

Chapter 2: Literature study

2.1. Parkinson's disease

2.1.1. Introduction

Parkinson's disease was first described in 1817 by James Parkinson in an essay dedicated to bring a group of symptoms, not yet classified at that time, to the attention of the medical community. He described the disease as an illness that affected the muscles without impairing the senses and intellectual capacity of the person (Parkinson, 1817; Antonelli *et al.*, 2006). In 1877 Charcot termed the disease "*maladie de Parkinson*" in acknowledgement of the efforts made by James Parkinson to bring the disease out of obscurity. Today, great advances are being made in the understanding and treatment of this disease, nevertheless, there is still no cure for Parkinson's disease (Lees, 2007).

There is an overall age standardised incidence of 1.0 - 1.6% for this disorder in subjects older than 65 years that is resident in Europe (De Rijk *et al.*, 1997; Van den Eeden *et al.*, 2003; Shook and Jackson, 2011). A lower prevalence rate is reported among African populations, but this remains debatable due to lower life expectancy owing to other causes, such as inaccurate diagnosis and unidentified environmental factors (McInerney-Leo *et al.*, 2004). While the mean age of onset is at 55 years, diagnosis is only made at an estimated age of 70.5 years. Untreated subjects have a three times higher mortality rate than their healthy peers of similar age (Van den Eeden *et al.*, 2003). Even when treated, the disease still decreases life expectancy and nearly always results in extremely disabling symptoms (Dauer and Przedborski, 2003).

The economic burden associated with Parkinson's disease can be divided into direct (inpatient care in hospitals, prescription drugs and outpatient care) and indirect costs (productivity loss and uncompensated caregiving by family members). The total cost related to Parkinson's disease in the United States alone, was estimated at \$23 billion for 2005, with 68% of this expenditure attributed to loss of productivity and uncompensated supervision by family. The number of persons in the age category of 65 years and older is expected to increase dramatically from 35 million, recorded in the 2000 census, to 80 million in 2040. Due to the greater prevalence of Parkinson's disease in people older than 65 years, it can be assumed that costs related to this disease will escalate dramatically (Huse *et al.*, 2005). Interestingly, the prevalence of Parkinson's disease is twofold higher in the male than in the female population (Van den Eeden *et al.*, 2003).

2.1.2. Clinical presentation of Parkinson's disease

The most prominent symptoms of this disease are tremor at rest, rigidity, akinesia (motor block) and bradykinesia (slowness of movement), while postural instability and non-motor symptoms follow in the more advanced stages of illness. Non-motor symptoms are mostly resistant to therapy and can become a substantial source of disability (Samii *et al.*, 2004; Jankovic, 2008). Examples of these non-motor symptoms are depression (most common non-motor symptom) as well as feelings of anxiety, apathy, insomnia, cognitive dysfunction and autonomic dysfunction (Lew, 2007). Non-motor complications of Parkinson's disease can, to a large extent, be prevented or delayed if neuroprotective treatment strategies are followed early on in disease management (Yacoubian and Standaert, 2009). The characteristic that differentiates Parkinson's disease from other movement disorders are the progressive nature of the disease and the asymmetric presentation of motor symptoms (Lew, 2007).

2.1.3. Neurochemical and neuropathological changes in Parkinson's disease

The pathological changes in the nervous system that result in Parkinson's disease, take years to reach the stage where significant clinical symptoms are distinguishable. The disorder is divided into a presymptomatic and symptomatic phase. When the symptomatic phase commences, nearly 80% of putamenal dopamine is exhausted and 60% of SNc dopaminergic neurons are destroyed (Braak *et al.*, 2004). One of the distinguishing, and most important neuropathological features of Parkinson's disease therefore is the degeneration of dopaminergic neurons in the SNc of the midbrain (Dauer and Przedborski, 2003). Depigmentation of the SNc follows the death of the highly pigmented dopaminergic neurons (Figure 2.1) (Lotharius and Brundin, 2002; Dauer and Przedborski, 2003).



Figure 2.1: A normal SNc on the left in comparison with depigmented SNc of the parkinsonian brain on the right (Youdim and Riederer, 1997).

A second characteristic pathological feature is the presence of Lewy bodies (Figure 2.2) in the parkinsonian brain (Dauer and Przedborski, 2003). Lewy bodies are spherical cytoplasmic aggregates that consist of a variety of proteins such as α -synuclein, parkin, ubiquitin and neurofilaments. They are present in all brain regions affected (Spillantini *et al.*, 1998; Lotharius and Brundin, 2002). It is suggested that Lewy bodies are formed during an active and tightly controlled attempt to contain the excess of proteins that are formed due to oxidative stress or other disease precipitators. These proteins are encapsulated for removal but the elimination mechanisms fail and they become permanent (Olanow *et al.*, 2004).

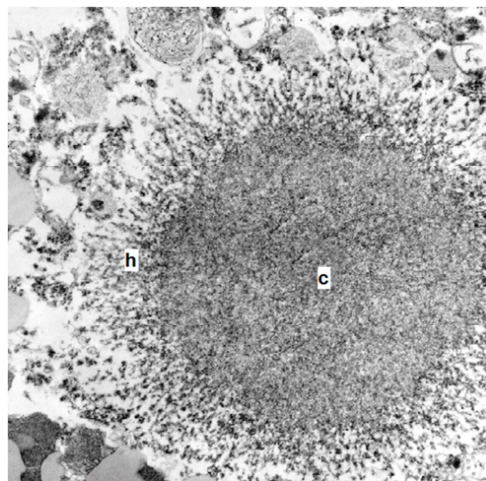


Figure 2.2: Electron micrograph of a Lewy body in the substantia nigra with (c) the Lewy body core with granular material and (h) the outer halo comprised of radiating filaments (Olanow *et al.*, 2004).

2.1.4. Apoptosis and Parkinson's disease

It has been suggested that cell death in Parkinson's disease is caused by apoptosis rather than necrosis (see Table 2.1 for a comparison between these two mechanisms). Normally, the function of apoptosis is to counterbalance excess cellular replication, but neuronal apoptosis can result from a variety of insults, many of which can be connected to the pathogenesis of Parkinson's disease (Olanow and Tatton, 1999).

