brain ratios with standard deviations (S.D.) were evaluated in each view for each of the procedures A to E, and compared for possible regional as well as procedural effects (Fig. 2). The comparisons were assessed for significant differences using Student's two-tailed  $t$ -test at 5 and 10% levels of confidence.

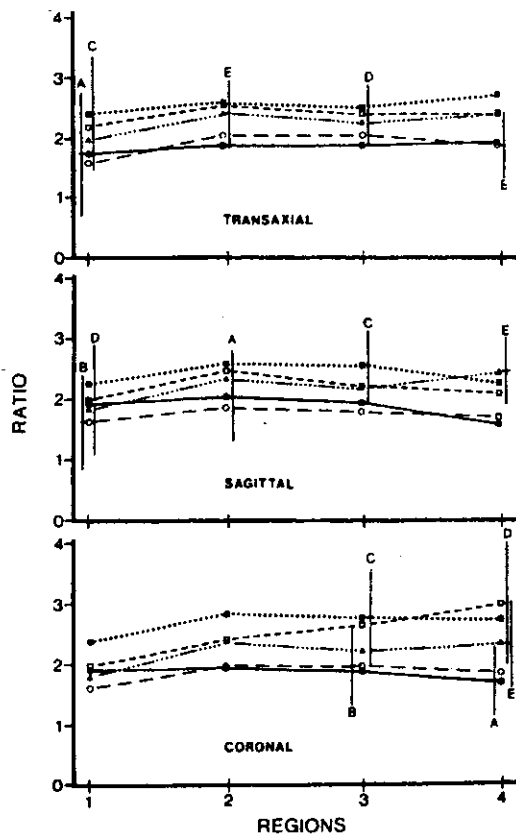


Fig. 2. Curves of ratio ( $R$ ) versus region number starting at frontal lobes to the occipital lobes transaxially, from right to left of the brain sagittally, and from the cerebellum to the dorsal slice of the cerebrum coronally. ●—● procedure A; ○—○ procedure B; ■—■ procedure C; □—□ procedure D; ▲—▲ procedure E. The largest standard deviations for each procedure are correspondingly indicated.

## Results and discussion

The results are summarized in Tables 1–3 and in Fig. 2. Table 1 presents mean regional and total brain ratios,  $R \pm$  S.D. in each view for all the procedures. In Table 2 the mean percentage increases ( $\pm$  S.D.) in  $R$  values are given between the various procedures, indicating percentage CBF changes because of the drug interventions.

No statistically significant regional effects were noted in any of the procedures ( $P > 0.05$ ). This is well illustrated in Fig. 2 where none of the curves showed any notable shape change.

Values of two (i.e. 2) for  $R$  would indicate no difference in rCBF observed between SPECT-1 and background corrected SPECT-2 data, assuming that CBF is the largely dominant factor in the HMPAO cerebral distribution [6, 9, 10].

The average total brain values of  $R$  approximating 1.9 in all views for the procedure A control study indicate possibly only a slight increase in CBF because of the ketamine hydrochloride [1]. This does not change with the low dose of acetazolamide (procedure B, Fig. 2) confirming no statistically significant effect ( $P > 0.10$ ) on CBF from such a low dose measured 5 min after its injection (Table 2).

Increased CBF as indicated by values of  $R$  larger than 2 was observed from procedure C (500 mg acetazolamide at 5 min postinjection; Table 1, Fig. 2). The average percentage increase measured from the total brain in all views was  $39 \pm 7\%$  with respect to the control study values. This result is in accordance with our previous observations [2]. Although substantial, this increase in CBF was only statistically significant ( $P < 0.05$ ) in the coronal view. At a 10% level of confidence, however, all three views transaxial, sagittal and coronal, had  $R$  values for procedure C significantly higher than the control values. This observation is also true for comparisons between effects of procedure C with the low dose

Table 1. Mean ( $n = 6$ ) regional and total brain ratios and standard deviations ( $R \pm$  s.d.) obtained in the three tomographic views from the various procedures.

Procedure		Transaxial regions					Sagittal regions					Coronal regions				
		1	2	3	4	Total brain	1	2	3	4	Total brain	1	2	3	4	Total brain
A	R	1.73	1.88	1.86	1.94	1.85	1.84	2.10	1.97	1.63	1.88	1.91	1.93	1.92	1.73	1.87
	s.d.	0.87	0.19	0.31	0.36	0.36	0.29	0.80	0.39	0.51	0.41	0.32	0.23	0.28	0.59	0.30
B	R	1.52	2.04	2.03	1.94	1.87	1.58	1.89	1.82	1.72	1.80	1.61	1.94	1.98	1.80	1.88
	s.d.	0.28	0.54	0.41	0.53	0.33	0.72	0.62	0.55	0.58	0.46	0.58	0.46	0.67	0.29	0.41
C	R	2.37	2.58	2.50	2.71	2.57	2.23	2.58	2.56	2.23	2.40	2.23	2.80	2.75	2.66	2.63
	s.d.	0.94	0.72	0.58	0.61	0.66	0.57	0.57	0.64	0.53	0.49	0.52	0.83	0.84	0.34	0.51
D	R	2.09	2.59	2.41	2.42	2.37	1.99	2.45	2.24	2.12	2.30	1.93	2.36	2.67	2.90	2.46
	s.d.	0.51	0.39	0.51	0.44	0.40	0.92	0.74	0.74	0.64	0.59	0.72	0.50	0.66	0.99	0.59
E	R	1.93	2.36	2.26	2.43	2.24	1.90	2.29	2.13	1.70	2.00	1.80	2.41	2.20	2.29	2.21
	s.d.	0.46	0.60	0.48	0.50	0.45	0.33	0.60	0.56	0.67	0.33	0.56	0.56	0.66	0.77	0.51

Table 2. Mean percentage cerebral blood flow (CBF) (total brain) changes and (s.d.) due to the various procedures.

With respect to baseline		Dose-dependent effects		Time and dose-dependent effects	
Procedures	%	Procedures	%	Procedures	%
A to B	2.0±1.7	B to C	37.0±1.5	B to E	20.0±3.0
A to C	39.0±7.0	B to D	27.6±2.0	C to E	-13.0±3.2
A to D	27.0±4.5	C to D	-8.6±1.5	E to D	10.3±5.0
A to E	18.3±10.7				

Percentage change expressed with respect to the procedure mentioned first.

procedure B, where the average increase because of the 500 mg acetazolamide amounts to 37% (Table 2).

High  $R$  values were also observed for procedure D (750 mg acetazolamide at 5 min; Table 1, Fig. 2). These were likewise elevated with respect to the control values pointing to an average CBF increase of  $27 \pm 4.5\%$ , i.e. not to the same extent as caused by procedure C. The CBF changes induced by procedure D do not differ statistically significantly from either control values, procedure B values or procedure C values ( $P > 0.05$ ). However, at a 10% level of confidence the increase of 27% in the CBF above the control values as obtained from procedure D was statistically significant, as was the difference with regard to the low dose values.

Although the effects from 500 mg acetazolamide at 15 min postinjection (procedure E) were lower by 13%

Table 3. Mean ( $n = 6$ ) haemodynamic and blood gas values ( $\pm$ s.d.) obtained from the various procedures respectively before (1) drug administration and as close to 5 min (or to 15 min for procedure E) after administration (2).

	Heart rate	Blood pressure	$p\text{CO}_2$	$p\text{O}_2$
A1	106±10	112±42	46.73±2.31	69.50±2.33 ( $n=3$ )
A2	107±12	118±39	45.17±2.33	75.93±6.77
B1	121±23	114±31	45.83±4.63	70.07±8.50
B2	122±27	122±38	44.67±5.15	76.41±11.08
C1	117±13	117±4	41.60±2.07	56.35±4.91 ( $n=4$ )
C2	112±17	110±5	40.08±4.87	76.43±7.47
D1	117±21	131±32	45.47±3.85	67.82±4.24
D2	116±24	133±39	47.95±2.05	70.75±9.86
E1	118±10	111±27	41.23±6.70	66.83±6.65
E2	114±20	110±15	42.00±4.50	82.37±3.29

Each value indicates the mean  $\pm$  s.d. of six experiments unless where indicated in parentheses.

than those from procedure C, i.e. when measured at 5 min, the changes were not statistically significant ( $P > 0.10$ ). These effects were also not statistically significantly different from control values (+18%;  $P > 0.10$ ), and lower than procedure D values (10%) from the high dose of acetazolamide (750 mg) 5 min postinjection.

The effect of acetazolamide used during the different procedures on the heart rate, blood pressure,  $p\text{CO}_2$  and

## Dose responses from drug interventions on CBF measured by HMPAO-SPECT

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$pO_2$  in the arterial line are summarized in Table 3. The heart rate and  $pCO_2$  values remained constant for all the procedures. Previously it was observed that acetazolamide initially induced a decrease in blood pressure followed by a subsequent increase after 25 min of acetazolamide administration [2]. In the present study no significant changes were observed for procedure E either (the late, 15 min, study). Acetazolamide increased the arterial  $pO_2$  parameter. This increase was, however, only statistically significant for procedure C (500 mg, 15 min) ( $P < 0.05$ ) and procedure E (500 mg, 5 min) ( $P < 0.05$ ). At a time 5 min after administration no significant differences in the  $pO_2$  were observed for procedures B and D. These results support previous suggestions that acetazolamide may have an effect on local cerebral oxygen metabolic rate [11, 12]. The  $pO_2$  effects are also dose dependent when the effects of the different dosages are compared. It was previously [5] reported that the maximum increase in CBF of humans was achieved 25 min after acetazolamide administration. However, the present study in the baboon indicates a maximum increase in CBF after 5 min, although the effect on the  $pO_2$  was found to last much longer. This further strengthens the suggestion that the vasodilatory properties of acetazolamide are unrelated to the inhibition of carbonic anhydrase [11, 12].

## Conclusion

In the current study the baboon model previously described by us was evaluated for time-dose effect sensitivity on CBF using the drug acetazolamide. Although acetazolamide, an acidic drug, binds strongly to plasma albumin (85–95%), no measurable displacement of any albumin bound HMPAO is foreseen, so as to change the availability of the lipophilic  $^{99m}Tc$ -HMPAO [5]. Acetazolamide is further tightly bound to carbonic anhydrase. Cells that are rich in carbonic anhydrase such as the erythrocytes and glial cells contain a higher concentration of acetazolamide than the plasma. Any possible acetazolamide-induced changes in blood cell labelling can only lead to small changes in blood radioactivity in the brain [13] since the volume of blood in the brain is small with respect to total blood volume. Furthermore, no changes in blood-brain barrier permeability and cardiac output have been reported for acetazolamide that could account for the observed increase of HMPAO level in the cerebrovascular system. The study is therefore describing a model sensitive to monitoring dose effect interventions of CBF. In particular it was noted for acetazolamide that a low dosage (250 mg) showed no effect while the high dosage (750 mg) induced changes in the CBF lower than those

observed for a 500 mg dosage at 5 min after administration. The model also confirmed that this last procedure has an optimal effect since 15 min postinjection the effect from 500 mg acetazolamide was again reduced.

## References

1. Dormehl IC, Redelinguys IF, Hugo N, Olivier D, Pilloy W. The baboon model under anaesthesia for *in vivo* cerebral blood flow studies using SPECT techniques. *J Med Prim* 1992; 21: 270–4.
2. Oliver DW, Dormehl IC, Redelinguys IF, Hugo N, Beverley G. Drug effects on cerebral blood flow in the baboon model. Acetazolamide and Nimodipine. *Am J Phys* in press.
3. Goodman LS, Gilman AG. The pharmacological basis of therapeutics, 8th edition. In: Gilman AG, Rall TW, Nies AS, Taylor P, eds. New York: Pergamon, 1990: 716–17.
4. Mithoefer J, Mayer P, Stocks J. Effects of carbonic anhydrase inhibition of the cerebral circulation of the anesthetized dog. *Ired Proc* 1957; 16: 88–9.
5. Bonte FJ, Devons MD, Reisch JS. The effect of acetazolamide on regional cerebral blood flow in normal human subjects as measured by single photon emission computed tomography. *Invest Radiol* 1988; 23: 564–8.
6. Bacciottini L, Pupi A, Formiconi AR, De Cristofaro MTR, Meldolesi U. Regional cerebral blood flow measurement in rat with HM-PAO and the indicator fractionation technique. *J Cereb Blood Flow Metab* 1989; 9: S414.
7. Pupi A, Bisi G, Siagra R, Santoro D, De Cristofaro MTR, Formiconi AR, Meldolesi U. The comparison of brain distribution of HM-PAO and microspheres in humans. *J Cereb Blood Flow Metab* 1989; 9: S411.
8. Wyper DJ, Hunter R, Patterson J, Goodwin G, McCulloch J. A split-dose technique for measuring changes in cerebral blood flow patterns. *J Cereb Blood Flow Metab* 1991; 11 (Suppl 2): S449.
9. Holm S, Madsen PL, Rubin P, Sperling B, Friberg L, Lassen N. Tc-99m HM-PAO activation studies: validation of the split-dose, image subtraction approach. *J Cereb Blood Flow Metab* 1991; 11 (Suppl 2): S766.
10. Pantano P, Di Piero V, Ricci M, Fieschi C, Bozzao L, Lenzi GL. Motor stimulation response by technetium-99m HMPAO split-dose method and single photon emission tomography. *Eur J Nucl Med* 1992; 19: 939–45.
11. Vorstrup S, Henriksen L, Paulson OB. Effect of acetazolamide on cerebral blood flow and cerebral metabolic rate for oxygen. *J Clin Invest* 1984; 74: 1634–9.
12. Laux BE, Raichle ME. The effect of acetazolamide on cerebral blood flow and oxygen utilization in the rhesus monkey. *J Clin Invest* 1978; 62: 585–92.
13. Neirinckx RD, Canning LR, Piper IM, Nowotnik DP, Pickett RD, Holmes RA, Volkert WA, Forster AM, Weisner PS, Marriott JA, Chaplin SB. Technetium-99m d,l-HM-PAO: a new radiopharmaceutical for SPECT imaging of regional cerebral blood perfusion. *J Nucl Med* 1987; 28: 191–202.

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## Drug Effects on Cerebral Blood Flow in the Baboon Model – Acetazolamide and Nimodipine

Single photon emission computed tomography (SPECT) has fully established itself as a useful technique for brain perfusion imaging (21). <sup>99m</sup>Tc-labelled radiopharmaceuticals are experiencing an ever increasing role in the non-invasive diagnosis of cerebral diseases (cerebral ischemia, dementia, epilepsy) and find application for subsequent monitoring of pharmacological interventions (18, 21).

The design and development of new pharmaceuticals that cross the blood brain barrier and show cerebral vascular smooth muscle and regional selectivity still pose a challenge. Animal models have been useful in the evaluation of promising novel structures for efficacy and toxicological information, particularly those that act on the central nervous system influencing cerebral blood flow (CBF) patterns. Results from such in vivo animal studies can provide important information in the development of new pharmaceuticals. Non-human primates are phylogenetically close to man and consequently facilitate meaningful extrapolation of results (8). A baboon model for SPECT brain imaging with <sup>99m</sup>Tc-HMPAO has been developed and found to be sensitive to the effects of anaesthesia on CBF (4). Anaesthesia is necessary for restraining the baboon during prolonged scintigraphy. The combination of ketamine and a barbiturate, with the ketamine used for darting of the ani-

### Key words

SPECT, acetazolamide, nimodipine, blood flow, baboon model

### Summary

The sensitivity of the baboon model under anaesthesia for single photon emission computed tomography (SPECT) of the brain with <sup>99m</sup>Tc-HMPAO, as recently developed by us to study cerebral blood flow patterns, was investigated using drugs that are known to increase cerebral blood flow, e.g. acetazolamide, the carbonic anhydrase inhibitor and nimodipine, the calcium channel blocker. Increases in cerebral blood flow for both acetazolamide and nimodipine were observed that correspond well with other studies. Statistically significant regional specificity was noted for acetazolamide and nimodipine. Interestingly a combination of these drugs did not enhance cerebral blood flow but rather decreased it in comparison with the individual drug responses. The results were correlated with arterial blood pressure, heart rate, pCO<sub>2</sub> and pO<sub>2</sub>. A blood pressure decrease was noted for both drugs, while acetazolamide had a marked influence on pO<sub>2</sub>. The results indicate that the baboon model is sensitive for evaluation of drug effects on cerebral blood flow.

### Schlüsselwörter

SPECT, Acetazolamid, Nimodipin, Hirndurchblutung, Pavian-Modell

### Zusammenfassung

Die Empfindlichkeit eines generell narkotisierten Pavian-Modells bei der Untersuchung mit SPECT mit <sup>99m</sup>Tc-HMPAO, einer kürzlich von uns entwickelten Methode zur Untersuchung der Hirndurchblutung (CBF), wird hier untersucht unter Verwendung von Pharmaka (Acetazolamid, Karboanhydrase-Inhibitor; Nimodipin, Kalziumkanalblocker), von denen bekannt ist, daß sie den humanen CBF steigern. Eine Steigerung des zerebralen Blutflusses wurde sowohl für Acetazolamid als auch für Nimodipin beobachtet, was mit den Ergebnissen anderer Studien gut übereinstimmt. Regionale Spezifität von statistischer Signifikanz wurde für Acetazolamid und Nimodipin festgestellt. Interessanterweise wurde der zerebrale Blutfluß durch eine Kombination der beiden Medikamente nicht verstärkt, sondern, verglichen mit der Wirkung des individuell gegebenen Medikaments, sogar verringert. Die Ergebnisse wurden mit arteriellem Blutdruck, Herzfrequenz, pCO<sub>2</sub> und pO<sub>2</sub> korreliert. Beide Mittel verringerten den Blutdruck, während Acetazolamid einen deutlichen Einfluß auf pO<sub>2</sub> zeigte. Die Ergebnisse machen deutlich, daß das Pavian-Modell unter Narkose empfindlich genug ist, um die Wirksamkeit eines Pharmakons auf den zerebralen Blutfluß zu bewerten.

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## Originalarbeit

mal, proves to be the anaesthesia of choice in CBF and regional CBF (rCBF) studies (4).

The purpose of the current study was to evaluate the sensitivity of the baboon model under anaesthesia using drugs that are known to increase cerebral blood flow, eg. acetazolamide (1-3, 7, 16) and nimodipine (6, 11, 12, 19). Additionally, with the effects from these established, the cerebrovascular dilatory response from a combination of acetazolamide and nimodipine was evaluated for possible beneficial implications in the treatment of cerebral disorders.

## Materials and Methods

Six adult male baboons (*Papio ursinus*, average mass 27 kg) were selected for this study. Anaesthesia was induced in each by darting with ketamine hydrochloride (Ketalar, Parke-Davis, S. A.: 10 mg/kg) and was followed immediately by an intravenous injection of  $^{99m}\text{Tc}$ -HMPAO (148 MBq), the cerebral distribution of which would then represent the effect of ketamine with no redistribution taking place. Five minutes later the baboon was intubated, maintained and controlled for the duration of the study under sodium thiopentone (Intraval, Maybaker, S. A.: 70 ml/h of a 0.5% solution). The subsequent SPECT acquisition to obtain the HMPAO distribution in the brain during ketamine was done with a Siemens Orbiter gamma camera coupled to an A<sup>3</sup> MDS computer using 32 views and 360° (10 sec/view). Following the first acquisition the baboon was reanesthetized with  $^{99m}\text{Tc}$ -HMPAO (296 MBq) and tomographed to detect the radionuclide distribution attained during what was then thiopentone anaesthesia. Care was taken to administer the two HMPAO injections at its latest within 30 min after reconstitution. Baboons were viewed in a supine position with a special head rest to ensure a reproducible position for comparison of tomographic slices.

The above procedure of two successive tomographic scans representing the effects of the two different forms of the anaesthesia in each baboon constitutes the control studies, and is called procedure A.

The effects of acetazolamide (Diamox, S. A. Cyanamid [Pty] [Ltd]) on cerebral blood flow were investigated (procedure B) in the following manner. The directions for the anaesthesia follow those for procedure A but the first SPECT acquisition was followed by an intravenous injection of 5 ml of acetazolamide (100 mg/ml), and only after another 5 min was the second double dose (296 MBq) administration of  $^{99m}\text{Tc}$ -HMPAO given, allowing adequate blood levels of acetazolamide to be reached. The animal was subsequently tomographed, according to procedure A, to obtain a tracer

distribution representative of the effect of acetazolamide when compared to procedure A.

Procedure C tested the effects of nimodipine (Bayer, Leverkusen) on the CBF of the baboon in a similar way as performed in procedure B, except that the first SPECT acquisition of the ketamine related cerebral distribution of  $^{99m}\text{Tc}$ -HMPAO (148 MBq) was followed by a very slow infusion (over 15 min) of nimodipine (1 µg/kg/min) (15) taking care to use only PVC-free tubing or a stainless steel needle in the administration to avoid any absorption resulting in a decrease in the concentration of nimodipine (10). After 10 min of nimodipine infusion the second injection of  $^{99m}\text{Tc}$ -HMPAO (296 MBq) followed, and tomography started after another 5 min at the time that the infusion was terminated (14, 15).

Procedure D investigated the effect of a combination administration of 5 ml acetazolamide (100 mg/ml) and nimodipine (1 µg/kg/min). The first SPECT acquisition was, as with procedure C, followed by a very slow infusion (over 15 min) of nimodipine. Five

minutes after starting the infusion the acetazolamide was injected, and the  $^{99m}\text{Tc}$ -HMPAO injection followed after yet another 5 min. Tomography began 5 min later when the infusion was stopped, and the HMPAO distribution then is, as in procedure B, representative of a combination situation 5 min after the injection of acetazolamide, and 10 min after the start of the nimodipine (see procedure C). The arterial blood pressures were recorded during all the procedures from a catheter in the femoral artery. Heart rates were also monitored as well as blood gases from an arterial line.

In order to check the possible influence of the two drugs on the  $^{99m}\text{Tc}$ -HMPAO input, a dynamic scintigraphic study was done on a count-down of isotope administration during ketamine anaesthesia (the control), again after acetazolamide, and eventually after nimodipine, before the above described SPECT acquisitions in each case were performed. Sixteen images of 15 sec were acquired in 64 × 64 word mode, and the subsequent time-activity curves compared for differences (Fig. 1).

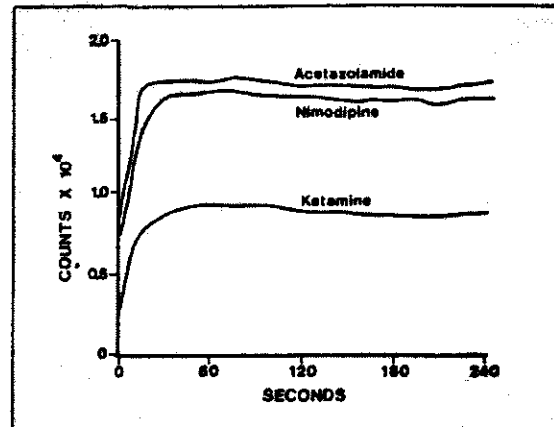


Fig. 1 Time-activity curves from the dynamic acquisition on count-down indicating uptake of  $^{99m}\text{Tc}$ -HMPAO under anaesthesia only (control), under acetazolamide, and nimodipine (the letter two from double dosages of HMPAO)

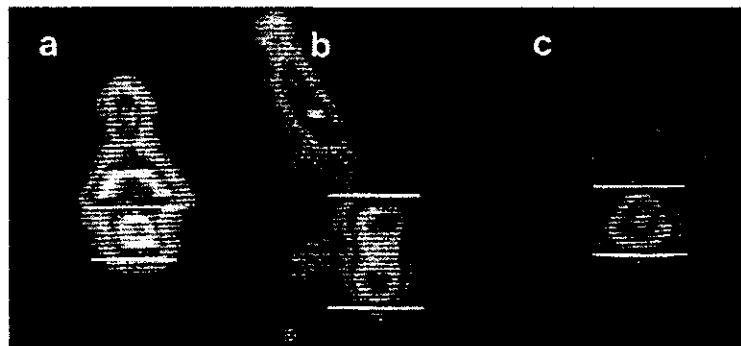
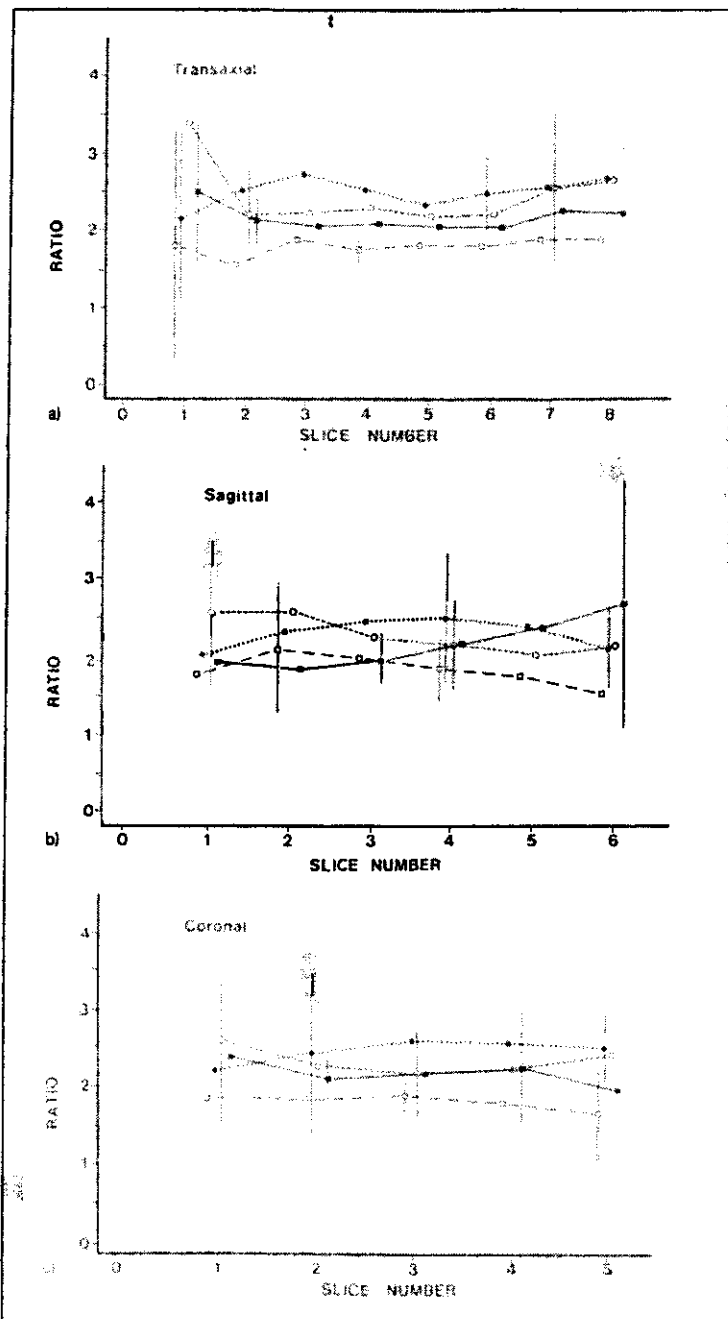


Fig. 2 a, b, c Typical tomographic brain slices in the coronal (a), sagittal (b) and transaxial (c) view indicating the regions of interest (ROI) i.e. the total brain between solid lines.



**Figs. 3a, b, c** Curves of mean ratios ( $n = 6$ ) vs slice number starting at the occipital lobes to the frontal lobes transaxially (a), from the right to the left of the brain sagittally (b), and from the cerebellum to the dorsal slice of the cerebrum coronally (c). The widely ( $\square$  ---  $\square$ ) spaced dashed line present the anaesthesia only experiment (Procedure A); the dotted ( $\bullet$  ---  $\bullet$ ) line the intervention with acetazolamide (Procedure B); the compactly spaced line ( $\circ$  ---  $\circ$ ) the intervention with nimodipine (Procedure C) and the solid line ( $\blacksquare$  ---  $\blacksquare$ ) the combination intervention (Procedure D)

### Statistical Methods

Mean ratios ( $n = 6$ ) and standard deviations (SD) were calculated per procedure for similar regions and for the total brain in the various projections. These were compared for inter-procedural effects as well as regional effects for a particular procedure. The comparisons were assessed for significant differences using Student's two-tailed *t*-test.

### Results

After backprojection and reconstruction the brain images consisted of transaxial, sagittal and coronal slices representing CBF and rCBF information during conditions prevailing under the procedure of anaesthesia only and under various drug interventions. Eight transaxial slices represented the whole brain, each of which was considered for count rate evaluation by the ROI (region of interest) feature. Similarly six sagittal slices and five coronal slices covering all of the brain were selected and analyzed. In subsequently placing the respective ROIs (Fig. 2a, b, c) for count rate/pixel values of the brain slices, care had to be taken to avoid the baboon sinus cavities and salivary glands. Count rate data were then inserted into the following equation to obtain the ratio *R*,

$$R = \frac{[\text{Intervention (2nd tracer injection)}] - [\text{Baseline (1st tracer injection)}]^*}{\text{Baseline (1st tracer injection)}}$$

which is an indication of the level change of the CBF during the drug intervention with respect to that attained during ketamine anaesthesia (baseline). The equation allows for subtraction of retained activity originating from the baseline study, (after decay correction[\*]). For all three views graphs were plotted of *R* vs slice numbers starting at the occipital lobes to the frontal lobes transaxially, from right to left sagittally, and from the cerebellum to the dorsal slice of the cerebrum coronally (see curves in Fig. 3a, b, c). Contrary to human data the coronal slices were not aligned to the axis of the brain (Fig. 4). The brain was subsequently divided into four equal regions using

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the curves (Fig. 3) and equal segments taken along the abscissa. Representative regional blood flow ratios were thus obtained for these regions for each procedure. Ratios thus became available for the control (anaesthesia only) study (procedure A) and for the pharmacological procedures B, C and D.

The input functions for  $^{99m}\text{Tc}$ -HMPAO, from the dynamic studies under ketamine, acetazolamide and nimodipine do not differ (Fig. 1). Tables 1, 2 and 3 present the mean ( $n = 6$ ) ratios (R) and SD obtained for the 4 brain regions as derived from Fig. 3, and for the total brain viewed respectively transaxially, sagittally and coronally under anaesthesia only, and after the various drug interventions.

The effects of acetazolamide (carbonic anhydrase inhibitor), nimodipine (calcium channel blocker) and a combination on the heart rate, blood pressure,  $\text{pCO}_2$  and  $\text{pO}_2$  in the arterial blood are summarized in Table 5 for pre-injection and post injection (10 min) values. The heart rate remained constant for all procedures. Table 5 indicates a decrease in blood pressure from acetazolamide ( $p < 0.05$ ) and nimodipine ( $p > 0.05$ ) but the combination showed no effect. The  $\text{pCO}_2$  values indicate no influence from any of the procedures. Acetazolamide and the drug combination procedure significantly increased the arterial  $\text{pO}_2$  ( $p < 0.05$ , for both).

## Discussion

The dynamic  $^{99m}\text{Tc}$ -HMPAO results (Fig. 1) clearly show a consistency in the uptake function which allows us to draw conclusions on the isotope distribution in the brain and cerebral blood flow under the various drug interventions.

Failure of the total brain and regional brain ratios to yield a value 2, which would correspond to the second double dose of  $^{99m}\text{Tc}$ -HMPAO points to intraprocedural anaesthesia variations as in procedure A, and additionally to the effect of the drugs as in procedures B, C and D.

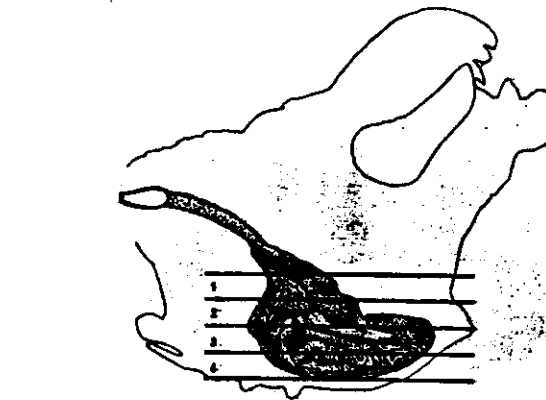


Fig. 4 The position of the coronal regions (slices) of the baboon brain in this study not aligned to the brain axis, and resembling transaxial slices of the human brain.

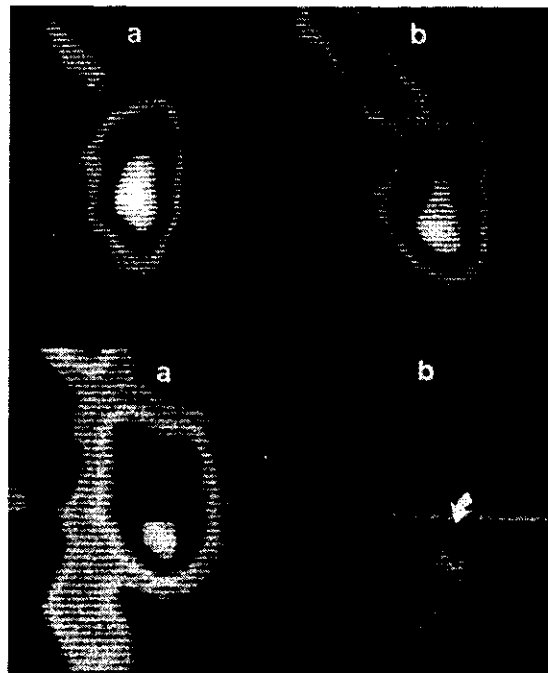


Fig. 5 Corresponding sagittal slices for baseline (a) and intervention (b) acquisition obtained from the anaesthesia only experiment (Procedure A: top) and (bottom) from the nimodipine experiment (Procedure C). Note for the latter procedure enhanced cerebellum (arrow) after nimodipine administration. This effect is not shown with procedure A (top).

Ketamine hydrochloride is known and was shown here to increase arterial blood pressure and heart rate (Table 4) leading to augmented CBV (4), which is in this study indicated by

lower ratios than 2 as were obtained from procedure A (see Tables 1 to 3).

For procedures A and B there are no statistically significant differences between the intraprocedural regional

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**Table 1** Mean ( $\pm$  SD) ratios from transaxial views of four equal cerebral slices and from total brain

	Region 1	Region 2	Region 3	Region 4	Total brain
Procedure A	1.73 $\pm$ .87	1.88 $\pm$ .19	1.86 $\pm$ .37	1.95 $\pm$ .36	1.86 $\pm$ .45
Procedure B	2.37 $\pm$ .94	2.58 $\pm$ .72	2.56 $\pm$ .84	2.23 $\pm$ .61	2.44 $\pm$ .73
Procedure C	3.30 $\pm$ .73	2.31 $\pm$ .63	2.33 $\pm$ .60	2.55 $\pm$ .77	2.62 $\pm$ .68
Procedure D	2.51 $\pm$ .96	2.14 $\pm$ .47	2.13 $\pm$ .46	2.50 $\pm$ .71	2.32 $\pm$ .65

Statistically significant differences for procedure C between region 1 and regions 2, 3, 4. Also between procedure A and C for region 1, ( $p < 0.05$ ).

**Table 2** Mean ( $\pm$  SD) ratios from sagittal views of four equal cerebral regions and the total brain

	Region 1	Region 2	Region 3	Region 4	Total brain
Procedure A	1.84 $\pm$ .29	2.10 $\pm$ .18	1.97 $\pm$ .39	1.83 $\pm$ .54	1.94 $\pm$ .35
Procedure B	2.23 $\pm$ .57	2.58 $\pm$ .57	2.56 $\pm$ .64	2.23 $\pm$ .53	2.40 $\pm$ .58
Procedure C	2.73 $\pm$ .88	2.46 $\pm$ .63	2.30 $\pm$ .53	2.25 $\pm$ .92	2.44 $\pm$ .75
Procedure D	2.06 $\pm$ 1.47	1.99 $\pm$ .47	2.31 $\pm$ .67	2.89 $\pm$ 1.87	2.31 $\pm$ 1.12

No statistically significant differences

**Table 3** Mean ( $\pm$  SD) ratios from coronal views of four equal cerebral regions and the total brain

	Region 1	Region 2	Region 3	Region 4	Total brain
Procedure A	1.91 $\pm$ .32	1.93 $\pm$ .23	1.92 $\pm$ .28	1.73 $\pm$ .59	1.87 $\pm$ .36
Procedure B	2.35 $\pm$ .52	2.80 $\pm$ .83	2.75 $\pm$ .84	2.66 $\pm$ .34	2.64 $\pm$ .63
Procedure C	2.68 $\pm$ 1.0	2.34 $\pm$ .60	2.40 $\pm$ .53	2.69 $\pm$ .78	2.53 $\pm$ .73
Procedure D	2.24 $\pm$ 1.0	1.99 $\pm$ .63	2.10 $\pm$ .76	1.96 $\pm$ .73	2.07 $\pm$ .78

Statistically significant differences between procedures A and B, region 4 ( $p < 0.05$ ).

**Table 4** Mean percentage (%) changes of ratios from the four brain regions ( $n = 4$ ) for the three views, comparing the different procedures with each other

Procedures	Transaxial	Sagittal	Coronal
Procedure A-B	32	24	41 (4:54%)
Procedure A-C	42 (1:91%)	26	36
Procedure A-D	25	20	11
Procedure B-C	9	2	-3
Procedure B-D	-4	-3	-21
Procedure C-D	-10	-4	-18

The parentheses contain the region number which had statistically significantly changed ( $p < 0.05$ ); the corresponding percentage change is also indicated. Negative values point to a CBF decrease.

**Table 5** Effects of procedures B (acetazolamide), C (nimodipine) and D (combination) on heart rate, blood pressure,  $pCO_2$  and  $pO_2$  in arterial blood in baboons

Measurement	Heart rate	Blood pressure	$pCO_2$	$pO_2$
B <sub>1</sub>	116.25 $\pm$ 12.74 (4)	118.00 $\pm$ 2.58 (4)	41.10 $\pm$ 2.37 (4)	56.35 $\pm$ 4.91 (4)
B <sub>2</sub>	111.50 $\pm$ 16.84 (4)	110.25 $\pm$ 4.50 (4)*	40.08 $\pm$ 4.87 (4)	78.43 $\pm$ 7.47 (4)*
C <sub>1</sub>	115.33 $\pm$ 22.38	132.50 $\pm$ 29.93	42.85 $\pm$ 7.55	61.12 $\pm$ 4.39
C <sub>2</sub>	116.33 $\pm$ 24.58	112.89 $\pm$ 20.26	40.50 $\pm$ 1.27 (2)	68.50 $\pm$ 5.37 (2)
D <sub>1</sub>	113.83 $\pm$ 18.95	108.50 $\pm$ 24.40	42.85 $\pm$ 7.55	61.12 $\pm$ 4.39
D <sub>2</sub>	117.83 $\pm$ 18.29	108.50 $\pm$ 32.49	38.90 $\pm$ 3.35	75.06 $\pm$ 9.23 (5)*

Each value indicates the mean  $\pm$  SEM of six experiments unless where indicated in parentheses. Subscripts 1 and 2 refer to the measurements respectively before and 10 min after injection of the drug. \*  $p < 0.05$  for post injection vs corresponding pre-injection values.

ratios ( $p > 0.05$ ), thus indicating no influence from procedure A and B on rCBF. Procedure B ratios tend to be larger regionally as well as for the total brain than those from procedure A (Tables 1 to 3); differences do not reach statistical significance ( $p > 0.05$ ) except for region 4 in the coronal view which is mainly a dorsal representation. The percentage increases (Table 4) correspond well with Bonte et al. (2), but in addition a high percentage increase in coronal region 4 was noted. Procedure C demonstrates a significantly higher ratio ( $p < 0.05$ ) for the cerebellum, represented in the transaxial region 1, than for any other region (Table 1). Ratios from procedure C also tend to be consistently higher regionally, and for the total brain than for the anaesthesia only procedure A. However, only region 1 transaxially (cerebellum) show a significantly and quite dramatically higher value ( $p < 0.05$ ) (Tables 1 to 4, and Fig. 3 and 5). No significant differences could be demonstrated between procedures B and C ( $p > 0.05$ ). Procedure D ratios can be seen from Tables 1 to 3 and from the curves (Fig. 3) to be predominantly depressed with respect to procedures B and C, occasionally taking on a value in between. Although the regional ratios from procedure D are higher than form the anaesthesia only (procedure A), indicating some effect, no statistically significant difference can be demonstrated ( $p > 0.05$ ).