Table 2.1: Comparison between apoptosis and necrosis of cells (Olanow and Tatton, 1999):

Apoptosis	Gradual form of cell death with marked cell shrinkage, preservation of plasma membranes, absence of inflammatory responses, cytoskeletal depolymerisation, fragmentation of nuclear DNA and chromatin condensation with formation of apoptotic bodies.
Necrosis	Rapid form of cell death with massive ionic influxes across the plasma membrane, widespread intracellular protein digestion by proteases, rupture of plasma membrane, inflammatory response and preservation of DNA.

Dopaminergic neurodegeneration is only a small part of a much broader spectrum of neurodegenerative processes present during Parkinson's disease. Pathology may begin in the brainstem and progress beyond the substantia nigra to the cortical and subcortical regions (Yacoubian and Standaert, 2009).

2.1.5. Aetiology and pathogenesis of Parkinson's disease

The direct cause of idiopathic Parkinson's disease remains unknown, but several hypotheses regarding the aetiology of the disorder exist.

a) Environmental hypothesis

The environmental hypothesis proposes that the neurodegeneration present in Parkinson's disease is the result of exposure to a dopaminergic neurotoxin. There are two possibilities, the first involves chronic exposure to a neurotoxin while the second entails limited exposure to a toxin that initiates a self-propagating cascade of destructive events that result in disease (Dauer and Przedborski, 2003).

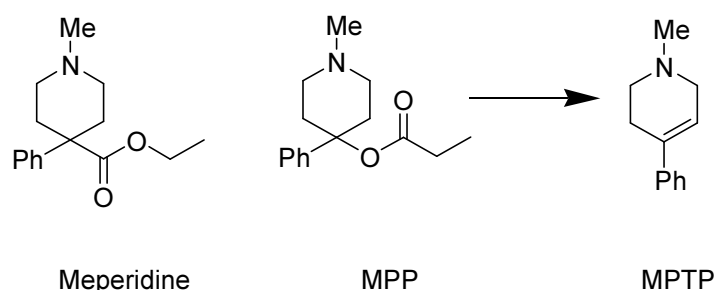


Figure 2.3: Meperidine and MPP, which is metabolised to neurotoxic MPTP (Silverman, 2004).

The environmental hypothesis has its origin in a syndrome identified by Langston and colleagues (1983) which manifested in four patients that misused 1-methyl-4-phenyl-4-

propionoxypiperidine (MPP, the reverse ester of meperidine, an abused analgesic) which contained traces of a neurotoxic degradation product, 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) (Figure 2.3). Consumption of MPTP produced a syndrome in these young drug addicts that closely mimicked the symptoms of Parkinson's disease. The mechanism of the neurotoxic effects of MPTP includes destruction of the substantia nigra and therefore the same pattern of neurodegeneration as observed during Parkinson's disease, albeit in an acute manner. Since MPTP is neutral, it easily crosses the blood-brain barrier (Figure 2.4) and is oxidised to two metabolites namely 1-methyl-4-phenyl-2,3-dihydropyridinium ion (MPDP⁺) and 1-methyl-4-phenylpyridinium ion (MPP⁺) within the brain. Once oxidised, the pyridinium ion (MPP⁺) can no longer diffuse out of the brain and it is therefore suggested that this ion is mainly responsible for the observed neurotoxicity. MPP⁺ is selectively taken up by dopaminergic cells and causes destruction of dopaminergic neurons through inhibition of mitochondrial complex 1 in the respiratory chain (Singer and Ramsay, 1990; Samii *et al.*, 2004; Silverman, 2004). This dysfunction of mitochondrial complex 1 leads to generation of reactive oxygen species, lowered cellular energy levels and ultimately cellular damage by oxidative stress and excitotoxicity (Dawson and Dawson, 2003). This discovery led to the hypothesis that Parkinson's disease is actually an environmental disease caused by ingestion or inhalation of neurotoxins similar to MPTP and initiated the search for environmental factors that can precipitate the disease (Dauer and Przedborski, 2003).

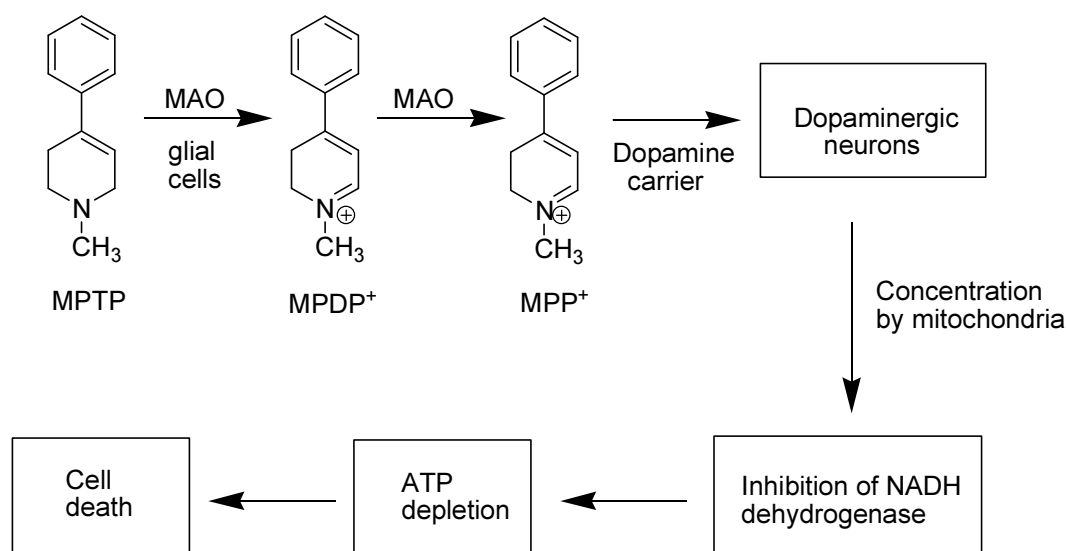


Figure 2.4: Mechanism of MPTP cytotoxicity (Singer and Ramsay, 1990).

Environmental pollution with certain pesticides and herbicides has also been implicated as causative factors of this disease. Human exposure to rotenone (an insecticide used in water

to remove unwanted fish species) led to development of Parkinson's disease in rural areas (Dauer and Przedborski, 2003; Lees *et al.*, 2009). Other exogenous toxins implicated in the development of Parkinson's disease are trace metals, cyanide, organic solvents, carbon monoxide and carbon disulphide (Olanow and Tatton, 1999).