The percentage changes in Table 4 indicate that the three drug procedures increase the mean regional ratios, less so for the combination

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drug, and that amongst themselves the drug procedures do not produce different results. None of these regional and total brain values differ statistically significantly, but it is interesting that the combination drug does not enhance CBF or rCBF at all, or proportionally to the effects of the drugs individually. Rather, the R values from the combination drug seem to follow the regional pattern obtained from nimodipine only, which is again different from the regional distribution pattern obtained from procedure B (Fig. 3).

Nimodipine was previously reported to increase the cerebral blood flow without significant influence on the blood pressure and arterial blood gases (14) and it was suggested that the cerebrovascular autoregulation is resistant to nimodipine (14). Niashikibe et al. (17) showed that the blood pressure is dose-dependently decreased by nimodipine (1–10 µg/kg).

It was previously shown that neither nimodipine nor acetazolamide significantly influence the pCO<sub>2</sub> (7, 10, 14, 17). Acetazolamide is known to produce metabolic acidosis due to its carbonic anhydrase activity giving rise to increased CO<sub>2</sub> tensions in the expired gas (5). However, the current dose and time scale of the determination of pCO<sub>2</sub> showed no significant change in the intravascular CO<sub>2</sub> tension, indicating that the increase in the cerebral blood flow observed for acetazolamide is unrelated to the principal local effect from a raise in the intravascular CO<sub>2</sub> tension. In accordance with other authors nimodipine showed no significant effect on the pO<sub>2</sub> (p > 0.05); only sporadically was the pO<sub>2</sub> increased in some animals (14, 17). Table 5 indicates that the increase in pO<sub>2</sub> due to acetazolamide alone (35%) is greater when compared to the acetazolamide-nimodipine combination (23%) suggesting that nimodipine attenuates the effect of acetazolamide. Hyperventilation was observed under acetazolamide treatment that could account for the higher pO<sub>2</sub> values.

It is interesting to note that the increased cerebral blood flow observed for acetazolamide and nimodipine alone individually is great-

ly diminished when the drugs are combined. These results strongly support the suggestion that acetazolamide may have an effect on the local cerebral metabolic rate for oxygen (13, 20). They may further indicate that the cerebral vasodilatory properties of acetazolamide are unrelated to its carbonic anhydrase inhibition. Also interesting is that although there is an initial decrease in blood pressure with acetazolamide the blood pressure was subsequently observed to increase to a maximum after 25 min. This time response was also observed for the pO<sub>2</sub>, coinciding with the maximum increase in cerebral blood flow previously reported (2).

## Conclusion

The current study has shown that the baboon model previously described (4) is sensitive to monitoring pharmacological interventions and to evaluate drug candidates for cerebral diseases. Some of the observations of this study will even add to the information available for the two drugs under consideration. It also becomes clear that a combination of acetazolamide and nimodipine used under the currently described time scheduling has no beneficial effects on cerebral blood flow pattern. The combination actually diminishes the positive effects of the individual drugs via a mechanism that cannot be readily explained at this time.

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## REFERENCES

- Bachmann A, Schürer L, Uterberg A, Wahl W, Staub F, Kempf O. Mediator-substanzen des Hirnödems bei der zerebralen Ischämie. *Arzneim Forsch/Drug Res* 1991; 41: 310–5.
- Bonte FJ, Devous MD, Reisch JS. The effect of acetazolamide on regional cerebral blood flow in normal human subjects as measured by single photon emission computed tomography. *Invest Radiol* 1988; 23: 564–8.
- Burt RW, Reddy RV, Mock BM, Wellman HN, Schanwecker DS, Witt R. Acetazolamide enhancement of HIPDM brain blood flow distribution imaging. *J Nucl Med* 1986; 27: 1627–31.
- Dormehl I, Redelinghuys F, Hugo N, Oliver DW, Pillay W. The baboon model under anaesthesia for in vivo cerebral blood flow studies using single photon emission tomographic (SPECT) techniques. *J Med Primatol* 1992; 21: 270–4.
- Goodman, Gilman's. *The Pharmacological Basis of Therapeutics*, 8th ed. Gilman AG, Rall TW, Nies AS, Taylor P (eds). New York: Pergamon Press 1990: 716–7.
- Harper AM, Craigen L, Kazda S. Effect of calcium antagonist, nimodipine, on cerebral blood flow and metabolism in the primate. *J CBF and Metab* 1981; 1: 349–56.
- Hauge A, Nicholaysen G, Thoresen M. Acute effects of acetazolamide on cerebral blood flow in man. *Acta Physiol Scand* 1983; 117: 233–9.
- Herman CLU, McKee AE, Chilling PW, Dickson LG, Hörwitz DL, Coran AG, Cryer PE, Capriva CJ, Forscher BK, Lillehei RC, Stubbs S. Baboon as a sub-human primate shock model in shock, high and low flow states. *Amsterdam Excerpta Medica* 1972: 42–8.
- Höllerhage H-G, Gaab MR, Zunkeller M, Walter GF. The influence of nimodipine on cerebral blood flow autoregulation and blood brain barrier. *J Neurosurg* 1988; 69: 919–22.
- Jakobsen R, Mikkelsen EO. Determination of nimodipine by gas chromatography using electron-capture detection; external factors influencing nimodipine concentrations during intravenous administration. *J Chromatogr* 1986; 374: 383–7.
- Kriegelstein J, Peruche B. *Pharmakologische Grundlagen der Therapie der zerebralen Ischämie*. *Arzneim-Forsch/Drug Res* 1991; 41: 303–9.
- Langley MS, Sorkin EM. Nimodipine. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic potential in cerebrovascular disease. *Drugs* 1989; 37: 669–99.
- Laux BE, Raichle ME. The effect of acetazolamide on cerebral blood flow and oxygen utilization in the rhesus monkey. *J Clin Invest* 1978; 62: 585–92.
- McGalden TA, Nath RG. Cerebrovascular autoregulation is resistant to calcium channel blockade with nimodipine. *Experientia* 1989; 45: 305–6.
- McGalden TA, Nath RG, Thiele K. The effects of a calcium antagonist (nimodipine) on basal cerebral blood flow and reactivity to various agonists. *Stroke* 1984; 15 (3): 527–30.
- Mithoefer J, Mayer P, Stocks J. Effect of carbonic anhydrase inhibition of the cerebral circulation of the anesthetized dog. *Fed Proc* 1957; 16: 88–9.
- Niashikibe M, Kamei K, Nagura J, Suzuki K. Effect of the newly synthesized calcium antagonist isopropyl methyl 2-carbamoyloxymethyl-6-methyl-4-(2,3-dichlorophenyl)-1,4-dihydropyridine-3,5-dicarboxylate



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## Effect of Sumatriptan on Cerebral Blood Flow in the Baboon Model

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### Summary

Changes in cerebral blood flow are implicated to be important in the pathophysiology of migraine. Furthermore, serotonin (5-HT) is known to be the most important substance in the etiology of migraine. Sumatriptan (CAS 103628-46-2), a 5-HT<sub>1D</sub> receptor agonist was recently introduced in the treatment of migraine. In the present study a baboon model was used to investigate the changes in cerebral blood flow due to anaesthesia and pharmacological interventions using <sup>99m</sup>Tc-labelled hexamethylpropylene amine oxime (<sup>99m</sup>Tc-HMPAO) and single photon emission computed tomography (SPECT). The effect of sumatriptan on cerebral blood flow was investigated after 10 min and again after 23 min, with the animal under anaesthesia, i.e. induction with ketamine and maintenance on thiopental. Sumatriptan did not alter the cerebral blood flow during the 10 min procedure. However, sumatriptan reversed the increased cerebral blood flow due to the prolonged anaesthesia (23 min), lowering the cerebral blood flow by more than 20%. No significant changes in the biochemical parameters (blood pressure, heart rate, pO<sub>2</sub> and pCO<sub>2</sub>) were observed. These results also suggest that sumatriptan reverses the increased cerebral blood flow most likely via 5-HT<sub>1D</sub> receptor stimulation.

### Zusammenfassung

Einfluß von Sumatriptan auf die Hirndurchblutung beim Schimpansen

### 1. Introduction

Migraine significantly affects the lives and productivity of about 10 to 15 % of adult population [1]. The development of effective treatment of a migraine attack has for many years been the focus of many researchers. An aura consisting of transient visual, sensory or motor symptoms may precede a migraine attack or may be absent. A focal reduction of regional cerebral blood flow (rCBF) initiates a migraine attack associated with an aura while the neurological symptoms of the aura have been implicated in the reduction of tissue perfusion [2-8]. Serotonin (5-HT), a mono-amine, exerts its physiological effects via stimulation of several 5-HT receptors. The role of serotonin in the pathogenesis of migraine attacks is now well accepted [9, 10, 11]. Sumatriptan (CAS 103628-46-2) a new 5-HT<sub>1D</sub> receptor agonist, has recently been added to the therapeutic arsenal in the treatment of acute migraine [12]. The antimigraine effect of sumatriptan appears to be via its stimulation of the 5-HT<sub>1D</sub> receptors. Friberg et al. recently reported that sumatriptan reversed the migraine pain that is associated with middle cerebral artery dilatation [13]. Diener et al.

Veränderungen in der Hirndurchblutung spielen eine wesentliche Rolle in der Pathophysiologie der Migräne. Es ist bekannt, daß Serotonin (5-HT) hierbei die wichtigste Substanz ist. Sumatriptan (CAS 103628-46-2), ein 5-HT<sub>1D</sub>-Rezeptoragonist, ist ein neues Migräne-Therapeutikum. In der vorliegenden Studie wurden beim Schimpansen Veränderungen in der Hirndurchblutung unter Narkose sowie entsprechende pharmakologische Interventionen mittels <sup>99m</sup>Tc-markiertem Hexamethylpropylenaminnoxim (<sup>99m</sup>Tc-HMPAO) und Single-Photon-Emissions-Computer-Tomographie (SPECT) untersucht. Der Einfluß von Sumatriptan auf die Hirndurchblutung wurde nach 10 bzw. 23 min untersucht, wobei die Narkose mit Ketamin eingeleitet und mit Thiopental aufrechterhalten wurde. Sumatriptan veränderte die Hirndurchblutung während der 10minütigen Testperiode nicht. Es induzierte jedoch eine Umkehr der unter der 23minütigen Narkose erhöhten Hirndurchblutung und erniedrigte diese um mehr als 20%. Die biochemischen Parameter (Blutdruck, Herzfrequenz, pO<sub>2</sub> und pCO<sub>2</sub>) waren nicht signifikant verändert. Diese Ergebnisse lassen vermuten, daß Sumatriptan eine Umkehr der erhöhten Hirndurchblutung wahrscheinlich via 5-HT<sub>1D</sub>-Rezeptor-Stimulation bewirkt.

**Key words:** Antimigraine drugs · CAS 103628-46-2 · Cerebral blood flow · Serotonin · Sumatriptan, pharmacology

further reported that sumatriptan did not significantly change the blood flow and velocities of the middle cerebral and basilar arteries and suggested that its action is mediated via mechanisms other than the well established vasoconstrictor actions on cerebral arteries [14]. Furthermore, sumatriptan was shown to selectively constrict the carotid arterial bed of anesthetized dogs and cats [15].

We recently developed a baboon model under anaesthesia to study the effects of drugs on the cerebral blood flow [16]. In continuation of our interest in drugs that exhibit cerebroconstrictor and dilatory effects we here report the study of the effects of sumatriptan on the cerebral blood flow in the baboon model using photon emission computed tomography (SPECT) and the radiopharmaceutical hexamethylpropylene amine oxime (<sup>99m</sup>Tc-HMPAO).

### 2. Materials and methods

#### 2.1. The animal study

Six adult male baboons (*Papio ursinus*, average weight 27 kg) were selected for this study. Each baboon was subjected to four

different procedures on different days, at least one month apart. Because of the necessity of handling the animals under anaesthesia, the first procedure, A, concerns a control CBF study with  $^{99m}\text{Tc}$ -HMPAO and SPECT under only the standard anaesthetic conditions of anaesthesia induction with ketamine hydrochloride (Ketalar<sup>®</sup>, Parke-Davis, S.A.; 10 mg/kg), followed by maintenance on thiopental sodium as previously described by us (Intraval<sup>®</sup>, 10 ml/h Maybaker, S.A.).

Procedures B and C investigated the CBF changes due to the sumatriptan (Imigran<sup>®</sup>, Glaxo, S.A.; 6 mg subcutaneous) at 10 and 23 min, respectively, after administration. Procedure D was a second control to evaluate the CBF during prolonged thiopental sodium anaesthesia after the ketamine blood levels had reached insignificant values, which was the case during procedure C.

### 2.2. Procedure A (control study)

After anaesthesia induction with ketamine chloride, each baboon received an i.v. injection of  $^{99m}\text{Tc}$ -HMPAO (148 MBq), the cerebral distribution of which would then represent the effect of ketamine on CBF. During the subsequent 5 min waiting time until the start of SPECT the baboon was intubated and the anaesthesia changed to a thiopental i.v. infusion (70 ml/h of a 0.5% solution), using an administration (drip) set. The following SPECT acquisition to obtain the HMPAO distribution in the brain during ketamine (SPECT-1) was done with a Siemens Orbitron gamma camera, coupled to a Sophy 250G computer, using 32 views,  $360^\circ$  (10 s/view), in  $64 \times 64$  word mode. Following the first acquisition, which with camera and computer readjustment, took approximately 8 min, and in addition a waiting time of 5 min, the baboon was reinjected with  $^{99m}\text{Tc}$ -HMPAO (296 MBq) and tomographed as above (SPECT-2) to detect the radionuclide distribution after 18 min of thiopental anaesthesia. This is the split-dose method of HMPAO application in CBF studies [17]. Ketamine, producing so-called dissociative anaesthesia is inappropriate during SPECT acquisitions because of undesirable involuntary movements of the animals. Procedure A is therefore the approach to expedite the start of an experiment and to allow correction for the influence that the ketamine might still have of CBF. The baboons were viewed in a supine position with a special head rest to ensure a reproducible position for comparable tomographic slices.

### 2.3. Procedure B (sumatriptan effect after 10 min)

The procedure B is similar as for procedure A, except for a delayed switch to thiopental anaesthesia after the first HMPAO injection in order to attain an 18 min duration of thiopental infusion at 10 min after the administration of sumatriptan immediately after SPECT-1, when the second (double dose) HMPAO injection is administered. This distribution of HMPAO as obtained from SPECT-2 will reflect on the effects of 18 min of thiopental anaesthesia as in the control study as well as the effect of sumatriptan after 10 min. Control ketamine-thiopental conditions were therefore maintained.

### 2.4. Procedure C (sumatriptan effect after 23 min)

Measuring the effect of sumatriptan after 23 min required a separate experiment as well as a changed procedure in order

not to exceed the maximum interval of 30 min between reconstitution of  $^{99m}\text{Tc}$ -HMPAO and its i.v. injection because of degradation of the radiopharmaceutical. The problem was overcome by comparing the effect of sumatriptan after 23 min to that after 10 min which follows from procedure B. After darning with ketamine the baboon is immediately placed on thiopental sodium as before and thus maintained for 20 min before the administration of sumatriptan (6 mg, s.c.), 10 min later (i.e. after 30 min of thiopental) the first HMPAO injection (148 MBq) was given and SPECT-1, as previously, followed 5 min later. These results will represent CBF influenced by 30 min of thiopental anaesthesia and after 10 min of sumatriptan. The second double dose of HMPAO (296 MBq) was injected directly at the completion of SPECT-1; this was 23 min after the sumatriptan injection and the subsequent distributions from SPECT-2 reflected on the effects of 43 min of thiopental and after 23 min of sumatriptan. Procedure C can obviously not yield results which could be compared to the control study procedure A, because of changed anaesthesia conditions. Therefore a second control study, was devised to take these changes into account (Procedure D).

### 2.5. Procedure D (control study with prolonged thiopental sodium)

Immediately after the ketamine induction the baboon was placed on sodium thiopental for 30 min at which stage the thiopental blood levels predominated.  $^{99m}\text{Tc}$ -HMPAO was injected at this stage to obtain, through SPECT-1, a distribution in the brain under influence of thiopental (30 min duration, as in procedure C). The baboon remained only on thiopental anaesthesia for the second double dose of HMPAO (after SPECT-1) at 43 min of thiopental. Thus the subsequent SPECT-2 would yield results according to the time scale of procedure C.

### 2.6. Data processing

After backprojection and reconstruction the brain images in all procedures consisted of transaxial, sagittal and coronal slices representing CBF and rCBF related information during conditions prevailing during various forms of anaesthesia and due to time dependent effects of sumatriptan. Eight slices of two pixels thickness represented the brain in all three views. Regions of interest (ROIs) were placed on the total brain (Fig. 1) and count rate data (counts/pixel) thus obtained were inserted into the following equation to obtain the ratio R:

$$R = \frac{(\text{SPECT-2})_{\text{data}} - (\text{SPECT-1})_{\text{data}}^*}{(\text{SPECT-1})_{\text{data}}}$$

where \* refers to decay corrected data from SPECT-1, present during SPECT-2 and which has to be subtracted from the SPECT-2 data, as background; and R is an indication of the level change of rCBF due to the changed conditions prevailing during the second HMPAO injection with respect to that of the first injection.

For procedure A the ratio R will reflect the change in rCBF during the anaesthesia change from ketamine to thiopental. A value of  $R = 2$  (due to the double second dose of  $^{99m}\text{Tc}$ -

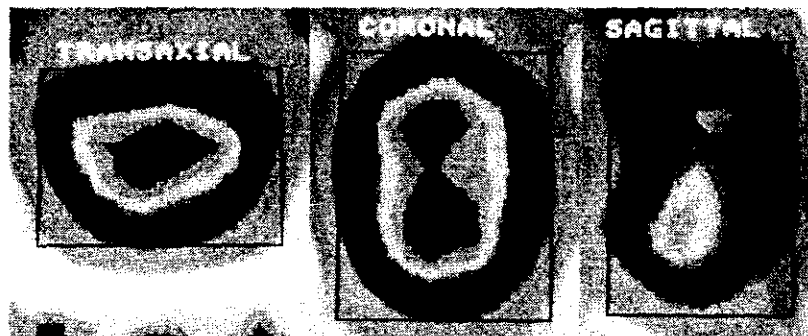


Fig. 1. Typical tomographic brain slices in the transaxial, coronal and sagittal views, with the position of the regions of interest (ROI), i.e. the total brain indicated within the rectangles.

HMPAO) will indicate no rCBF change during procedure A due to changed anaesthesia. R for procedure B will additionally reflect on changes due to the sumatriptan 10 min after administration and was compared to R (procedure A) to assess the effects of the sumatriptan. For procedure C the ratio R will compare the effects of sumatriptan after 10 min and 23 min if the R-value for prolonged thiopental anaesthesia prevailing during procedure C is known. This value was available from procedure D. Comparisons of R-values between procedures A and B and between procedures C and D were done by a Student two-tailed t-test on a 5% level of confidence.

Blood pressure, heart rate and blood gases ( $pO_2$  and  $pCO_2$ ) were measured before each HMPAO injection during each of the procedures to determine the effects of the different interventions and anaesthetic procedures on these parameters.

### 3. Results

The R-values for each of the eight slices in each view are presented in Fig. 2 and 3: Fig. 2 (a, b, c) compares the control R-values of procedure A and the R-values of sumatriptan at 10 min after the injection, and Fig. 3 (a, b, c) compares the R-values from procedure C (23 min post sumatriptan) with those from the prolonged thiopental anaesthesia, procedure D. From the maximum and minimum standard deviation bars indicated in the figures no statistically significant ( $p > 0.05$ ) differences were found between the various slices in any one particu-

Table 1

Procedure	Mean CBF and SD (n = 6)
A	1.89 ± 0.12
B	1.96 ± 0.10
C	1.82 ± 0.08
D	2.30 ± 0.27

lar procedure, indicating no obvious regional effect on CBF during any of the four procedures. The values for the three views also do not differ statistically significantly, which confirms a lack of regional influence on the CBF. It therefore becomes sufficient to measure the effects of the four procedures by global or total brain CBF changes (rather than the slices), in which manner the information will also improve statistically.

Global CBF as a mean (n = 6) and SD for each procedure appears in Table 1. No statistically significant difference is found between procedures A and B ( $p > 0.05$ ), but procedure C significantly lowers the R-values which otherwise follow from the control study under prolonged anaesthesia (procedure D) ( $p < 0.05$ ).

The blood pressure, heart rate and blood gases ( $pO_2$  and  $pCO_2$ ) were monitored for any influence during the different procedures. Transient marginal increases were ob-

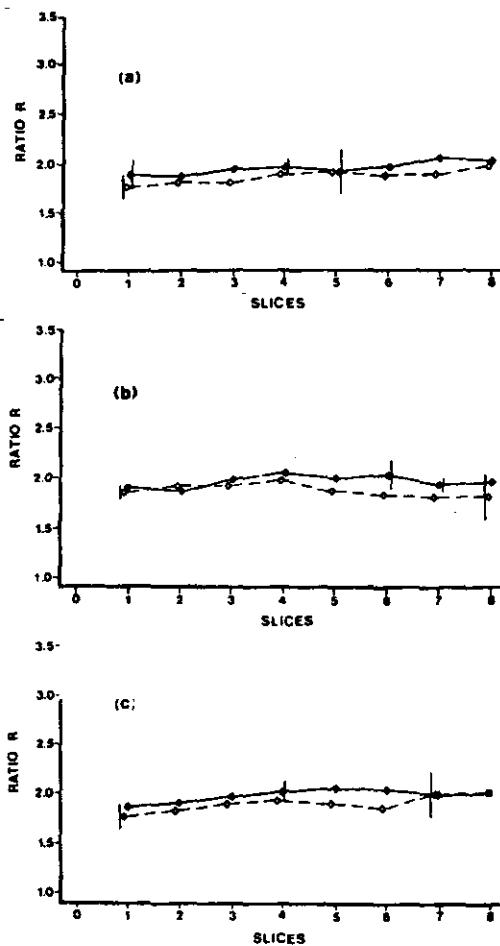


Fig. 2: R-values per slice (means; n = 6) for procedure A, (solid line) and procedure B (dashed line) with maximum and minimum SDs indicated, in (a) transaxial, (b) coronal and (c) sagittal views.

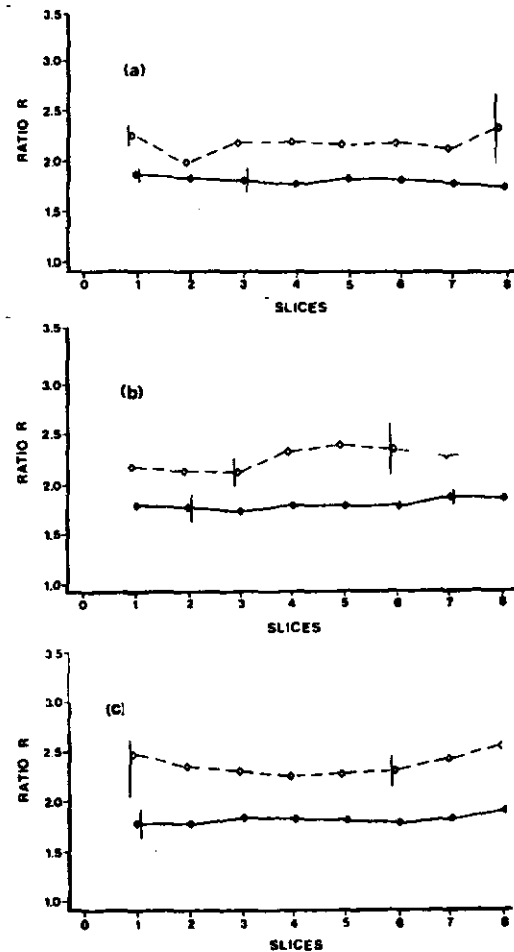


Fig. 3: R-values per slice (means; n = 6) for procedure C (solid line) and procedure D (dashed line) with maximum and minimum SDs indicated in (a) transaxial, (b) coronal and (c) sagittal views.

served for blood pressure, heart rate and  $p\text{CO}_2$  at the 10 min interval (procedure C) of the sumatriptan intervention. A non-significant increase in the  $p\text{CO}_2$  level was observed at the 23 min sumatriptan intervention.

#### 4. Discussion

The R-values for the ketamine-thiopentone control study (procedure A) are lower than the value 2 (two), i.e. they relate to an augmented CBF during ketamine (from the equation) which corresponds to the increase in arterial BP and HR [16, 18, 19].

The prolonged maintenance of the animals under thiopentone in procedure D could account for the ratios R in this case being distinctly larger than 2. The barbiturates are known to accumulate in the muscle and subsequently in fatty tissue and an enhanced  $p\text{CO}_2$  effect could result from their release during a prolonged study, leading to increased CBF from additional vasodilation (seen in the interventional phase of procedure D) in the absence of controlled ventilation [20].

The effect of sumatriptan after 10 min (procedure B) does not change the control values from procedure A significantly ( $p > 0.05$ ) although the values tend to be somewhat higher, pointing towards an increased CBF from the sumatriptan at 10 min. 23 min after the administration of sumatriptan, R-values (procedure C) are obtained which differ statistically significantly from the control value from procedure D. However, the R-values of procedure B and procedure C, i.e. 10 min and 23 min sumatriptan respectively are not significantly different. This means that the relationship

sumatriptan related CBF count at 23 min

sumatriptan related CBF count at 10 min

introduces a factor into the R-values from procedure D which reduces these significantly. This factor has to be less than 1, and thus points to a higher CBF at 10 min than at 23 min. Also the effect of sumatriptan at 23 min is enhanced compared to the 10 min study. At 10 min after sumatriptan there was indeed no effect observed with relation to control R-values, (procedure A), whereas at 23 min a significant change (reduction) of 22% was measured (procedure D). This result suggests that sumatriptan returns the CBF to normality.

A transient slight increase in the peripheral blood pressure [21] and marginally lower heart rate, were observed at 10 min, which was not present at the 23 min interval. Slightly, but non-significant increases in the  $p\text{CO}_2$  and  $p\text{O}_2$  levels were recorded for the procedures B and C under sumatriptan. No correlation is implicated in these slight changes in biochemical parameters.

This study shows that a substance with cerebrovasoconstrictor activities such as sumatriptan is beneficial in normalizing the effect, i.e. the increased CBF, of the anaesthetic regime used in this baboon model. It was recently demonstrated that the dilatation of the middle cerebral arteries, during a headache phase of a migraine attack, was reversed by sumatriptan [13, 22]. The present results may indicate that the serotonin,  $5\text{-HT}_{1D}$  receptors are likely to be involved in the normalization of the cerebral blood flow during our anaesthetic procedure with the sumatriptan intervention.

Investigation of other agents acting on the cerebrovascular serotonergic receptors can give further insight on the changes in the cerebral blood flow that occur during a migraine attack and the changes that were observed during the anaesthesia in our baboon model.

#### 5. References

- [1] Kenyon, J. (ed.), *Drugs and Therapy Perspectives*, Vol. 1, pp. 1-4 Aids International, Auckland, New Zealand (1993) - [2] Skinhoj, E., *Arch. Neurol.* 29, 95 (1973) - [3] Olesen, J., Larsen, B., Lauritzen, M., *Ann. Neurol.* 9, 344 (1981) - [4] Lauritzen, M., Skyhoj, Olsen, T., Lassen, N. A., Paulson, O. B., *Ann. Neurol.* 13, 633 (1983) - [5] Skyhoj, Olsen, T., Friberg, L., Lassen, N. A., *Arch. Neurol.* 44, 156 (1987) - [6] Lauritzen, M., Olesen, J., *Brain* 107, 447 (1984) - [7] Olesen, J., Friberg, J., Olsen, T. S., *Ann. Neurol.* 28, 791 (1990) - [8] Friberg, L., Skyhoj, Olsen, T., Roland, P. E., Lassen, N. A., *Brain* 110, 917 (1987) - [9] Humphrey, P. P. A., Feniuk, W., Marriot, A. S., Tanner, R. J. N., Jackson, M. R., *Eur. Neurol.* 31, 282 (1991) - [10] Lance, J. W., Lambert, G. A., Goadsby, P. J., Zagami, A. S., *Cephalalgia* 9, 7 (1989) - [11] Overmeyer, R. H., *Tomorrow's Med. Today* 12, 33 (1990) - [12] Doenicke, A., Brand, J., Perrin, V., *Lancet* I, 1309 (1988) - [13] Friberg, L., Olesen, J., Iversen, H. K., Sperling, B., *Lancet* 338, 13 (1991) - [14] Diener, H.-C., Peters, C., Rudzio, M., Noe, A., Dichgans, J., Haux, R., Ehrmann, R., Tfelt, Hansen, P. J., *Neurol.* 238, 245 (1991) - [15] Humphrey, P. P. A., Feniuk, W., Perren, M. J., Oxford, A. W., Brittain, R. T., *Drugs Future* 14, 35 (1989) - [16] Dormehl, I., Redelinguys, F., Hugo, N., Oliver, D. W., Pilloy, W., *J. Med. Primatol.* 21, 270 (1992) - [17] Pantano, T., Du Piero, V., Ricci, M., Fieschi, C., Bozzao, L., Lizenzi, C. N. L., *Eur. J. Nucl. Med.* 19, 939 (1992) - [18] Lassen, N. A., Christensen, M. S., *Brit. J. Anaesth.* 48, 719 (1976) - [19] Wood, M., Wood, A. J. J., *Drugs Anaesth.* 2, 255 (1990) - [20] Kuschinsky, W., *Arzneim.-Forsch./Drug Res.* 41 (1), 284 (1991) - [21] Fowler, P. A., Lacey, L. F., Thomas, M., *Eur. Neurol.* 31, 291 (1991) - [22] Moskowitz, M. A., Henrikson, B. M., Markowitz, S., Saito, K., in: *Basic mechanisms of headache*, J. Olesen, L. Edvinsson (eds.), pp. 429-437, Elsevier, Amsterdam (1988)

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## Cerebral Blood Flow Effects of Sumatriptan in Drug Combinations in the Baboon Model

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### Summary

Sumatriptan (CAS 103628-46-2, Imigran<sup>®</sup>) has established itself as an important therapeutic agent in the treatment of migraine. Although considerable understanding of, in particular, the vascular pathophysiology of migraine has been gained during the past decade, the pathophysiology and mediators involved in the pain experience during migraine are not yet fully explained. The mechanisms behind the pharmacological effects of sumatriptan are still only partially understood. In the present study the effects of sumatriptan on drug induced cerebral blood flow increases in the baboon model were investigated using <sup>99m</sup>Tc-HMPAO (hexamethylpropylene amine oxime) and SPECT (single photon emission computed tomography). Sumatriptan selectively reduced drug induced cerebral blood flow increases. The effects of halothane anaesthesia and acetazolamide on cerebral blood flow were not reversed by sumatriptan, while the effect of nimodipine was attenuated by 47% (to the level of cerebral blood flow below the normal flow baseline). These results support multiple mechanisms for sumatriptan involving vascular neurotransmission and neurogenic inflammatory responses via serotonin receptor stimulation and Ca<sup>2+</sup> mobilization. Drug-drug interactions are further implicated through this study.

### Zusammenfassung

Einfluß von Sumatriptan in Arzneimittelkombinationen auf die Hirndurchblutung beim Schimpansen

### 1. Introduction

Sumatriptan (CAS 103628-46-2) has effectively been used in the treatment of acute migraine and is furthermore a useful tool to investigate and elucidate drug mechanisms in migraine and related cerebrovascular headaches. We have recently successfully utilized a baboon model in the monitoring of pharmacological interventions [1-4], using <sup>99m</sup>Tc-labelled hexamethylpropylene amine oxime (HMPAO). Increased cerebral blood flow (CBF) was observed after treatment with the carbonic anhydrase inhibitor, acetazolamide, and the calcium channel blocker, nimodipine [2, 3]. Sumatriptan reversed the increased cerebral blood flow induced by the prolonged anaesthesia [4]. It was also observed that a combination treatment of acetazolamide and nimodipine had no significant beneficial effect and showed a lower increase in the cerebral blood flow when compared with the individual drug responses. The current study focussed on the effects of combination drug treatment of sumatriptan with acetazolamide and sumatriptan with nimodipine in the baboon model, using single photon emission computed tomography (SPECT) techniques, specifically concentrating on the increases and reduc-

Sumatriptan (CAS 103628-46-2, Imigran<sup>®</sup>) ist ein bedeutendes Therapeutikum in der Behandlung der Migräne. Obwohl die Pathophysiologie der Migräne relativ gut bekannt ist, sind die Pathophysiologie und Mediatoren der Schmerzempfindung während der Migräne noch nicht vollständig aufgeklärt. Die Mechanismen der pharmakologischen Wirkungen von Sumatriptan sind ebenfalls nur teilweise bekannt.

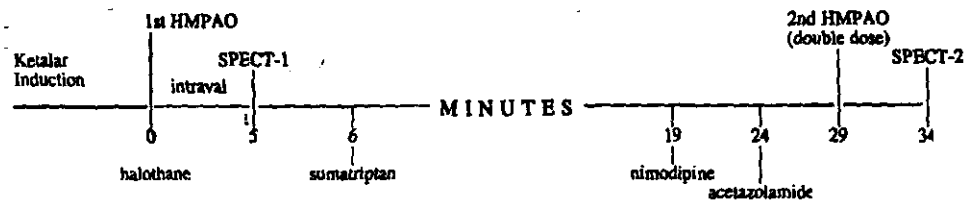
In der vorliegenden Studie wurde der Einfluß von Sumatriptan auf die Arzneistoff-induzierte Erhöhung der Hirndurchblutung am Schimpansenmodell unter Verwendung von <sup>99m</sup>Tc-HMPAO (Hexamethylpropylenaminnoxim) und SPECT (Single-Photon-Emission-Computertomographie) untersucht. Sumatriptan verringerte die Arzneistoff-induzierte Zunahme der Hirndurchblutung. Die Wirkungen einer Halothan-Narkose und von Acetazolamid auf die Hirndurchblutung wurden durch Sumatriptan nicht umgekehrt, während die Wirkung von Nimodipin um 47% verlangsamt wurde (bis die Hirndurchblutung unter den normalen Ausgangswert fiel). Diese Ergebnisse deuten auf mehrfache Mechanismen von Sumatriptan hin: vaskuläre Neurotransmission und neurogene inflammatorische Antworten via Serotonin-Rezeptor-Stimulation und Ca<sup>2+</sup>-Mobilisierung. Darüber hinaus waren Arzneistoff-Interaktionen relevant.

**Key words:** Acetazolamide · Antimigraine drugs · CAS 103628-46-2 · Cerebral blood flow · Halothane · Imigran<sup>®</sup> · Nimodipine · Sumatriptan, pharmacology

tions in CBF previously observed [2, 3, 4]. The objective of the present study was to determine and evaluate possible drug interactions of these combinations in the clinical application and from these results to further gain insight into the pharmacology of these drugs. In addition to the above described procedures the effect of sumatriptan on the increased CBF, which has been observed during halothane anaesthesia [1], was also assessed in order to establish if sumatriptan is able to reverse this effect of halothane and to gain further information on specificity of the pharmacological action of sumatriptan and the interaction with specific anaesthesia regimes.

### 2. Materials and methods

Twelve adult male baboons (*Papio ursinus*, average weight 27 kg), divided into two groups of six each, were used for the investigation. The ages of the baboons are uncertain but they are mature adults of similar age which have been in captivity for four years. They are housed, maintained and cared for according to the guidelines laid down in the National Code for Animal Use in Research, Education, Diagnosis and Testing of Drugs and Related Substances in South Africa. These guidelines are in line with international standards.



min	Procedure A	Procedure B	Procedure C	Procedure D	Procedure E	Procedure F	Procedure G	Procedure H
	ketamine	ketamine	ketamine	ketamine	ketamine	ketamine	ketamine	ketamine
							Halothane	Halothane
0	1st HMPAO	1st HMPAO	1st HMPAO	1st HMPAO	1st HMPAO	1st HMPAO	1st HMPAO	1st HMPAO
	intraval	intraval	intraval	intraval	intraval	intraval		
5	SPECT-1	SPECT-1	SPECT-1	SPECT-1	SPECT-1	SPECT-1	SPECT-1	SPECT-1
6		sumatriptan		sumatriptan		sumatriptan		sumatriptan
19					nimodipine	nimodipine		
24			acetazolamide	acetazolamide				
29	2nd HMPAO	2nd HMPAO	2nd HMPAO	2nd HMPAO	2nd HMPAO	2nd HMPAO	2nd HMPAO	2nd HMPAO
34	SPECT-2	SPECT-2	SPECT-2	SPECT-2	SPECT-2	SPECT-2	SPECT-2	SPECT-2

Fig. 1: Time schedule for the various procedure protocols indicating time of each intervention.

Each animal was subjected to four different procedures (A to D for the one group, and E to H for the second group) (Fig. 1), with at least a six-week interval between the consecutive procedures.

Procedure A was the control study under anaesthesia only [1] in which induction was obtained with ketamine hydrochloride (10 mg/kg<sup>-1</sup> i.v.) followed immediately by an i.v. injection of 148 MBq of <sup>99m</sup>Tc-HMPAO.

The animal was then maintained on an infusion of thiopentone sodium (70 ml/h of 0.05% solution), followed after 5 min by the first single photon emission tomography acquisition (SPECT-1). The data of SPECT-1 represent the HMPAO distribution (uptake and retention) in the brain, resulting from CBF during ketamine sedation [5, 6]. The acquisition with a Siemens Orbiter gamma camera in 64 × 64 word mode, used 32 projections during a 360° rotation, allowing 15 per projection, i.e. about 12 min per rotation. 12 min after the end of tomography, a second i.v. administration of <sup>99m</sup>Tc-HMPAO (296 MBq - double the first dosage) followed, i.e. the split-dose method [7, 8], and 5 min later the second acquisition (SPECT-2) as before. These data represent a CBF pattern of HMPAO under thiopental anaesthesia, but with a background from the first HMPAO distribution under ketamine. After backprojection and reconstruction (Hamming/Hamm) the brain images consisted of two sets (SPECT-1 and SPECT-2), each of four compacted transaxial, sagittal and coronal slices. The two sets represented CBF patterns respectively related to ketamine and to thiopental anaesthesia. Regions of interest (ROIs) were placed on the SPECT-1 and SPECT-2 data, in each view, and counts per pixel obtained from each slice. These values for each slice were inserted into the following equation.

$$R = \frac{[\text{SPECT-2}] (\text{counts/pixel}) - [\text{SPECT-1}] (\text{counts/pixel})}{\text{SPECT-1} (\text{counts/pixel})}$$

R represents the level changes of rCBF during the second anaesthesia, thiopentone with respect to rCBF during ketamine (the first anaesthesia) after subtraction of the background (\*), having been corrected for decay.

Procedure B followed the same protocol as above, except for an additional i.m. injection of sumatriptan (Imigran<sup>®</sup>), 1 min into

the SPECT-1 (ketamine related) acquisition. The double dose of HMPAO administered 12 min after the end of SPECT-1, will therefore represent the rCBF distribution due to sumatriptan (23 min p.i.), when compared to Procedure A. Also for Procedure C the protocol of the baseline Procedure A was followed, but 7 min after completion of SPECT-1, i.e. 5 min before the second double dose of HMPAO, 500 mg of acetazolamide (100 mg/ml) was i.v. administered. Thus SPECT-2 reflected the rCBF 5 min after the acetazolamide injection. In Procedure E, the drug intervention was by nimodipine, which started as an infusion (1 µg/kg/min) 10 min before the second HMPAO injection, and 2 min after the completion of SPECT-1, and continued for 5 min until SPECT-2 started. SPECT-2 would then reflect on rCBF after 10 min of nimodipine.