Certain environmental factors can also play a protective role and diminish the risk for developing Parkinson's disease. Both smoking and coffee consumption lowers the risk of developing the disease substantially (Hernan *et al.*, 2002). A diet rich in vegetables, fruits and fish is also associated with a reduction in the risk of developing the disease (Okubo *et al.*, 2011).

b) Glutamate hypothesis

Glutamate is required for normal brain function but excessive amounts of this excitatory neurotransmitter can lead to excitotoxic cell death (Lipton and Rosenberg, 1994). Glutamate receptors of the *N*-methyl-*D*-aspartate (NMDA) type regulate influx of Ca^{2+} which in excessive amounts, activates a variety of potentially destructive processes. Excitotoxic injury is implicated in chronic neurodegenerative disorders although exact mechanisms are yet to be established (Standaert and Young, 2006).

c) Genetic hypothesis

Mutations in certain genes encoding for proteins such as parkin, α -synuclein and ubiquitin C-terminal hydrolase L1 (part of the ubiquitin-proteasome system) have been linked to an increased risk of developing levodopa-responsive Parkinson's disease. These proteins are involved in the phagocytosis of dysfunctional mitochondria by autophagosomes and the loss of function contributes to increased oxidative stress and consequently increases the risk for developing Parkinson's disease (Dawson and Dawson, 2003; Lees *et al.*, 2009). Contrastingly, studies in monozygotic twins dispute a convincing link between genetic origins and sporadic Parkinson's disease (De Lau and Breteler, 2006). A study that compared the genetic hypothesis with the environmental hypothesis suggested that evidence substantiating a genetic link may in fact be environmental of origin. Families share the same environment and this makes it difficult to differentiate between environmental and genetic influences (Fuente-Fernandez and Calne, 2002). Indeed it seems that most cases of Parkinson's disease are sporadic without any genetic link (McNaught *et al.*, 2001).

d) Misfolded protein hypothesis

As discussed in the genetic hypothesis, mutations in the ubiquitin-proteasome system have been implicated in the development of Parkinson's disease. This system is indispensable for clearance of mislocated, misfolded, mutant and damaged proteins in eukaryotic cells. Impeded protein clearance in the nigral dopaminergic neurons leads to the formation of protein aggregates that can cause direct damage by distorting the cell, or disturbing intracellular processes (Dauer and Przedborski, 2003), subsequently resulting in the degeneration of these neurons. The presence of Lewy bodies is therefore a clear indication that altered protein handling is present. Even in sporadic disease where no genetic connection has been established, there is evidence of inadequate processing of misfolded proteins (McNaught *et al.*, 2001).

e) Energy metabolism

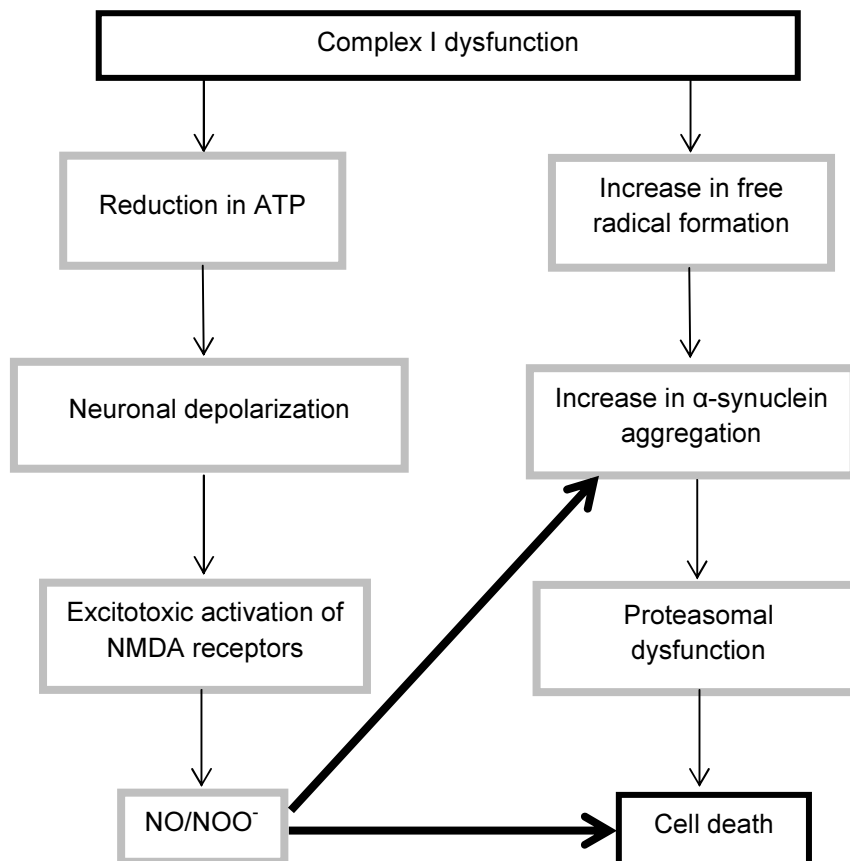


Figure 2.5: Results of dysfunction of mitochondrial electron transport chain complex I (Dawson and Dawson, 2003).

The capacity for oxidative metabolism in neurons declines with age, however, in the parkinsonian brain the severity of defects in energy metabolism is far greater than in a normal brain of a similar age. The most notable defect is a reduction in the function of

complex I of the mitochondrial electron-transport chain, resulting in low levels of adenosine-triphosphate (ATP) and generation of reactive oxygen species (Figure 2.5). This disturbance in energy metabolism may cause selective degeneration of neurons that underlie the pathology of Parkinson's disease (Dawson and Dawson, 2003; Standaert and Young, 2006)

f) Toxic metabolism of dopamine hypothesis

Dopamine and its metabolites are thought to exert a cytotoxic effect that results in compromised cellular integrity and consequently cellular death (Hattori *et al.*, 2009). Dopamine is metabolised (Figure 2.6) to 3,4-dihydroxyphenylacetic acid (DOPAC), a reaction catalysed by monoamine oxidase (MAO) (Standaert and Young, 2006).

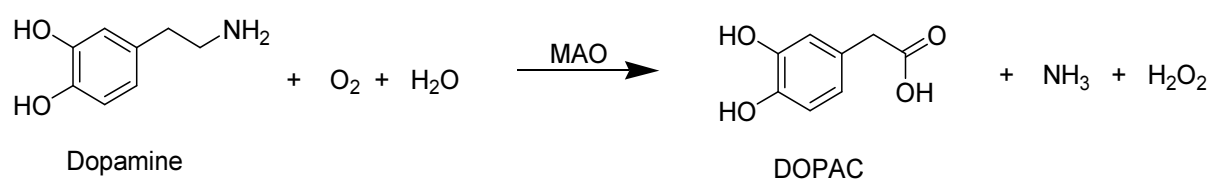


Figure 2.6: Catabolism of dopamine to DOPAC (Standaert and Young, 2006).

The metabolic reduction of oxygen during the catabolism of dopamine results in the formation of hydrogen peroxide. The hydrogen peroxide is subsequently converted into reactive oxygen species (ROS) such as the hydroxyl radical (OH[•]) in the presence of metal ions (Figure 2.7), in what is called the Fenton reaction (Olanow and Tatton, 1999).

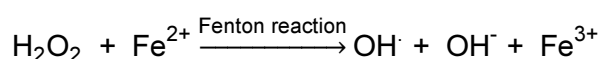


Figure 2.7: Formation of ROS (Standaert and Young, 2006).

ROS damage cellular components such as proteins, lipids and DNA. Because of the high metabolic rate of the brain and a reduced capacity for cellular repair, it is more susceptible to damage created by ROS (Olanow and Tatton, 1999; Dauer and Przedborski, 2003; Andersen, 2004).

g) Viral hypothesis

Because of the increased concentration of certain antibodies in the parkinsonian brain, similar to that observed after a viral infection, there is a hypothesis that infection by a known or unknown virus may contribute to the pathogenesis of this illness. Indeed, cases of post encephalitic Parkinson's disease have been reported. However, further research is required

to establish whether a virus infection can result in an increased vulnerability in developing this disease (Hirsch and Hunot, 2009).

h) Inflammation hypothesis

Neuro-inflammation plays a significant role in disease development and all parkinsonian brains show superior activity of brain and peripheral inflammatory cells and factors (Hirsch and Hunot, 2009). If normal mechanisms of control are lost, toxic factors are produced that can promote neurodegeneration (Armentero *et al.*, 2011).

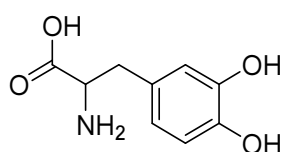
It is not yet clear at present whether inflammation causes Parkinson's disease or if it only serves as a disease promoter in the presence of other causative mechanisms (Armentero *et al.*, 2011).

2.2. Current treatment strategies in Parkinson's disease

Current therapy is inadequate and involves symptomatic treatment only (as none of the drugs currently used as antiparkinsonian agents have been approved as neuroprotective agents). The focus of therapies for Parkinson's disease is aimed at amplification of dopaminergic signalling in the striatum by the following mechanisms (Xu *et al.*, 2005):

- Increasing formation of dopamine (levodopa)
- Simulation of dopamine (dopamine agonists)
- Blocking degradation of dopamine (MAO-B inhibitors, COMT inhibitors) (Díaz-Cabiale *et al.*, 2002).

2.2.1. Levodopa



18

Levodopa (**18**) is still, after 40 years, the most effective and valuable therapeutic agent available in the arsenal of Parkinson's disease treatments (Chen and Swope, 2007; Pedrosa and Timmermann, 2013). Inescapably, all patients will eventually use it, regardless of the therapeutic agents used as initial treatment. Levodopa is the immediate precursor of dopamine (Lang and Lees, 2002; Pedrosa and Timmermann, 2013). After administration it crosses the blood brain barrier and is decarboxylated to dopamine, resulting in an increase

in the amount of dopamine available for binding to D₁ and D₂ receptors (Figure 2.8) (Chen and Swope, 2007; Chen *et al.*, 2011). Dopamine activity is terminated by reuptake of dopamine into the presynaptic neuron or inactivation by the COMT and MAO enzymes (Chen and Swope, 2007).

A disadvantage associated with levodopa therapy is motor complications that develop with long-term therapy. The two side effects predominantly experienced are motor fluctuations (at the end of each dose interval) and dyskinesia (involuntary jerking movements) (Hauser *et al.*, 2006; Pedrosa and Timmerman, 2013).

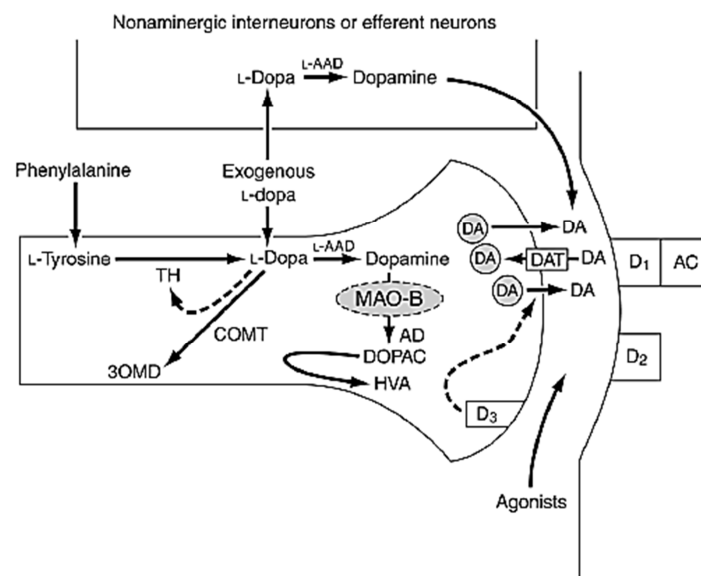


Figure 2.8: Exogenous levodopa and metabolism in the presynaptic dopaminergic neuron (Chen and Swope, 2007).