Procedures D and F concerned the two drug combinations, respectively acetazolamide plus sumatriptan and nimodipine plus sumatriptan as follows: For Procedure D a combination protocol of Procedure B and C is followed, i.e. an injection of sumatriptan 1 min into SPECT-1, and an injection of acetazolamide 7 min after SPECT-1, so that by the time of the second double dosage of HMPAO, 23 min will have passed after sumatriptan administration and 5 min after acetazolamide. Therefore SPECT-2 will reflect rCBF due to a drug combination along the lines of the protocols of the single drugs in Procedures B and C.

Similarly Procedure F (Procedure B and E combined) will have SPECT-1 information on rCBF related to the ketamine anaesthesia as do all other procedures, but SPECT-2 will reflect on the effect of the 10 min nimodipine infusion which will start 2 min after SPECT-1 had ended, plus the effect of sumatriptan after 23 min, having been injected 1 min into SPECT-1.

The last two procedures G and H concerned the possible role of the halothane anaesthesia and the interaction with sumatriptan. The animals were placed on halothane (2% halothane/oxygen, Boyle's machine), immediately after having been immobilized by ketamine. The first injection of <sup>99m</sup>Tc-HMPAO under halothane was followed five minutes later by SPECT-1, as above. 12 min after SPECT-1 another injection of HMPAO (double dose) was given and SPECT-2 followed 5 min later. This procedure was the baseline for Procedure H, which followed the same course but with an injection of sumatriptan 1 min into SPECT-1 and 23 min before the second HMPAO injection.

During all procedures the blood pressure (BP), heart rates (HR) and blood gases were monitored before each drug intervention and before the second HMPAO injection.

<sup>1)</sup> Manufacturer: Glaxo S.A., Midrand (Republic of South Africa).

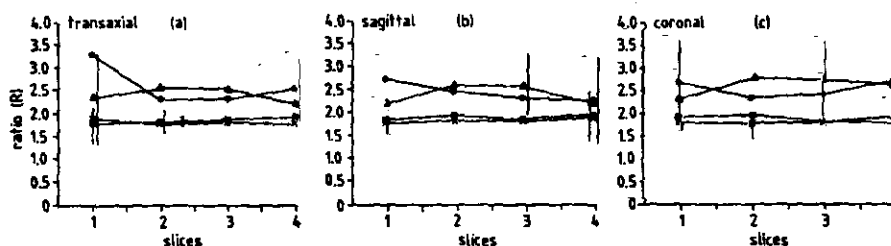


Fig. 2: Curves of mean ratio (R) ( $n = 6$ ) versus slice number starting at the frontal to the occipital lobes transaxially, from right to left of the brain sagittally, and from the cerebellum to the dorsal slice of the cerebrum coronally. ■ Procedure A; × Procedure B; ▲ Procedure C; ● Procedure E. This figure compares single drug interventions with anaesthesia (ketamine-thiopental) baseline. The largest standard deviations for each procedure are indicated.

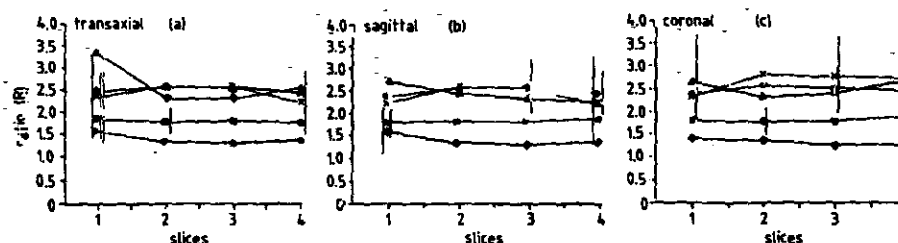


Fig. 3: As in Fig. 2. ■ Procedure B; ▲ Procedure D; ● Procedure F; × Procedure C; ◆ Procedure E. This figure illustrates drug interactions and comparative changes with respect to single drug effects.

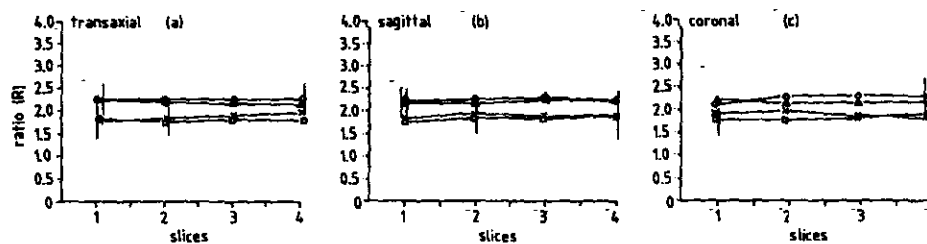


Fig. 4: As in Fig. 2 and 3. × Procedure A; ■ Procedure B; ▲ Procedure G; ● Procedure H. The influence of the nature of anaesthesia is here illustrated.

### 2.1. Statistical methods

Mean ratios  $R$  ( $n = 6$ ) and standard deviations were calculated per procedure for similar regions (slices). An average  $R$ -value was obtained for the total brain for a particular procedure by considering all slices of all views. These were compared for interprocedural effects and the comparisons assessed for significant differences, using Student's two-tailed  $t$ -test for paired variables on a 5% confidence level.

### 3. Results

The results are presented in Fig. 2-4, where the mean ratio ( $\pm$  SD) ( $n = 6$ ) for each slice is depicted in each procedure.

Fig. 2 displays Procedure A (control), and Procedures B, C and E, which are the single drug interventions respectively of sumatriptan, acetazolamide and nimodipine, in the transaxial (a), sagittal (b), and coronal (c) views. In Fig. 3 the single drug sumatriptan intervention (Procedure B) is also compared to the combination drug intervention, Procedures D and F.

The possible role that the anaesthesia can play is illustrated in Fig. 4, where the two control studies without interventions viz the ketamine-thiopentone study (Procedure A) and the halothane study (Procedure G) are compared to corresponding studies with the sumatriptan interventions, i.e. Procedures B and H.

In Table 1 means ( $n = 6$ )  $\pm$  SD of the ratio  $R$  are presented for the total brain (obtained by averaging  $R$  from all slices and all views in a procedure), with percentage changes between procedures, as well as (when applicable) significant changes in HR, BP,  $PCO_2$  and  $PO_2$ .

From Fig. 2 and Table 1 it is clear that the sumatriptan intervention did not change control  $R$ -values statistically significantly ( $1.87 \pm 0.36$  vs  $1.82 \pm 0.22$ ,  $p > 0.05$ ); also not with halothane anaesthesia ( $2.18 \pm 0.32$  vs  $2.23 \pm 0.28$ ,  $p > 0.05$ ; Fig. 4).

Single drug interventions with acetazolamide and nimodipine increased CBF, as reflected in the  $R$ -values, by 32% and 35%, respectively, with respect to the control values of Procedure A. These increases are statistically significant ( $p < 0.05$ ). The drug combinations of sumatriptan plus acetazolamide (Procedure D) and sumatriptan plus nimodipine (Procedure F), influenced CBF in different ways. According to Procedure D the sumatriptan had no statistically significant effect ( $p > 0.05$ ) on the acetazolamide induced increases obvious from Procedures C and A. However, the sumatriptan administered in Procedure F reduced the nimodipine induced increases in CBF from Procedure E statistically significantly ( $2.53 \pm 0.4$  vs  $1.34 \pm 0.32$ ,  $p < 0.05$ ). These reduced  $R$  values from Procedure F are even significantly below the control values of Procedure A ( $p < 0.05$ ).

No significant changes occurred for any of the interventional procedures in HR and  $PCO_2$  taken just before

Table 1: Mean ( $\pm$  SD) ( $n = 6$ ) ratios R for total brain as averaged from all slices and all view for all procedures, percentage changes of these R-values and parameter changes when statistically significant ( $P < 0.05$ ).

	R $\pm$ SD	% Change in R	HR	BP	PCO <sub>2</sub>	PO <sub>2</sub>
Procedure A (Baseline)	1.87 $\pm$ 0.36		-	-	-	
Procedure B Sumatriptan	1.82 $\pm$ 0.22		-	-	-	
Procedure C Acetazolamide	2.49 $\pm$ 0.34		-	-7	-	+20
Procedure D Acetazolamide + Sumatriptan	2.54 $\pm$ 0.32		-	-	-	+7
Procedure E Nimodipine	2.53 $\pm$ 0.40		-	-18	-	
Procedure F Nimodipine + Sumatriptan	1.34 $\pm$ 0.31		-	-55	-	
Procedure G Halothane Baseline	2.18 $\pm$ 0.32		-	-	-	
Procedure H Halothane + Sumatriptan	2.23 $\pm$ 0.28		-	-	-	
Proc A to C <sup>a)</sup>		+32				
Proc A to D		+36				
Proc A to E		+35				
Proc A to F		-28				
Proc B to D		+40				
Proc B to F		-26				
Proc E to F		-47				

<sup>a)</sup> The percentage change is given with respect to the procedure mentioned first.

drug administration and just before the second HMPAO injection. Significant reductions ( $p < 0.05$ ) in BP occurred with the acetazolamide intervention (Procedure C;  $\Delta$ BP = -7), as well as with nimodipine (Procedure E;  $\Delta$ BP = -18), and the combination Procedure F of sumatriptan and nimodipine (-55). PO<sub>2</sub> increased significantly with acetazolamide (+20), and also when in combination with sumatriptan (+7).

#### 4. Discussion

The results clearly indicate that sumatriptan had no effect (potentiation or antagonism) on the increased cerebral blood flow induced by acetazolamide within the current time scale and dose regimes. Similarly, sumatriptan did not influence the halothane anaesthesia, even though halothane pointed to increased CBF, when compared to the ketamine-thiopental alternative [1]. Conversely sumatriptan significantly influenced the increased cerebral blood flow induced by nimodipine. The increased blood flow effect of nimodipine was attenuated by 47% ( $p < 0.05$ ) by simultaneous treatment with sumatriptan. The reduced CBF-values noted in this procedure were also statistically significantly lower than the control values without drug treatment ( $p < 0.05$ ). These results clearly indicate that sumatriptan is a potent antagonist of nimodipine and that the pharmacological reversal of the induced increase in the CBF occurs not for all drug combinations. These results may further suggest that cerebral blood flow attenuation (vascular mechanism) is but one component of the pharmacology of sumatriptan as was previously outlined by Moskowitz [9]. The current data may give further insight into other mechanisms of action of sumatriptan and the pathological mechanisms in migraine.

The current research protocols for acetazolamide and halothane anaesthesia indicate no drug-drug interactions with simultaneous administration of sumatriptan.

The increased CBF due to the action of acetazolamide and halothane was maintained. The blood pressure and oxygen (PO<sub>2</sub>) changes for the single drugs procedures are similar to those previously observed [2, 3]. Interesting is the marked drop in blood pressure in the sumatriptan-nimodipine combination study while a drop in cerebral flow is observed. Compensation responses could explain this occurrence.

Nimodipine, a dihydropyridine calcium channel antagonist has been shown to dilate cerebral arterioles and to increase cerebral blood flow (see review of nimodipine [10]). It has been suggested that nimodipine at a dose of 120 mg/day can effectively be used in the prophylactic treatment of migraine [11]. The potent antagonism by sumatriptan of the nimodipine induced increase in CBF argues against simultaneous treatment of these two drugs. The combination could result in attenuation or diminishing of the effects of nimodipine. This result is however of importance to gain further knowledge on the actions of these drugs in the treatment of migraine.

Serotonin (5-HT) and some specific 5-HT-receptors are implicated in migraine (see reviews of 5-HT and the classification of receptors of 5-HT [12, 13]). Sumatriptan exhibits agonist activity at the 5-HT<sub>1D</sub>- and 5-HT<sub>1</sub> like-receptors [13]. 5-HT<sub>1D</sub>-receptors have been demonstrated in human cerebral arteries [14, 15] and to be of the type 5-HT<sub>1D $\beta$</sub> . Activation of these receptors results in the inhibition of forskolin-stimulated adenylyl cyclase activity [16]. However these receptors have been found to link positively and negatively to adenylyl cyclase depending on the cell line used for the expression of these receptors. Zgombick et al. have recently observed inhibition of adenylyl cyclase and stimulation of calcium mobilization [17]. The marked effect of sumatriptan to reduce the increased CBF due to nimodipine supports the involvement of Ca<sup>2+</sup> in the pharmacology of sumatriptan. This result suggests that sumatriptan is able to normalize the cerebrovascular bed via multiple mechanisms that included amongst others vasoconstriction and influencing Ca<sup>2+</sup> mobilization that might be important in the inflammatory and pain responses that occur through mediators such as nitric oxide (NO), histamine and other neurotransmitters and -peptides.

Moskowitz et al. have researched the neurogenic and vascular mechanisms of sumatriptan in the migraine [18-25]. It was concluded that sumatriptan blocks the vascular headaches through blockade of neural transmission and neurogenic inflammatory responses and that post- and prejunctional 5-HT<sub>1D</sub>- and 5-HT<sub>1</sub> like-receptors are implicated in the pharmacology of sumatriptan. It has been suggested that perivascular neurogenic inflammation around the dural and meningeal arteries could account for the migraine pain and that the process is associated with release of neuropeptide transmitters from perivascular trigeminal nerve endings [18]. Olesen et al. [26] have recently suggested that nitric oxide (NO) may play an important role in migraine and other vascular headaches. It was observed that glyceryl trinitrate (which can be regarded as a pro-drug for NO [27]) causes a reduction in the blood velocity in the middle cerebral artery [28]. No effect on regional cerebral blood flow was observed after glyceryl nitrate treatment indicating an increase dilatation of the middle cerebral artery in migraine sufferers [29]. Furthermore, histamine-induced headache occurs most likely due to the formation of NO in the cranial blood vessels [30, 31]. NO through guanylate cyclase coupling has been postulated to trigger a chain of events that induce smooth muscle relaxation via decreases in cytosolic Ca<sup>2+</sup> concentrations [28, 29]. Sumatriptan apart from its vasoconstrictor effects might exert its therapeutic effects on the pain component of migraine via other mechanisms that might involve Ca<sup>2+</sup> mobilization. The findings that NO could

be involved in migraine through vasodilatation and the induction of migraine-like headaches could be an important mechanism to explain pharmacology of sumatriptan. An inhibitory effect of sumatriptan on NO would result in the antagonism of both the vasodilatation and the possible effects on perivascular sensory nerves of NO. The effect on the calcium concentrations resulting in dilatation of cerebral blood arteries upon NO release could be antagonized by sumatriptan as was observed during the nimodipine combination procedure.

### 5. Conclusion

The current study strongly suggests that sumatriptan is able to reverse induced increases in cerebral blood flow due to pharmacological interventions. However, this reversal clearly does not occur in all the cases and is more specific than initially anticipated. The inability of sumatriptan to influence the acetazolamide and the halothane increases in CBF supports the specificity of sumatriptan, particularly in view of the marked effect on nimodipine. This study was able to establish possible drug-drug interactions involving sumatriptan. The data clearly supports multiple mechanisms of action for sumatriptan during the treatment of migraine that involves both direct action on cerebral blood vessels via stimulation of amongst others 5-HT<sub>1D</sub>-receptors and effects of Ca<sup>2+</sup> mobilization. Sumatriptan's action could also involve the inhibition of endogenous substances, such as NO, histamine and other mediators, that are active in vascular neurotransmission and neurogenic inflammatory responses during migraine. The effect of sumatriptan on these substances is currently being investigated.

### 6. References

- [1] Dormehl, I. C., Redelinghuys, I. F., Hugo, N., Oliver, D. W., Pilloy, W. J., *J. Med. Prim.* 21, 270 (1992) - [2] Oliver, D. W., Dormehl, I. C., Redelinghuys, I. F., Hugo, N., Glass, B., *Nuklear Medizin* 32, 292 (1993) - [3] Dormehl, I. C., Oliver, D. W., Hugo, N., *Nucl. Med. Comm.* 14, 573 (1993) - [4] Oliver, D. W., Dormehl, I. C., Hugo, N., *Arzneim.-Forsch./Drug Res.* 44 (II), 925 (1994) - [5] Bacciottini, L., Pupi, A., Formiconi, A. R., De Cristofaro, M. R. T., Meldolesi, V., *J. Cereb. Blood Flow Metab.* 9, 5414 (1989) - [6] Pupa, A., Bisi, G., Siagara, R., Santoro, D., De Christofaro, M. T. R., Formiconi, A. R., Meldolesi, V., *J. Cereb. Blood Flow Metab.* 9, 5411 (1989) - [7] Wyper, D. J., Hunter, R., Patterson, J., Goodwin, G., McCulloch, J., *J. Cereb. Blood Flow Metab.* 11 (Suppl. 2), 5449 (1991) - [8] Holm, S., Madsen, P. L., Rubin, P., Sperling, B., Friberg, L., Lassen, N., *J. Cereb. Blood Flow Metab.* 11 (Suppl. 2), S766 (1991) - [9] Moskowitz, M. S., *TIPS* 13, 307, 1992 - [10] Langley, M. S., Sorkin, E. M., *Drugs* 27, 669 (1989) - [11] Gawel, M. A., *Cephalgia* 7, 453 (1987) - [12] Humphrey, P. P. A., Apperley, E., Feniuk, W., Perren, M. J., in: *Cardiovascular Pharmacology of 5-Hydroxytryptamine*, pp. 417-431, Kluwer Academic, Dordrecht, The Netherlands (1991) - [13] Hoyer, D., Clarke, D. E., Fozard, J. R., Hartig, P. R., Martin, G. R., Mylcharene, E. J., Saxena, P. R., and Humphrey, P. P. A., *Pharmacol. Rev. Pharmacol. Rev.* 46, 157 (1994) - [14] Hamel, E., Grègore, L., Lau, B., *Eur. J. Pharmacol.* 242, 75 (1993) - [15] Hamel, E., Fan, E., Linville, D., Ying, V., Villemure, J. G., Chia, L. S., *Mol. Pharmacol.* 44, 242 (1993) - [16] Hoyer, D., Schoeffter, P., *Eur. J. Pharmacol.* 147, 145 (1988) - [17] Zgombick, J. M., Borden, L. A., Cochran, T. L., Kucharewicz, S. A., Weinschank, R. L., Branchek, T. A., *Mol. Pharmacol.* 44, 575 (1993) - [18] Moskowitz, M. A., Macfarlane, R. C., *Metab. Rev.* 3, 1 (1993) - [19] Moskowitz, M. A., *Cephalgia* 12, 5, 1992 - [20] Buzzi, M. G., Sakas, D. E., Moskowitz, M. A., *Eur. J. Pharmacol.* 165, 251 (1989) - [21] Buzzi, M. G., Moskowitz, M. A., *Br. J. Pharmacol.* 99, 202 (1990) - [22] Saito, K., Markowitz, S., Moskowitz, M. A., *Ann. Neurol.* 224, 732 (1988) - [23] Markowitz, S., Saito, K., Moskowitz, M. A., *J. Neurosci.* 7, 4129 (1987) - [24] Matsubara, T., Moskowitz, M. A., Byun, B. I., *Br. J. Pharmacol.* 104, 3 (1991) - [25] Nozaki, K., Moskowitz, M. A., Boccacini, P., *Br. J. Pharmacol.* 106, 405 (1992) - [26] Olesen, J., Thomsen, L. L., Iversen, H. K., *TIPS* 15, 149 (1994) - [27] Rand, M., *Clin. Exp. Pharmacol. Physiol.* 19, 147 (1992) - [28] Thomsen, L. L., Iversen, H. K., Brinck, T. A., Olesen, J., *Cephalgia* 13, 395 (1993) - [29] Dahl, A., Russel, D., Nyberg-Hansen, R., Rootwell, K., *Stroke* 20, 173 (1989) - [30] Toda, N., *Am. J. Physiol.* 258, H311 (1990) - [31] Ottosson, A. L. P., Jansen, I., Langemark, M., Olesen, J., Edvinsson, L., *Cephalgia* 11, 183 (1991)

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## A Comparative Cerebral Blood Flow Study in a Baboon Model with Acetazolamide Provocation: $^{99m}\text{Tc}$ -HMPAO vs $^{123}\text{I}$ (IMP)

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Pharmacological interactions are important when nuclear medical procedures are applied to patients under drug therapy, or drug provocation. This study compares in baboon models (regional) cerebral blood flow [(r)CBF] results from  $^{99m}\text{Tc}$ -HMPAO and  $^{123}\text{I}$ -iodoamphetamine [ $^{123}\text{I}$ (IMP)] each with and without acetazolamide, the latter a suggested drug for testing cerebrovascular reserve. Expected differences in cerebral uptake were observed between the two radio-tracers without acetazolamide. The increase in tracer uptake resulting from acetazolamide is significantly enhanced for  $^{123}\text{I}$ (IMP), which could have diagnostic implications.

### Introduction

Drug combinations when administered simultaneously have the potential to interact with each other on two main pharmacological levels, viz pharmacokinetically and/or pharmacodynamically (Stockley, 1991). Interactions are, therefore, also to be expected for radiopharmaceuticals when administered with drugs. This should be considered when nuclear medical procedures are applied to patients under drug therapy, when drugs are used provocatively to facilitate nuclear medical diagnosis (e.g. acetazolamide and cerebrovascular reserve, Devous *et al.*, 1992), and also when used to evaluate drugs in pharmaceutical development and for pharmacological responses.

Comparisons of the drug-induced behaviour of different radiopharmaceuticals during the above procedures are important in order to interpret differences in radiopharmaceutical responses, to understand and anticipate possible interactions and to acquire and quantify datasets obtained from different radiopharmaceuticals for practical diagnostic applications. With the above in mind a study was done to assess the results obtained from (regional) cerebral blood flow [(r)CBF] measurements using  $^{99m}\text{Tc}$ -hexamethylpropylene amine oxime (HMPAO) and *N*-isopropyl-*p*-(123)-iodoamphetamine,  $^{123}\text{I}$ (IMP), with and without acetazolamide [Diamox, S.A.

Cyanamid (Pty) Ltd] in a baboon model. Differences are explained in terms of induced metabolic acidosis, and alkaline urine, following from acetazolamide.

### Materials and Methods

Six adult male baboons (average weight, 27 kg) were used for the investigation. The studies were performed after approval by the Ethics Committee of the University of Pretoria, according to the guidelines of the National Code for Animal Use in Research, Education Diagnosis and Testing of Drugs and Related Substances in South Africa. Each animal was subjected to four different procedures (A-D), with at least a six-week interval between consecutive procedures. Procedures A and C were control studies under anaesthesia only (Dormehl *et al.*, 1994) in which induction was performed with ketamine hydrochloride (10 mg/kg i.v.) (Ketalar, Parke Davis, Cape Town) and immediately followed by a maintained controlled infusion of thiopentone sodium (Intraval, Sandoz S.A., Randburg) (70 mL/hr of a 0.5% solution) using an administration (drip) set. After a 10 min stabilization period under thiopentone, procedure A continued with an i.v. injection of 148 MBq of  $^{99m}\text{Tc}$ -HMPAO. Five minutes later the first SPECT acquisition (SPECT-1) followed with a Siemens Orbiter gamma camera, using 32 projections during a 360° rotation (10 per view). The baboons were viewed in a supine position with a special head rest to ensure a reproducible position to compare tomographic

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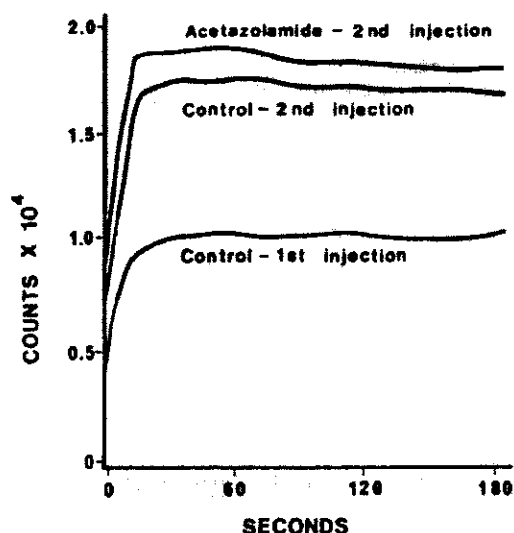


Fig. 1. Time-activity curves representing input functions from the dynamic acquisition on countdown of the first  $^{99m}\text{Tc}$ -HMPAO injection (control anaesthesia, first injection), of the second  $^{99m}\text{Tc}$ -HMPAO injection (control anaesthesia, second injection), and of the second  $^{99m}\text{Tc}$ -HMPAO injection with acetazolamide, the latter two from double dosages of the tracer.

slices (Dormehl *et al.*, 1992). SPECT-1 was immediately followed by a second i.v. administration of  $^{99m}\text{Tc}$ -HMPAO (296 MBq, i.e. double the first dose), and 5 min later by the second similar SPECT acquisition (SPECT-2): the split-dose method (Holm *et al.*, 1991; Wyper *et al.*, 1991). The data from SPECT-1 and SPECT-2 represent HMPAO distributions (uptake and retention) in the brain resulting from CBF at two different times of prolonged thiopentone anaesthesia (Bacciottini *et al.*, 1989; Pupi *et al.*, 1989). SPECT-2 data also contain background from the first HMPAO distribution.

Procedure C differed from procedure A by replacement of the two administrations of  $^{99m}\text{Tc}$ -HMPAO by, respectively, 148 and 296 MBq of  $^{123}\text{I}$ (IMP) at corresponding times (as in procedure A) during the thiopentone anaesthesia.

Procedures B and D followed the same protocols, respectively, as procedures A and C until completion of the first SPECT acquisition (SPECT-1) but then this was immediately succeeded by an intravenous injection of 500 mg of acetazolamide (Oliver *et al.*, 1993; Dormehl *et al.*, 1993). Procedures B and D were continued 10 min later by, for procedure B, a second HMPAO injection (296 MBq), and similarly for procedure D, 296 MBq of  $^{123}\text{I}$ (IMP), to be followed after 5 min by SPECT-2 in both cases. Procedures B and D will, therefore, reflect on the effect of acetazolamide (10 min post administration) with respect to anaesthesia-only baselines using the two tracers HMPAO (procedure A) and amphetamine (procedure C) (Oliver *et al.*, 1994).

The split-dose method is based on the chemical properties of the tracer that crosses the blood-brain barrier and is trapped in brain cells. With HMPAO it becomes important to check radiochemical purity before its first application (within 5 min of preparation with a fresh eluate). For kit preparation each vial of HMPAO was reconstituted with 1110 MBq (30 mCi) of saline diluted  $^{99m}\text{Tc}$  in a 5 mL syringe. The lipophilic complex which determines the required lipophilic character and subsequent high brain retention (Ell *et al.*, 1985a, b; Holmes *et al.*, 1985; Nowotnik *et al.*, 1985) was never found to be below 90%. This was checked in each case using chromatography on ITLC SG strips with methylethylketone (MEK) and saline as solvent.  $^{99m}\text{Tc}$ -HMPAO as a lipophilic complex runs with the solvent front in MEK but remains at the origin in saline. With time it could convert to a secondary complex which remains at the origin in both

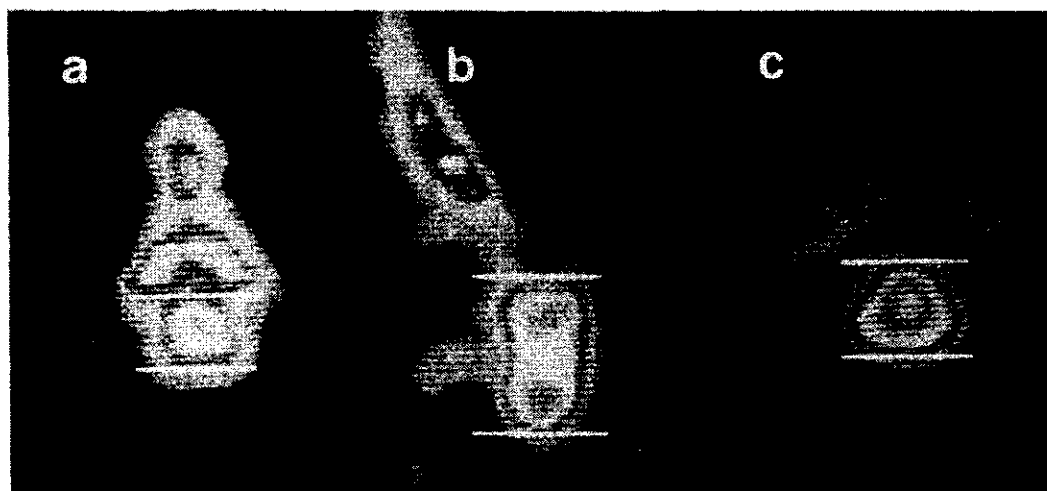


Fig. 2. Typical tomographic brain slices in the coronal (a), sagittal (b), and transaxial (c) views, indicating the regions of interest (ROI), i.e. the total brain.

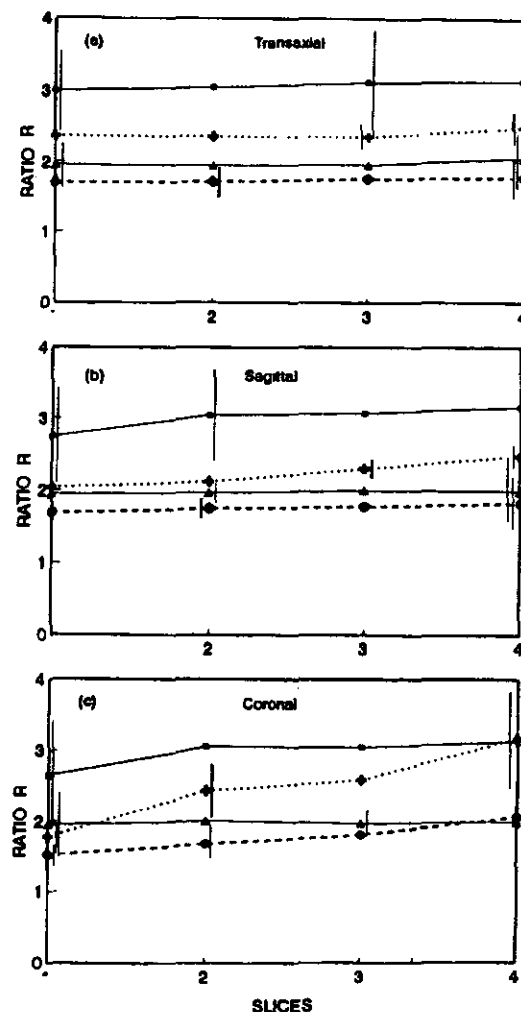


Fig. 3. Curves of the mean ratio  $R$  ( $n = 6$ ) versus slices number (region) starting at the frontal lobes to the occipital lobes transaxially (a), from right to left of the brain sagittally (b), and from the cerebellum to the dorsal slice of the cerebrum coronally (c). For each procedure the smallest and largest standard deviations are indicated. Ratio  $R$  represents the level of change of rCBF during prolonged anaesthesia as a baseline, or during prolonged anaesthesia and acetazolamide intervention. Procedure A (●), Procedure B (+), Procedure C (▲), Procedure D (■).

systems. The second injection of HMPAO following the first by 20–30 min (depending on the procedure) was from the same  $^{99m}\text{Tc}$ -HMPAO preparation as the first and was accompanied on a count down by a dynamic data acquisition (15 s per image for 4 min). Such a dynamic acquisition was also done with the first injection of HMPAO (Fig. 1). The shape of the time-activity curves thus obtained would reflect on the possibility of reduction of lipophilic  $^{99m}\text{Tc}$ -HMPAO. A tendency to wash out would prompt the termination of the study. Unchanged input functions with respect to the first HMPAO injection for SPECT-1 as seen in Fig. 1 for the second double dose injection (with and

without diamox) permitted continuation with SPECT-2.

The efficacy of  $^{123}\text{I}$ (IMP) is also a consequence of its lipophilicity, the initial uptake being a measure of perfusion while progressive brain accumulation is probably a combined sequence of intravascular/extravascular intracerebral pH gradients, favourable brain lipid/aqueous partition coefficients, and the affinity for high-capacity, relatively non-specific binding sites for amines located in the brain and/or brain capillary endothelium. High levels of brain activity are maintained for several hours (Winchell *et al.*, 1980a, b).

Following back-projection and reconstruction the brain images consisted of two sets of four compacted slices in the transaxial, sagittal and coronal views, the two sets representing rCBF patterns from SPECT-1 and SPECT-2 for each procedure. Regions of interest (ROIs) were placed for the brain in the slices from SPECT-1 and -2 data in each view, for all the procedures, and counts per pixel obtained from each slice (Fig. 2). These values for each slice were inserted into the following equation, for each procedure:

$$R =$$

$$\frac{[\text{SPECT-2}] (\text{counts/pixel}) - [\text{SPECT-1}]^* (\text{counts/pixel})}{\text{SPECT-1} (\text{counts/pixel})}$$

$R$  represents the level of change of rCBF during prolonged anaesthesia as a baseline, or during prolonged anaesthesia and acetazolamide intervention.  $[\text{SPECT-1}]^*$  is decay corrected (Dormehl *et al.*, 1992).

From the individual baboon values mean regional and total brain ratios  $R$  were evaluated in each view for each of the procedures A–D, and compared for possible regional as well as procedural effects. The comparisons were assessed for significant differences using Student's two-tailed  $t$ -test at 1 and/or 5% level of confidence. The regional information refers to the tomographic slices rather than to anatomical structures.

## Results

The results are summarized in Fig. 3a–c, where the mean ( $n = 6$ ) ratios  $R$ , indicating level changes in HMPAO and IMP uptake and retention during prolonged thiopentone anaesthesia, and because of the administration of acetazolamide are shown for four compacted slices of the brain in three views:

Table 1. Percentage changes\* in total brain uptake of HMPAO and IMP because of the various procedures

Procedures	Percentage change	Statistical significance $P$ -values
		$P > 0.05$
		$P < 0.01$
		$P < 0.01$
		$P > 0.05$
		$P < 0.01$
		$P < 0.05, P > 0.01$

\*Percentage changes are expressed with respect to the procedure mentioned first.

transaxial, sagittal, and coronal. The smallest and largest standard deviations are given for each procedure.

Since no statistically significant regional differences ( $P > 0.05$ ) became apparent from the data (i.e. no statistically significant relative region-related changes in the curves, also not for procedure B in the dorsal region, Fig. 3c, the uptake and retention of the tracer in each procedure was equated to the average  $R$ -value from all the slices in all views, and referred to as the total brain value for that procedure. Changes in these average total brain  $R$ -values are summarized in Table 1 as percentage differences due to the various procedures.

It becomes apparent from Table 1 that the cerebral uptake of  $^{123}\text{I}(\text{IMP})$  is higher than of  $^{99\text{m}}\text{Tc-HMPAO}$  by about 13% ( $P > 0.05$ ) which agrees well with known data (Winchell *et al.*, 1980a, b; Dormehl *et al.*, 1994). No meaningful regional differences appear in the intraprocedural dis-

tribution of the two tracers, also not inter-procedural.

The influence of acetazolamide on rCBF when using  $^{99\text{m}}\text{Tc-HMPAO}$  as a tracer is one of a 37% increase (Table 1; Fig. 4a). This is significant ( $P < 0.01$ ) and in line with previous observations (Dormehl, 1993; Oliver *et al.*, 1993). Interesting is, however, the tendency of elevated rCBF (although not statistically significant,  $P > 0.05$ ) owing to acetazolamide in slice number 4 of the coronal view (Fig. 3c). This region is a dorsal representation of the brain and the observation has previously also been made (Dormehl, 1993), but remains unexplained, and maybe even meaningless.

The increased CBF owing to acetazolamide, when using  $^{123}\text{I}(\text{IMP})$  is enhanced (52%;  $P < 0.01$ ), with respect to  $^{99\text{m}}\text{Tc-HMPAO}$  (Fig. 4b). The difference 37 vs 52% is statistically significant ( $P < 0.01$ ). Regional patterns do not seem to be present.

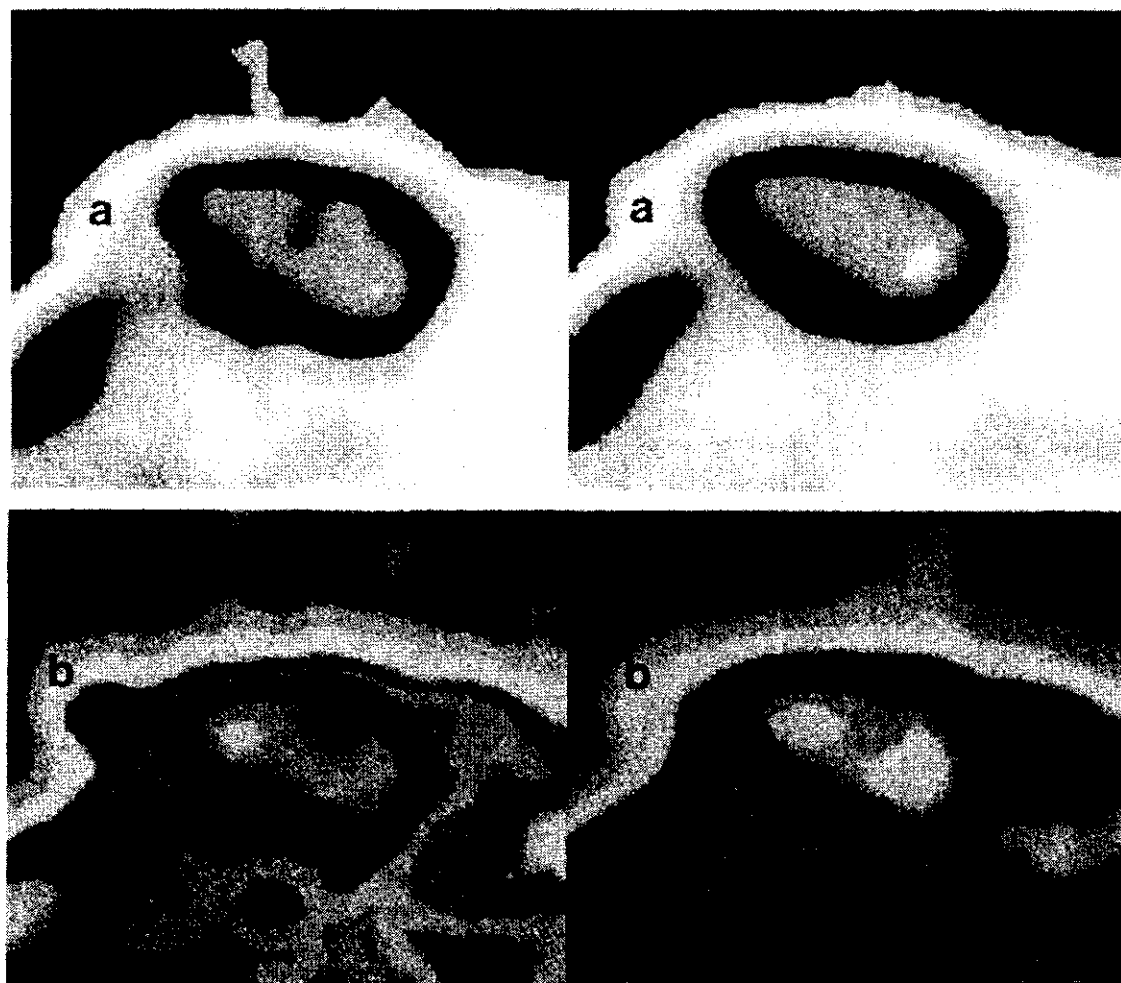


Fig. 4. Sample images in the sagittal view for Procedure B, with  $^{99\text{m}}\text{Tc-HMPAO}$  (a) and for Procedure D, with  $^{123}\text{I}(\text{IMP})$  (b). The first image in each pair, from the splitdose method, represents the distribution in the brain from the first injection (SPECT-1). The second image represents the subtracted image (SPECT-2 - SPECT-1), to illustrate the effect of acetazolamide.