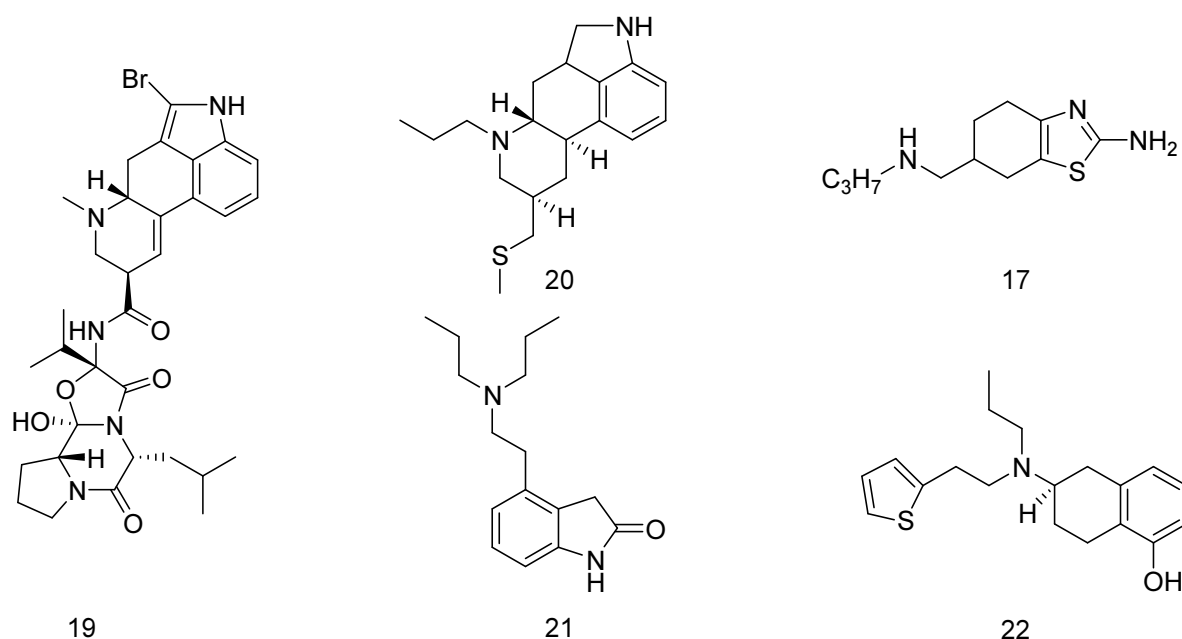
Dyskinesia (which can be even more disabling than the disease itself) occurs in 68% of patients after 5 years of levodopa therapy. These involuntary movements occur when striatal dopamine levels peak, due to overstimulation of dopamine receptors. Lowering the dose of levodopa is often beneficial, while surgery could be considered for severe disabling dyskinesia (Gillespie, 2008).

Motor fluctuations are characterised by periods of normal movement (“on”-times), followed by periods where the motor symptoms of Parkinson’s disease namely tremor, rigidity and bradykinesia (“off”-times) are experienced. This is due to the increasing loss of neuronal dopamine storage capacity and the short half-life of levodopa. Initially, the neurons have the capacity to store dopamine, resulting in adequate dopamine availability during the whole dose interval. With the progressive death of the SNc-neurons, this capability is lost and function becomes dependent on the exogenous source of dopamine, namely the

administered levodopa to provide an adequate dopamine concentration in the SNc alone. Thus, as the disease progresses, the duration of action of levodopa will shorten and it will have to be administered more frequently (Chen and Swope, 2007). The drug also possesses an unpredictable pharmacokinetic profile and accurate dosing is difficult (Gillespie, 2008).

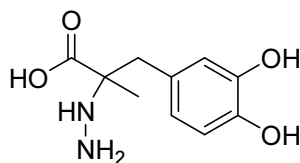
A further serious complication of therapy with levodopa is the potential of the drug to be auto-oxidized to a quinone that leads to the generation of ROS. Levodopa can therefore enhance oxidative stress and disease progression (Hattori *et al.*, 2009).

2.2.2. Dopamine agonists

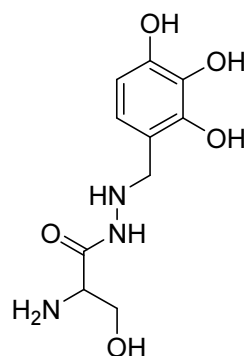


Dopamine agonists are divided into two categories namely, ergot derivatives (bromocriptine (**19**) and pergolide (**20**)) and non-ergot derivatives (pramipexole (**17**), ropinirole (**21**) and rotigotine (**22**)). Bromocriptine and pergolide are no longer used due to pulmonary and heart complications (Chen and Swope, 2007). Since there is a reduced risk for motor complications when dopamine agonists are used as initial therapy, compared to initial therapy with levodopa (Rascol *et al.*, 2000; Hely *et al.*, 2000; Holloway *et al.*, 2004), the consensus is that dopamine agonists should be used initially in young patients. Levodopa however, remains the mainstay of therapy for geriatric patients due to the high susceptibility of this group of patients to the side effects (such as dizziness) associated with the use of dopamine agonists (Hely *et al.*, 2000; Chen and Swope, 2007; Pedrosa and Timmermann, 2013).

2.2.3. Carbidopa and benserazide



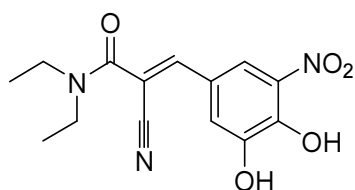
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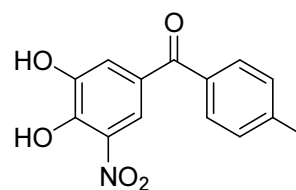
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Carbidopa (**23**) and benserazide (**24**) are inhibitors of aromatic-L-amino-acid decarboxylase and are incorporated in levodopa formulations to decrease the peripheral conversion of levodopa to dopamine. This inhibition lessens adverse effects associated with peripheral dopamine metabolism and increases the amount of levodopa that crosses the blood brain barrier (Chen *et al.*, 2011).

2.2.4. Catechol-O-methyltransferase (COMT) inhibitors



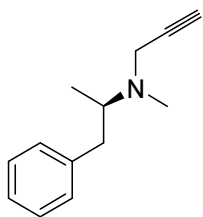
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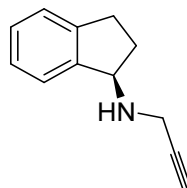
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The main indication for the use of COMT inhibitors, such as entacapone (**25**) and tolcapone (**26**) are the control of motor fluctuations associated with levodopa therapy (Pahwa *et al.*, 2006). They elevate levodopa bioavailability in the brain but have no effect on Parkinson's disease in the absence of dopamine. Use has been limited by reports of fatal hepatotoxicity (Chen and Swope, 2007).

2.2.5. MAO-B inhibitors



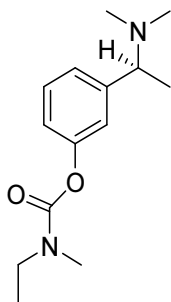
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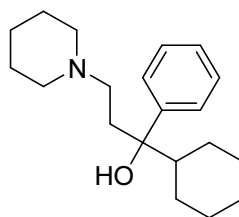
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Two selective MAO-B inhibitors are clinically used, namely selegiline (deprenyl) (**27**) and rasagiline (**28**). The inhibition of MAO-B in the brain decreases the metabolism of dopamine and prolongs activity of available dopamine (Chen and Swope, 2007). MAO-B inhibitors can be used as monotherapy to improve motor function in early disease, as well as in conjunction with levodopa therapy to diminish motor fluctuations associated with long term levodopa use (Miyasaki *et al.*, 2002; Suchowersky *et al.*, 2006). Unfortunately, selegiline may intensify dyskinesia due to an increase in the peak effects of levodopa (Chen and Swope, 2007). MAO-B inhibitors may have additional neuroprotective effects attributed to their inhibition of dopamine metabolism, a process which can produce oxygen free radicals that lead to destruction of nigrostriatal neurons (Chen and Swope, 2007).

2.2.6. Anticholinergic drugs



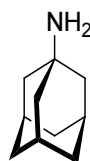
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In Parkinson's disease, the striatal dopamine deficiency leads to an increase in cholinergic interneuron activity which presents symptomatically as tremor. Inhibition of cholinergic activity is therefore of therapeutic value, although the use of anticholinergic drugs like rivastigmine (**29**) and thrihexyphenidyl (**30**) are hampered by adverse effects such as vision disturbances, confusion, constipation, xerostoma, sedation and urinary retention. Anticholinergic drugs can be used as monotherapy or as add-on therapy to other agents (Chen and Swope, 2007).

2.2.7. Amantadine



31

Amantadine (**31**) is moderately effective in the symptomatic relief of Parkinson's disease through an undetermined mechanism. The inhibition of NMDA receptors may be involved (Chen and Swope, 2007).

2.2.8. Neuroprotective therapy

Although present therapy therefore provides symptomatic relief, disease progression is not adequately addressed. Initially, after starting treatment, a dramatic improvement in symptoms is thus experienced, inevitably followed by a relapse or worsening of symptoms as the disease progresses. The search for neuroprotective therapies are the main focus of present research. A few antiparkinsonian agents, for example selegiline and rasagiline (registered MAO-B inhibitors), may possess neuroprotective properties. The following section describes some of the developments in this field.

a) Monoamine oxidase inhibitors

Clinical studies have shown that selegiline (**27**), a selective irreversible MAO-B inhibitor delays disease progression due to inhibition of dopamine metabolism, while rasagiline (**28**), also exhibits positive neuroprotective properties (Olanow *et al.*, 2008; Yacoubian and Standaert, 2009).

b) Dopaminergic drugs

In vitro and animal studies (for example bromocriptine in mice, Ogawa *et al.*, 1994) have shown that dopamine receptor agonists can reduce dopaminergic cell death. The mechanism of action may be by stimulating D₂ autoreceptors on SNc terminals resulting in a reduced dopamine release, with a concomitant theoretical decrease in oxidative stress. Conclusive evidence for neuroprotection by these agents is yet to be produced (Yacoubian and Standaert, 2009).

c) Adenosine receptor antagonists

The neuroprotective effects of caffeine and other adenosine A_{2A} antagonists, such as istradefylline, have been illustrated in the presence of neurotoxins, such as MPTP in animals (Kalda *et al.*, 2006). However, none of the adenosine A_2 antagonists are currently in clinical use. For a detailed discussion of adenosine and adenosine receptor antagonists see section 2.3.

d) Other agents

Coenzyme Q_{10} is a cofactor in the electron transport chain in mitochondria and is presently being investigated as a prospective neuroprotective agent in Parkinson's disease (Shults *et al.*, 2002). Another prospective neuroprotective agent is creatinine, which promotes mitochondrial ATP production (LeWitt and Taylor, 2008). Although glutathione is given intravenously in the clinical setting as a neuroprotective agent, no scientific evidence for this proposed effect exists to date (Samii *et al.*, 2004).

Neurotrophic factors, such as glial cell derived neurotrophic factor (GDNF) are important for the maintenance of dopaminergic neurons. GDNF has been shown to be neuroprotective, to encourage fibre outgrowth and improve neuronal function in animals. Delivery of GDNF has unfortunately been problematic and several strategies, such as a bolus injection into the cerebral ventricles, chronic infusion pump and live replication-deficient viral particles have been explored to overcome this problem (Gill *et al.*, 2003).

As inflammation plays an important role in the progression of Parkinson's disease, anti-inflammatory agents like non-steroidal anti-inflammatory drugs (NSAIDs) and minocycline have been investigated as a means to halt disease progression (Yacoubian and Standaert, 2009).

2.2.9. Diverse treatments

Surgery can alleviate motor deficiencies produced by levodopa therapy and surgical procedures are presently being reintroduced after falling into disuse (Laitinen *et al.*, 1992). Adverse effects of surgery include brain haemorrhage, infarct and seizures and even death. Deep brain stimulation with high frequencies mimics surgery through reduction of neural activity, with less irreversible brain trauma (Samii *et al.*, 2004).

2.2.10. Shortcomings of current therapy

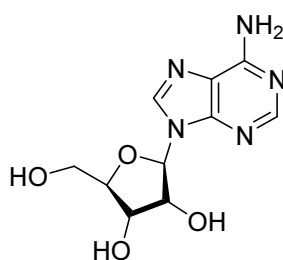
In addition to affording inadequate neuroprotection against disease advancement, the other shortcoming of antiparkinsonian therapy is the adverse effects associated with the use of these drugs (Xu *et al.*, 2005).