### Discussion

Amphetamine-like drugs are all basic compounds that are able to penetrate the blood-brain barrier owing to their fairly high degree of lipophilicity. Drugs of this type are primarily excreted by the kidneys and reabsorption through the kidney tubules can occur which is dependent on the urinary pH. In acid urine these basic compounds are ionized (lipid insoluble) and are unable to diffuse back, and are subsequently excreted in the urine. Conversely alkaline urine would result in an increase of the serum levels of basic drugs owing to increase in the reabsorption with subsequent higher concentrations in the central nervous system compartment. Acetazolamide, a carbonic anhydrase inhibitor is known to induce metabolic acidosis and alkaline urine (Gerhardt *et al.*, 1969). It has previously been reported that the interaction between acetazolamide and quinidine may lead to quinidine intoxication due to increased serum levels because of the alkaline urine induced by acetazolamide (Goodman and Gilman, 1990). A similar mechanism could account for the higher values observed for IMP. IMP is, therefore, more susceptible to possible drug interactions (in particular those with marked influences on systemic and urine pH) than technetium-labelled HMPAO, and this could explain the enhanced CBF-effect with IMP and acetazolamide.

The mean *R*-values for the two acetazolamide procedures B and D respectively using HMPAO and IMP to measure rCBF, differ by about 26% ( $P > 0.05$ ). Procedure D yields the higher values and the percentage change exceeds that found between the baseline procedures A and C, i.e. 26 vs 13%, which is statistically significant. This corresponds with the enhanced effect of acetazolamide on rCBF when measured with IMP.

In the light of these observations greatly over- and under-estimated changes in CBF can be expected, respectively, between procedures A and D, and C and B. This would logically argue against dual-isotope studies [ $^{99m}\text{Tc}$ -HMPAO and  $^{123}\text{I}$ (IMP)] in brain-stress testing after pharmacological intervention with acetazolamide for evaluating vasodilatory reserve.

Furthermore, the extent and reason for the acetazolamide-induced effect on CBF, which differ for  $^{99m}\text{Tc}$ -HMPAO and  $^{123}\text{I}$ (IMP) should also be considered when selecting the appropriate tracer for the diagnostic investigation or for experimental investigative purposes.

### Conclusions

The current study clearly illustrates the importance of comparative nuclear medical studies in order to gain better insight into pharmacodynamical and pharmacokinetic interactions between drugs and radiopharmaceuticals. These data further emphasize that drugs that influence metabolic parameters

systemically and urinary could indeed alter the biodistribution of radiopharmaceuticals. Even when such changes are subtle they could still be meaningful and important.

### References

- Bacciottini L., Pupi A., Formiconi A. R., De Cristofaro M. T. R. and Meldolesi U. (1989) Regional cerebral blood flow measurement in rat with HM-PAO and the indicator fractionation technique. *J. Cereb. Blood Flow Metab.* 9, S414.
- Bonte F. J., Devous M. D. and Reisch J. D. (1988) The effect of acetazolamide on regional cerebral blood flow in normal human subjects as measured by single photon emission computed tomography. *Inves. Radiol.* 23, 564-568.
- Devous M. D., Payne K. J. and Lowe J. L. (1992) Dual-isotope brain SPECT imaging with technetium-99m and iodine-123: clinical validation using xenon-133 SPECT. *J. Nucl. Med.* 33, 1919-1924.
- Dormehl I. C., Redelinghuys I. F., Hugo N., Oliver D. and Pilloy W. (1992) The baboon model under anesthesia for *in vivo* cerebral blood flow studies using SPECT techniques. *J. Med. Prim.* 21, 270-274.
- Dormehl I. C., Oliver D. W. and Hugo N. (1993) Dose response from pharmacological interventions for CBF changes in a baboon model using  $^{99m}\text{Tc}$ -HMPAO and SPECT. *Nucl. Med. Commun.* 14, 573-577.
- Dormehl I. C., Pilloy W. P., Hugo N. and Rossouw D. T. (1994) Regional cerebral blood flow patterns using  $^{123}\text{I}$ (IMP) and  $^{99m}\text{Tc}$ -HMPAO with SPECT in the normal anaesthetised baboon. *Eur. J. Nucl. Med.* 21, 848.
- Ell P. J., Cullum I., Costa D. C., Jarritt P. H., Hocknell J. M. L. and Lui D. (1985a) Regional cerebral blood flow mapping with a new Tc-99m labelled compound. *The Lancet* 50-51.
- Ell J. P., Hocknell J. M. L., Jarritt P. A., Pickett R. D., Canning L. R., Cullum I., Lui D., Compas-Costas D., Nowotnik D. P. and Neirinckx R. D. (1985b) A Tc-99m-labelled radiotracer for the investigation of cerebral vascular disease. *Nucl. Med. Commun.* 6, 437-441.
- Gerhardt, R. E., Knouss R. F., Thyrium P. T., Luchi R. J. and Morris J. J. (1969) Quinidine excretion in aciduria and alkaluria. *Ann. Inter. Med.* 71, 927.
- Goodman L. S. and Gilman A. G. (1990) In *The Pharmacological Basis of Therapeutics*, 8th Edn (Edited by Gillman A. G., Rall T. W., Nies A. S. and Taylor P.), pp. 716-717. Pergamon Press, New York.
- Holm S., Madsen P. L., Rubin P., Sperling B., Friberg L. and Lassen N. (1991) Tc-99m HM-PAO activation studies: validation of the split-dose, image subtraction approach. *J. Cereb. Blood. Flow. Metab.* 11, S766.
- Holmes R. A., Chaplin S. B., Royston K. G., Hoffman T. J. and Volkert W. A. (1985) Cerebral uptake and retention of Tc-99m hexamethylpropylene amine oxime (Tc-99m HM-PAO). *Nucl. Med. Commun.* 6, 443-447.
- Nowotnik D. P., Canning L. R., Cumming S. A., Harrison R. C., Higley B., Nechvatal G., Pickett R. D., Piper I. M., Bayne V. J., Forster A. M., Weisner P. S. and Neirinckx R. D. (1985) Development of a  $^{99m}\text{Tc}$ -labelled radiopharmaceutical for cerebral blood flow imaging. *Nucl. Med. Commun.* 6, 499-506.
- Oliver D. W., Dormehl I. C., Redelinghuys I. F., Hugo N. and Beverley G. (1993) Drug effects on cerebral blood flow in the baboon model. Acetazolamide and nimodipine. *Nucl. Med.* 32, 292-298.
- Pupi A., Bisi G., Siagra R., Santoro D., De Cristofaro M. T. R., Formiconi A. R. and Meldolesi U. (1989) The comparison of brain distribution of HM-PAO and microspheres in humans. *J. Cereb. Blood. Flow Metab.* 9, S411.

- Stockley I. H. (1991) *Drug Interactions, A Sourcebook of Drug Interactions, Their Mechanisms, Clinical Importance and Management*, 2nd Edn. Blackwell Scientific Publications, London.
- Winchell H. S., Horst W. D., Braun L., Oldendorf W. H. and Hattner R. (1980a) *N*-Isopropyl-(I-123) *p*-iodoamphetamine; single pass brain uptake and washout; binding to brain synaptosomes and localisation in dog and monkey brain. *J. Nucl. Med.* 21, 947-953.
- Winchell H. S., Baldwin R. M. and Lin T. H. (1980b) Development of I-123-labelled amines for brain studies: Localization of I-123-iodophenylalkyl amines in rat brain. *J. Nucl. Med.* 21, 940-946.
- Wyper D. J., Hunter R., Patterson J., Goodwin G. and McCulloch J. (1991) A split-dose technique for measuring changes in cerebral blood flow patterns. *J. Cereb. Blood Flow Metab.* 11, S449.

- to detect hemodynamic compromise in occlusive cerebrovascular disease. *Stroke* 1992;23:1733-1742.
6. Layton WM Jr, Hallery DW. Deformity of forelimb in rats: association with high doses of acetazolamide. *Science* 1965;149:306-308.
7. Karasawa J, Kikuchi H, Yamagata S, et al. Cerebral hemodynamics in moyamoya disease. Recovery of cerebral blood flow after hyperventilation. *Neurol Med Chir* 1988;28:327-332.
8. Shiv KS, Donald HW, Noor MG, et al. Epidural anesthesia for a patient with moyamoya disease presenting for cesarean section. *Anesth Analg* 1994;79:183-185.
9. Harald D, Siegfried E, Annemarie B, et al. Radiopharmacokinetics and radiation dose from  $^{99m}\text{Tc}$ -HM-PAO (preliminary report). *Eur J Nucl Med* 1987;13:429-431.
10. United Nations Scientific Committee on the Effects of Atomic Radiation. Sources and effects of ionizing radiation. UNSCEAR 1993 Report to the General Assembly with Scientific Annexes. New York: United Nations; 1993.
11. Cosor M, Steven F, John EP, et al. Hepatic uptake of technetium-99m HM-PAO in a fetus. *J Nucl Med* 1990;31:237-239.

## Technetium-99m-HMPAO, Technetium-99m-ECD and Iodine-123-IMP Cerebral Blood Flow Measurements with Pharmacological Interventions in Primates

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Technetium-99m-bicisate ethyl cysteinyl dimer (ECD) presents a different pattern from cerebral blood flow (CBF) in the subacute phase of cerebral infarction, as measured by PET, perhaps due to lack of oxygen and enzyme activity; this pattern is contrary to that of hexamethyl-propyleneamine oxime (HMPAO) but similar to that of N-isopropyl- $^{123}\text{I}$ -iodoamphetamine ( $^{123}\text{I}$ IMP). This study explores possible CBF differences among HMPAO, ECD and IMP, with various relevant drug interventions. **Methods:** Anesthetized adult baboons were used in these SPECT studies. Four studies ( $n = 6$  baboons for each study), one control study and three intervention studies involving intravenous acetazolamide, nimodipine infusion and intramuscular sumatriptan, were followed with  $^{99m}\text{Tc}$ -HMPAO,  $^{99m}\text{Tc}$ -ECD and  $^{123}\text{I}$ IMP. The split-dose method was used as follows. For each tracer, intervention data from the second SPECT (SPECT-2) after the second tracer injection (444 MBq) reflected a change in CBF with respect to the baseline SPECT (SPECT-1) data from the initial injection (222 MBq). These changes as a ratio,  $R$  ( $R = \text{SPECT-2/SPECT-1}$ ), for each study, and the  $R$  values for each tracer were compared to  $R$  values from the corresponding control studies, yielding a quantitative estimate of drug effects. **Results:** There were no significant differences ( $p > 0.05$ ) between HMPAO and ECD for the control, acetazolamide and sumatriptan studies, but there was indeed a difference between the two for the nimodipine study, indicating a nimodipine-dependent underestimation of CBF with ECD (and also with IMP), with respect to HMPAO. A further significant difference was that larger CBF increases were observed with acetazolamide, as measured with  $^{123}\text{I}$ IMP. **Conclusion:** This is a crucial observation for the clinical interpretation of CBF SPECT data and should direct the choice of tracer for a specific examination.

**Key Words:** drug-tracer interaction; CBF SPECT; baboon model  
*J Nucl Med* 1997; 38:1897-1901

There is considerable interest in the development of tracers to measure cerebral blood flow (CBF) with SPECT. Such tracers should be trapped in the brain long enough so that their distribution can be quantitated and should demonstrate good spatial resolution.

Among the tracers that have been found useful are the iodine-labeled amines, e.g., N-isopropyl- $^{123}\text{I}$ -iodoamphetamine ( $^{123}\text{I}$ IMP) (1). Its uptake linearly corresponds to a wide range of flow, as assessed by microspheres (2). The brain

retention of IMP will be a balance of washin and washout, which in turn will be influenced by blood flow, a retention mechanism that is stereoselective, and by metabolism of the tracer (3). Despite its widespread use as a cerebral blood perfusion agent, IMP appears to redistribute in the brain with time (4). Of several  $^{99m}\text{Tc}$ -labeled compounds synthesized as cerebral perfusion agents,  $^{99m}\text{Tc}$ -hexamethyl-propyleneamine oxime ( $^{99m}\text{Tc}$ -HMPAO) has been used extensively, in spite of its unfavorable stability after preparation. Its retention in the brain is limited to the enzymatic reactions with glutathione, of which there is a high prevalence (5,6). The CBF agent N,N'-1,2-ethylene-di-yl-bis-L-cysteinyl diethyl ester, labeled with  $^{99m}\text{Tc}$ -bicisate ethyl cysteinyl dimer (ECD), has a high initial brain extraction with a slow clearance (7). The retention in the brain is associated with stereospecific deesterification to hydrophilic acid derivatives (8,9). As a CBF agent in healthy subjects, it corresponds well with  $^{133}\text{Xe}$  (10), although ECD underestimates higher flow rates, as HMPAO is also known to do. However, in cases of subacute stroke, ECD failed to show reflow hyperemia in the infarct area, contrary to the action of HMPAO (11,12) but similar to the known action (albeit to a lesser extent) of  $^{123}\text{I}$ IMP (13).

It is important to know and understand quantitative and qualitative differences that are related to CBF, as measured by the various CBF agents. Such differences between the tracers may occur during various pathological conditions, as well as after relevant pharmacological interventions. Changes in the metabolic states of the brain appear to influence the kinetics and net accumulation of  $^{99m}\text{Tc}$ -HMPAO at the cellular level by modifying the uptake, the backdiffusion or both (14). Studies comparing these tracers under pharmacological intervention conditions have not yet been reported, and these comparisons were the aim of this study.

The drugs used for this purpose were chosen from previous studies reported in the literature. Acetazolamide has been used frequently in neuro-SPECT studies to evaluate cerebrovascular reserve. The recently developed lipophilic dihydropyridine calcium channel blocker, nimodipine, demonstrates superiority in its influence on CBF compared to other calcium channel blockers and has been used for migraine and dementia (15). The recent introduction of the 5-HT<sub>1D</sub>-agonist, sumatriptan, for the treatment of migraine has been a therapeutic breakthrough, with its undoubtable influence on abnormal CBF.

Drug intervention on CBF can ideally be investigated by the

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split-dose technique, whereby two doses of the tracer are administered within a ~1-hr interval (16,17), with the scan after the first administration acting as a control for the scan after the second administration, which is made with the subject at the appropriate response time of the drug to be evaluated. The baboon model, together with the split-dose method used in this study, has repeatedly proven to be ideally suited to the investigation of pharmacological interventions (18-21).

This study reports the results of the above-mentioned pharmacological interventions on CBF as measured by the tracers  $^{99m}\text{Tc}$ -HMPAO,  $^{99m}\text{Tc}$ -ECD and [ $^{123}\text{I}$ ]IMP, using the baboon model and split-dose SPECT.

#### MATERIALS AND METHODS

Adult male baboons (*Papio ursinus*; average weight, 27 kg) were used for this investigation. The studies performed were approved by the Ethics Committee of the University of Pretoria, according to the guidelines of the National Code for Animal Use in Research, Education and Testing of Drugs and Related Substances in South Africa.

Four different procedures, with six animals per procedure, were performed for each of the three CBF tracers,  $^{99m}\text{Tc}$ -HMPAO,  $^{99m}\text{Tc}$ -ECD and [ $^{123}\text{I}$ ]IMP.

Procedure A was a control experiment with no pharmacological intervention, in which an intravenous injection of 222 MBq of  $^{99m}\text{Tc}$ -HMPAO or, alternatively,  $^{99m}\text{Tc}$ -ECD was administered at time zero into a baboon sedated with 10 mg/kg ketamine hydrochloride intramuscularly (Ketalar; Parke Davis, Cape Town, South Africa). This served as the induction of anesthesia and was followed immediately by a maintained controlled infusion of a 0.5% solution of thiopentone sodium [Intraval, Rhône-Poulenc, Rorer, South Africa (Pty) Ltd.] at 70 ml/hr using an administration (drip) set. Five minutes after the tracer injection, the first SPECT acquisition (SPECT-1) began. The Siemens Orbiter gamma camera performed 32 projections during a 360° rotation, at 20 sec per view. The baboons were viewed in the supine position with a special head rest to ensure reproducible repositioning for comparable tomographic slices. SPECT-1 was followed by a second intravenous administration of tracer  $^{99m}\text{Tc}$ -HMPAO or  $^{99m}\text{Tc}$ -ECD, at double the first dose (i.e., 444 MBq) and at a time 29 min after the first injection and 5 min before a second similar SPECT acquisition, SPECT-2: this procedure is known as the split-dose method (16,17). The data from SPECT-1 and SPECT-2 represent HMPAO or ECD distribution (uptake and retention) in the brain, resulting from CBF during ketamine hydrochloride and maintained (~29 min) thiopentone anesthesia, respectively. Procedure A, therefore, served as the procedure that represented the effects of anesthesia on CBF (18) and explained bioavailability changes of the tracer when comparisons were drawn between the interventional studies.

Procedure B was the same as Procedure A until the completion of SPECT-1, which was then succeeded by an intravenous injection of 500 mg of acetazolamide (Diamox; South Africa Cyanamid (Pty) Ltd.), at 24 min, i.e., 5 min before the second tracer administration. Thus, SPECT-2, which again followed 5 min after the second  $^{99m}\text{Tc}$ -HMPAO/ECD injection, as in Procedure A, reflected the influence of acetazolamide (5-min response time) on CBF.

Procedure C had the same protocol as Procedure B, but the intervention was an infusion of nimodipine (Nimotop IV; Bayer (Pty) Ltd.), at 1 µg/kg/min, which started 10 min before the second tracer injection and continued as an infusion for a total of 15 min. SPECT-2 as before, began at this stage.

During Procedure D, the effect of sumatriptan (Imigran; Glaxo (Pty) Ltd.), was investigated. A response time of 23 min was chosen to permit (because of stability limitations) the use of the



FIGURE 1. Typical coronal (a and b) and sagittal (c and d) views, representing, in each view, baseline and changed postnimodipine CBF patterns obtained with  $^{99m}\text{Tc}$ -HMPAO.

same vial of HMPAO for the second injection at 29 min. The intramuscular administration of sumatriptan, therefore, took place at 6 min, i.e., 1 min after SPECT-1 had commenced. SPECT-2 thus reflected the effect of sumatriptan at the 23-min response time, which is close to the time that leads to optimal effect.

After backprojection and reconstruction of SPECT-1 and SPECT-2 data, the brain images in all procedures consisted of transaxial, sagittal and coronal slices, representing CBF- and regional CBF-related information. Eight slices of one pixel thickness represented the brain in all three views [Fig. 1, coronal (a and b) and sagittal views (c and d)].

Regions of interest were placed on the total brain, as viewed in each slice, and counting rate data (counts/pixel) thus obtained were inserted into the following equation to obtain the ratio R:

$$R = \frac{(\text{SPECT-2}) - (\text{SPECT-1})^*}{\text{SPECT-1}}$$

where \* refers to decay-corrected data from SPECT-1 that is present during SPECT-2 and has to be subtracted from the SPECT-2 data as a background correction. R is an indication of the level change of regional CBF during extended anesthesia or in addition, because of the drug interventions, as measured with  $^{99m}\text{Tc}$ -HMPAO or  $^{99m}\text{Tc}$ -ECD. A value of R = 2 indicates no change during the procedure. The arterial blood pressures (BPs) were recorded during all the procedures from a catheter in the femoral artery. Heart rates were also monitored, as were blood gases from an arterial line.

Procedures A-D were repeated using [ $^{123}\text{I}$ ]IMP (National Accelerator Center, Faure, South Africa) as tracer for both injections (i.e., 148-296 MBq) in the protocols described above.

The R values for eight slices in transaxial, sagittal and coronal view could be compared between control and interventional studies for each tracer and also between tracers for similar procedures. A two-tailed Student's t-test for paired variables was used, with a 5% level of confidence.

#### RESULTS

The results are summarized as curves of mean (n = 6) R values versus slice number in the transaxial view for all procedures and tracers (Fig. 2). Values from the sagittal and

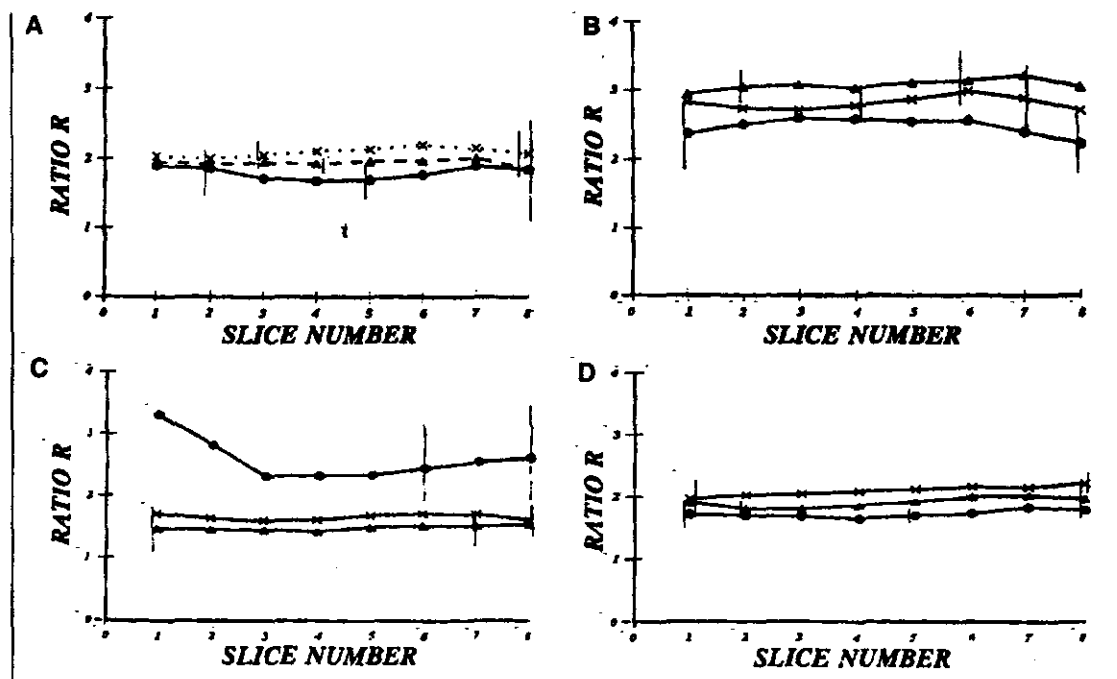


FIGURE 2. (A-D) Mean ratios  $R$  ( $n = 6$ ) versus tomographic slice number in the transaxial views (occipital to frontal) comparing  $^{99m}\text{Tc}$ -HMPAO (●),  $^{99m}\text{Tc}$ -ECD (○) and  $^{123}\text{I}$ IMP (x) for the control (A) and each intervention, acetazolamide (B), nimodipine (C) and sumatriptan (D).

coronal views in all cases confirm the measured drug effects, as represented by the transaxial view, and were, therefore, not included in the figure.

Regional effects are only crudely represented by the slice-related information of this method. No such regional effects could be established from any of the mean  $R$  versus slice number curves as being meaningful interslice differences, except for nimodipine-induced changes, as measured by  $^{99m}\text{Tc}$ -HMPAO. In this case, the increased CBF was significantly more pronounced in the cerebellum [Fig. 1, sagittal view (c and d)]. Average  $R$  values were thus calculated using all slices to represent total brain  $R$  values and are presented in Table 1. Percentage differences based on these  $R$  values are presented for each procedure with respect to the control of that particular tracer (Table 1). For all three tracers, CBF increases were noted after acetazolamide intervention. Technetium-99m-HMPAO measured a CBF increase after nimodipine intervention, but  $^{99m}\text{Tc}$ -ECD and  $^{123}\text{I}$ IMP measurements showed decreases in CBF under similar conditions. Changes in the physiological parameters due to the drug interventions are presented in Table

2. The only statistically significant changes ( $p < 0.05$ ) were seen in  $p\text{O}_2$  and  $B_p$  for acetazolamide (+20 and -8, respectively).

#### DISCUSSION

To date, various comparisons have been drawn between cerebral perfusion imaging agents for SPECT (13,22-24), but the general conclusion remains that all currently available radiopharmaceuticals, including  $^{99m}\text{Tc}$ -HMPAO,  $^{99m}\text{Tc}$ -ECD and  $^{123}\text{I}$ IMP, are far from ideal. Although all three of the above tracers are neutral and lipophilic, their kinetic behavior and trapping mechanisms differ. Consequently, there are certain conditions where they have been found to give different images; among these are cases of stroke and neurophysiological stimulations (11,25). From this study, it appears that interpretation of data from pharmacological interventions could also be a challenge and should be treated with caution.

$R$  values, as defined in this study, should ideally reach the value of two (18), to confirm the second double dose of tracer if no change in CBF or influencing metabolism had occurred

TABLE 1  
Mean Ratios from the Total Brain Region as Obtained from Various Interventions and Percentage Change from Each with Respect to Its Own Control

Intervention	Mean ratio ( $R$ ) $\pm$ s.d. ( $n = 6$ )			% difference with respect to control		
	Technetium-99m-HMPAO	Technetium-99m-ECD	Iodine-123-IMP	Technetium-99m-HMPAO	Technetium-99m-ECD	Iodine-123-IMP
	1.79 $\pm$ 0.13	2.07 $\pm$ 0.24				
	2.53 $\pm$ 0.15	2.78 $\pm$ 0.13		41.3%	34.3%	54.9%
	2.51 $\pm$ 0.14	1.67 $\pm$ 0.13		40.2%	-19.3%	-24.1%
	1.74 $\pm$ 0.10	2.09 $\pm$ 0.08		-3%	1%	-2.5%

TABLE 2  
Effects of Procedures B (Acetazolamide), C (Nimodipine) and D (Sumatriptan) on Heart Rate, Blood Pressure, pCO<sub>2</sub> and pO<sub>2</sub> in Arterial Blood in Baboons

Procedure	Heart rate	Blood pressure	pCO <sub>2</sub>	pO <sub>2</sub>
B <sub>1</sub>	116.25 ± 12.74			56.35 ± 4.81
B <sub>2</sub>	111.50 ± 16.84			76.43 ± 7.47*
C <sub>1</sub>	115.33 ± 22.38			61.12 ± 4.39
C <sub>2</sub>	116.33 ± 24.58			68.50 ± 5.37
D <sub>1</sub>	115.31 ± 11.05			59.17 ± 8.39
D <sub>2</sub>	114.31 ± 14.13			66.55 ± 9.32

\*p < 0.05, for postintervention compared to corresponding preintervention values.

Each value indicates the mean ± s.d. of six experiments. Subscript 1 and 2 refer to the measurements before the intervention and at the chosen response time, respectively.

before the second tracer injection. R values from the interventional procedures can be compared to their corresponding controls to establish the effect of each intervention, which will be CBF- and/or metabolism-related, while controlling for common anesthesia effects and tracer effects.

Of the mean control R values obtained in this study, those obtained by <sup>99m</sup>Tc-ECD and [<sup>123</sup>I]IMP reach the value two more closely and are also not significantly statistically different (p > 0.05) from each other. Neither do they differ statistically significantly from the mean control R value obtained by the <sup>99m</sup>Tc-HMPAO studies (p > 0.05), although the last appears to be lower. By now, this low R value has been repeatedly reproduced with this experimental model and can be explained in terms of backdiffusion of the tracer (26) and the ketamine hydrochloride anesthesia, which leads to increased BP (+30) and heart rate (+15) during the first <sup>99m</sup>Tc-HMPAO administration and is not maintained during the second barbiturate phase of the study (18).

The lower <sup>99m</sup>Tc-ECD back-flux from the brain leads to a higher retention of <sup>99m</sup>Tc-ECD than <sup>99m</sup>Tc-HMPAO, which may contribute to the higher control R values obtained with <sup>99m</sup>Tc-ECD (2.07 ± 0.24 compared to 1.79 ± 0.13) (26). The time to reach the steady state is the same for these two tracers and will not contribute to the difference (26).

All three tracers measure the familiar increase of CBF by acetazolamide (12,21,27,28). In comparison the highest CBF increase (+54.9%) is measured by [<sup>123</sup>I]IMP, which has previously been reported (19) and has been partly attributed to a pH effect. Alkaline urine, which results from a carbonic anhydrase inhibitor such as acetazolamide (ΔpCO<sub>2</sub> = 0; ΔpO<sub>2</sub> = +20; ΔBP = -8) (21), leads to reabsorption and an increase of the serum levels of basic drugs, with subsequent higher concentrations in the central nervous system compartment (29,30). N-Isopropyl-β-iodoamphetamine as a basic compound is, therefore, more susceptible to possible drug interactions (in particular, those with marked influences on systemic and urine pH) than are <sup>99m</sup>Tc-HMPAO and <sup>99m</sup>Tc-ECD, explaining the significantly enhanced CBF effect with [<sup>123</sup>I]IMP and acetazolamide. N-Isopropyl-β-iodoamphetamine could, thus, be the more sensitive tracer to assess cerebrovascular reserve through acetazolamide intervention.

The underestimation of acetazolamide-induced CBF increases, which would normally have been expected with <sup>99m</sup>Tc-HMPAO due to high flow, was not obvious in this study (41% compared to 35%) (26,31) but was indicated with <sup>99m</sup>Tc-ECD (34.3%) (32). The first-pass extraction rate is flow-dependent for <sup>99m</sup>Tc-ECD and <sup>99m</sup>Tc-HMPAO, and back-flux affects <sup>99m</sup>Tc-HMPAO retention more than it does <sup>99m</sup>Tc-ECD retention. Therefore, the question arises of whether a certain degree

of saturation of enzymatic reactions had occurred that might have accompanied the high rate of bioavailability of <sup>99m</sup>Tc-ECD, as follows with a high CBF from acetazolamide. Protein binding plays an important role in the distribution of drugs, and the difference in binding between <sup>99m</sup>Tc-HMPAO and <sup>99m</sup>Tc-ECD could contribute to different concentrations of un-ionized hydrophobic metabolites so that a metabolic parameter, such as saturation, becomes a factor, particularly at higher flow rates. Saturation is not expected with <sup>99m</sup>Tc-HMPAO (11).

The Ca<sup>2+</sup>-blocker nimodipine was shown to increase CBF by 40.2% for <sup>99m</sup>Tc-HMPAO and decrease it by 19.3% and 24.1% for <sup>99m</sup>Tc-ECD and [<sup>123</sup>I]IMP, respectively; all changes were statistically significant (p < 0.05) (Fig. 2). The larger effect measured by [<sup>123</sup>I]IMP as compared to that of <sup>99m</sup>Tc-ECD was not significantly different (p > 0.05). The difference in R values among the tracers after the nimodipine intervention, especially with <sup>99m</sup>Tc-HMPAO, immediately warns that a drug-tracer interaction must be considered and allowed for when measuring CBF with SPECT. The role of cofactors in metabolism, such as magnesium cations, is well-established, and drugs that influence such factors could indeed contribute to changed accumulation of compounds that are dependent on these factors for their metabolism. Nimodipine, as a calcium-channel blocker, may exert its effects through such a mechanism, resulting in unexpected blood flow patterns that manifest as being tracer-selective. It is unclear why nimodipine should influence the transport of <sup>99m</sup>Tc-HMPAO across the blood-brain barrier differently than it does the transport of <sup>99m</sup>Tc-ECD and [<sup>123</sup>I]IMP.

It should, however, be noted that, for nimodipine, not only have CBF increases been measured (33) but also, at higher doses than those in the present experiment, CBF reductions have been accompanied by reduced BP (34), suggesting a loss of autoregulation at high doses. Studies indicate that even low doses of nimodipine can inhibit the autoregulatory adjustment to altered BP (33,35), depending on the size of the BP stimulus. The large s.d., which is more obvious for nimodipine, suggest a contribution in this study from biological variability of BP responses to the results.

The intervention with sumatriptan had no effect on normal CBF, as measured by all three tracers (p > 0.05).

#### CONCLUSION

The most obvious observations noted in the CBF values, as measured by [<sup>123</sup>I]IMP, are the close approximation to R = 2 in the control study and the high R values with acetazolamide. Furthermore, [<sup>123</sup>I]IMP measures, after nimodipine intervention, a reduction in CBF, as does <sup>99m</sup>Tc-ECD, contrary to <sup>99m</sup>Tc-HMPAO. It is known that [<sup>123</sup>I]IMP follows a pattern of

linearity with flow over a wider flow range than do  $^{99m}\text{Tc}$ -HMPAO and  $^{99m}\text{Tc}$ -ECD, and it could, therefore, be regarded as the truer CBF agent. On the other hand, as a basic drug, amphetamine uptake is influenced by urinary and intracellular pH, as is seen when it is used with acetazolamide (increased uptake) and in subacute stroke (decreased uptake), respectively. Technetium-99m-ECD also demonstrates hypoactivity in subacute stroke, which is linked to altered esterase function in hypoxia, and its low CBF values, measured after the nimodipine intervention (which [ $^{123}\text{I}$ ]IMP also measures), could, therefore, be seen as relating to a drug-tracer interaction, in which metabolic processes play a role. Technetium-99m-HMPAO shows a focal area of hyperactivity during subacute stroke. The inclination to caution due to drug-tracer interactions with CBF SPECT measurements is, therefore, not unfounded. The familiar differences of back-flux and, possibly, saturation between  $^{99m}\text{Tc}$ -HMPAO and  $^{99m}\text{Tc}$ -ECD were also observed, which could influence the diagnostic sensitivity of the particular tracer.

It is, in addition, an interesting finding that sumatriptan does not appear to change normal CBF.

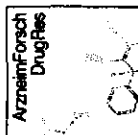
This study confirms that the interpretation of CBF SPECT data after pharmacological interventions could be a challenge and should be viewed with cognizance of tracer and drug characteristics.

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#### REFERENCES

- Winchell HS, Baldwin RM, Lin TH. Development of I-123-labeled amines for brain studies: localization of I-123 iodo-phenylalkyl amines in rat brain. *J Nucl Med* 1980;21:940-947.
- Kuhl DE, Barrio JR, Huang SC, et al. Quantifying cerebral blood flow by N-isopropyl- $^{123}\text{I}$ -iodoamphetamine (IMP) tomography. *J Nucl Med* 1982;23:196-203.
- Nishizawa S, Tanada S, Yonekura Y, et al. Regional dynamics of N-isopropyl-( $^{123}\text{I}$ )-iodo-amphetamine in human brain. *J Cereb Blood Flow Metab* 1989;30:150-156.
- Lassen NA, Henriksen L, Holm S, et al. Cerebral blood flow tomography: xenon-133 compared with isopropyl-amphetamine-iodine-123. *J Nucl Med* 1983;27:17-21.
- Lassen NA, Anderson AR, Friberg L, Paulson OB. The retention of [ $^{99m}\text{Tc}$ ]-d,l-HMPAO in the human brain after intracarotid bolus injection: a kinetic analysis. *J Cereb Blood Flow Metab* 1988;8(suppl):S13-S22.
- Neirinckx RD, Burke JF, Harrison RC, Forster AM, Andersen AR, Lassen A. The retention mechanism of technetium-99m-HMPAO: intracellular reaction with glutathione. *J Cereb Blood Flow Metab* 1988;8(suppl):S4-S12.
- Walovitch RC, Hill TC, Garrity ST, et al. Characterization of technetium-99m-L,L-ECD for brain perfusion imaging, part I: pharmacology of technetium-99m ECD in non-human primates. *J Nucl Med* 1989;30:1892-1901.
- Holman LB, Hellman RS, Goldsmith SJ, et al. Biodistribution, dosimetry and clinical evaluation of technetium-99m ethyl cysteinate dimer in normal subjects and in patients with chronic cerebral infarction. *J Nucl Med* 1989;30:1018-1024.
- Vallabhajosula S, Zimmermann RE, Picard M, et al. Technetium-99m ECD: a new brain imaging agent. In vivo kinetics and biodistribution studies in normal human subjects. *J Nucl Med* 1989;30:599-604.
- Huglo D, Rousseaux M, Leys D, Fialdes P, Steinling M. Regional cerebral blood flow imaging: a quantitative comparison of 99m-Tc-bicisate with  $^{133}\text{Xe}$  using single photon emission computed tomography. *J Cereb Blood Flow Metab* 1994;14(suppl 1):S76-S83.
- Lassen NA, Sperling B. 99m-Tc-bicisate reliably images CBF in chronic brain diseases but fails to show reflow hyperemia in subacute stroke: report of a multicenter trial of 105 cases comparing  $^{133}\text{Xe}$  and 99m-Tc-bicisate (ECD, Neuroline) measured by SPECT on the same day. *J Cereb Blood Flow Metab* 1994;14(suppl 1):S44-S48.
- Nakagawara J, Nakamura J, Takeda R, et al. Assessment of postischemic reperfusion and diamox activation test in stroke using  $^{99m}\text{Tc}$ -ECD SPECT. *J Cereb Blood Flow Metab* 1994;14(suppl 1):S49-S57.
- Moretti J-L, Defer G, Tamgac F, Weinmann CB, Cesaro P. Comparison of brain SPECT using 99m-Tc-bicisate (L,L-ECD) and [ $^{123}\text{I}$ ]IMP in cortical and subcortical strokes. *J Cereb Blood Flow Metab* 1994;14(suppl 1):S84-S90.
- Aha CS, Tow DE, Yu CC, Greene RW. Effect of metabolic alterations on the accumulation of Tc-99m-labeled D,L-HMPAO in slices of rat cerebral cortex. *J Cereb Blood Flow Metab* 1994;14:324-331.
- Höllerhage H-G, Gaab MR, Zunkeller M, Walter GF. Nimodipine treatment has been implicated in impaired CBF autoregulation. *J Neurosurg* 1988;69:919-922.
- Holm S, Madsen PL, Sperling B, Lassen NA. Use of 99m-Tc-bicisate in activation studies by split-dose technique. *J Cereb Blood Flow Metab* 1994;14(suppl 1):S115-S120.
- Wyper DJ, Hunter R, Patterson J, Goodwin G, McCulloch J. A split-dose technique for measuring changes in cerebral blood flow patterns [Abstract]. *J Cereb Blood Flow Metab* 1991;11(suppl):S449.
- Dormehl IC, Redelinghuys IF, Hugo N, Oliver D, Pillay W. The baboon model under anaesthesia for in vivo cerebral blood flow studies using SPECT techniques. *J Med Prim* 1992;21:270-274.
- Dormehl IC, Oliver DW, Hugo N. Dose response from pharmacological interventions for CBF changes in a baboon model using  $^{99m}\text{Tc}$ -HMPAO and SPECT. *Nucl Med Commun* 1995;14:573-577.
- Dormehl IC, Oliver DW, Hugo N, Rossouw D. A comparative cerebral blood flow study in a baboon model with acetazolamide provocation:  $^{99m}\text{Tc}$ -HMPAO vs. [ $^{123}\text{I}$ ]IMP. *Nucl Med Biol* 1994;22:373-378.
- Oliver DW, Dormehl IC, Redelinghuys IF, Hugo N, Beverley G. Drug effects on cerebral blood flow in the baboon model: acetazolamide and nimodipine. *Nuklearmedizin* 1993;32:292-298.
- Moretti JL, Caglar M, Weinmann P. Cerebral perfusion imaging tracers for SPECT: which one to choose? *J Nucl Med* 1995;36:359-363.
- Suzuki S, Sakai F, Akutsu T, Tazaki Y. Tracer kinetics of [ $^{123}\text{I}$ ]IMP, Tc-99m-HM-PAO and Tc-99m-ECD: measurements of temporal changes in arterial and jugular venous radioactivity [Abstract]. *J Cereb Blood Flow Metab* 1991;11(suppl 2):S774.
- Yonekura Y, Tsuchida T, Saito N, et al. Brain perfusion SPECT with  $^{99m}\text{Tc}$ -bicisate: comparison with PET measurement and linearization based on permeability-surface area product model. *J Cereb Blood Flow Metab* 1994;14(suppl 1):S58-S65.
- Holm S, Madsen PL, Rubin P, Sperling B, Friberg L, Lassen N. Tc-99m HMPAO activation studies: validation of the split-dose, image subtraction approach [Abstract]. *J Cereb Blood Flow Metab* 1991;11(suppl):S766.
- Murase K, Tanada S, Inoue T, et al. Kinetic behavior of Tc-99m ECD in the human brain using compartment analysis and dynamic SPECT: comparison with Tc-99m HMPAO. *J Nucl Med* 1992;33:135-143.
- Bonte FJ, Devous MD, Reisch JS. The effect of acetazolamide on regional cerebral blood flow in normal human subjects as measured by single photon emission computed tomography. *Invest Radiol* 1988;23:564-568.
- Nakagawara J, Takeda R, Tanaka Y, Nakamura J, Suematsu K. Assessment of hemodynamic reserve in candidates for carotid bypass surgery using SPECT and [ $^{123}\text{I}$ ]IMP activated with acetazolamide test. *Adv Funct Neuroimaging* 1989;2:8-15.
- Gerhardt RE, Knouss RF, Thyrium PT, Luchi RJ, Morris JJ. Quinidine excretion in aciduria and alkaluria [Abstract]. *Ann Intern Med* 1969;71:927.
- Goodman LS, Gilman AG. In: Gilman AG, Rall TW, Nies AS, Taylor P, eds. *The pharmacological basis of therapeutics*, 8th Ed. New York: Pergamon Press; 1990: 716-717.
- Vorstrup S. Tomographic cerebral blood flow measurements in patients with ischemic cerebrovascular disease and evaluation of the vasodilator capacity by the acetazolamide test. *Acta Neurol Scand* 1988;77(suppl 144):1-48.
- Friberg L, Anderson AR, Lassen NA, Holm S, Dam M. Retention of  $^{99m}\text{Tc}$ -bicisate in the human brain after intracarotid injection. *J Cereb Blood Flow Metab* 1994; 14(suppl):S19-S27.
- McGalden TA, Nath RG. Cerebrovascular autoregulation is resistant to calcium channel blockade by nimodipine. *Experientia* 1989;45:305-306.
- Haws CW, Heistad DD. Effects of nimodipine on cerebral vasoconstrictor responses [Abstract]. *Am J Phys* 1984;247:H170.
- Sahlin C, Brismar J, Delgado T, Owman C, Salford LG, Svenggaard NA. Cerebrovascular and metabolic changes during the delayed vasospasm following experimental subarachnoid hemorrhage in baboons, and treatment with a calcium antagonist. *Brain Res* 1987;403-413.



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# Cerebral Blood Flow Effects of Sodium Valproate in Drug Combinations in the Baboon Model

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### Summary

Sodium valproate (CAS 1069-66-5, Epilim<sup>®</sup>) has been used in the management of epilepsy during the last three decades. Although important information on the pharmacological actions and efficacy of sodium valproate has accrued to date, limited research has been conducted on its effects on cerebral blood flow. In recent years, with the aid of SPECT (single photon emission computed tomography) and PET (positron emission tomography) it has been shown that marked cerebral blood flow changes occur in epileptic patients. Furthermore it was established recently that sodium valproate influences the cerebral blood flow in children by decreasing the flow significantly. The present study investigated the effects of sodium valproate on the cerebral blood flow, using <sup>99m</sup>Tc-HMPAO (hexamethylpropylene amine oxime) and SPECT, in a primate model, as well as the effects of its drug interactions with therapeutic agents that influence cerebrovascular dynamics, e.g. sumatriptan, nimodipine and acetazolamide. The current study using single dose treatment with sodium valproate did not detect a decrease or increase of the cerebral blood flow when compared with control baseline results. Drug interaction between sodium valproate and nimodipine may occur as a reduction of 25 % in cerebral blood flow from the baseline control was observed in this case. The effects observed for the combinations of sodium valproate respectively with sumatriptan and acetazolamide are attributed to the influences of the sumatriptan (decrease) and acetazolamide (increase) alone. The cerebral blood flow effects of these drugs and possible interactions during an acute epileptic seizure need to be investigated.

### Zusammenfassung

#### *Einfluß von Natriumvalproat in Arzneimittelkombinationen auf die Hirndurchblutung beim Schimpansen*

Natriumvalproat (CAS 1069-66-5, Epilim<sup>®</sup>) wird seit drei Jahren zur Behandlung von Epilepsie benutzt. Obgleich während dieser Zeit wichtige Informationen über die pharmakologischen Wirkungen und Effizienz von Natriumvalproat erhalten wurden, erfolgten bis heute nur sehr begrenzte Forschungen bezüglich seines Einflusses auf die zerebrale Durchblutung. In den letzten Jahren wurde mit Hilfe von SPECT (Single-Photon-Emission-Computertomographie) und PET (Positron-Emission-Tomographie) gezeigt, daß beträchtliche Schwankungen in der zerebralen Durchblutung von epileptischen Patienten auftraten. Weiterhin wurde in letzter Zeit festgestellt, daß Natriumvalproat die zerebrale Durchblutung bei Kindern signifikant herabsetzte. Die vorliegende Studie untersuchte den Effekt von Natriumvalproat auf die zerebrale Durchblutung mit Hilfe von <sup>99m</sup>Tc-HMPAO (Hexamethylpropylenaminnoxim) und SPECT am Primatenmodell. Zusätzlich wurde seine Wechselwirkung mit Medikamenten untersucht, die die zerebrovaskuläre Dynamik beeinflussen, wie z. B. Sumatriptan, Nimodipin und Acetazolamid. Die vorliegende Studie, die eine Einzeldosisbehandlung von Natriumvalproat beinhaltete, ergab weder einen Anstieg noch einen Abfall der zerebralen Durchblutung verglichen mit den Kontrollwerten. Wechselwirkung zwischen Natriumvalproat und Nimodipin könnte der Grund für eine 25%ige Reduktion der zerebralen Durchblutung im Vergleich zu Kontrollergebnissen sein, wie sie in diesem Fall beobachtet wurden. Die beobachteten Effekte für die Kombination von Natriumvalproat mit Sumatriptan und auch mit Acetazolamid werden den Einflüssen der Einzelsubstanzen (Abfall durch Sumatriptan und Anstieg durch Acetazolamid) zugeschrieben. Die zerebralen Durchblutungseffekte dieser Arzneimittel und die möglichen Wechselwirkungen während eines akuten epileptischen Anfalls erfordern noch weitere Untersuchungen.

**Key words** Acetazolamide · CAS 1069-66-5 · Epilim<sup>®</sup> · Nimodipine · Sodium valproate, cerebral blood flow effects, drug interactions, primate model · Sumatriptan

## 1. Introduction

Sodium valproate (CAS 1069-66-5) has been introduced into the arsenal of anti-epileptic drugs about three decades ago in Europe and was approved by the Food and Drug Administration in the United States in 1978 [1]. Its effectiveness as an anti-epileptic drug is well established and it has been shown to inhibit seizures in a variety of models. Also its modes of action are well documented [1-5]. Recently an intravenous preparation of sodium valproate has been developed and is currently marketed in South Africa as Epilim<sup>®</sup>1). Drug interactions of sodium valproate with significant clinical implications have been demonstrated: amongst others the plasma concentration of phenobarbital may rise by as much as 40% when valproate is administered concurrently, which may involve inhibition of the metabolism of phenobarbital [1]. Valproate also competes for plasma protein binding sites with subsequent increased plasma concentration of free (unbound) tolbutamide. The development of cerebral blood flow tracers such as technetium labelled <sup>99m</sup>Tc-HMPAO (hexamethylpropylene amine oxime) and <sup>99m</sup>Tc-ECD (ethyl cysteinate dimer) has made it possible to investigate the cerebral blood flow dynamics under various conditions, e.g. during pharmacological drug interventions, anaesthesia and in drug combination studies [6-11]. A non-human primate model developed by us [6] has proven to be adequately sensitive for measuring the effects of anaesthesia, pharmacological interventions, and drug combination on cerebral blood flow (CBF) using single photo emission computed tomographic techniques and the above mentioned cerebral blood flow tracers.

It has recently been reported that there is a significant (although slight) reduction in cerebral blood flow in children upon treatment with sodium valproate and carbamazepine [12]. Several studies have indicated that cerebral blood flow abnormalities are present in epileptic patients [13-18]. The monitoring of cerebral blood flow of epileptic patients during therapy as well as during drug combination administrations is becoming more important for our understanding of the mechanisms involved in epilepsy. The objective of the current study was to investigate the possible drug interactions of sodium valproate with other cerebrovasoactive drugs, i.e. sumatriptan (CAS 103628-46-2) (used during the treatment of migraine), nimodipine (CAS 66085-59-4) (a calcium channel blocker), acetazolamide (CAS 59-66-5) (the standard drug for investigating cerebrovascular reserve, a carbonic anhydrase inhibitor). Clinical important information on the influence of these combinations on cerebral blood flow could be gained from these studies. They could also add to the knowledge of the pharmacology of these drugs.

<sup>1)</sup> Manufacturer: R & C Pharmaceuticals, a Division of Reckitt and Coleman, Merebank (Republic of South Africa).

## 2. Materials and methods

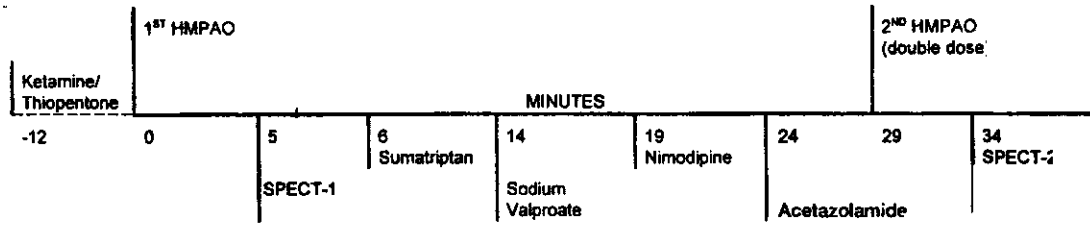
Adult male baboons (*Papio ursinus*, average weight 27 kg) were used for this study. The animals were obtained from Mr. E. Venter, Vaalwater, Northern Province (Republic of South Africa). The studies were performed after approval from the Ethics Committee of the University of Pretoria, according to the guidelines of the National Code for Animal Use in Research, Education and Testing of Drugs and Related Substances in South Africa. These guidelines are in line with international standards.

Five different procedures (A-E) with six animals per procedure were carried out (Fig. 1). Procedure A was a control study where changes in CBF which occurred during anaesthesia of 29 min were measured. This was also the duration of the anaesthesia used for procedures B-E when CBF changes were measured after sodium valproate (obtained from the manufacturer) only (B) and after various double drug interventions at appropriate response times. The drug interventions studied were: sodium valproate (100 mg/ml/min i.v. infused over 10 min; procedure B) at response time  $t = 15$  min; and then drug combination interventions which consisted of sodium valproate (100 mg/ml/min i.v. infused over 10 min) with one of either acetazolamide (100 mg/ml i.v. [5 ml]; procedure C), nimodipine (1  $\mu$ g/kg/min infused over 10 min; procedure D), and sumatriptan (6 mg i.m.; procedure E), each injected so as to allow measurement at the appropriate response time  $t = 15$  min for sodium valproate,  $t = 5$  min for acetazolamide,  $t = 10$  min for nimodipine;  $t = 23$  min for sumatriptan.

For each study the baboon was sedated with ketamine hydrochloride (10 mg/kg i.m.) (Anaket-V<sup>®</sup>, Centaur Labs, Bryanston, Gauteng, SA). Was followed immediately by maintained and controlled infusion of thiopentone sodium (70 ml/h of 0.5% solution) (Intraval<sup>®</sup>, Rhône-Poulenc Rorer S.A., Mirand, Gauteng, SA) using an administration (drip) set. After a 12-min-stabilisation period under thiopentone, procedure A, the control study under anaesthesia, started with an i.v. injection of 222MBq of <sup>99m</sup>Tc-HMPAO at time  $t = 0$  (see Fig. 1). 5 min later the first SPECT acquisition (SPECT-1) followed with a Siemens Orbiter gamma camera, using 32 projections of 20 s during a 360° rotation.

The baboons were always positioned in the supine position with a special head rest to ensure reproducible and comparable tomographic slices for all procedures. A second i.v. administration of <sup>99m</sup>Tc-HMPAO (444 MBq, i.e. double the first dose) was administered at  $t = 29$  min taking care not to exceed the 30-min time limit of use after reconstitution and allowing for pharmacologically appropriate response times as described above. 5 min later a similar SPECT acquisition, SPECT 2 followed (the split dose method), which measures anaesthesia related changes in CBF taking place in between the two HMPAO administrations. Procedure B is similar to Procedure A described above but includes an intervention with sodium valproate at  $t = 14$  min. Procedure C, D and E describe combination drug interventions with sodium valproate ( $t = 14$  min), vz. acetazolamide ( $t = 24$  min) (procedure C), nimodipine ( $t = 19$  min) (procedure D) and sumatriptan ( $t = 6$  min) (procedure E). The arterial blood pressures were recorded during all the procedures from a catheter in the femoral artery. Heart rates were monitored as well.

After back projection and reconstruction the brain images consisted of transaxial, sagittal and coronal slices representing CBF and rCBF information during conditions prevailing under the procedure of anaesthesia only, and under the various drug interventions. Eight transaxial slices represented the whole brain each of which was



min	Procedure A	Procedure B	Procedure C	Procedure D	Procedure E
	Ketamine	Ketamine	Ketamine	Ketamine	Ketamine
-12	thiopentone	thiopentone	thiopentone	thiopentone	thiopentone
0	1 <sup>st</sup> HMPAO	1 <sup>st</sup> HMPAO	1 <sup>st</sup> HMPAO	1 <sup>st</sup> HMPAO	1 <sup>st</sup> HMPAO
5	SPECT-1	SPECT-1	SPECT-1	SPECT-1	SPECT-1
6					Sumatriptan
14		Sodium Valproate	Sodium Valproate	Sodium Valproate	Sodium Valproate
19				Nimodipine	
24			Acetazolamide		
29	2 <sup>nd</sup> HMPAO	2 <sup>nd</sup> HMPAO	2 <sup>nd</sup> HMPAO	2 <sup>nd</sup> HMPAO	2 <sup>nd</sup> HMPAO
34	SPECT-2	SPECT-2	SPECT-2	SPECT-2	SPECT-2

Fig. 1: The time schedule for the various drug interventions procedure protocols indicating the time of each intervention.

Table 1a: The mean R ratios ( $\pm$  SD) (n = 6) from transaxial views of eight equal cerebral regions and total brain for the five different procedures with the slice number starting from the frontal to the occipital lobes.

Procedure	Region 1	Region 2	Region 3	Region 4	Region 5	Region 6	Region 7	Region 8	Total brain
	2.25 $\pm$ 0.32	2.12 $\pm$ 0.18	2.10 $\pm$ 0.13	2.14 $\pm$ 0.08	2.13 $\pm$ 0.16	2.09 $\pm$ 0.11	2.09 $\pm$ 0.08	2.00 $\pm$ 0.19	
	2.19 $\pm$ 0.24	2.12 $\pm$ 0.17	2.05 $\pm$ 0.15	2.08 $\pm$ 0.13	2.09 $\pm$ 0.16	2.15 $\pm$ 0.15	2.14 $\pm$ 0.18	2.14 $\pm$ 0.12	
	2.79 $\pm$ 0.48	2.76 $\pm$ 0.35	2.73 $\pm$ 0.30	2.81 $\pm$ 0.34	2.94 $\pm$ 0.39	2.96 $\pm$ 0.43	2.88 $\pm$ 0.44	2.85 $\pm$ 0.46	
	1.67 $\pm$ 0.40	1.59 $\pm$ 0.32	1.53 $\pm$ 0.31	1.53 $\pm$ 0.29	1.55 $\pm$ 0.30	1.56 $\pm$ 0.31	1.54 $\pm$ 0.34	1.54 $\pm$ 0.32	
	2.05 $\pm$ 0.17	1.93 $\pm$ 0.14	1.94 $\pm$ 0.15	1.96 $\pm$ 0.14	2.00 $\pm$ 0.17	2.03 $\pm$ 0.19	2.01 $\pm$ 0.14	1.99 $\pm$ 0.14	

Statistically significant differences between Procedure A and C (p < 0.05) for the transaxial views of the total brain.

Table 1b: The mean R ratios ( $\pm$  SD) (n = 6) from saggital views of eight equal cerebral regions and total brain for the five different procedures with the slice number from left to right of the brain.

Procedure	Region 1	Region 2	Region 3	Region 4	Region 5	Region 6	Region 7	Region 8
	2.25 $\pm$ 0.32	2.12 $\pm$ 0.18	2.10 $\pm$ 0.13	2.14 $\pm$ 0.08	2.13 $\pm$ 0.06	2.09 $\pm$ 0.11	2.09 $\pm$ 0.08	
	2.40 $\pm$ 0.44	2.26 $\pm$ 0.25	2.20 $\pm$ 0.17	2.13 $\pm$ 0.18	2.07 $\pm$ 0.12	2.04 $\pm$ 0.13	2.01 $\pm$ 0.19	
	3.15 $\pm$ 0.72	2.94 $\pm$ 0.50	2.93 $\pm$ 0.42	2.91 $\pm$ 0.43	2.89 $\pm$ 0.39	2.83 $\pm$ 0.40	2.76 $\pm$ 0.31	
	1.78 $\pm$ 0.32	1.63 $\pm$ 0.31	1.55 $\pm$ 0.30	1.52 $\pm$ 0.29	1.54 $\pm$ 0.29	1.56 $\pm$ 0.31	1.63 $\pm$ 0.34	
	1.93 $\pm$ 0.26	1.93 $\pm$ 0.18	1.95 $\pm$ 0.16	1.95 $\pm$ 0.14	1.96 $\pm$ 0.15	2.02 $\pm$ 0.17	2.04 $\pm$ 0.17	

Statistically significant differences between Procedure A and C (p < 0.01), and A and D (p < 0.05) for the saggital views of the total brain.

Table 1c: The mean R ratios ( $\pm$  SD) (n = 6) from coronal views of eight equal cerebral regions and total brain for the five different procedures with the slice number from the cerebellum to the dorsal slice of the cerebrum.

Procedure	Region 1	Region 2	Region 3	Region 4	Region 5	Region 6	Region 7	Region 8
	2.06 $\pm$ 0.33	2.10 $\pm$ 0.19	2.12 $\pm$ 0.11	2.08 $\pm$ 0.14	2.14 $\pm$ 0.23	2.17 $\pm$ 0.24	2.19 $\pm$ 0.36	
	2.01 $\pm$ 0.28	2.05 $\pm$ 0.24	2.06 $\pm$ 0.17	2.09 $\pm$ 0.12	2.11 $\pm$ 0.13	2.11 $\pm$ 0.14	2.14 $\pm$ 0.17	
	2.65 $\pm$ 0.2	2.70 $\pm$ 0.28	2.72 $\pm$ 0.31	2.82 $\pm$ 0.37	2.86 $\pm$ 0.42	2.91 $\pm$ 0.47	2.89 $\pm$ 0.48	
	1.57 $\pm$ 0.44	1.52 $\pm$ 0.37	1.52 $\pm$ 0.33	1.54 $\pm$ 0.29	1.60 $\pm$ 0.30	1.60 $\pm$ 0.27	1.65 $\pm$ 0.30	
	1.88 $\pm$ 0.17	1.92 $\pm$ 0.14	1.96 $\pm$ 0.11	2.02 $\pm$ 0.16	2.02 $\pm$ 0.16	2.00 $\pm$ 0.17	2.03 $\pm$ 0.21	

Statistically significant differences between Procedure A and C (p < 0.01), and A and D (p < 0.05) for the coronal views of the total brain.

considered for count rate evaluation by the ROI (region of interest) feature. Similarly eight sagittal and eight coronal slices covering all of the brain were selected and analysed.

Count rate data for each slice in each view were then inserted into the following equation to obtain a corresponding ratio R, for each procedure:

$$R = \frac{(\text{SPECT-2}) - (\text{SPECT-1})^*}{(\text{SPECT-1})}$$

R is an indication of the level change of the CBF during the thiopentone anaesthesia (procedure A), or, in addition, due to the drug interventions (procedures B to E), measured by SPECT-1 and SPECT-2, at the selected response times. The equation allows for the subtraction of retained activity originating from the SPECT-1 study after decay correction (\*). For all three views graphs were plotted of R vs. slice number starting at the occipital lobes to the frontal lobes transaxially, from right to left sagittally and from the cerebellum to the dorsal slice of the cerebrum coronally (Table 1a-c).

### 2.1. Statistical methods

Mean ratios R (n = 6) and standard deviations were calculated per procedure for similar regions (slices). An average r-value was obtained for the total brain for a particular procedure by considering all the slices of all the views. These were compared for interprocedural effects and the comparisons assessed for significant differences using Student's two-tailed t-test for paired variables on a 5% confidence limit.

### 3. Results

The curves of R-values (mean  $\pm$  SD) vs. slice number in all three views are plotted for each procedure in Table 1a-c for procedures A to E, respectively. With no intra-procedural regional, slice dependent differences statistically significant, mean R-values were obtained and used to represent the total brain for each procedure (Table 2). Percentage changes with respect to the control anaesthesia only study (procedure A) are compared in Table 2.

Haemodynamic changes for Procedures A-E appear in Table 3. The control R value for cerebral blood flow under thiopentone anaesthesia obtained during the current study is similar to previous data [6] vs. R = 2.13 (Table 1a-c). From Table 1a-c and Table 2 it is clear that sodium valproate did not change the control R-values at all and therefore did not influence the cerebral blood flow. The drug combination interventions with sodium valproate and sumatriptan, or acetazolamide, or nimodipine reveal that the cerebral blood flow is differently influenced by these combinations. The combination (Procedure C) of sodium valproate and acetazolamide (Table 1a-c and Table 2) yielded total brain R-values which were statistically significantly increased (34%) when compared to both the control and the single sodium valproate drug intervention (2.84  $\pm$  0.38 vs. 2.13  $\pm$  0.12, p < 0.01). Conversely, the combination (Procedure D) of sodium valproate and nimodipine (Table 1a-c and Table 2) yielded total brain R-values which were statistically significantly decreased (25%) when compared to both the control and the single

Table 2: The mean R value  $\pm$  SD (n = 6) for total brain as averaged from all slices and all views for each procedure, percentage changes of these R-values with respect to control and significance.

Procedure	R-value	% Change <sup>a)</sup>	Significance <sup>a)</sup>
Procedure A Control	2.13 $\pm$ 0.12		
Procedure B Sodium valproate	2.13 $\pm$ 0.12	0	p > 0.05
Procedure C Sodium valproate + Acetazolamide	2.84 $\pm$ 0.38	+34 %	p < 0.05 p < 0.01
Procedure D Sodium valproate + Nimodipine	1.59 $\pm$ 0.31	-25 %	p < 0.05 p < 0.01
Procedure E Sodium valproate + Sumatriptan	1.90 $\pm$ 0.14	-11 %	p > 0.05 p < 0.1

<sup>a)</sup> With respect to control.

Table 3: The effect of all the procedures on peripheral blood pressure.

Procedure	$\Delta$ BP % 0-29 min	$\Delta$ CBF %	$\Delta$ BP % 14-29 min (sodium valproate) <sup>a)</sup>
Procedure A Control	+12	0	+7
Procedure B Sodium valproate	+19	0	+7
Procedure C Sodium valproate + Acetazolamide	+23	+34	+11
Procedure D Sodium valproate + Nimodipine	+9	-25	-3
Procedure E Sodium valproate + Sumatriptan	+19	-11	

<sup>a)</sup> With respect to control.

sodium valproate drug intervention (1.59  $\pm$  0.31 vs. 2.13  $\pm$  0.12, p < 0.01). From Procedure E, i.e. the combination of sodium valproate and sumatriptan (Table 1a-c and Table 2) showed a slight decrease (11%) in cerebral blood flow approaching statistical significance difference when compared to both the control and the single sodium valproate intervention (1.90  $\pm$  0.14 vs. 2.13  $\pm$  0.12, p > 0.05 to p < 0.1).

No significant changes occurred for any of the interventions in the heart rate taken just before drug administration versus just before the second HMPAO injection. Changes in blood pressure with respect to initial values (before drug intervention) (see Table 3) occurred with sodium valproate (increased 19%), combination of sodium valproate and acetazolamide (increased 23%), combination of sodium valproate and nimodipine combination (increased 9%) and combination of sodium valproate and sumatriptan (increased 19%). It was previously shown that drug interventions, such as acetazolamide, and combinations of sumatriptan may influence blood pressure [19]. Furthermore,

anaesthesia is also known to influence the blood pressure [6, 20, 21]. The increase in blood pressure observed for the current anaesthetic regime (see Table 3), i.e. the control Procedure A, was found to be approximately 12% with respect to the initial value at the beginning of the procedure. Based on the effect of the anaesthesia on blood pressure the following blood pressure effects can be deduced for the different procedures: Procedure B (sodium valproate) increase of 12%; Procedure C (sodium valproate and acetazolamide) increase of 11%; Procedure D (sodium valproate and nimodipine) decrease of -3%; Procedure E (sodium valproate and sumatriptan) increase 7%.

#### 4. Discussion

The results clearly indicate that sodium valproate had no effect on the cerebral blood flow, both on the regional and total brain flow. This is in contrast to the significant decrease (although slight) in cerebral blood flow reported in children upon treatment with sodium valproate [12]. The difference in the study procedures (chronic vs. acute) between the latter and the current study may explain the different outcomes. It was previously observed that acetazolamide increased the cerebral blood in the baboon model by approximately 35% [7, 9]. This increase is nearly identical to the 34% observed for the drug combination of sodium valproate and acetazolamide in the current study, indicating the increase in cerebral blood flow is only due to the acetazolamide and supports the previous conclusion that sodium valproate does not influence the cerebral blood flow.

Nimodipine, the dihydropyridine calcium channel blocker, has recently been shown to diminish the increased cerebral blood flow induced by acetazolamide [9, 19] and that a combination of nimodipine and sumatriptan decreased the cerebral blood flow markedly [19]. The present study using nimodipine and sodium valproate is in line with the previous two findings and clearly shows that cerebral blood flow is decreased below the control baseline. Nimodipine has however previously been reported in a study with the same tracer to dilate the cerebral arterioles and thus to increase cerebral blood flow [19, 22]. The current study therefore points to an interaction of sodium valproate and nimodipine, and therefore calls for caution when simultaneously used.

Sumatriptan has been shown to reverse the increased cerebral blood flow due to prolonged anaesthesia [10]. The nearly significant ( $0.05 > p < 0.1$ ) decrease in cerebral blood flow upon simultaneous treatment with sodium valproate and sumatriptan suggests that the decrease may be due to the reversal of the anaesthesia induced increase [10] cerebral blood flow by sumatriptan. Drug interaction between sumatriptan and sodium valproate is therefore not implicated by this study. Although changes in the blood pressures (Table 3) were observed during these studies, the effect were

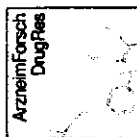
only a slight increase which could be attributed to the action of sodium valproate. These were generally smaller than those observed during anaesthesia alone. No significant changes in blood pressure were noted for the combination studies with sodium valproate when compared to the anaesthesia control and no peripheral drug interaction is therefore envisaged.

#### 5. Conclusion

The present study clearly indicated that sodium valproate exhibits no acute effects on cerebral blood flow. The current investigation did not address the effects and drug interactions upon chronic treatment with sodium valproate. The changes in the blood pressure observed during these studies were only insignificantly different than the increase during anaesthesia alone. No drug interactions with respect to cerebral blood flow dynamics are envisaged when sodium valproate and acetazolamide or sumatriptan is administered simultaneously in acute treatment. Possible drug interaction may occur between sodium valproate and nimodipine as shown in the current data upon single dose treatment. The current study could be follow-up using halothane for anaesthesia, due to its marked induced increases in cerebral blood flow. The decrease in cerebral blood flow previously reported for sodium valproate might then be noticeable using halothane.

#### 6. References

- [1] McNamara, J. O., in: Goodman and Gilman's The Pharmacological Basis of Therapeutics 9<sup>th</sup> Edition, J. G. Hardman, L. E. Limbird, P. B. Molinoff et al., (eds), pp. 476, McGraw-Hill, New York (1996)
- [2] Chapman, A., Keane, P. E., Meldrun, B. S. et al., *Prog. Neurobiol.* **19**, 315 (1982)
- [3] Phillips, N. I., Fowler, L. J., *Biochem. Pharmacol.* **31**, 2257 (1982)
- [4] Mclean, M. J., Macdonald, R. L., *J. Pharmacol. Exp. Ther.* **238**, 727 (1986)
- [5] Coulter, D. A., Huguenard, J. R., Prince, D. A., *Br. J. Pharmacol.* **100**, 800 (1990)
- [6] Dormehl, I. C., Redelinghuys, F., Hugo, N. et al., *J. Med. Primatol.* **21**, 270 (1992)
- [7] Dormehl, I. C., Oliver, D. W., Hugo, N., *Nucl. Med. Commun.* **14**, 573 (1993)
- [8] Dormehl, I. C., Lipp, M. D. W., Hugo, N. et al., *Eur. J. Nucl. Med.* **20**, 11 (1993)
- [9] Oliver, D. W., Dormehl, I. C., Redelinghuys, F. et al., *Nucl. Med.* **32**, 292 (1993)
- [10] Oliver, D. W., Dormehl, I. C., Hugo, N., *Arzneim.-Forsch./Drug Res.* **44** (II), 925 (1994)
- [11] Jordaan, B., Oliver, D. W., Dormehl, I. C. et al., *Arzneim.-Forsch./Drug Res.* **46** (II), 844 (1996)
- [12] Futagi, Y., Otani, K., Imai, K., *Epilepsia* **35**, 827 (1994)
- [13] Feggi, L., Scarpa, P., Calzolari, F., *Clin. Nucl. Med.* **20**, 42 (1995)



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# Cerebral Blood Flow Effects of the Nitric Oxide Donor, Nitroglycerin and its Drug Combinations in the Non-Human Primate Model

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### Summary

Nitroglycerin (CAS 55-63-0, Nitrocel<sup>®</sup>) has successfully been used in the management of angina during the last several decades. Although important information on the pharmacological actions and efficacy of nitroglycerin have been extracted, to date, limited research has been conducted on its effects on cerebral blood flow. In recent years, with the aid of SPECT (single photon emission computed tomography) and PET (positron emission tomography) it has been shown that marked cerebral blood flow changes occur under treatment of a wide variety of drugs. Ilucidation of the pharmacological mode of action of nitroglycerin has gained momentum with the discovery of nitric oxide (NO) as an endogenous mediator and with the knowledge that nitroglycerin acts as a NO donor. The present study investigated the effects of nitroglycerin (0.25 µg/kg/min over 10 min) on the cerebral blood flow, using <sup>99m</sup>Tc-HMPAO (hexamethylpropylene amine oxime) and SPECT, in an anaesthetised primate model, as well as the effects of its drug interactions with therapeutic agents that influence cerebrovascular dynamics, e.g. sumatriptan, nimodipine and acetazolamide. The present study with nitroglycerin indicates that the response time to measure cerebral blood flow effects seems to be present and an important factor as the transient is relatively short. The current treatment regime with nitroglycerin indicates a slight increase, when compared with control control results, although not significant, except for regional significant increases in particular the occipital regions of the brain. Drug interaction between nitroglycerin and nimodipine may occur as a reduction of 20 % in cerebral blood flow from the control control was observed in this case. The results for the combination of nitroglycerin with sumatriptan showed a further increase of the cerebral blood flow to near significance, when compared with the control results and is significantly increased (+27 %) when compared with sumatriptan treatment alone. Effective treatment with sumatriptan may therefore be compromised with simultaneous administration of nitroglycerin or NO donor drugs. The combination of nitroglycerin and acetazolamide suggested that the increase in cerebral blood flow is primarily attributed to the influence of acetazolamide. The cerebral blood flow effects of these drugs and possible interactions during an angina attack need to be investigated.

### Zusammenfassung

#### *Einfluß des Stickoxid-Donator Nitroglycerin allein oder in Kombination auf die Hirndurchblutung bei Pavianen*

Nitroglycerin (CAS 55-63-0, Nitrocel<sup>®</sup>) ist erfolgreich bei der Behandlung von Angina während der letzten Jahrzehnten angewandt worden. Obwohl wichtige Informationen über die pharmakologische Funktion und Wirkung von Nitroglycerin erhalten worden sind, ist bis heute nur eine begrenzte Forschung bezüglich seiner Effekte auf den zerebralen Blutfluß erfolgt. Mit Hilfe von SPECT (single photon emission computed tomography) und PET (positron emission tomography) konnte in den letzten Jahren gezeigt werden, daß deutliche Änderungen im zerebralen Blutfluß bei der Behandlung mit einer großen Vielfalt von Medikamenten erfolgten. Die Aufklärung der pharmakologischen Wirkungsweise von Nitroglycerin verstärkte sich mit der Entdeckung von Stickoxid (NO) als einem endogenen Mediator sowie mit der Erkenntnis, daß Nitroglycerin als ein NO-Donator fungiert. In der vorliegenden Studie wurden sowohl die Wirkungen von Nitroglycerin (0.25 µg/kg/min über einen Zeitraum von 10 min) auf die zerebrale Durchblutung unter Benutzung von <sup>99m</sup>Tc-HMPAO und SPECT in einem anästhesierten Primatenmodell (Papio Ursinus) als auch die Effekte seiner Wechselwirkungen mit solchen Substanzen, die die zerebrovaskuläre Dynamik beeinflussen, gemessen: z. B. Sumatriptan, Nimodipin und Acetazolamid. Die vorliegende Studie mit Nitroglycerin weist darauf hin, daß seine Ansprechzeit für die Messungen des zerebralen Blutflusses einen wichtigen Faktor darstellt, da der transiente Effekt relativ kurz ist. Die gegenwärtige Behand-

lung mit Nitroglycerin weist, verglichen mit Basiskontrollen unter Anästhesie, auf eine leichte Zunahme hin, obwohl das nur für regionale Anstiege speziell in den occipitalen Hirnregionen statistisch signifikant ist. Wirkstoffwechselwirkungen zwischen Nitroglycerin und Nimodipin erschienen als eine 20%ige Reduktion in der zerebralen Durchblutung, verglichen mit der in diesem Fall beobachteten Basiskontrolle. Die Ergebnisse für die Kombination von Nitroglycerin mit Sumatriptan zeigten einen weiteren nahezu signifikanten Anstieg des zerebralen Blutflusses, verglichen mit den Kontrollwerten, und einen signifikanten Anstieg (+27 %) gegenüber ausschließlicher Behandlung mit Sumatriptan. Eine effektive Behandlung mit Sumatriptan könnte daher bei einer gleichzeitigen Verabreichung von Nitroglycerin oder NO-Donatoren beeinträchtigt werden. Die Kombination aus Nitroglycerin und Acetazolamid ließ darauf schließen, daß ein Ansteigen des zerebralen Blutflusses in erster Linie dem Einfluß von Acetazolamid zuzuschreiben ist. Die zerebralen Durchblutungseffekte dieser Substanzen sowie die möglichen Wechselwirkungen während eines Anginaanfalls müssen noch untersucht werden.

**Key words** Acetazolamide · CAS 55-63-0 · Nimodipine · Nitroce® · Nitroglycerin, cerebral blood flow effects, drug interactions, primate model · Sumatriptan

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## 1. Introduction

Since the discovery, identification and subsequent characterisation of the nitric oxide, NO (CAS 10102-43-9) as an important endogenous vascular and inflammatory mediator in living organisms, NO has received ever increasing attention from researchers investigating its wide range of biological effects and its biochemistry [1, 2]. NO is synthesised from L-arginine and molecular oxygen by nitric oxide synthase (NOS) through enzymatic deamination of L-arginine to L-citrulline. Several isoforms of NOS have been sought and discovered [3, 4]. Subsequently novel structures have been searched for and have been discovered, such as the nitric oxide synthase inhibitors, such as methyl arginine and nitroarginine, that influence the vast array of responses observed for NO [1]. The effect of NO or NO donors on cerebral blood flow has recently been the focus of various studies using a variety of experimental models [5-8]. Part of the pharmacological mode of action of the classical cardiovascular drugs, such as nitroglycerin and sodium nitroprusside is now attributed to their decomposition and subsequent action as NO donors [1, 9, 10, 11]. They therefore are able to mimic some of the effects of endogenously released nitric oxide.

The success of the non-human primate model developed by the present authors [12-21], to investigate drug interventions prompted the study of the effects of the NO donor, nitroglycerin (CAS 55-63-0) on the cerebral blood flow. The baboon (*Papio ursinus*) model has previously been used, for single photon emission computed tomography (SPECT) brain imaging utilising the radiopharmaceutical, Tc-99m hexamethylpropylene amine oxime (<sup>99m</sup>Tc-HMPAO), to measure cerebral blood flow during pharmacological interventions with drugs used in different pharmacological fields, i.e. migraine (serotonin receptors), cardiovascular disease (calcium channel blockers), epilepsy, cognitive disorders and glaucoma (carbonic anhydrase inhibi-

tion) [12-21]. The current study reports the cerebrovascular effects of the NO donor, nitroglycerin, also in its drug combinations with other known cerebrovascular drugs, i.e. sumatriptan (CAS 103628-46-2), nimodipine (CAS 66085-59-4) and acetazolamide (CAS 59-66-5) in the primate model, using the split-dose [22, 23] method.

## 2. Materials and methods

Six adult male baboons (*Papio ursinus*, average weight 27 kg) were used for this investigation. The animals were obtained from Mr. E. Venter, Vaalwater, Northern Province (Republic of South Africa). The baboons were housed, maintained and cared for according to the guidelines laid down in the National Code for Animal Use in Research, Education, Diagnosis and Testing of Drugs and related substances in South Africa. These guidelines comply with international standards. The studies were performed after the approval of the Ethics committee of the University of Pretoria. Each baboon was subjected to 5 different procedure (A-E) at least six weeks apart (Table 1).

As limited dose-response data have been reported using nitroglycerin with respect to the current baboon model, a preliminary study was conducted to investigate the effect of dose-time responses upon treatment with nitroglycerin in order to select a treatment regime which could be followed during the current study. The following dose-time intervals were investigated for cerebral blood flow effects: 0.03 µg/kg/min at 12 min; 0.166 µg/kg/min at 5 min; 0.166 µg/kg/min at 10 min; 0.25 µg/kg/min at 2 min; 0.25 µg/kg/min at 5 min; 0.25 µg/kg/min at 10 min; 1.0 µg/kg/min, at 5 min. For all the above described regimes the nitroglycerin was initially administered over 10 min before the time intervals as indicated were started. Based on the results presented in Table 1, a dose-time regime of 0.25 µg/kg/min at 2 min was selected for the present study, i.e. procedures B-E.

Procedure A concerned the control cerebral blood flow (CBF) study using the <sup>99m</sup>Tc-HMPAO split-dose method [22, 23] with two respective SPECT acquisitions after two consecutive <sup>99m</sup>Tc-HMPAO administrations at chosen times (see Table 1), under standard anaesthesia conditions. This entails induction with ketamine hydrochloride (Anaket-V®, Centaur Labs, Bryanston, Gaut-

Table 1: The time schedule for the various drug intervention protocols indicating the time of each intervention.

1st HMPAO						2nd HMPAO double dose					
min											
-12	0	5	6	17	19	24	29	34			
			Sumatriptan	Nitroglycerine	Nimodipine	Acetazolamide					
		SPECT-1							SPECT 2		
min	Procedure A	Procedure B	Procedure C	Procedure D	Procedure E						
-12	ketamine thiopentone	ketamine thiopentone	ketamine thiopentone	ketamine thiopentone	ketamine thiopentone						
0	1st HMPAO	1st HMPAO	1st HMPAO	1st HMPAO	1st HMPAO						
5	SPECT-1	SPECT-1	SPECT-1	SPECT-1	SPECT-1						
6											
17		nitroglycerine	nitroglycerine	nitroglycerine	nitroglycerine						
19				nimodipine	nimodipine						
24			acetazolamide								
29	2nd HMPAO	2nd HMPAO	2nd HMPAO	2nd HMPAO	2nd HMPAO						
34	SPECT-2	SPECT-2	SPECT-2	SPECT-2	SPECT-2						

eng, S.A.; 10 mg/kg), followed by maintenance on thiopentone sodium (Sandothal<sup>®</sup>, Sandoz Products, Randburg, Gauteng, S.A.; 70 ml/h of a 0.5% solution, Procedure A). A period of 12 min stabilisation under anaesthesia followed before the start of the each study.

Procedures B, C, D and E investigated the CBF changes at 2 min post the end of alternatively administration of nitroglycerine (Nitrocene<sup>®</sup>, Schwarz Pharma AG, Randburg, S.A.; 0.25 µg/kg/min for 10 min, Procedure B), a combination of nitroglycerin (0.25 µg/kg/min for 10 min) and sumatriptan (6 mg, Procedure C), a combination of nitroglycerin (0.25 µg/kg/min over 10 min) and nimodipine (1 µg/kg/min over 10 min, Procedure D), and a combination of nitroglycerin (0.25 µg/kg/min over 10 min) and acetazolamide at 24 min (500 mg/5 ml, Procedure E). The test substances were obtained from the respective manufacturers.