Central nervous system side effects, in particular cognitive disturbances, impede the therapeutic use of anticholinergics and antiglutamatergic drugs while the most restricting side effect of dopaminergic therapy is the occurrence of dyskinesia. An increase in adenosine A_{2A} receptor binding and mRNA levels in the putamen have been reported in patients who develop levodopa induced dyskinesia. Furthermore, the increase in adenosine A_{2A} receptors seems to be a long lasting adaptive alteration. Levodopa treatment further increases the ratio of adenosine A_{2A} heteromers vs. A_{2A}/D₂ heteromers which leads to increased A_{2A} receptor signalling (Antonelli *et al.*, 2006). It is therefore postulated that adenosine A_{2A} receptor overexpression is involved in the pathogenesis of levodopa induced dyskinesia in Parkinson's disease (Calon *et al.*, 2004; Xu *et al.*, 2005).

The distinctive distribution of adenosine A_{2A} receptors in the brain, especially in the striatum, where it plays an important role in movement, as well as its role in development of dyskinesia indicates that this receptor may be an attractive non-dopaminergic target for therapy in Parkinson's disease (Xu *et al.*, 2005).

2.3. Adenosine, adenosine receptors and its role in Parkinson's disease

2.3.1. Introduction



32

Adenosine (**32**) is an endogenous purine nucleoside ubiquitously present in the extracellular space (Nakav *et al.*, 2008). It is important for energy transfer in the forms of adenosine triphosphate (ATP) and diphosphate (ADP) as well as during signal transduction as cyclic adenosine monophosphate (cAMP) (Wadkins and Lehninger, 1958). Adenosine further regulates the

release of central nervous system neurotransmitters presynaptically, and is therefore also classified as a neuromodulator (Cunha, 2005). Peripherally, adenosine plays a role in the cardiovascular system, the gastro-intestinal system, pain perception, cell growth, cell proliferation, the immune system and apoptosis (Schulte and Fredholm, 2003). In the central nervous system, it is implicated in sleep, arousal, locomotion, basal ganglia function, cerebral blood flow, nociception and drug addiction (Xu *et al.*, 2005; Jaakola *et al.*, 2008a).

The adenosine receptor family is part of the heterotrimeric guanine nucleotide-binding protein (G-protein) coupled receptor (GPCR) group (Pirainen *et al.*, 2011) and four mammalian adenosine receptor subtypes, namely the A₁, A_{2A}, A_{2B} and A₃ receptors have been identified (see Table 2.2) (Xu *et al.*, 2005). There is high conservation of amino acid sequence identity between human adenosine receptor subtypes (Pirainen *et al.*, 2011). Adenosine A_{2A} and A_{2B} receptors are coupled to G_s (stimulatory G-protein) subunits and mediate an increase in cAMP, while adenosine A₁ and A₃ receptors are coupled to G_i (inhibitory G-protein) subunits and mediate a decrease in cAMP (Schulte and Fredholm, 2003).

Table 2.2: Properties and distribution of human adenosine receptor subtypes (Xu *et al.*, 2005; Fredholm *et al.*, 2001):

	A ₁	A _{2A}	A _{2B}	A ₃
G-protein	G _i	G _s	G _s	G _i
Signaling pathway	↓cAMP ↑IP ₃ /DAG*	↑cAMP ↑IP ₃	↑cAMP ↑IP ₃ /DAG	↓cAMP ↑IP ₃ /DAG
Tissue function	Bradycardia, inhibition of lipolysis, glomerular filtration reduction, tubero-glomerular feedback, antinociception, sympathetic and parasympathetic activation, neuronal hyperpolarization, ischemic preconditioning.	Sensorimotor integration in basal ganglia, inhibition of platelet aggregation and polymorphonuclear leukocytes, vasodilatation, defence against ischemic damage, activation of sensory nerve activity.	Relaxation of smooth muscle in vasculature and intestine, inhibition of monocytes and macrophages, activation of mast cell mediator release.	Boost mediator release from mast cells, preconditioning.
Distribution in the brain	Widespread (particularly in cortex, cerebellum, and hippocampus).	Restricted (Striatum, nucleus accumbens, olfactory tubercle).	Widespread (low concentration in all areas).	Widespread (higher concentration in cerebellum and hippocampus).

*IP₃ = inositol triphosphate, DAG= diacylglycerol

2.3.2. The structure of the adenosine A_{2A} receptor

The adenosine A_{2A} receptor distinguishes itself by selective localization in the striatum and therefore shows a high involvement in basal ganglia function and disorders of this area. It is a glycoprotein with a single carbohydrate chain and a molecular mass of 45 kDa (Xu *et al.*, 2005).

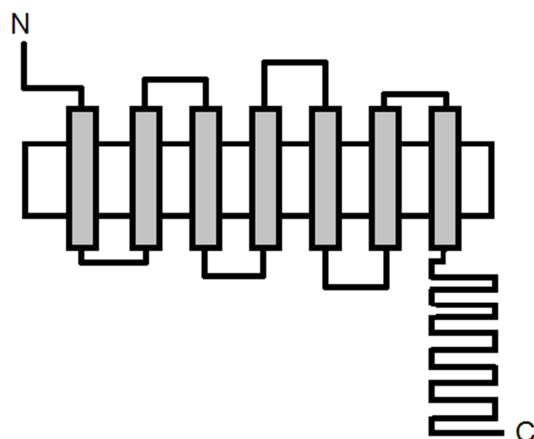


Figure 2.9: A basic representation of the structure of the adenosine A_{2A} receptor in cellular membrane (adapted from Xu *et al.*, 2005).

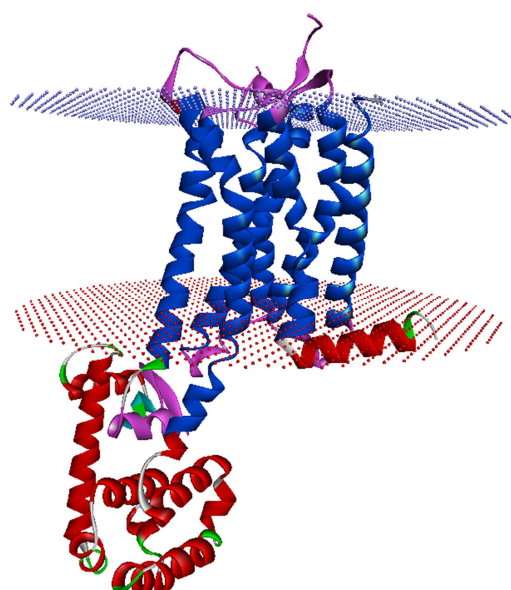


Figure 2.10: Crystal structure of the adenosine A_{2A} receptor adapted from Jaakola *et al.* (2008a). The view of the adenosine A_{2A} receptor is perpendicular to the plasma membrane. The helices are coloured blue and the extracellular and intracellular loops are pink. The membrane boundaries were obtained from the OPM database (OPM database, 2012) and the PDB file was obtained from the RCSB Protein data bank (Jaakola *et al.*, 2008b).