### 2.1. Procedure A (control study)

Anaesthesia was induced in each animal by darting with ketamine hydrochloride, and maintained with thiopental sodium, using an administration (drip) set. The baboon is placed in the supine position, positioned with a special head rest to ensure a reproducible position to compare tomographic slices. After a period of 12 min stabilisation, the control study under anaesthesia (thiopental - thiopental), started at time T = 0 with an i.v. injection of 222 MBq <sup>99m</sup>Tc-HMPAO. The baboon was maintained and controlled with thiopental for the duration of the study (Table 1). The first acquisition, SPECT-1, was performed at 5 min with a Siemens Orbiter gamma camera coupled to a Sophy 256G computer using thirty-two 20 s views and a 360° rotation, in 64 × 64 word mode with the baboon in a supine position. At 29 min, the baboon was reinjected with <sup>99m</sup>Tc-HMPAO (444 MBq, i.e. double the first dosage) and SPECT-2 performed at 34 min [22, 23]. These data represented the CBF pattern of <sup>99m</sup>Tc-HMPAO under thiopental anaesthesia to be compared with the patterns after the drug interventions.

### 2.2. Procedure B (nitroglycerin)

Procedure B (nitroglycerin) followed the same protocol as described for the control study except that the nitroglycerin (0.25 µg/kg/min over 10 min) was i.v. administered at 17 min post administration of the first <sup>99m</sup>Tc-HMPAO injection (Table 1). The 2nd <sup>99m</sup>Tc-HMPAO administration and 2nd scan followed the same protocol as the control study. Results for Procedure B would depict cerebral blood flow changes due to the nitroglycerin intervention, 2 min after completion of the drug intervention.

### 2.3. Procedures C (nitroglycerin and sumatriptan), Procedure D (nitroglycerin and nimodipine), Procedure E (nitroglycerin and acetazolamide)

The drug combination Procedures C, D, and E followed the same sequence as procedure B, the nitroglycerin study (administered at 17 min over 10 min), but with the additional administration of an i.m. injection of sumatriptan, 6 mg at 6 min of the study, i.e. 23 min drug response time (Procedure C), administration of nimodipine, 1 µg/kg/min over 10 min starting at 19 min of the study (Procedure D), and administration of acetazolamide, 500 mg i.v. at 24 min of the study, i.e. 5 min drug response time (Procedure E). The 2nd <sup>99m</sup>Tc-HMPAO administration (at 29 min) and 2nd scan, SPECT-2 (at 34 min) followed again the same protocol as the control study (Table 1). The results from these Procedures C to E, would represent the effects of these drug combinations on the cerebral blood flow in the baboon.

The arterial blood pressures were recorded during all the procedures via a catheter in the femoral artery. Heart rates were monitored together with a 12 lead ECG.

After backprojection and reconstruction (filter: Butterworth 4.25), the brain images in all procedures consisted of transaxial, sagittal and coronal slices representing rCBF related information due to the above mentioned drug interventions. Eight slices of one pixel thickness each, represented the brain area in each slice, and in all three views. Regions of interest (ROIs) were placed on the total brain area and count rate data (counts/pixel)

thus obtained were inserted into the following equation to obtain the ratio R:

$$R = \frac{(SPECT-2) - (SPECT-1)^*}{(SPECT-1)}$$

where \* refers to decay corrected data from SPECT-1, present during SPECT-2, which has to be subtracted from the SPECT-2 data, as background; R is an indication of the level change of rCBF due to the changed conditions prevailing during the second HMPAO injection with respect to that of the first injection, i.e. due to prolonged anaesthesia alone (Procedure A), or additionally with drug interventions.

A value of R = 2 (due to the double second dose of <sup>99m</sup>Tc-HMPAO) will indicate no rCBF change during procedure A due to prolonged anaesthesia. R for procedure B, C, D and E will additionally reflect on changes due to the nitroglycerin (B), the combination of nitroglycerin and sumatriptan (C), of nitroglycerin and nimodipine (D) and the nitroglycerin and acetazolamide (E), and was compared to R (Procedure A as control) to assess the effects of these drugs.

2.4. Statistical analysis

Mean ratios (n = 6) and standard deviations (SD) were calculated per procedure for similar regions (slices) and for the total brain in the various projections. These were compared for interprocedural effects as well as regional effects for a particular procedure. The comparisons were assessed for significant differences using Student's two-tailed t-tests on a 1% and 5% level of confidence.

3. Results

Table 2 presents the data reflecting the time-dose cerebral blood flow responses upon treatment with nitroglycerin. Based on these preliminary results the treatment regime for the current study was determined (i.e. 0.25 µg/kg/min administered over 10 min with 2 min response time, after the end of the nitroglycerin administration, before the 2nd HMPAO injection at 29 min). The data are presented in Tables 3, 4 and 5 and Fig. 1 and 2.

Tables 3 and 4 present the mean ratios (R ± SD) (n = 6) for the total brain under anaesthesia only (Procedure A: control), and after the various drug interventions (Procedure B: nitroglycerin (NO), Procedure C: nitroglycerin and sumatriptan, Procedure D: nitroglycerin and nimodipine, Procedure E: nitroglycerin and acetazolamide). Mean percentage (%) changes of ratios from the total brain, comparing the different procedures with each other, are also reflected in Tables 3 and 4. Table 4

Table 2: The mean ratios for the time-dose cerebral blood flow responses at time (min) post the administration of nitroglycerin (µg/kg/min over 10 min) interventions compared to control.

Dose	Time (min)	R ratio
control	-	2.13 ± 0.11
0.03	12	2.11 ± 0.16
0.166	10	2.09 ± 0.04
0.166	20	2.03 ± 0.00
0.25	2	2.23 ± 0.12
0.25	5	2.06 ± 0.18
0.25	10	1.74 ± 0.25
1.0	5	2.05 ± 0.02

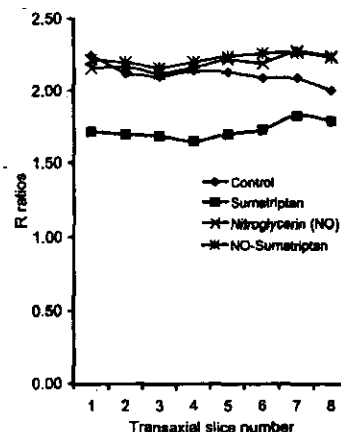


Fig. 1a: Transaxial curves of mean ratio (R) (n = 6) versus slice number starting at the frontal to the occipital lobes for the control, nitroglycerin, sumatriptan and combination of sumatriptan and nitroglycerin.

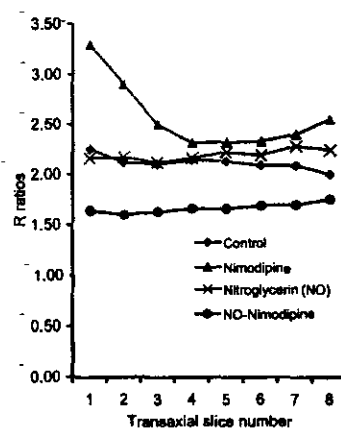


Fig. 1b: Transaxial curves of mean ratio (R) (n = 6) versus slice number starting at the frontal to the occipital lobes for the control, nitroglycerin, nimodipine and combination of nimodipine and nitroglycerin.

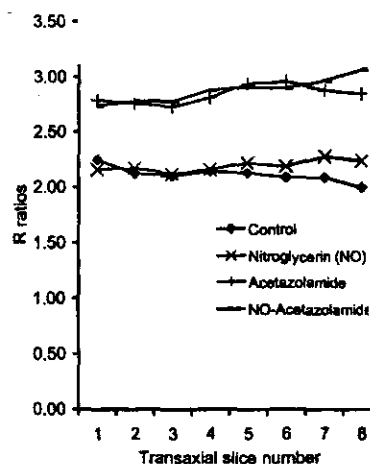


Fig. 1c: Transaxial curves of mean ratio (R) (n = 6) versus slice number starting at the frontal to the occipital lobes for the control, nitroglycerin, acetazolamide and combination of acetazolamide and nitroglycerin.

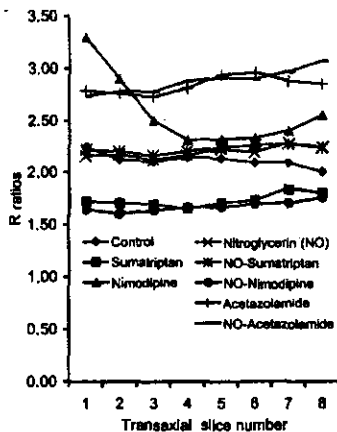


Fig. 2a: Curves of mean ratio (R) (n = 6) versus slice number of all procedures and single drug interventions in the transaxial view starting from the frontal to the occipital lobes.

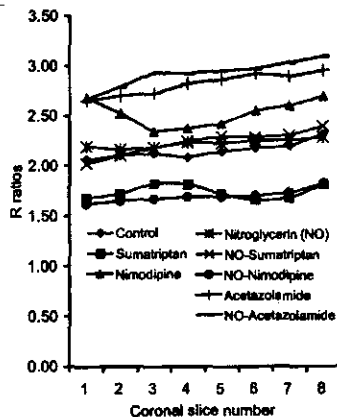


Fig. 2b: Curves of mean ratio (R) (n = 6) versus slice number of all procedures and single drug interventions in the coronal view from the cerebellum to the dorsal slice of the cerebrum.

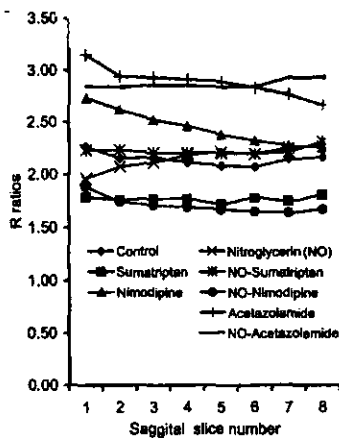


Fig. 2c: Curves of mean ratio (R) (n = 6) versus slice number of all procedures and single drug interventions in the saggital view from left to right of the brain.

presents the mean percentage (%) changes of the ratios, comparing with the drugs previously investigated as single drug administrations, i.e. sumatriptan, nimodipine and acetazolamide.

Table 5 presents the effects of these drug interventions during the different procedures A to E and sumatriptan, nimodipine and acetazolamide, on the cardiovascular parameters, i.e. heart rate and blood pressure. The data has been transformed to difference data and compared with the control data. Fig. 1a, b, c present R-values vs. slice number for the transaxial views for the different Procedures. Fig. 2a, b, c present R-values vs. slice number for the transaxial, coronal and saggital views for the combined data for all the Procedures. Only the transaxial views (Fig. 1a, b, c) are presented here as the saggital and coronal views presented similar ratios.

It is clear from Table 2 and Fig. 1 and 2 that the nitroglycerin (R value  $2.19 \pm 0.08$ ) did not significantly alter the cerebral blood flow when compared with the control R value of  $2.13 \pm 0.12$ , although an increasing trend was noted. The drug combination interventions with nitroglycerin and sumatriptan (Procedure C), or nimodipine (Procedure D), or acetazolamide (Procedure E) reveal distinct different influences on the cerebral blood flow by these combinations (see Table 3 for percentage changes with respect interprocedural differences). The combination of nitroglycerin and acetazolamide (Procedure E, Table 3) significantly increased the cerebral blood flow (total brain R values increased by 35%) when compared with both the control ( $2.89 \pm 0.14$  vs.  $2.13 \pm 0.12$ ,  $p < 0.01$ ) and the nitroglycerin response alone ( $2.89 \pm 0.14$  vs.  $2.19 \pm 0.08$ ,  $p < 0.01$ ). Conversely, the combination of nitroglycerin and nimodipine (Procedure D, Table 3) yielded total brain R values which were statistically significantly decreased when compared with both the control ( $-20\%$ ;  $1.69 \pm 0.18$  vs.  $2.13 \pm 0.11$ ,  $p < 0.01$ ) and the single nitroglycerin drug intervention ( $-22\%$ ;  $1.69 \pm 0.18$  vs.  $2.19 \pm 0.08$ ,  $p < 0.01$ ). The combination of nitroglycerin and sumatriptan (Procedure C, Table 3) did not significantly alter the R values when compared with both the control ( $2.22 \pm 0.07$  vs.  $2.13 \pm 0.11$ ,  $p > 0.05$ ) and the nitroglycerin alone ( $2.22 \pm 0.08$  vs.  $2.19 \pm 0.08$ ,  $p > 0.05$ ).

Table 4 presents the total brain R values for all the Procedures in the current study (A to E), compared to single drug interventions with sumatriptan, nimodipine and acetazolamide [15, 16, 19]. Comparative % changes of all these data sets will follow in the Discussion.

No significant changes occurred for any of the interventions in the heart rate for the Procedures A to E (Table 5). The largest increase in heart rate (8%) was noted for the combination of nitroglycerin and nimodipine but was statistically insignificant. Changes of blood pressure of more than 10% with respect to initial values (before drug intervention) (Table 5) occurred with nitroglycerin (Procedure B) (increase 16%) alone. It is known that anaesthesia also influences the blood pressure and it was previously shown that the current anaes-

Table 3: The mean R value  $\pm$  SD (n = 6) for total brain as averaged from all slices and all views for each procedure, percentage changes of these R-values with respect to the different procedures, and the statistical significance.

Procedure	R-value	% Change with respect to control	% Change with respect to nitroglycerine	% Change with respect to nitroglycerine and sumatriptan	% Change with respect to nitroglycerine and nimodipine
Procedure A Control	2.13 $\pm$ 0.11				
Procedure B Nitroglycerin	2.19 $\pm$ 0.08	+2.8 (p > 0.05)			
Procedure C Nitroglycerin + Sumatriptan	2.22 $\pm$ 0.07	+4.2 (p > 0.05 p < 0.10)	+1.4 (p > 0.05)		
Procedure D Nitroglycerin + Nimodipine	1.69 $\pm$ 0.18	-20.7 (p < 0.01)	-22.8 (p < 0.01)	-23.9 (p < 0.01)	
Procedure E Nitroglycerin + Acetazolamide	2.89 $\pm$ 0.14	+35.7 (p < 0.01)	+32.0 (p < 0.01)	+30.2 (p < 0.01)	+71 (p < 0.01)

Table 4: The mean R value  $\pm$  SD (n = 6) for total brain as averaged from all slices and all views for each procedure, percentage changes of these R-values with respect to sumatriptan, nimodipine and acetazolamide only interventions, and statistical significance.

Procedure	R-value	% Change with respect to control (significance)	% Change with respect to nitroglycerin (significance)	% Change with respect to sumatriptan (significance)	% Change with respect to nimodipine (significance)	% Change with respect to acetazolamide (significance)
Procedure A Control	2.13 $\pm$ 0.11					
Procedure B Nitroglycerin	2.19 $\pm$ 0.08	+2.8 (p > 0.05)				
Procedure C Nitroglycerin + Sumatriptan	2.22 $\pm$ 0.07	+4.2 (p > 0.05 p < 0.10)	+1.4 (p > 0.05)	+27.6 (p < 0.01)	-11.6 (p < 0.02)	-21.8 (p < 0.01)
Procedure D Nitroglycerin + Nimodipine	1.69 $\pm$ 0.18	-20.7 (p < 0.01)	-22.8 (p < 0.01)	-2.9 (p > 0.05)	-32.6 (p < 0.01)	-40.5 (p < 0.01)
Procedure E Nitroglycerin + Acetazolamide	2.89 $\pm$ 0.14	+35.7 (p < 0.01)	+32.0 (p < 0.01)	+66.1 (p < 0.01)	+13.1 (p < 0.05)	+0.02 (p > 0.05)
Sumatriptan [16]	1.74 $\pm$ 0.10	-18.3 (p < 0.05)	-20.5 (p < 0.01)			
Nimodipine [15]	2.51 $\pm$ 0.14	+17.8 (p < 0.01)	+14.6 (p < 0.02)	+44.3 (p < 0.01)		
Acetazolamide [19]	2.84 $\pm$ 0.38	+33.3 (p < 0.01)	+29.7 (p < 0.01)	+63.2 (p < 0.01)	+13.1 (p < 0.05)	

Table 5: The effect of all the procedures on heart rate and blood pressure with respect to control.

Procedure	$\Delta$ HR	$\Delta$ HR %	$\Delta$ BP	$\Delta$ BP%
Procedure B Nitroglycerine	0	0	+13 $\pm$ 4	+16
Procedure C Nitroglycerin + Sumatriptan	+0.2 $\pm$ 5.8	+ 0.2	+4.2 $\pm$ 5.4	+5
Procedure D Nitroglycerin + Nimodipine	+9.2 $\pm$ 21	+8	-134 $\pm$ 9.8	-7
Procedure E Nitroglycerin + Acetazolamide	-1.6 $\pm$ 3.6	-1.4	+10.8 $\pm$ 11.6	+9
Sumatriptan [16]	-1.5 $\pm$ 5	-1.4	+5.3 $\pm$ 3.8	+5
Nimodipine [15]	+4.5 $\pm$ 7.9	+4	-13 $\pm$ 7.9	-7
Acetazolamide [19]	-5.0 $\pm$ 14.0	-4	-7.8 $\pm$ 3.5	-7

thetic regime for control Procedure A increased the blood pressure with approximately 12% with respect to the initial value at the beginning of the procedure [19]. The increase (16%) observed after the single nitroglycerine intervention is therefore not meaningful.

#### 4. Discussion

It was previously demonstrated that various forms of anaesthesia [13, 24, 25] influence cerebral blood flow. Sumatriptan [16], nimodipine [15] and acetazolamide [14, 15] also caused statistically significant changes in the cerebral blood flow with respect to control (see Table 4). However, contrary to a literary report [26], sodium valproate did not

influence the cerebral blood flow in the primate model [19]. It was suggested that chronic treatment used in the literature report [26] compared to the acute single dose approach used in the primate study [19] might explain the inconsistencies in cerebral blood flow effects.

Although in the current study only a slight cerebral blood flow increase of the total brain was observed for nitroglycerin, when compared with the control study, it was not statistically significant with respect to the control ( $R = 2.19$  vs.  $2.13$ ). However, statistically significant regional increases in cerebral blood flow was observed for the occipital areas of the brain, i.e. slice 7 for the transaxial view, with an increase of approximately to 10% ( $R = 2.24$  vs.  $2.0$ ;  $p < 0.05$ ). Similar increases were observed in the occipital regions for the combination of sumatriptan and nitroglycerin ( $R = 2.23$  vs.  $2.0$ ). Diverse cerebral blood flow responses have been reported after nitroglycerin administration. A study in cynomolgus monkeys suggests that tonic production of NO contributes to the control of cerebral blood flow during isoflurane anaesthesia, which is decreased by nitric oxide synthase inhibition, i.e. with N omega-nitro-L-arginine methyl ester (L-NAME) [6]. This study was performed by firstly NOS inhibition by L-NAME, and secondly followed by L-arginine administration which reversed the initial decreased cerebral blood flow [6]. This reversal was however not above the control values. Studies have also showed decreases in cerebral blood flow after inhibition of nitric oxide synthase for different anaesthetic regimes [5, 8]. It was also shown that nitroglycerin increases local cerebral blood flow in man [27]. Furthermore, it was shown that cerebral blood flow was unchanged in beagle dogs after nitroglycerin treatment [28]. From the above and our preliminary investigation it is clear that nitroglycerine treatment regime can be of importance in the clinical situation; furthermore that nitroglycerin related increases in cerebral blood flow may be more evident through the reversal of decreased cerebral blood flow after NOS inhibition.

For the drug combinations it was found that nitroglycerin reversed the nimodipine-induced increase in cerebral blood flow to below the control control value ( $R = 2.51$  vs.  $1.69$ ). An increase in cerebral blood flow after nimodipine administration has also been reported previously [29]. A similar reversal was recently observed upon the combination treatment with sodium valproate and nimodipine [19]. This marked influence supports the importance of calcium and the action of nitroglycerine through the NO process. Drug interaction from simultaneous administration of these two drugs is therefore implicated by these results.

The sumatriptan-induced decreased cerebral blood flow [16] was reversed by nitroglycerin to values that are slightly higher than the control but not statistically significantly. This result indicates that sumatriptan did not influence the cerebral blood

flow due to nitroglycerin treatment which may suggest that sumatriptan would not be effective in treating cerebral blood flow increases due to nitroglycerin therapy, i.e. the nitroglycerin induced headache [9, 10]. These results may also suggest that in so far as the action of sumatriptan is concerned in the treatment of migraine, NO seems to play a lesser role in the cerebrovascular pharmacological mode of action of sumatriptan. However the effective treatment of migraine with sumatriptan may be compromised from simultaneous treatment with nitroglycerin as was indicated by reversal of the effect of sumatriptan.

No significant interaction between nitroglycerin and acetazolamide is envisaged and the effects observed are primarily attributed to acetazolamide for the drug combination. A similar observation was reported in the drug combination of acetazolamide with the anti-epileptic drug, sodium valproate [19] with no drug interactions envisaged.

Nitroglycerin treatment alone exhibited the largest influence on the arterial blood pressure, i.e. an increase of 16% with respect to the control. The increase is insignificant when the increase (12%) due to the anaesthesia alone [19] is taken into consideration. The increase in blood pressure was lower for all the combination studies with nitroglycerin and a decrease was even observed for the combination of nitroglycerin and nimodipine. The heart rates were only marginally influenced with the largest effect being an increase of 8% for the combination of nitroglycerin and nimodipine. The dosage regimes used in the present study for nitroglycerin and nimodipine. The dosage regimes used in the present study for nitroglycerin and the other drugs therefore exhibit insignificant cardiovascular effects when compared with the control.

## 5. Conclusion

The current study was emphasised the clinical importance of a NO donor drug in drug combinations with respect to cerebrovascular effects. The study has shown that, although only marginal total brain cerebral blood flow increases were observed on nitroglycerin treatment, significant regional increases in the occipital areas were noted. Nitroglycerin influences the effects of various cerebrovascular drugs administered simultaneously, in different ways: drug interactions seem possible between nitroglycerin and nimodipine; nitroglycerin treatment may compromise migraine treatment with sumatriptan; the combination of acetazolamide and nitroglycerin was found to be acceptable with respect to cerebral blood flow responses. The influences of the drug combinations of nitroglycerin on the haemodynamic parameters (blood pressure and heart rate) were found to be insignificant with respect to anaesthesia control data. The cerebral blood flow effects of NO synthase inhibitors, such as L-NAME is currently being under investigation.

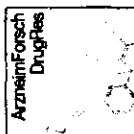
## 6. References

- [1] McNamara, J. O., in: Goodman and Gilman's The Pharmacological Basis of Therapeutics, 9th ed., J. G. Hardman, L. E. Limbird, P. B. Molinoff et al. (eds.), pp. 137, 254, 286, 827, 830, McGraw-Hill, New York (1996)
- [2] Moncada, S., Palmer, R. M. J., Higgs, E. A., *Pharmacol. Rev.* **43**, 109 (1991)
- [3] Bredt, D. S., Snyder, S. H., *Ann. Rev. Biochem.* **63**, 174 (1994)
- [4] Marletta, M. A., *J. Biol. Chem.* **268**, 12231 (1993)
- [5] Todd, M. M., Wu, B., Warner, D. S., Maktabi, M., *Anesthesiology* **80**, 1128 (1994)
- [6] McPherson, R. W., Kirsch, J. R., Tobin, J. R. et al., *Anesthesiology* **80**, 1320 (1994)
- [7] Trucco, M., Cananzi, C., Salvadori, P. R. et al., *Funct. Neurol.* **9**, 247 (1994)
- [8] Hylland, P., Nilsson, G. E., Lutz, P. L., *J. Cereb. Blood Flow Metab.* **16**, 290 (1996)
- [9] Thomsen, L. L., Iversen, H. K., Brinck, T. A. et al., *Cephalgia* **13**, 396 (1993)
- [10] Iversen, H. K., *Cephalgia* **15**, 281 (1995)
- [11] Olesen, J., Iversen, H. K., Thomsen, L. L., *Neuroreport* **4**, 1027 (1993)
- [12] Dormehl, I. C., Oliver, D. W., Hugo, N., in: SPECT in Neurology and Psychiatry, P. P. de Deyn, R. A. Driecks, A. Alavi et al. (eds.), Chapter 62, pp. 521-535, John Libbey and Company, Amsterdam (1997)
- [13] Dormehl, I. C., Redelinghuys, F., Hugo, N. et al., *J. Med. Primatol.* **21**, 270 (1992)
- [14] Oliver, D. W., Dormehl, I. C., Redelinghuys, I. F. et al., *Nukl. Med.* **32**, 292 (1993)
- [15] Dormehl, I. C., Oliver, D. W., Hugo, N., *Nucl. Med. Comm.* **14**, 573 (1993)
- [16] Oliver, D. W., Dormehl, I. C., Hugo, N., *Arzneim.-Forsch./Drug Res.* **44 (II)**, 925 (1994)
- [17] Dormehl, I. C., Oliver, D. W., Hugo, N., *Arzneim.-Forsch./Drug Res.* **45 (II)**, 952 (1995)
- [18] Jordaan, B., Oliver, D. W., Dormehl, I. C., Hugo, N., *Arzneim.-Forsch./Drug Res.* **46 (II)**, 844 (1996)
- [19] Oliver, D. W., Dormehl, I. C., *Arzneim.-Forsch./Drug Res.* **48 (II)**, 1058 (1998)
- [20] Dormehl, I. C., Oliver, D. W., Hugo, N. et al., *Nucl. Med. Biol.* **22**, 373 (1995)
- [21] Dormehl, I. C., Oliver, D. W., Langen, K.-J. et al., *J. Nucl. Med.* **38**, 1897 (1997)
- [22] Wyper, D. J., Hunter, R., Patterson, J. et al., *J. Cereb. Blood Flow Metab.* **11**, S449 (1991)
- [23] Holm, S., Madsen, P. L., Rubin, P. et al., *J. Cereb. Blood Flow Metab.* **11**, S766 (1991)
- [24] Lassen, N. A., Christensen, M. S., *Br. J. Anaesth.* **48**, 719 (1976)
- [25] Wood, M., Wood, A. J. J., *Drugs Anaesth.* **2**, 179 (1990)
- [26] Futagi, Y., Otani, K., Imai, K., *Epilepsia* **35**, 827 (1994)
- [27] Abe, K., *Can. J. Anaesth.* **40**, 775 (1993)
- [28] Hamaguchi, M., Ishibashi, T., Katsumata, N. et al., *Cardiovasc. Drugs Ther.* **6**, 611 (1992)
- [29] Langley, M. S., Sorkin, E. M., *Drugs* **27**, 669 (1989)

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# Effect of Cyclodextrin Complexation on the in vivo Disposition of the Brain Imaging Radiopharmaceutical $^{99m}\text{Tc}$ Ethyl Cysteinate Dimer ( $^{99m}\text{Tc}$ -ECD)

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## Summary

The brain imaging radiopharmaceutical,  $^{99m}\text{Tc}$  ethyl cysteinate dimer ( $^{99m}\text{Tc}$ -ECD,  $^{99m}\text{Tc}$ -bicisate) is the most recent addition to the available set of radiopharmaceuticals for measuring cerebral blood flow. Ideally radiotracers should be trapped in the brain long enough so that their distribution can be quantitated and should demonstrate good spatial resolution. Furthermore, the stability (chemical and metabolic) and bioavailability of radiopharmaceuticals have in general proved to be a challenge during development and clinical administration. In view of these challenges and background, this study with  $^{99m}\text{Tc}$ -ECD is presented. The aims of this research program were to develop novel approaches to improve the chemical and metabolic stability and the bioavailability of  $^{99m}\text{Tc}$ -ECD across the blood brain barrier for cerebral blood flow determinations, using the well known non-human primate in vivo baboon model. These aims were addressed by investigating the influence of cyclodextrin -  $^{99m}\text{Tc}$ -ECD complexation on normal cerebral blood flow patterns, using two different cyclodextrins, i.e.,  $\gamma$ -cyclodextrin (CAS 17465-86-0) and  $\beta$ -trimethylcyclodextrin (CAS 55216-11-0). The effect of incubation of  $^{99m}\text{Tc}$ -ECD (with or without cyclodextrin complexation) in plasma, on metabolic esterase action, was also investigated. Possible protection against plasma esterase by acetylcholine (CAS 51-84-3) of  $^{99m}\text{Tc}$ -ECD was further determined. The current study has shown that cyclodextrin complexation of  $^{99m}\text{Tc}$ -ECD indeed offers a useful approach to improve the stability of the radiopharmaceutical against peripheral metabolism. The acetylcholine shows also potential to protect  $^{99m}\text{Tc}$ -ECD. However, it is clear from the current data that the choice of cyclodextrin is of utmost importance, as has been observed from significantly reduced the bioavailability of  $^{99m}\text{Tc}$ -ECD when complexed with  $\beta$ -trimethylcyclodextrin. The plasma incubation procedures showed that  $\gamma$ -cyclodextrin offers protection with only slightly reduced bioavailability. This study has indicated that novel approaches, such as cyclodextrin technologies, indeed show potential to modify the performance in its currently available  $^{99m}\text{Tc}$ -ECD form.

## Zusammenfassung

### *Einfluß der Cyclodextrin-Komplexierung auf die In-vivo-Verteilung des Hirnszintigraphie-Radiopharmakons <sup>99m</sup>Tc-Ethylcysteinat-Dimer (<sup>99m</sup>Tc-ECD)*

Technetium-<sup>99m</sup>-Ethylcysteinat-Dimer (<sup>99m</sup>Tc-ECD) ist das jüngste Radiopharmakon zur Messung des zerebralen Blutflusses. Im Idealfall sollten Radiotracer ausreichend lange im Gehirn gespeichert werden, um eine Qualifizierung ihrer Verteilung bei guter räumlicher Auflösung zu gewährleisten. Eine besondere Herausforderung während der Entwicklung solcher Radiopharmazeutika ist die generelle Gewährleistung ihrer Stabilität (chemisch und metabolisch) sowie ihrer Bioverfügbarkeit. Die vorliegende Studie mit <sup>99m</sup>Tc-ECD konzentriert sich auf diese Probleme. Neue Annäherungen zur Verbesserung der metabolischen Stabilität und Bioverfügbarkeit durch die Blut-Hirn-Schranke zur Messung des zerebralen Blutflusses werden vorgestellt. Dabei wurde das bewährte Primatenmodell benutzt. Im einzelnen wurde 1. der Einfluß der Cyclodextrin-<sup>99m</sup>Tc-ECD-Komplexierung, einerseits mit  $\gamma$ -Cyclodextrin, andererseits mit  $\beta$ -Trimethylcyclodextrin, und 2. der Effekt der Inkubation von <sup>99m</sup>Tc-ECD (mit oder ohne Cyclodextrin-Komplexierung) in Plasma auf die metabolische Esterase-Aktivität untersucht. Dabei ergab sich ein gewisser Schutzeffekt von <sup>99m</sup>Tc-ECD durch Acetylcholin. Die Ergebnisse zeigen, daß 1. die Cyclodextrin-Komplexierung die Stabilität des Radiopharmakons im peripheren Metabolismus erhöht, 2. die  $\gamma$ -Cyclodextrin-Komplexierung dabei nur zu einer geringen, die  $\beta$ -Trimethylcyclodextrin-Komplexierung aber zu einer deutlichen Reduktion der Bioverfügbarkeit führt, 3. auch Acetylcholin einen Schutzeffekt auf <sup>99m</sup>Tc-ECD hat. Die Studie zeigt, daß die Cyclodextrin-Technologie in der Tat geeignet ist, die gegenwärtig vorhandene Form von <sup>99m</sup>Tc-ECD für die Hirnszintigraphie deutlich zu verbessern.

**Key words** Brain imaging · Cyclodextrin, complexation with technetium-<sup>99m</sup> ethyl cysteinat dimer · Radiopharmaceuticals · Technetium-<sup>99m</sup> ethyl cysteinat dimer, complexation with cyclodextrin

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## 1. Introduction

Several radiopharmaceutical agents, e.g., N-isopropyl-<sup>123</sup>I]-iodoamphetamine ([<sup>123</sup>I]IMP), <sup>99m</sup>technetium hexamethyl-propyleneamine oxime (<sup>99m</sup>Tc-HMPAO) and <sup>99m</sup>technetium ethyl cysteinat dimer (<sup>99m</sup>Tc-ECD) have found useful application as tracers to measure cerebral blood flow (CBF) [1, 2]. Ideally these tracers should be trapped in the brain long enough so that their distribution can be quantitated, and should demonstrate good spatial resolution. The abovementioned agents each show unique properties that, although they are far from the optimal, warrant their clinical application in nuclear medicine. Despite its widespread use, the iodine labeled amphetamine, IMP appears to redistribute in the brain with time [3], and its retention mechanism is stereoselective which depends on its metabolism [4]. Furthermore, the stability (chemical and metabolic) and bioavailability of radiopharmaceuticals have in general proved to be a challenge during development and clinical administration. The retention of <sup>99m</sup>Tc-HMPAO in the brain is further limited to the enzymatic reactions with glutathione [5, 6]. <sup>99m</sup>Tc-ECD exhibits a high initial brain extraction with a slow clearance [7] with brain metabolism yielding hydrophilic monoacid esters trapped in the primate brain [8]. Peripheral systemic enzymatic esterase metabolism [9, 10] of <sup>99m</sup>Tc-ECD, further negatively impacts on the amount of brain extraction for the tracer, due

the formation of hydrophilic acid derivatives, unable to cross the hydrophobic blood brain barrier. Recently, improved stability was demonstrated with <sup>99m</sup>Tc-labeled liposomes through formation of a hydrazino nicotyl derivative [11]. Moretti's group recently investigated the uptake of liposome-encapsulated <sup>99m</sup>Tc-MIBI by both sensitive and multi-drug-resistant tumour cell lines [12]. Cyclodextrin technologies have frequently been employed during the last decade to improve the bioavailability of poor water soluble drugs, i.e. miconazole, lorazepam, cyclosporin A and others [13–15]. In view of these challenges and background, the current study with <sup>99m</sup>Tc-ECD is presented. The aims of this research program were to develop novel approaches to improved the metabolic stability and the bioavailability of <sup>99m</sup>Tc-ECD across the blood brain barrier for cerebral blood flow determinations, using the well known non-human primate in vivo baboon model [1]. These aims were addressed using cyclodextrin complexation technologies (using two different cyclodextrins, i.e.,  $\gamma$ -cyclodextrin (CAS 17465-86-0) and  $\beta$ -trimethylcyclodextrin, i.e. heptakis 2,3,6-tri-O-methyl- $\beta$ -cyclodextrin (CAS 55216-11-0)) and incubating <sup>99m</sup>Tc-ECD in plasma with or without cyclodextrin complexation prior to in vivo administration. It was further assessed if acetylcholine (CAS 51-84-3) was able to protect <sup>99m</sup>Tc-ECD from peripheral esterase metabolisms

## 2. Materials and methods

Six adult male baboons (*Papio ursinus*, average weight 25 kg) were used for this study. The animals were obtained from Mr. E. Venter, Northern Province (Republic of South Africa). The studies were performed after approval by the Ethics Committee of the University of Pretoria, according to the guidelines of the National Code for Animal Use in Research, Education and Testing of Drugs and Related Substances in South Africa. These guidelines are in line with international standards. Previously conducted studies used the sensitive familiar baboon model developed for cerebral blood flow determinations with single photon emission computed tomography [16-23]. An identical approach was followed for the current investigation.

Six different procedures (A-F) were carried out on each of the six baboons with three week intervals between. These procedures were: five modifications in the use of  $^{99m}\text{Tc}$ -ECD as CBF tracer with hopefully higher bioavailability to the brain than is the case of unmodified, conventional  $^{99m}\text{Tc}$  labelled ECD which made out the sixth study (see Procedure A in Table 1).

For the control study (Procedure A), each baboon was sedated with ketamine hydrochloride (10 mg/kg i.m)

(Anaket-V<sup>®</sup>, Centaur Labs, Bryanston, Gauteng, SA). This was followed immediately by maintained and controlled infusion of thiopentone (thiopental) sodium (70 ml/h of 0,5 % solution) (Intraval<sup>®</sup>, Rhône-Poulenc Rorer S.A., Midrand, Gauteng, SA). After a 12-min stabilisation period under thiopentone, Procedure A, the control study started at  $t = 0$  with an i.v. injection of 222 MBq of  $^{99m}\text{Tc}$ -Ethyl Cysteinate Dimer (ECD), Neurolite<sup>®</sup>, Du Pont Pharma). The ECD was labelled according to the manufacturer's directions. Five min after the tracer injection for Procedure A, the first SPECT acquisition (SPECT-1) followed with a Siemens Orbiter gamma camera, using 32 projections of 20 s per view during a 360 ° rotation. The baboons were always positioned in the supine position with a special head rest to ensure reproducible and comparable tomographic slices for all procedures. SPECT-1 was followed by a second intravenous administration of tracer  $^{99m}\text{Tc}$ -ECD of double the radioactivity dose (i.e. 444 MBq) at  $t = 20$  min. After another 5 min a similar SPECT acquisition, SPECT-2 followed (the split dose method), which, for Procedure A, measures the anaesthesia related CBF changes taking place in between the two ECD administrations. Procedure B, was the same as Procedure A, except that the second tracer application was of  $^{99m}\text{Tc}$ -

Table 1: The time schedule for the various tracer procedure protocols indicating the time of each procedure.

min	Procedure A	Procedure B	Procedure C	Procedure D	Procedure E	Procedure F
	ketamine	ketamine	ketamine	ketamine	ketamine	ketamine
-12	thiopental	thiopental	thiopental	pentobarbital	pentobarbital	
	1st $^{99m}\text{Tc}$ -ECD	1st $^{99m}\text{Tc}$ -ECD	1st $^{99m}\text{Tc}$ -ECD	1st $^{99m}\text{Tc}$ -ECD	1st $^{99m}\text{Tc}$ -ECD	
	SPECT-1	SPECT-1	SPECT-1			
		mix ECD + plasma				
7			mix + plasma + acetylcholine			
8		incubation (37 °C, 10 min)	incubation (37 °C, 10 min)			
18		mix $^{99m}\text{Tc}$ + ECD + plasma	mix $^{99m}\text{Tc}$ + ECD + acetylcholine + plasma			
20	injection 2nd $^{99m}\text{Tc}$ -ECD	injection 2nd $^{99m}\text{Tc}$ -ECD + plasma	injection 2nd $^{99m}\text{Tc}$ -ECD + acetylcholine + plasma			
25	SPECT-2	SPECT-2	SPECT-2			
				complexation 2nd $^{99m}\text{Tc}$ -ECD + $\gamma$ -cyclodextrin	complexation 2nd $^{99m}\text{Tc}$ -ECD + $\beta$ -trimethyl-cyclodextrin	complexation 2nd $^{99m}\text{Tc}$ -ECD + $\gamma$ -cyclodextrin
45				SPECT-1	SPECT-1	SPECT-1 and mix $^{99m}\text{Tc}$ -ECD + $\gamma$ -cyclodextrin + plasma
47						
60				injection 2nd $^{99m}\text{Tc}$ -ECD + $\gamma$ -cyclodextrin	injection 2nd $^{99m}\text{Tc}$ -ECD + $\beta$ -trimethyl-cyclodextrin	
105				SPECT-2	SPECT-2	

ECD incubated in 2 ml plasma, which was obtained from 10 ml of the baboon's blood which was previously drawn and centrifuged. The ECD and plasma were mixed at 6 min and at  $t = 8$  min the incubation period for 10 min ( $37^\circ\text{C}$ ) started. At 18 min double the first activity dose of  $^{99\text{m}}\text{Tc}$  (444 MBq) was mixed with ECD in plasma and the second i.v. administration of  $^{99\text{m}}\text{Tc}$ -ECD in plasma took place at  $t = 20$  min. Thus, SPECT-2, which again followed 5 min after the second injection at  $t = 25$  min reflected the influence of the plasma incubation of ECD in relation to its bioavailability to the brain.

Procedure C had the same protocol as Procedure B, but the second application was an infusion of Tc-labelled ECD, plasma and acetylcholine (1% solution) the latter to act as an esterase substrate in the plasma. This tracer modification was achieved as follows: at  $t = 7$  min the plasma and acetylcholine were mixed and incubation ( $37^\circ\text{C}$ ) started at  $t = 8$  min, for 10 min. At 18 min double the first dose of  $^{99\text{m}}\text{Tc}$ -ECD (444 MBq) was mixed with the plasma and acetylcholine. This was injected i.v. as a second tracer at  $t = 20$  min. SPECT-2 followed 5 min later ( $t = 25$  min). SPECT-2 should reflect improved brain bioavailability with respect to SPECT-1, if the acetylcholine protected the ECD from degradation by plasma esterases.

Procedure D and E concerned complexation of the ECD into two different cyclodextrin formulations,  $\gamma$ -cyclodextrin and  $\beta$ -trimethylcyclodextrin, i.e. heptakis 2,3,6-tri-O-methyl- $\beta$ -cyclodextrin. For both these procedures the maintaining anaesthesia was changed to the long acting sodium pentobarbitone (30 ml/h of a 0.8% solution) (Sagatal<sup>®</sup>, Kyron Laboratories Pty Limited, Benrose, Gauteng, SA), due to the longer time involved, to allow for release of the ECD from the complex to cross the blood brain barrier (see Table 1).  $^{99\text{m}}\text{Tc}$ -ECD was complexed with  $\gamma$ -cyclodextrin by incubating the  $^{99\text{m}}\text{Tc}$ -ECD with a 10-30 molar excess ( $\gamma$ -CD: ECD) for 30 min at room temperature in physiological normal saline solution. In Procedure E, the  $\gamma$ -cyclodextrin was replaced by  $\beta$ -trimethylcyclodextrin and the complexation performed as before. Split dose SPECT was performed as in Procedures A-C, except that SPECT-1 followed 45 min after the first i.v. administration of  $^{99\text{m}}\text{Tc}$ -ECD, and the second SPECT-2, likewise 45 min after either ECD +  $\gamma$ -cyclodextrin, or ECD +  $\beta$ -trimethylcyclodextrin, labelled with the double dose of  $^{99\text{m}}\text{Tc}$ -ECD, was administered. Additionally Procedure F was eventually performed, similarly to Procedure D, but the second injection was of  $^{99\text{m}}\text{Tc}$ -ECD +  $\gamma$ -cyclodextrin with additional incubation done in 2 ml plasma for 10 min, starting at 47 min, injected at 60 min and scanning started at 105 min.

During all the above procedures arterial blood pressures were recorded from a catheter in the femoral artery and heart rates were monitored.

After backprojection and reconstruction of SPECT-1 and SPECT-2 data, the brain images in all procedures consisted of transaxial, sagittal and coronal slices, representing total brain CBF and some regional related CBF information. Eight slices of one pixel thickness each represented the brain in all three views, as mentioned above.

Regions of interest were placed on the total brain, as viewed in each slice and count rate data (counts/pixel) thus obtained were inserted into the following equation to obtain the ratio R:

$$R = \frac{(\text{SPECT-2}) - (\text{SPECT-1})^*}{\text{SPECT-1}}$$

where \* refers to decay-corrected data from SPECT-1 which is present during SPECT-2 and has to be subtracted from the SPECT-2 data as a background correction.

R is an indication of the level change of (r)CBF during extended anaesthesia using  $^{99\text{m}}\text{Tc}$ -ECD, or in addition, because of modifications of the second tracer in each study.

### 2.1. Statistical methods

The R values for eight slices in transaxial, sagittal and coronal view could be compared between control and modified studies and between modified tracers studies. A two-tailed Student's t-test for paired variables was used, to express significance with a 5% level of confidence.

### 3. Results

R values (mean  $\pm$  SD) are presented in Tables 2-4 (transaxial, sagittal and coronal), for each of the eight slices, and each Procedure A-F. It is clear from Tables 2-4 that the biodisposition of the Tc-ECD radiopharmaceuticals is influenced differently. With no intraprocedural regional, slice dependent differences showing statistical significance (see Fig. 1-transaxial), mean R-values including all slices and all views were obtained, and used to represent the total brain for each procedure, as shown in Table 4. Percentage changes due to the tracer modification ( $\Delta R$ ) are given between the different procedures in Table 5. No significant haemodynamic changes were experienced for Procedures A-F. The control R value for cerebral blood flow under thiopentone anaesthesia, and measured with  $^{99\text{m}}\text{Tc}$ -ECD in the normal way (Procedure A) is similar to previous data [2], viz.  $R = 2.07 \pm 0.15$ . From Table 5 it is clear that incubating the ECD with the animal's plasma for 10 min before injection for the 2nd scan (Procedure B) significantly reduced ( $R = 1.75 \pm 0.11$ ) the bioavailability to the brain by 15.5% ( $p < 0.05$ ), and that this attenuation was only slightly altered (-13%) with the addition of acetylcholine (Procedure C) to the plasma ( $R = 1.80 \pm 0.20$ %, change -13%,  $p > 0.05$ ,  $p < 0.10$  with respect to Control). The ef-

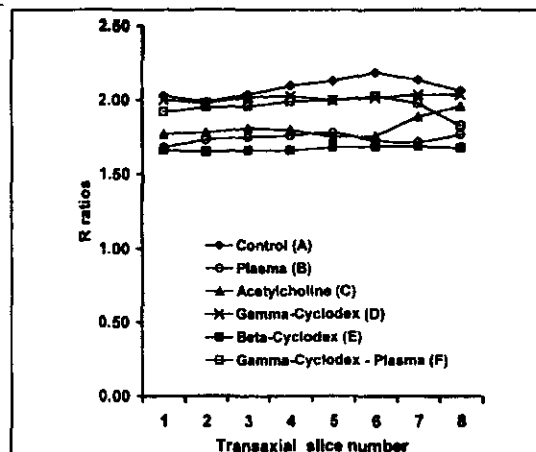


Fig. 1. Curves of the mean ratio (R) ( $n = 6$ ) versus slice number of all the procedures (A-F) in the transaxial view starting from the frontal to occipital lobes.

Table 2: The mean R-ratios ( $\pm$  SD) (n = 6) from transaxial views of eight equal cerebral slices for the six different procedures with the slice number starting from the frontal to the occipital lobes.

Procedure	Slice 1	Slice 2	Slice 3	Slice 4	Slice 5	Slice 6	Slice 7	Slice 8
A	2.03 $\pm$ 0.15	1.99 $\pm$ 0.21	2.05 $\pm$ 0.26	2.10 $\pm$ 0.28	2.13 $\pm$ 0.30	2.19 $\pm$ 0.28	2.14 $\pm$ 0.28	2.07 $\pm$ 0.36
B	1.68 $\pm$ 0.13	1.73 $\pm$ 0.10	1.75 $\pm$ 0.14	1.76 $\pm$ 0.12	1.78 $\pm$ 0.10	1.73 $\pm$ 0.12	1.72 $\pm$ 0.10	1.77 $\pm$ 0.15
C	1.77 $\pm$ 0.04	1.78 $\pm$ 0.11	1.81 $\pm$ 0.16	1.80 $\pm$ 0.22	1.75 $\pm$ 0.01	1.76 $\pm$ 0.15	1.89 $\pm$ 0.08	1.96 $\pm$ 0.01
D	2.00 $\pm$ 0.46	1.98 $\pm$ 0.34	2.02 $\pm$ 0.31	2.03 $\pm$ 0.32	2.00 $\pm$ 0.30	2.02 $\pm$ 0.31	2.04 $\pm$ 0.18	2.04 $\pm$ 0.15
E	1.66 $\pm$ 0.08	1.65 $\pm$ 0.10	1.66 $\pm$ 0.12	1.66 $\pm$ 0.12	1.68 $\pm$ 0.10	1.69 $\pm$ 0.09	1.69 $\pm$ 0.10	1.68 $\pm$ 0.02
F	1.92 $\pm$ 0.23	1.95 $\pm$ 0.28	1.96 $\pm$ 0.15	1.99 $\pm$ 0.30	2.00 $\pm$ 0.30	2.03 $\pm$ 0.30	1.98 $\pm$ 0.23	1.83 $\pm$ 0.26

Table 3: The mean R-ratios ( $\pm$  SD) (n = 6) from sagittal views of eight equal cerebral slices for the six different procedures with the slice number from the left to right.

Procedure	Slice 1	Slice 2	Slice 3	Slice 4	Slice 5	Slice 6	Slice 7	Slice 8
A	2.18 $\pm$ 0.40	2.12 $\pm$ 0.30	2.12 $\pm$ 0.27	2.09 $\pm$ 0.26	2.10 $\pm$ 0.15	2.07 $\pm$ 0.30	2.06 $\pm$ 0.30	2.03 $\pm$ 0.15
B	1.75 $\pm$ 0.07	1.74 $\pm$ 0.07	1.74 $\pm$ 0.11	1.81 $\pm$ 0.13	1.78 $\pm$ 0.16	1.74 $\pm$ 0.11	1.75 $\pm$ 0.11	1.74 $\pm$ 0.12
C	1.59 $\pm$ 0.37	1.70 $\pm$ 0.25	1.75 $\pm$ 0.13	1.79 $\pm$ 0.05	1.86 $\pm$ 0.01	1.81 $\pm$ 0.01	1.94 $\pm$ 0.16	2.04 $\pm$ 0.35
D	1.73 $\pm$ 0.85	2.05 $\pm$ 0.24	2.02 $\pm$ 0.27	2.00 $\pm$ 0.28	2.00 $\pm$ 0.27	2.01 $\pm$ 0.25	1.98 $\pm$ 0.24	1.96 $\pm$ 0.27
E	1.83 $\pm$ 0.20	1.71 $\pm$ 0.13	1.68 $\pm$ 0.12	1.66 $\pm$ 0.11	1.66 $\pm$ 0.12	1.67 $\pm$ 0.11	1.67 $\pm$ 0.09	1.68 $\pm$ 0.07
F	2.08 $\pm$ 0.63	1.87 $\pm$ 0.27	1.83 $\pm$ 0.27	1.92 $\pm$ 0.27	1.95 $\pm$ 0.28	1.96 $\pm$ 0.28	1.92 $\pm$ 0.27	1.84 $\pm$ 0.28

Table 4: The mean R-ratios ( $\pm$  SD) (n = 6) from coronal views of eight equal cerebral slices for the six different procedures with the slice number from the cerebellum to the dorsal slice of the cerebrum.

Procedure	Slice 1	Slice 2	Slice 3	Slice 4	Slice 5	Slice 6	Slice 7	Slice 8
A	2.12 $\pm$ 0.45	2.15 $\pm$ 0.41	2.11 $\pm$ 0.33	2.11 $\pm$ 0.26	2.06 $\pm$ 0.26	2.03 $\pm$ 0.24	1.92 $\pm$ 0.27	1.71 $\pm$ 0.68
B	1.77 $\pm$ 0.12	1.75 $\pm$ 0.08	1.76 $\pm$ 0.11	1.75 $\pm$ 0.08	1.75 $\pm$ 0.13	1.73 $\pm$ 0.10	1.73 $\pm$ 0.10	1.72 $\pm$ 0.10
C	1.45 $\pm$ 0.78	1.38 $\pm$ 0.51	1.57 $\pm$ 0.30	1.71 $\pm$ 0.08	1.85 $\pm$ 0.04	1.95 $\pm$ 0.07	2.08 $\pm$ 0.30	2.32 $\pm$ 0.60
D	1.94 $\pm$ 0.28	1.99 $\pm$ 0.26	2.04 $\pm$ 0.28	2.01 $\pm$ 0.24	2.04 $\pm$ 0.24	2.01 $\pm$ 0.26	2.04 $\pm$ 0.24	2.02 $\pm$ 0.24
E	1.63 $\pm$ 0.09	1.65 $\pm$ 0.10	1.66 $\pm$ 0.10	1.67 $\pm$ 0.10	1.69 $\pm$ 0.11	1.70 $\pm$ 0.11	1.71 $\pm$ 0.12	1.71 $\pm$ 0.15
F	2.01 $\pm$ 0.37	2.09 $\pm$ 0.34	2.07 $\pm$ 0.31	1.96 $\pm$ 0.25	1.82 $\pm$ 0.25	1.74 $\pm$ 0.25	1.63 $\pm$ 0.26	1.51 $\pm$ 0.46

fect of cyclodextrin complexation with the radiotracer is clearly evident in the results obtained from Procedures D and E. Complexing ECD with  $\gamma$ -cyclodextrin for SPECT-2 as described in Procedure D, yielded an R-value of  $2.00 \pm 0.30$ , which although 3.4% below the control of Procedure A,

was not statistically significantly different from the control value ( $p > 0.05$ ). On the other hand, the complex between ECD and  $\beta$ -trimethylcyclodextrin resulted in significantly decreased R-value when compared to both the control, Procedure A ( $R = 1.68 \pm 0.11$  vs.  $2.07 \pm 0.15$ ,  $p < 0.02$ ) and the  $\gamma$ -cyclodextrin complex, Procedure D ( $R = 1.68 \pm 0.11$  vs.  $2.00 \pm 0.30$ ,  $p < 0.05$ ). The changes are -18.8% and -16% between Procedures A and D and Procedures B and D, respectively. The result from Procedure F, where the complex of ECD and  $\gamma$ -cyclodextrin was incubated in plasma shows a R-value of  $R = 1.91 \pm 0.12$ . This result was significantly higher (+9%) than the  $R = 1.80 \pm 0.20$  observed in Procedure B, for uncomplexed ECD that was incubated in plasma. Conversely, the result from Procedure F is a slightly but not significantly lower (-7.7%) R-value than the control result ( $R = 2.07 \pm 0.15$ ). The results from Procedures D ( $2.00 \pm 0.30$ ) and F ( $1.91 \pm 0.12$ ) are similar with no significant difference.

Table 5: The mean R-values  $\pm$  SD (n = 6) for total brain as averaged from all slices and all views for each Procedure. percentage changes ( $\Delta R$ ) of these R-values with respect to the procedure indicated in brackets.

Procedure	R-value	Change % ( $\Delta R$ )	Significance
Procedure A Control	2.07 $\pm$ 0.15		
Procedure B ECD in plasma incubated	1.75 $\pm$ 0.11	-15.5% (A)	$p < 0.05$
Procedure C ECD + plasma + acetylcholine incubated	1.80 $\pm$ 0.20	-13.0% (A) +3.0% (B)	$p < 0.10$ , $p > 0.05$ $p > 0.05$
Procedure D ECD + $\gamma$ -cyclo- dextrin	2.0 $\pm$ 0.30	-3.4% (A) +8.6% (B)	$p > 0.05$ $p > 0.05$ , $p < 0.10$
Procedure E ECD + $\beta$ -tri- methylcyclodex- trin	1.68 $\pm$ 0.11	-18.8% (A) -16.0% (D)	$p < 0.02$ $p < 0.05$
Procedure F ECD + $\gamma$ -cyclo- dextrin in plasma incubated	1.91 $\pm$ 0.12	-7.7% (A) +9.0% (B) -4.5% (D)	$p > 0.05$ $p < 0.05$ $p > 0.05$

#### 4. Discussion

The current study indicated that the metabolic stability and central nervous system bioavailability across the blood-brain barrier are important components for cerebral blood flow radioactive tracers, such as technetium labeled ECD. Furthermore, these aspects can be influenced, e.g. to improve the brain imaging properties of the radiopharmaceu-

tical. The present study focussed on the effect of complexation of the ECD with two different cyclodextrins during cerebral flow determinations. The instability of ECD due to metabolism by plasma esterases was further pointed out by incubating ECD in vitro in plasma with or without being complexed with the cyclodextrin prior in vivo SPECT investigation.

The marked reduction (-15.5%) in the R-value for Procedure B (plasma incubation) when compared with the control (Procedure A) clearly indicate that the incubation of ECD in plasma adversely affected the ECD metabolic stability. This additional exposure of ECD to the plasma esterases *ex vivo* before the in vivo administration, clearly resulted in significant additional esterase metabolism of ECD. Inoue and co-workers [24] earlier reported the in vitro metabolism of ECD in blood. They concluded that the majority of the enzyme that is involved in the metabolism of ECD exists in the red blood cells. An approach to protect ECD from this esterase degradation was attempted with Procedures C (adding acetylcholine) and Procedures D and E (using different cyclodextrins complexes). The improvement of 3% in the case of Procedure C (adding acetylcholine) above Procedure B is measurable and maybe indicative of the slight protection of acetylcholine towards the esterase degradation of Tc-ECD, but insignificant ( $p > 0.05$ ) under the current dosage regimes.

On the other hand complexing ECD with  $\gamma$ -cyclodextrin for SPECT-2 as described in Procedure D, yielded an R-value of  $2.00 \pm 0.30$ , which although 3.4% below the control of Procedure A, was not statistically significantly different from it ( $p > 0.05$ ). The cyclodextrin complexation showed protection of ECD when compared with Procedure B. An improvement of 8.6% was observed for the complexed ECD over the ECD not being complexed with the cyclodextrin and incubated in plasma. This finding is further supported by the result obtained from Procedure F, i.e. that  $\gamma$ -cyclodextrin offered some protection to the ECD against degradation. This can be seen from the R-value,  $R = 1.91 \pm 0.12$  for Procedure F, where the incubation of  $\gamma$ -cyclodextrin complexed ECD in plasma did not show the same degree of degradation as in Procedure B ( $\Delta R = +9\%$ ,  $p < 0.05$ ), while not significantly differing from Procedure A ( $\Delta R = -7.70\%$ ,  $p > 0.05$ ) or from Procedure D ( $\Delta R = -4.5\%$ ,  $p > 0.05$ ) where no plasma incubation was involved.

The somewhat lower bioavailability of  $\gamma$ -cyclodextrin complexed ECD to the brain (Procedure D vs. Procedure A,  $\Delta R = -3.4\%$ ) although not significant, could point to a slightly decreased release of the  $^{99m}\text{Tc}$ -ECD from the complex, to cross the blood brain barrier. This seems to be confirmed by the results from Procedure E, where complexation of  $^{99m}\text{Tc}$ -ECD with the other cyclodextrin, i.e.  $\beta$ -trimethylcyclodextrin for the SPECT-2 injection rendered R values significantly lower than in the control Procedure A ( $R = 1.68 \pm 0.11$ ,  $\Delta R = -18.80\%$ ,  $p < 0.02$ ). The  $\beta$ -trimethylcyclodextrin was shown to display a binding by a factor of ap-

proximately 66% stronger than  $\gamma$ -cyclodextrin ( $\Delta R = 16\%$  between Procedure D and E,  $p < 0.05$ ) (J.-L. Moretti, personal communication). A marked reduction in the release of the  $^{99m}\text{Tc}$ -ECD from the  $\beta$ -trimethylcyclodextrin complex or a too slow release from the complex could account for the significantly lower R-value for Procedure E. The complex of ECD with  $\beta$ -trimethylcyclodextrin therefor reduces the availability of ECD for crossing the blood-brain barrier during the course of the experiment, while protection against the esterase degradation is observed through the complex formation.

## 5. Conclusion

The complex of ECD with  $\gamma$ -cyclodextrin showed similar R ratios as both the control and when the complex was incubated in plasma. In support of this finding, incubation of ECD alone showed significantly lower R ratios. This study therefor revealed that biodegradation can indeed be reduced with the complexation approach using cyclodextrin technologies. The  $\beta$ -trimethylcyclodextrin however, reduced the availability of the ECD significantly, resulting in low R ratio values. Therefor, this study has also shown that the availability of the radiotracer from the complex is of significant importance with respect to the timely release of the radiopharmaceutical, which is critical as not to negatively influence its availability and clinical application. The choice of cyclodextrin for the complexation with ECD must therefore be direct to protect the ECD against metabolic degradation as well as to ensure timely release for successful imaging characteristics.

## 6. References

- [1] Dormehl, I. C., Oliver, D. W., Hugo, N., in: SPECT in Neurology and Psychiatry, P. P. de Deyn, R. A. Driecks, A. Alavi et al. (eds.), Chapter 62, pp. 521-535, John Libbey and Company, Amsterdam (1997)
- [2] Dormehl, I. C., Oliver, D. W., Langen, K.-J. et al., Technetium-99Tc-HMPAO, Technetium-99mTc-ECD and Iodine-123-IMP Cerebral Blood Flow Measurements with Pharmacological Interventions in Primates. *J. Nucl. Med.* 38, 1897 (1997)
- [3] Nishizawa, S., Tanda, S., Yonekura, Y. et al., Regional dynamics of N-isopropyl-( $^{123}\text{I}$ )-iodo-amphetamine in human brain. *J. Cerebr. Blood Flow Metab.* 30, 150 (1989)
- [4] Lassen, N. A., Henriksen, L., Holm, S. et al., Cerebral blood flow tomography xenon-133 compared with isopropyl-amphenamine-iodine-123. *J. Nucl. Med.* 27, (1983)
- [5] Lassen, N. A., Anderson, A. R., Friberg, L. et al., The retention of [ $^{99m}\text{Tc}$ ]-d,l-HMPAO in the human brain after intracarotid bolus injection: a kinetic analysis. *J. Cerebr. Blood Flow Metab.* 8 (Suppl.), S13 (1988)
- [6] Neirinckx, R. D., Burke, J. F., Harrison, R. C. et al., Andersen, A. R., Lassen, A., the retention mechanism of technetium- $^{99m}$ -HMPAO-intracellular reaction with glutathione. *J. Cerebr. Blood Flow Metab.* 8 (Suppl.) S4 (1988)

- [7] Walovitch, R. C., Hill, C. T., Garrity, S. T. et al., Characterization of technetium-<sup>99m</sup>-L.L.-ECD for brain perfusion imaging, part I: pharmacology of technetium-<sup>99m</sup>-ECD in non-human primates. *J. Nucl. Med.* **30**, 1892 (1989)
- [8] Walovitch, R. C., Cheesman, E. H., Maheu, L. J. et al., Studies of the retention mechanism of the brain perfusion imaging agent <sup>99m</sup>Tc-bicisate (<sup>99m</sup>Tc-ECD). *J. Cereb. Blood Flow Metab.* **14**, S4 (1994)
- [9] Holman, L. B., Hellman, R. S., Goldsmith, S. J. et al., Biodistribution, dosimetry and clinical evaluation of technetium-<sup>99m</sup> ethyl cysteinyl dimer in normal subjects and in patients with chronic cerebral infarction. *J. Nucl. Med.* **30**, 1018 (1989)
- [10] Vallabhajosula, S., Zimmerman, R. E., Pickard, M. et al., Technetium-<sup>99m</sup> ECD: a new brain imaging agent. In vivo kinetic and biodistribution studies in normal human subjects. *J. Nucl. Med.* **30**, 599 (1989)
- [11] Laverman, P., Dams, E. T., Oyen, W. J. et al., A novel method to label liposomes with <sup>99m</sup>Tc by the hydrazino nicotinyl derivative. *J. Nucl. Med.* **40**, 192 (1999)
- [12] Duran Cordobes, M., Moretti, J.-L., De Beco, V. et al., Uptake of liposome-encapsulated <sup>99m</sup>Tc<sup>nl</sup>-MIBI by sensitive and multidrug-resistant tumour cell lines. *Nucl. Med. Comm.* **20**, 433 (1999)
- [13] Jenjarla, S., Puranjoti, P., Kasina, R. et al., Preparation, characterization and evaluation of miconazole-cyclodextrin complexes for improved oral and topical delivery. *J. Pharm. Sci.* **87**, 425 (1998)
- [14] Miyake, K., Arima, H., Irie, T. et al., Enhanced absorption of cyclosporin A by complexation with dimethyl-beta cyclodextrin in bile duct-cannulated and noncannulated rats. *Biol. Pharm. Bull.* **22**, 66 (1999)
- [15] Mahrrouk, G. M., Al-Angary, A. A., Al-Meshal, M. A., Modified beta-cyclodextrin lorazepam inclusion complexation. *Pharm. Ind.* **56**, 1080 (1994)
- [16] Dormehl, I. C., Redelinghuys, I. F., Hugo, N. et al., the baboon model under anaesthesia for in vivo cerebral blood flow studies using SPECT techniques. *J. Med. Prim.* **21**, 270 (1992)
- [17] Oliver, D. W., Dormehl, I. C., Redelinghuys, I. F. et al., Drug effects on cerebral blood flow in the baboon model: acetazolamide and nimodipine. *Nucl. Med.* **32**, 292 (1993)
- [18] Dormehl, I. C., Oliver, D. W., Hugo, N., Dose response from pharmacological interventions for CBF changes in a baboon model using <sup>99m</sup>Tc-HMPAO and SPECT. *Nucl. Med. Comm.* **14**, 573 (1993)
- [19] Oliver, D. W., Dormehl, I. C., Hugo, N., The effect of sumatriptan on cerebral blood flow in the baboon model. *Arzneim.-Forsch./Drug Res.* **44** (II), 952 (1995)
- [20] Dormehl, I. C., Oliver, D. W., Hugo, N., Cerebral Blood Flow effects of Sumatriptan in Drug Combinations in the Baboon Model. *Arzneim.-Forsch./Drug Res.* **45** (II), 952 (1995)
- [21] Jordaan, B., Oliver, D. W., Dormehl, I. C. et al., Cerebral blood flow effects of piracetam, pentifylline, and nicotinic acid in the baboon model compared with the known effect of acetazolamide. *Arzneim.-Forsch./Drug Res.* **45** (II), 844 (1996)
- [22] Oliver, D. W., Dormehl, I. C., Cerebral blood flow effects of Sodium Valproate and its drug combinations in the baboon model. *Arzneim.-Forsch./Drug Res.* **48** (I), 692 (1998)
- [23] Oliver, D. W., Dormehl, I. C., Cerebral blood flow of the nitric oxide donor, nitroglycerin and its drug combinations in the non-human primate model. *Arzneim.-Forsch./Drug Res.* **49** (II), 732 (1999)
- [24] Inoue, Y., Momose, T., Amo, K. et al., Metabolism of <sup>99m</sup>Tc-ethyl cysteinyl dimer (<sup>99m</sup>Tc-ECD) in blood-mainly in vitro. *Kaku Igaku* (Japan)

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## The Primate Model in Neuropharmacology for Cerebral Blood Flow Determinations with HMPAO SPECT

## Chapter 62

I.C. Dormehl, D.W. Oliver\* and N. Hugo

### Animal models in medical research

Early man obtained information by observing his environment. Animals, as an integral part of the environment, contributed to stimulate the understanding and creativity of reasoning man to improve his way of life.

Early recordings of serious investigations on human biology and anatomy point towards initial dissections on human corpses and cadavers, until these fell into disfavour and were banned in the Middle Ages. The emphasis for research in the medical sciences then reverted to the deliberate use of a variety of animals. Richard Lower successfully performed blood transfusions on dogs in the middle of the 17th century. In June 1667, the first blood transfusion on a human was carried out in France by Jean Baptiste Denis who transfused sheep's blood into two boys, both of whom subsequently died. It was much later that the research activities of Nobel Prize winner, Karl Landsteiner (1868-1943) eventually provided blood transfusions with sound scientific basis, and thus confirmed the impact of early animal research on the shaping of civilisation.

In the 1800s, David Ferrier stimulated animal cortex to localise brain functions, and around 1892 animal seizure models were demonstrated using a variety of techniques. Such animal research, made with circumspection, with care to avoid suffering, with respect for life, and upholding scientific merit, is still justifiable, and continuing even if under the watchful eye of concerned animal protection groups. Among others, animal models have been useful in pharmacology for evaluating drug efficacy, and for supplying information on the toxicity of a wide variety of pharmaceutical agents, including those active on the central nervous system (CNS) and influencing cerebral blood flow (CBF) patterns.

The use of non-human primates for medical research has been documented already in the early years of this century, when Robert Koch used monkeys to study the pattern of infection with human trypanosomiasis. Non-human primates are morphologically and often functionally similar to man. The morphological and functional superiority of the non-human primate is represented by its human-like brain, hands and uterus. The kidney of the non-human primate is also unique among animals, because only man and the non-human primate have kidneys with a multi-papillar structure, and microscopic examination of nephritis in rhesus monkeys confirmed identical results to those in man. Also the ECG of the non-human primate has been identified to be similar to that of man<sup>1</sup>.

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variations in rCBF in healthy brains, and in patients with functional psychiatric disease are of smaller magnitude, 2 - 35% of resting flow<sup>10</sup>. In the normal brain as well as in neuropsychiatric pathology, rCBF is coupled to metabolic demand and changes in CBF could reflect on neuronal metabolism.

Local reductions in CBF in Alzheimer's Disease (AD) may point to reductions in neuronal activity in relevant areas due to cell loss through atrophy and loss of synapses. In the early phase of AD, abnormal patterns of perfusion, i.e. hypoperfusion have been established in the temporo parietal cortex. The predictive value of this observation is high in AD patients with memory and cognitive impairment<sup>11</sup>.

Using HMPAO SPECT, it was found that increasing age (range 21-80 years) was associated with both an increase in side-to-side asymmetry and a preferential decline of rCBF in the frontal cortex<sup>12,13</sup>.

CBF evaluations with HMPAO SPECT of patients with schizophrenia indicated a physiological dysfunction of the prefrontal cortex and sometimes the temporal lobe, at the onset of the illness prior to neuroleptic treatment<sup>14</sup> (Figure 2).

Paralimbic hypoperfusion was detected in unipolar depression from HMPAO SPECT studies, although the psychiatric rating scales correlated poorly with CBF (Figure 3). Major differences in functional images are found in cases of epilepsy, depending on whether the patient is in the interictal, ictal or postictal state (Figure 4). Hyperfusion at the focus during the ictal state is to 95% positively predictive, but the study is logistically difficult to perform, especially with HMPAO. <sup>99m</sup>Tc-ECD with its longer *in vitro* consistency appears to be a better proposition for use in patients with epilepsy<sup>15,16</sup>.

Cerebral toxoplasmosis is a common opportunistic CNS infection in AIDS patients. Neuropathological findings include inflammatory infiltrates with polymorphonuclear leukocytes, in addition to lymphocytes, plasma cells and histocytes. In the later stages, cysts and areas of necrosis are present. Initially no abnormalities may be observed on a CT scan. AIDS patients present with abnormal HMPAO SPECT indicating the early stage of a CNS toxoplasma lesion<sup>17</sup>.

Patients with AIDS dementia complex (ADC - an AIDS-related dementia) were found, using HMPAO SPECT, to have exclusive cortical CBF derangements and maximum hypoperfusion in the frontal and parietal lobes. The degree of hypoperfusion significantly correlated with the severity of the dementia complex<sup>18</sup>. Cocaine-induced seizures occur due to the local

anaesthetic actions of this compound. Severe vasospasm is caused by the brain catecholamines leading to reduced CBF<sup>19</sup>. Various resting patterns of disruption of rCBF and metabolism as observed in functional imaging are associated with Parkinsonian disorders. Temporal frontal lobe perfusion seems to be affected.

There is increasing evidence that HMPAO SPECT for rCBF determinations is a viable technique for detecting cortical lesions following traumatic brain injury (TBI) (moderate and mild), revealing a greater number of lesions than CT or MRI<sup>20</sup>, especially during the first hours following an ictus (Figure 5). In a study on patients with chronic traumatic brain injury, brain SPECT was found to correlate with performance on a number of neurophysiological tests<sup>21</sup>.

A combination of HMPAO SPECT and carotid angiography increased the reliability of the Matas test as a means of determining the effect of carotid artery ligation<sup>22</sup>. CBF changes relating to neurotoxicity from various industrial substances, e.g. Cd, Mn, were detected with SPECT and correlated to behavioural abnormalities<sup>23</sup>. It is also true that confirmed normal CBF coupled to changed receptor distributions for e.g. D<sub>2</sub>-distribution, measured in the basal ganglia by IBZM, contributes to the diagnostic result<sup>24</sup>.

The evidence is, therefore, that SPECT has obvious diagnostic potential, and with pharmacological interventions and computer analysis, is a new tool in the assessment of brain disease. Although the literature clearly abounds with applications of SPECT in neuropathology, only small numbers of patients are being scanned in nuclear medicine facilities compared with the large numbers treated for neurological disorders. Clinicians are still sceptical about the diagnostic sensitivity and specificity of brain SPECT.

### Cerebral blood flow from HMPAO SPECT data

Fundamentally, all medical imaging involves visual comparisons. When used for diagnosis, images are frequently compared with mental pictures, which in any event are the interpreter's concepts of normality and different diseased states. For prognosis or treatment, monitoring comparisons often focus on changes in serial studies in the same patient, and depend on patterns seen in the images.

In nuclear medicine, these patterns represent spatial and temporal arrangements and rearrangements of the physiological and biochemical processes under investigation. The imaging techniques, should have

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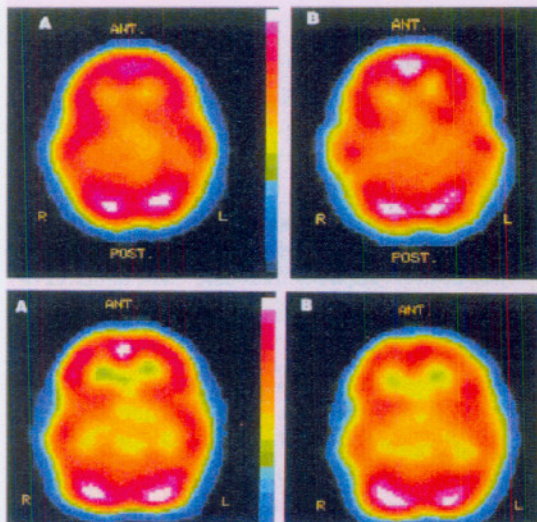


Figure 2 HMPAO SPECT slices at 36 mm above the frontocerebellar line in (top) a representative control subject, and (bottom) a representative schizophrenic patient. a, Resting scan; b, Wisconsin Card Sorting Test (WCST). An increase in prefrontal rCBF is noticed in the control during WCST. For the patient prefrontal hyperperfusion is present at rest, and a decrease in rCBF for this region follows during WCST (reference 14)

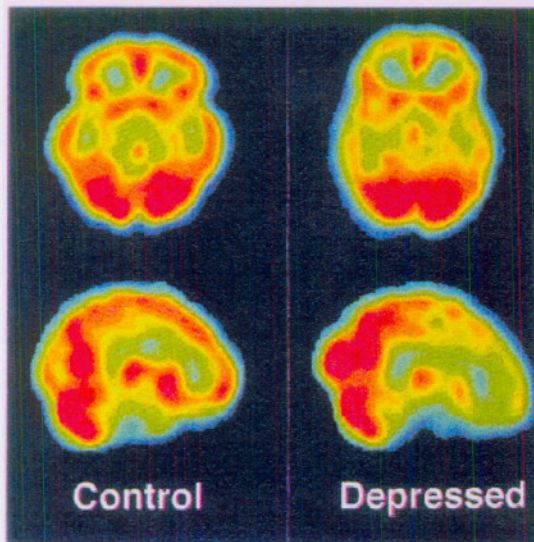


Figure 3 Transaxial (upper) and sagittal (lower) views of HMPAO SPECT images from a depressed patient and an age-matched, sex-matched, non-depressed control (taken from Mayberg *et al. J. Nucl. Med.* 1994)

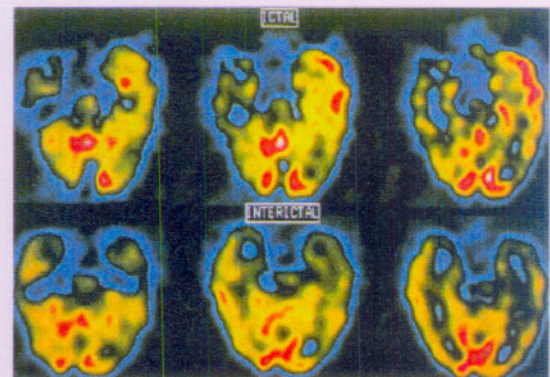


Figure 4 Ictal and interictal perfusion patterns in a 38-year-old woman with bilateral EEG foci and suspected left temporal sclerosis.  $^{99m}\text{Tc}$ -HMPAO SPECT shows decreased perfusion in the left temporal lobe interictally and markedly increased perfusion in the left temporolateral and temporopolar region, and slightly increased perfusion in the mesial structures during the seizure (taken from Grünwald *et al. J. Nucl. Med.* 1994)

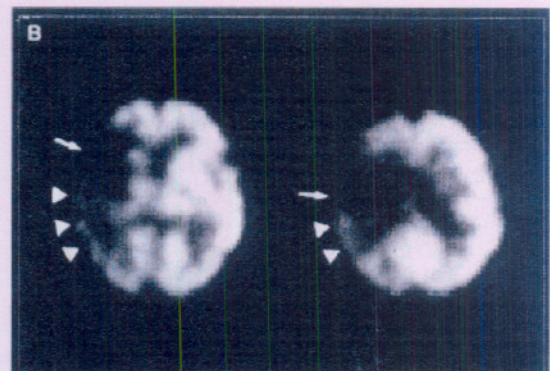


Figure 5 A 65-year-old woman with sudden onset of total aphasia: transaxial SPECT images obtained at 1.8 h after onset demonstrate an area of severe low perfusion (arrows) enclosed by a large area of mild hypoperfusion (arrowheads) which is consistent with the territory of the right middle cerebral artery (taken from Shimosegawa *et al. J. Nucl. Med.* 1994)

adequate spatial and time resolution to capture the available information in as much detail as possible.

A methodology for brain SPECT has been defined for establishing consensus among experts - a kind of modified Delphi-technique for tracer preparation and optimal imaging<sup>25</sup>, but still no formal guidelines or recommendations exist whereby the diagnostic efficacy could be universally validated.

A key test of computer-based techniques for qualitative as well as quantitative image information will be their ability to detect lesions at low thresholds without loss of specificity. Image manipulation, such as filtering and smoothing should be standardised, as should be slice orientation. Computer-assisted image interpretation follows three routes: comparing activity in one part of the image with another, looking for ratios; comparing activity in the regions of interest (ROI) with a normal data base; and generating kinetic data. These procedures introduce a degree of quantitation into the SPECT technique. Quantitation is desirable above visual-only interpretation for better differentiation, e.g. between AD and other possible treatable dementia-related diseases, and also for sensitive monitoring of therapy.

Although it is known that counts/pixel in regions of the brain are related to blood flow in the region<sup>26,27</sup>, absolute blood flow non-invasively with radiopharmaceuticals such as HMPAO is not easily attainable. The approach of calculating ratios could vary: the ratio of counts in a particular ROI to the counts in an identical contralateral region as in unilateral TBI, or to the counts in the unaffected hemisphere<sup>26</sup>, even to counts in the cerebellum which could be corrected for early diffusion of tracer<sup>27,28</sup>. The last ratio depends on the assumption that the cerebellum is itself not activated by the test procedure. In AD, the basal ganglia can be the reference structure as it is in this case usually spared. A lesion is identified if the ratio is below a certain threshold value. This threshold is chosen by defining a range of values from 'normal' volunteers.

Normals, especially where age-matching is needed, are not always adequately available. Furthermore, what is normal? A clear and unequivocal knowledge of normal brain perfusion is an absolute prerequisite to objective interpretation of scans and assessment of the accuracy of such interpretations. Normal perfusion and the acceptable limits at each region must be mapped and recognised. The appreciation of normal patterns and variability is the accumulated result of a large number of normal observations.

With respect to the analysis of SPECT images, the limited anatomical information derived from blood

flow images renders it difficult to localise the anatomical site of interest. Hence the ability of the technique to give information about small areas of brain is limited not only by intrinsic properties such as spatial resolution and the partial volume effect, but also by the reliable identification of precisely the brain regions observed. The extent of anatomic variability must be recognised and accounted for.

To aid interpretation of normals, an atlas of normal structures was defined and correlated with the widely recognised Talairach Tournoux stereotactic neurosurgical atlas<sup>29,30</sup>. Hereby the brain image is mathematically resized into a defined stereotactic space, following which the use of the corresponding coordinate system allows identification of specific areas (Figure 6). All normals are, however, not alike, not only anatomically, but also there are age-related changes in cortical perfusion, and gender differences<sup>31</sup>. A brain SPECT database from different participating institutions is needed. Important advances at various centres have been made in defining normal brain

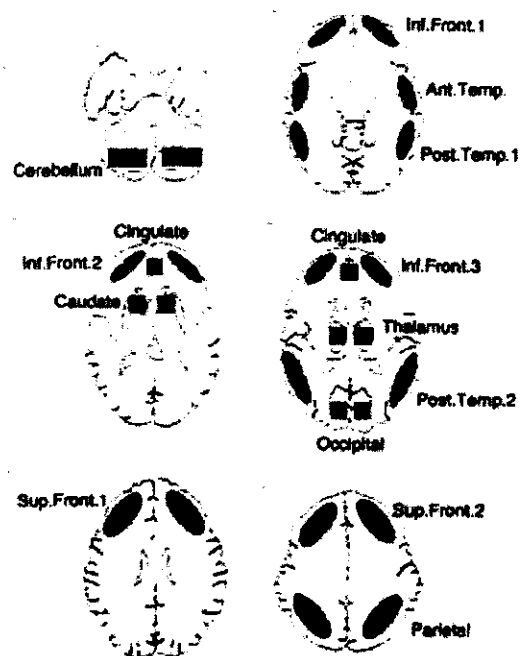


Figure 6 SPECT region of interest template: transaxial views at six standardised brain levels. The rCBF was measured in standardised brain regions by using generalised template modifications from the Matsui and Hirano brain atlas (taken from Matsui *et al. Igaku-Shoin (New York)* 1978. The regions of interest were defined in cortical and subcortical regions (taken from Mayberg *et al. J. Nucl. Med.* 1994)

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perfusion and condensing such descriptions into usable digital formats - also identifying the perfusion patterns associated with various disease entities and correlating these patterns with clinical outcome. A multicentre interobserver agreement on dementia studied with HMPAO SPECT was published<sup>32</sup>, with encouraging discrimination of subtypes of dementia. More imaging centres are developing the resources necessary to superimpose functional images onto similarly orientated cranial MR images of the same subject. This technique currently represents the best way to obtain precise anatomical localisation in functional brain images.

Shapes of ROIs are also not consistent. Sometimes predetermined and regular shapes are used, in other cases irregular shapes designed to enclose a specific structure. The placement of regions can likewise vary, i.e. either individually and manually, or with the aid of a standardised computer algorithm. Region placement is normally planned before results become available for objectivity (Figure 6).

The reality, however, remains that threshold values of ratios are observer-dependent and also display-dependent. Work is in progress on the accuracy of various methods of image display and colour scale mapping<sup>33</sup>. However, one has to recognise a high level of complexity and normal variability in brain structure and functional interrelationships which make brain SPECT difficult to interpret with the unaided eye, and the techniques for analysis are not yet adequately standardised.

Automated computer pattern recognition could be the alternative. Given one set of images from a normal population and another from patients (e.g. with AD), the artificial neural network (ANN) will find the best means of differentiating these two image patterns with no interference from pathophysiological knowledge of the disease process. A neural network algorithm<sup>34</sup> proved effective at recognising the pattern of AD - more than just a measure of parietal uptake or an asymmetry index. An image is reviewed for patterns, not a single finding but a constellation of findings, individually non-specific but diagnostic when taken together. This is also the way the human mind works. However it provides neither a quantitative measure of distinguishing between groups for comparisons nor kinetic data, and therefore does not yet fully tap the diagnostic potential of SPECT. Nor could more than two different groups be handled successfully.

The sensitivity and specificity of diagnostic brain SPECT is therefore still an unknown, and a reason why clinicians do not in general involve this modality in their diagnostic procedures. Interpretation and techniques applied to that effect are also not simple.

### Measurement and interpretation of CBF changes following interventions

Although tracer uptake in the brain is influenced by various parameters such as cardiac output<sup>35</sup>, retention fraction<sup>36</sup>, and lipophilic and hydrophilic fractions<sup>37</sup>, a numerical value that could monitor brain uptake of HMPAO would be useful for follow-up, activation and pharmacological studies. Quantification of absolute CBF or uptake has been pointed out to be problematic and not yet a routine diagnostic clinical practice. Various methods have been suggested to convert brain counts into brain uptake, such as using calibrated point sources as external standard<sup>38</sup>, but these have not yet found easy application.

Follow-up studies, however, measuring changes in CBF and HMPAO uptake in the same patient after an intervention do not demand absolute values, e.g. relative rCBF increases could be measured after shunt surgery in patients with normal pressure hydrocephalus, and some of these regional improvements correlated with improvement in psychiatric symptomatology. Successful follow-up CBF-measurements after vascular reconstructive surgery in moyamoya disease have also been reported.

Measures of pharmacological modulation of rCBF have been used to learn about cerebral activity of the administered drug. It provides the ideal scenario of the follow-up study to assess pharmacological effect and treatment efficacy in neurological and psychiatric pathologies. Another example would be to involve activation paradigms whereby the magnitude of CBF change could be monitored after a pharmacological challenge<sup>39</sup>. This could show how reversible the deficits are and how treatable a patient might be. Acetazolamide is a drug often used to determine cerebral reserve before and after a surgical intervention<sup>40</sup>.

Alignment of multiple studies for comparison is a major problem. Pelizzari<sup>41</sup> looks at surface contours, others at the anterior commissure - posterior commissure line for reference<sup>42</sup>. Alternatively, external fiducial markers can be used<sup>43</sup>. To avoid subjectivity, alignment of scans can be performed by an automated computer algorithm. However, with each patient as his own control, the trained human observer is still very good at compensating for artefacts and recognising anatomical variability and functional changes.

Semiquantification of CBF changes, induced pharmacologically or otherwise, becomes comparatively easy and still reliable, using the split-dose method<sup>44,45</sup>. It is an ideal method to use in drug development where acute effects of the drug need to be monitored, as is the case with cerebral infarction. An

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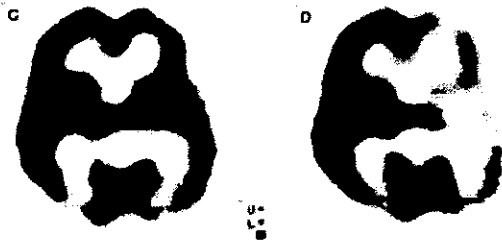


Figure 7 Patient with narrowing of the right carotid-fork IMP SPECT image before acetazolamide (C). Activity slightly decreased in the entire left cerebral hemisphere. (D) IMP SPECT image after acetazolamide. Markedly decreased activity in the entire left cerebral hemisphere (taken from Hoshi *et al.* *J. Nucl. Med.* 1994)

Acetazolamide stress testing has also been used to assess cerebrovascular reserve in patients with epilepsy and dementia<sup>57</sup>. In these instances the purpose of the test is to determine whether alterations in resting rCBF are of neuronal or vascular origin. Primary neuronal dysfunction (such as AD), produces rCBF abnormalities only as an epiphenomenon, through autoregulation. Such areas have a normal acetazolamide response.

CBF changes measured after pharmacological intervention can be assessed for their therapeutic significance in various diseases, e.g. the use of calcium channel blockers in stroke patients, where the drug might only be effective prior to induced or spontaneous reperfusion. If given acutely, some antidepressants administered to patients with affective disorders inhibit the re-uptake of noradrenaline and/or serotonin in the synapse, while others block inactivation<sup>58</sup>. Possible CBF changes induced in this manner and their significance could be studied by the split-dose method of HMPAO SPECT. Research has also demonstrated that many antidepressants exhibit effects on receptor sensitivity after chronic administration. A decrease in beta-adrenergic receptor sensitivity and enhanced responsiveness to serotonergic and alpha-adrenergic stimulation has been noted. Monoamine oxidase inhibitors block the inactivation of biogenic amines and thus increase the concentration of those neurotransmitters<sup>59</sup>. Therefore monitoring changes of rCBF in patients with mood disorders may be useful to direct therapy. It could also be applied in the development of appropriate pharmacological agents, and for better understanding of the pathophysiology of depression, the latter especially when correlated to cognitive behavioural changes. A clear correlation between pathological HMPAO SPECT findings and an

increasing Hamilton depression rating scale (HDRS) was reported<sup>60</sup>, which was not the case in patients with normal brain SPECT.

It appears that deranged CBF and cerebral metabolism are present in schizophrenia<sup>61</sup>, and subsyndromes could be regionally correlated in the brain. It has been hypothesised that in schizophrenia there is an overactivity of the dopaminergic system, with higher D<sub>2</sub> receptor densities in the left than right putamen, not found in controls<sup>62</sup>. This agrees with the work showing increased perfusion and metabolism in the basal ganglia on the left side. The phenomenon will find application in the field of pharmacology, i.e. studying receptor systems and cerebral metabolism and the perfusion response to pharmacological interaction by agonists and antagonists<sup>63</sup>. This research provides exciting opportunities to test hypotheses relating to antipsychotic pharmacodynamics and clinical response in living humans.

Preliminary results from a group of normal ageing patients under extended therapy with oral piracetam, or alternatively a combination of nicotinic acid with pentifylline, point towards improved rCBF and corresponding improvement in memory. Drugs may increase blood flow without altering cerebral metabolism. A significant increase in glucose metabolism was found with PET after piracetam treatment in an AD group of patients<sup>64</sup>. The small number of patients, however, prohibits a conclusion on the therapeutic relevance of metabolic effects in this case.

Although neurotransmitter and receptor interactions with drugs can be effectively monitored *in vivo* with SPECT and PET, leading to dose determinations and investigations of the impact of pharmacological manipulations on receptor systems, the arrival of both the tracer and the drug is blood flow and brain perfusion dependent, and this must be taken into consideration, if not also measured.

The split-dose method with HMPAO lends itself ideally to neuro-activation studies<sup>65,66</sup>. In order to observe the pattern of CBF associated with a task, it is necessary to perform that task only during the relatively short fixation period of the tracer, starting shortly before administration of HMPAO and continuing for approximately 4 min. This minimises problems such as initial learning effects in cognitive tasks and the time needed for the development of a physiological response after the HMPAO injection.

The verbal fluency task was found to be associated with a small but significant increase in rCBF in the left dorsolateral prefrontal cortex, which did not always prove to be the case with the Wisconsin Card Sorting Test<sup>66</sup>, despite it being considered equally robust in

testing this cerebral region. A SPECT study of schizophrenic patients performing a verbal fluency task showed that compared with a control group, the schizophrenic sample had decreased HMPAO uptake in left frontal cortical and left posterior cortical regions<sup>67</sup>.

Two tests were also performed during the CBF-imaging of 20 medication-free schizophrenics and 25 normals, viz the Wisconsin Card Sorting Test, and a simple number matching test. In the controls, the flow to the dorsolateral prefrontal cortex was clearly increased during the card test, not so in the patients. Number matching did not affect flow to this area for either group<sup>68</sup>.

A split-dose subtraction HMPAO SPECT study was performed on schizophrenic patients in collaboration with a verbal memory activation test to look for deficiencies in temporal lobe function (Busatto, unpublished results). Despite low frontal lobe flow and significantly poorer performance on the memory task, the degree of medial temporal activation measured in the schizophrenic group was not significantly different from that of the control group. Grossly, the brain is only subtly abnormal in schizophrenia. It is instructive in this regard to note that the frontal lobe, which has the more subtle pathology, tends to be more disordered functionally.

Based upon the premise that increased neuronal (synaptic) activity leads to an increase in regional cerebral blood flow, activation studies for Parkinson's disease patients have been developed in which subjects are scanned under two or more differing conditions e.g. at rest and while moving a limb in order to identify brain regions whose blood flow differs between the conditions. From this, it is inferred that localised changes in neuronal activation are responsible for, or at least involved in, the human activity under study.

Interactions between cognitive and pharmacological manipulations (i.e. neuro-modulation) and measured by SPECT and PET can also be very informative. Certain drugs were found to augment task-induced increases in rCBF e.g. buspirone and apomorphine acting on memory-induced rCBF increases in the retrosplinal region and dorsolateral prefrontal cortex respectively<sup>69</sup>. The particular experimental model can be chosen for pharmacological reasons, i.e. to find the effect of a drug on cognition, or for neuropsychological purposes where a task will be selected if it is known to implicate a particular neurotransmitter system. Methodological problems are matching the time-course of drug activity with that of tracer uptake and washout<sup>63</sup>, and analysing data to distinguish between global and regional drug effects on blood flow/metabolism.

A recent example of this strategy is the reaction of akinetic Parkinson's patients before and after a subcutaneous injection of apomorphine (a dopamine agonist), during a finger opposition task<sup>70</sup>. The akinetic patients, unlike normal controls, failed to increase rCBF to contralateral primary sensory areas and the supplementary motor cortex during the task. Following apomorphine, akinesia resolved and rCBF to motor regions in patients resembled that of controls. This was the evidence for functional differentiation of motor areas in akinetic patients, which was shown to be reversible by a dopaminergic drug.

It was also found that schizophrenic patients did not activate frontal regions following apomorphine - unlike controls. Schizophrenic patients are known to perform poorly on the Wisconsin Card Sorting Test, which is a measure of prefrontal function and have relatively low flow to frontal regions<sup>68</sup>. These data provide support for the hypothesis of a relative frontal cortical dopamine deficiency in schizophrenia (Pedro, unpublished results).

The importance of the methods of neuropharmacology outlined above is clear. In future brain SPECT and PET studies will have a necessary role to play in the development and evaluation of novel and classical psychotropic drugs, and some of this of necessity involves animal experimentation.

### The non-human primate and split-dose HMPAO SPECT

Although it is now possible to relate the basic molecular structure of a receptor to its cellular, biochemical and biophysical responses and pharmacological specificity in the test-tube, animals have a major use to enable understanding drug action on neurophysiology. Behavioural responses of animals to psychotropic drugs provide some indication of neuroreceptor function *in vivo* but extrapolation to human neuropsychology, even from the non-human primate, has limitations. For one there are no appropriate animal models for schizophrenia, but models of traumatic brain injury and seizures have been used, as were primates which were rendered parkinsonian by administration of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)<sup>71</sup>.

The baboon model has found frequent application in receptor imaging with SPECT.

In developing a baboon model for CBF determinations with SPECT imaging and <sup>99m</sup>Tc-HMPAO, it was found to be meaningfully sensitive to the effects of anaesthesia on CBF<sup>72</sup>.

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Anaesthesia is necessary for restraining the animal during scintigraphy, and it becomes critical when the procedure becomes prolonged. The split-dose method used with the model compares rCBF before and after a drug intervention, (i.e. from two sets of SPECT data, viz SPECT-1 and SPECT-2), and the result is given as a ratio (R) of the two determinations once the background contribution from SPECT-1 to the SPECT-2 data had been subtracted. To assess and understand the results from this model, i.e. the R values, a control representation for each investigation is required which describes through R values, rCBF changes taking place during an anaesthesia-only experimental run, and choosing a corresponding point in time for the second HMPAO injection to match the intervention response in the subsequent drug-intervention study. Anaesthesia is chosen to least disturb normal CBF, and to be easy to maintain and monitor. A useful form of anaesthesia would be ketamine-barbiturate combinations. The ketamine (10 mg/kg of ketamine hydrochloride) is necessary for induction, but not useful for maintenance because of the possibility of convulsions during longer studies. Thiopentone sodium can be used for maintenance (70 ml/h of a 0.5% solution) for shorter studies (1 h), and pentobarbitone for studies of 2 h and longer. The baseline study will then have the first HMPAO injection with the baboon still under the influence of ketamine from darting, and the second double dose of HMPAO after SPECT-1, (performed under barbiturate maintenance), and injected to match the intervention of the experimental protocol.

Table 1 gives R values from various anaesthesia combinations for tomographic slices as acquired in the transaxial, sagittal and coronal views, and for the global brain<sup>72</sup>. The ratio R is an indication of the level change of rCBF at a chosen time during the second anaesthesia with respect to the first. Curves plotted of R versus slice number (Figure 8) display some regional information from the various slices as they pertain predominantly to anatomical structures.

If no changes in CBF occur during the switch to a second anaesthesia (e.g. ketamine-thiopentone), or during prolonged anaesthesia, (e.g. thiopentone-thiopentone) (Table 1), a value of  $R = 2$  would be expected because of the double dose of HMPAO for the second injection. The mean value of  $R = 1.79 \pm 0.03$  for ketamine-thiopentone from many studies is statistically meaningfully less than two ( $R < 2$ ). Ketamine hydrochloride is known, and has been shown in correlation studies, to increase blood pressure and heart rate, leading to augmented CBF which is not maintained during the barbiturate phase, and this will reduce the R values.

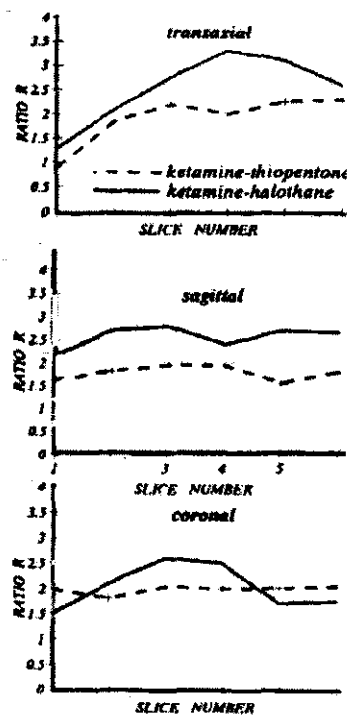


Figure 8 A set of typical curves of ratio (R) versus slice number, starting at a, the occipital lobes transaxially, b, from right to left of the brain sagittally and c, from the cerebellum to the dorsal slice of the cerebrum coronally. Dotted lines represent ketamine-thiopentone data; solid lines ketamine-halothane data

The model under halothane anaesthesia (ketamine-halothane; 1.5% halothane in oxygen) renders R values significantly  $> 2$ . Halothane reduces blood pressure and heart rate. Inhibition of the autoregulation of CBF in response to blood pressure changes leads to the vasodilatory effects of halothane, and changed blood flow distribution to various organs, with specifically decreased cerebral vascular resistance, which explains the increased CBF measured in this case.

With the cerebrovascular effects of the anaesthesia known, the model has found application for assessing acute responses from drug intervention. A study with acetazolamide (500 mg) produced a similar increase in CBF after 10 min, i.e. 32% as has been shown in humans<sup>73</sup>. An infusion of the calcium channel blocker nimodipine increased CBF globally after 10 min by approximately 35%, also known for humans; in addition it has a distinct regional effect with a near to 90% increase of rCBF in the cerebellum. These two

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Table 1 Mean ( $\pm$  s.d.) ratios from transaxial, sagittal and coronal views of four equal cerebral regions and from the total brain regions

	Region 1	Region 2		Region 3	Region 4	Total Brain
Procedure A	2.27 $\pm$ 0.62	2.17 $\pm$ 0.36	Transaxial	2.16 $\pm$ 0.33	2.13 $\pm$ 0.22	2.18 $\pm$ 0.06
Procedure B	1.73 $\pm$ 0.87	1.88 $\pm$ 0.19		1.86 $\pm$ 0.37	1.95 $\pm$ 0.36	1.86 $\pm$ 0.09
Procedure C	1.82 $\pm$ 0.52	2.06 $\pm$ 0.35		1.92 $\pm$ 0.29	2.18 $\pm$ 0.36	2.00 $\pm$ 0.16
Procedure D	2.21 $\pm$ 0.50	3.04 $\pm$ 0.18		2.82 $\pm$ 0.28	2.74 $\pm$ 0.31	2.70 $\pm$ 0.35
			Sagittal			
Procedure A	2.35 $\pm$ 0.83	2.25 $\pm$ 0.36		2.30 $\pm$ 0.29	2.56 $\pm$ 0.67	2.37 $\pm$ 0.14
Procedure B	1.84 $\pm$ 0.29	2.10 $\pm$ 0.18		1.97 $\pm$ 0.39	1.83 $\pm$ 0.54	1.94 $\pm$ 0.13
Procedure C	2.06 $\pm$ 0.49	2.06 $\pm$ 0.42		1.82 $\pm$ 0.55	1.62 $\pm$ 0.38	1.89 $\pm$ 0.21
Procedure D	2.43 $\pm$ 0.70	2.90 $\pm$ 0.22		2.99 $\pm$ 0.19	2.86 $\pm$ 0.32	2.80 $\pm$ 0.25
			Coronal			
Procedure A	2.18 $\pm$ 0.48	2.08 $\pm$ 0.27		2.71 $\pm$ 0.90	2.47 $\pm$ 0.48	2.36 $\pm$ 0.29
Procedure B	1.91 $\pm$ 0.32	1.93 $\pm$ 0.23		1.92 $\pm$ 0.28	1.73 $\pm$ 0.59	1.87 $\pm$ 0.10
Procedure C	1.96 $\pm$ 0.24	2.04 $\pm$ 0.35		2.09 $\pm$ 0.45	2.30 $\pm$ 0.52	2.10 $\pm$ 0.15
Procedure D	2.20 $\pm$ 0.37	2.99 $\pm$ 0.34		2.99 $\pm$ 0.22	2.66 $\pm$ 0.51	2.71 $\pm$ 0.37

A, Prolonged thiopentone (1.79  $\pm$  0.03 (n = 48)); B, ketamine-thiopentone; C, ketamine-pentobarbitone; D, ketamine-halothane

results help to validate the baboon model for HMPAO SPECT and the split-dose method. In addition it was observed that, although acetazolamide is known to produce metabolic acidosis due to its carbonic anhydrase activity, the increase in CBF did not correlate with an increase in PaCO<sub>2</sub>, but rather with an increase in PaO<sub>2</sub> (35%). The increased CBF from nimodipine was achieved without influence on blood pressure and arterial blood gases. It is known that cerebrovascular autoregulation is resistant to nimodipine.

An investigation of the combined effect of acetazolamide and nimodipine on the CBF in the baboon model showed a suppression of CBF with respect to the individual responses; also the increase in PaO<sub>2</sub> due to acetazolamide, was reduced to 23%, suggesting that nimodipine attenuates the effect of acetazolamide, and that acetazolamide may have an effect on the local cerebral metabolic rate for oxygen. This observation was substantiated in a subsequent study where dose and time responses of acetazolamide were investigated with this model<sup>74</sup>.

The antimigraine drug sumatriptan, a 5-HT serotonergic agonist, yielded with this model no or an insignificant effect (slight increase) on CBF, 10 min

after being administered intramuscularly<sup>75</sup>. However, after 25 min, a significant decrease (20%) was observed in the CBF, when compared with an anaesthesia-only baseline study which showed a marked increase in CBF during the prolonged anaesthesia. It was proposed that sumatriptan only had an effect when an abnormal increased CBF existed. Still using the split-dose HMPAO SPECT method, a combination study of sumatriptan (at 25 min) with nimodipine showed a significant decrease in the nimodipine-induced high CBF, but no effect on the increased CBF due to acetazolamide. There was also no effect on increases found under halothane anaesthesia. There is therefore a specificity in the action of sumatriptan, which implicates Ca<sup>2+</sup>, and an indication that the serotonin receptors are likely to be involved in the normalisation of CBF (Dormehl, unpublished results).

The baboon model was modified to investigate the effect of various forms of local anaesthesia on CBF in the acute phase. It allowed for control of PaCO<sub>2</sub> by ventilation under general anaesthesia. This model describes the functional dependence of  $\Delta$ CBF versus  $\Delta$ PaCO<sub>2</sub> using SPECT HMPAO and the split-dose method together with induced PaCO<sub>2</sub> changes between the two HMPAO injections. Normal reactivity to

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PaCO<sub>2</sub> changes were validated (Figure 9). The model with thiopentone anaesthesia and controlled ventilation was used to investigate the mechanisms of CBF increase known to follow on the local anaesthetic lidocaine (6 mg/kg). The baboons were maintained at constant respiratory rate and volume. The results indicate that the correlation between ΔCBF and the corresponding change in PaCO<sub>2</sub> deviated from the baseline pattern of the model, and had only a slight dependence on ΔPaCO<sub>2</sub>, confirming a cerebrovascular contribution by the lidocaine. The increase in CBF (24%) at around 2 min after lidocaine administration, agrees with known information<sup>76</sup>. The increased slope of the CO<sub>2</sub> reactivity curve might indicate an increased sensitivity to CO<sub>2</sub> after administration of lidocaine<sup>77</sup>.

The use of <sup>123</sup>I-iodoamphetamine (IMP) with the split-dose technique was explored. The question was whether it provides the same information as does HMPAO - can the two agents be interchanged at will? Comparing R values (i.e. rCBF changes) as measured by the HMPAO, and by the IMP split-dose method during prolonged thiopentone anaesthesia, produced 13% higher values as determined by IMP, which were not statistically significant, but could relate to the i.v. IMP needing 20 min to reach a steady state. However, doing the same comparison but with an acetazolamide intervention, increased CBF as measured with IMP by 52%, with respect to the IMP anaesthesia-only study, compared with the 37% from the HMPAO measurement to its own control. This could have positive diagnostic implications identifying IMP as the more sensitive drug to check CBF changes in a test of cerebrovascular reserve. The induced metabolic acidosis and alkaline urine from acetazolamide had

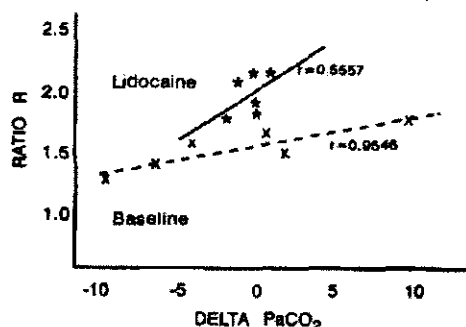


Figure 9 Linear regression lines to fit the raw data of CBF changes (R) versus PaCO<sub>2</sub> changes for the baseline study ( $r = 0.9546$ ,  $p < 0.00001$ ) and the lidocaine study ( $r = 0.5557$ ,  $p = 0.0001$ )

Table 2 Percentage changes\* in total brain uptake of HMPAO and IMP due to various procedures

Procedures	Percentage change	Statistical significance p-values
A to C	+ 13 ± 1	p > 0.05
A to B	+ 37 ± 8	p < 0.01
C to D	+ 52 ± 3	p < 0.01
B to D	+ 26 ± 8	p > 0.05
A to D	+ 74 ± 4	p < 0.01
C to B	+ 21 ± 6	p < 0.05, p > 0.01

\*Percentage changes are expressed with respect to the procedure mentioned first; A, thiopentone anaesthesia and <sup>99m</sup>Tc-HMPAO for both tracer injections; B, thiopentone anaesthesia <sup>99m</sup>Tc-HMPAO for both tracer injections and acetazolamide (500 mg and 10 min response time); C, thiopentone anaesthesia, <sup>123</sup>I(IMP) for both tracer injections; D, as above with C, but with acetazolamide (500 mg, 10 min response time)

apparently altered biodistribution of the two radiopharmaceuticals to different degrees. A dual-isotope study, split-dose with the two tracers combined for whichever reason will, according to this study, give incorrect values (Table 2)<sup>78</sup>.

### Conclusion

The diagnostic and investigative importance of cerebral blood flow measurements, using SPECT techniques with lipophilic tracers such as HMPAO has been shown here. It is also apparent that both diagnosis and investigation benefit tremendously when pharmacological modulation of rCBF is applied. Semiquantitative data on cerebral function can be gained from the split-dose method. This method is also ideal to assess the therapeutic significance and directions of pharmacological interventions in neuropathology and psychiatric disease. It is especially here, in drug research and development, that the baboon model as described can find wide application.

### References

- Cheetam, R.W.S. Premature artificial systoles in the baboon. *S. Afr. Med. J.* 1934, 8, 739
- Wagenen, G. Proceedings conference on nonhuman primate toxicology, U.S. Gov. Print Office, 1966, p. 103
- Wilson, J.G. Proceedings conference on nonhuman primate toxicology, U.S. Gov. Print Office, 1966, p. 114

## Chapter 62 - The Primate Model in Neuropharmacology for Cerebral Blood Flow Determinations with HMPAO SPECT

- 4 Hill, W.C.O. in *Primates: comparative anatomy and taxonomy*, volume 8: cynopithecinae, Wiley Interscience, New York, 1970, p. 5
- 5 Connolly, C.J. in 'External morphology of the primate brain', Charles C. Thomas Co., Springfield, USA, 1950, p. 3
- 6 Le Gros Clark, W.E. in 'The antecedents of man: an introduction to the evolution of the primates', Quadrangle Books, Chicago, USA, 1960, p. 262
- 7 Lassen, N. and Ingvar, D. Regional cerebral blood flow measurement in man: a review. *Arch. Neurol.* 1963, 9, 615
- 8 Veall, N. and Mallett, B.L. The two-compartment model using  $^{133}\text{Xe}$  inhalation and external counting. *Acta Neurol. Scand.* 1965, 14, 83
- 9 Warkentin, S., Nilsson, A., Risberg, J. *et al.* Regional cerebral blood flow in schizophrenia: repeated studies during a psychotic episode. *Psychiatr. Res. Neuroimaging* 1990, 35, 27
- 10 Fox, P.T. Functional brain mapping with positron emission tomography. *Semin. Neurol.* 1989, 9, 323
- 11 Holman, B.L., Johnson, K.A., Gerada, B. *et al.* The scintigraphic appearance of Alzheimer's disease: a prospective study using technetium-99m-HMPAO SPECT. *J. Nucl. Med.* 1992, 33, 181
- 12 Markus, H.S., Costa, D.C., Lennox, G. *et al.*  $^{99\text{m}}\text{Tc}$ -HMPAO rCBF SPECT in corticobasal degeneration. Abstract of 20th Annual Meeting of the British Nuclear Medicine Society. *Nucl. Med. Commun.* 1992, 13
- 13 Waldemar, G., Hasselbach, S.G., Anderson, A.R. *et al.*  $^{99\text{m}}\text{Tc}$ -d,l-HMPAO and SPECT of the brain in normal ageing. *J. Cereb. Blood Flow Metab.* 1991, 11, 508
- 14 Catafau, A.M., Parellada, E., Lomena, F.J. *et al.* Prefrontal and temporal blood flow in schizophrenia: resting and activation technetium-99m-HMPAO SPECT patterns in young neuroleptic-naive patients with acute disease. *J. Nucl. Med.* 1994, 35, 935
- 15 Kuikka, J.T. and Berkovic, S.F. Localization of epileptic foci by single-photon emission tomography with new radiotracers. *J. Nucl. Med.* 1994, 21, 1173
- 16 Menzel, C., Grünwald, F., Pavics, L. *et al.* Brain single-photon emission tomography using technetium-99m bicisate (ECD) in a case of complex partial seizure. *J. Nucl. Med.* 1994, 21, 1243
- 17 Catafau, A.M., Sol, M., Lomena, F.J. *et al.* Hyperfusion and early technetium-99m HMPAO SPECT appearance of central nervous system toxoplasmosis. *J. Nucl. Med.* 1994, 35, 1041
- 18 Maini, C.L., Pigorini, F., Pau, F.M. *et al.* Technetium-99m-HMPAO brain SPECT in acquired immune deficiency syndrome (AIDS). *Eur. J. Nucl. Med.* 1989, 15, 417
- 19 Miller, B.L., Mena, I., Giombetti, R. *et al.* Neuropsychiatric effects of cocaine: SPECT measurements. *J. Addict. Dis. (US)* 1992, 11, 47
- 20 Jacobs, A., Put, E., Ingels, M. and Bassuyt, A. Prospective evaluation of technetium-99m-HMPAO SPECT in mild and moderate traumatic brain injury. *J. Nucl. Med.* 1994, 35, 942
- 21 Ichise, M., Chung, D.-G., Wang, P. *et al.* Technetium-99m-HMPAO SPECT, CT and MRI in the evaluation of patients with chronic traumatic brain injury: a correlation with neuropsychological performance. *J. Nucl. Med.* 1994, 35, 217
- 22 Matsuda, H., Higashi, S., Neshandar, I. *et al.* Evaluation of cerebral collateral circulation by technetium 99m-HMPAO brain SPECT during Matas test: report of three cases. *J. Nucl. Med.* 1988, 29, 1724
- 23 Lill, D.W., Mounz, J.M. and Darji, J.T. Technetium-99m-HMPAO brain SPECT evaluation of neurotoxicity due to manganese toxicity. *J. Nucl. Med.* 1994, 35, 863
- 24 Ichise, M., Toyama, H., Fornazzari, L. *et al.* Iodine-123-IBZM dopamine D2 receptor and technetium-99m-HMPAO brain perfusion SPECT in the evaluation of patients with and subjects at risk for Huntington's disease. *J. Nucl. Med.* 1993, 34, 1274
- 25 Fletcher, J.W., Wolf, S.H. and Royal, H.D. A consensus-development method for producing diagnostic procedure guidelines: SPECT brain perfusion imaging with Tc-99m-HMPAO. *J. Nucl. Med.* 1994, 35, 2003
- 26 Andersen, A.R., Friberg, H.H., Schmidt, J.F. and Hasselbalch, S.G. Quantitative measurements of cerebral blood flow using SPECT and  $^{99\text{m}}\text{Tc}$ -d,l-HMPAO compared to xenon-133. *J. Cereb. Blood Flow Metab.* 1988, 8, 569
- 27 Lassen, N., Andersen, A., Friberg, L. and Paulson, O. The retention of  $^{99\text{m}}\text{Tc}$ -d,l-HMPAO in the human brain after intracarotid bolus injection: a kinetic analysis. *J. Cereb. Blood Flow Metab.* 1988, 8, 513
- 28 Matsuda, H., Tsuji, S., Shuke, N. *et al.* A quantitative approach to technetium-99m-hexamethylpropylene amine oxime. *Eur. J. Nucl. Med.* 1992, 19, 195
- 29 Minoshima, S., Koeppe, R.A., Fray, K.A. *et al.* Stereotactic PET atlas of the human brain: aid for visual interpretation of functional brain images. *J. Nucl. Med.* 1994, 35, 949
- 30 Talairach, J. and Tournoux, P. in 'Co-planar stereotaxic atlas of the human brain', Thieme, New York, USA, 1988
- 31 Levin, J.M., Holman, B.L., Mendelson, J.H. *et al.* Gender differences in cerebral perfusion in cocaine abuse:  $^{99\text{m}}\text{Tc}$ -HMPAO SPECT study of drug abusing women. *J. Nucl. Med.* 1994, 35, 1902
- 32 Hellman, R.S., Tikofsky, R.S., Van Heertum, R. *et al.* A multi-institutional study of interobserver agreement in the evaluation of dementia with rCBF/SPET technetium-99m exametazime (HMPAO). *Eur. J. Nucl. Med.* 1994, 21, 306
- 33 Stapleton, S.J., Caldwell, C.B., Leonhardt, C.L. *et al.* Determination of thresholds for detection of cerebellar blood flow deficits in brain SPECT images. *J. Nucl. Med.* 1994, 35, 1547
- 34 Kippenhan, J.S., Barker, W.W., Nagel, J. *et al.* Neural network classification of normal and Alzheimer's disease subjects using high-resolution and low-resolution PET cameras. *J. Nucl. Med.* 1994, 35, 7

## Chapter 62 - The Primate Model in Neuropharmacology for Cerebral Blood Flow Determinations with HMPAO SPECT

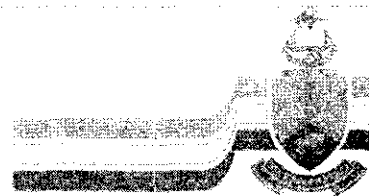
- 67 Lewis, S.H., Ford, R.A., Syed, G.M. *et al.* A controlled study of  $^{99m}\text{Tc}$ -HMPAO single photon emission imaging in chronic schizophrenia. *Psychol. Med.* 1992, 22, 27
- 68 Weinberger, D.R., Berman, K.F., Zec, R.F. *et al.* Physiologic dysfunction of dorsolateral prefrontal cortex in schizophrenia I. Regional cerebral blood flow evidence. *Arch. Gen. Psychiatr.* 1986, 43, 114
- 69 Friston, K.J., Frith, D.C., Liddle, P.F. *et al.* Comparing functional (PET) images: the assessment of significant change. *J. Cereb. Blood Flow Metab.* 1991, 11, 690
- 70 Rascol, O., Sabatini, U., Chollett, F. *et al.* Supplementary and primary sensory motor area activity in Parkinson's disease. *Arch. Neurol.* 1992, 32, 749
- 71 Hantraye, P., Brownweil, A.L., Elmalch, D. *et al.* Dopamine fibre detection by [ $^{11}\text{C}$ ]-CFT and PET in a primate model of parkinsonism. *Neuro Report* 1992, 3, 265
- 72 Dormehl, I.C., Redelinghuys, F., Hugo, N. *et al.* The baboon model under anaesthesia for in vivo cerebral blood flow studies using SPECT techniques. *J. Med. Prim.* 1992, 21, 270
- 73 Oliver, D., Dormehl, I., Redelinghuys, F. *et al.* Drug effects on cerebral blood flow in the baboon model - Acetazolamide. *Nucl. Med.* 1993, 32, 292
- 74 Dormehl, I.C., Oliver, D.W. and Hugo, N. Dose response from pharmacological interventions for CBF determinations in a baboon model using  $^{99m}\text{Tc}$ -HMPAO and SPECT. *Nucl. Med. Comm.* 1993, 14, 573
- 75 Oliver, D., Dormehl, I. and Hugo, N. Effects of sumatriptan on CBF in the baboon model. *Arzneimittel Forschung/Drug Research* 1994, 8, 925
- 76 Dutka, A.J., Mink, R., McDermot, J. *et al.* Effect of lidocaine on somatosensory evoked response and cerebral blood flow after canine cerebral embolism. *Stroke* 1992, 23, 1515
- 77 Dormehl, I.C., Lipp, M.D.W., Hugo, N. *et al.* Influence of intravenously administered lidocaine on cerebral blood flow in a baboon model standardized under controlled general anaesthesia using single photon emission tomography and technetium-99m hexamethylpropylene amine oxime. *Eur. J. Nucl. Med.* 1993, 20, 1095
- 78 Dormehl, I.C., Oliver, D.W., Hugo, N. and Rossouw, D. A comparative cerebral blood flow study in a baboon model with acetazolamide provocation:  $^{99m}\text{Tc}$ -HMPAO versus  $^{123}\text{I}$ (IMP). *Nucl. Med. Biol.* 1995, 22, 373

# Letter of Consent

## Prof IC Dormehl

# Appendix

## 4



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Geagte Professor Koeleman

#### INSAKE : DW Oliver PhD Studies

Sedert die tagtiger jare het daar 'n navorsingsamewerkingverbintenis ontwikkel tussen myself en Prof Douglas Oliver op die gebied van *in vivo* nie-menslike primaat navorsing. Ons het in die negentiger jare gefokus op serebrale bloedvloei-navorsing. U is ook goed vertrouwd met hierdie samewerking tussen ons. Hierdie verbintenis het gelei tot die ontwikkeling van verskeie navorsingsprojekte wat uiters suksesvol uitgevoer is en besondere internasionale erkenning geniet. Navorsing op hierdie terrein het hoofsaaklik bygedra dat beide Professor Oliver en myself B evalueeringsstatus by die NRF verwerf het vir navorsing waarvoor, beide van ons baie trots is. Soos u ook bewus is, is van die navorsing Farmakologies van aard.

Professor Oliver het my genader oor die moontlikheid om van hierdie resultate vir 'n PhD-studie, wat hy beoog, aan u Universiteit aan te bied. Hy het voorts versoek dat ek as een van sy promoters sal optree vir hierdie beoogde studie. Dr Linda Brand, vakhof van Farmakologie aan die PUK sal optree as die ander promotor.

Ons het hierdie beoogde studie bespreek en gevind dat die studie inderdaad aan die vereistes van 'n PhD voldoen gesien in die lig van al die internasionale bydraes wat gemaak is.

In die lig van bogenoemde tree ek graag op as promotor van Prof Oliver en gee ek hiermee toestemming dat hy die resultate van ons samewerking kan aanwend vir die beoogde PhD studie. Die keuse van die data vir insluiting in die proefskrif sal deur konsultasie tussen Dr Brand, Prof Oliver en myself geskied.

Prof Oliver word alle sterkte toegewens vir die PhD.

Kontak my gerus indien daar enige navrae is.

Met vriendelike groete

PROF. I.C. DORMEHL  
DIREKTEUR  
2002-01-10