The adenosine A_{2A} receptor exhibits other characteristics (Figure 2.9) similar to G-protein coupled receptors in the A class, including 7 transmembrane α -helices, one short membrane associated helix (helix 8), an extracellular amino-terminus (N-terminus), a cytosolic carboxy terminus (C-terminus), three extracellular loops (ECL 1-3) and three intracellular loops (ICL 1-3) (Piirainen *et al.*, 2011). The adenosine A_{2A} receptor binds with high affinity to adenosine ($K_d = 0.1\text{-}1.0\ \mu\text{M}$) (Xu *et al.*, 2005). Recently, a crystal structure has been obtained for this receptor (Figure 2.10) (Jaakola *et al.*, 2008a).

2.3.3. The ligand-binding cavity

Residues that are conserved in all adenosine receptor subtypes are Phe 168, Glu 169, Asn 253 and Leu 249 (Jaakola *et al.*, 2010; Piirainen *et al.*, 2011).

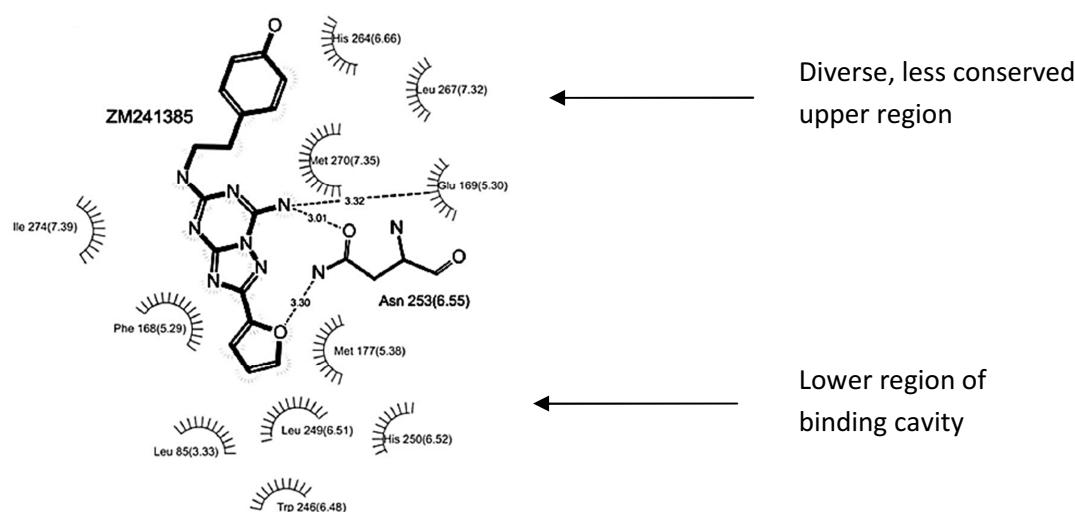


Figure 2.11: The ligand binding pocket of adenosine A_{2A} receptor with ligand ZM241385 (Jaakola *et al.*, 2010).

Interactions that determine selectivity of the ligand for a certain receptor subtype occur mainly with residues present in the less conserved upper region of the binding cavity (Figure 2.11), while interactions between the ligand and residues in the lower part of the binding cavity determine affinity to a large extent (Jaakola *et al.*, 2010).

For ZM241385, a known adenosine A_{2A} receptor antagonist, the bicyclic triazolotriazine core is positioned in the centre of the binding cavity with an orientation almost perpendicular to the membrane plane (Jaakola *et al.*, 2008a; Piirainen *et al.*, 2011). The aromatic stacking interactions that occur between Phe 168 and the triazolotriazine core is of great importance as this type of interaction is observed for all high affinity receptor agonists and antagonists (Jaakola *et al.*, 2010). A common feature of many adenosine A_{2A} receptor antagonists is a furan ring. The furan ring of ZM 241385 interacts with the adenosine A_{2A} receptor (through a

water-mediated interaction) to produce restriction of the movement of the Trp 254 residue. This residue acts as a “toggle switch” and its movement has an interaction with the rest of the receptor to mediate conversion between the active and inactive states of the receptor. Restriction of movement produced will hinder the structural rearrangement essential for activation of this receptor (Jaakola *et al.*, 2008a; Piirainen *et al.*, 2011).

The availability of the crystal structure enables the researcher to predict the viability of a proposed structure as a receptor ligand.

2.3.4 Adenosine A₁ receptor

There is no crystal structure available for the adenosine A₁ receptor but as discussed before, amino acid residues seem to be well conserved between the adenosine A₁ and A_{2A} receptor subtypes. It is therefore hypothesized that very small structural differences in the receptors exist that enable some ligands to be selective for one of these receptors. These differences will only be clear once the crystal structure of adenosine A₁ receptors have been deduced. However, because a dual adenosine A₁/A_{2A} antagonist would be advantageous (see section 2.5.1), the highly conserved binding cavity between the receptor subtypes is a positive factor (Ijzerman *et al.*, 1992; Jaakola *et al.*, 2010).

2.3.5. Adenosine A_{2A} receptor and the motor pathway

One of the advantages of targeting adenosine A_{2A} receptors as antiparkinsonian therapy is their unique restrictive distribution in the striatum. The striatum is connected to the output structures of the basal ganglia by the direct, striatonigral and the indirect, striatopallidal pathways. Both the stimulatory direct pathway and the inhibitory indirect pathway are activated during movement and the two pathways must therefore be in balance to initiate movement (Figure 2.12A). Dopamine facilitates motor activity by exciting D₁ receptor-expressing neurons in the direct pathway and by inhibiting D₂ receptor-expressing neurons in the indirect pathway. Adenosine has opposing effects on motor function to that of dopamine, performing a neuromodulatory role on the function of dopamine. Adenosine A₁ receptors are expressed by the direct pathway and have an inhibitory role here while adenosine A_{2A} receptors on the indirect pathway have enhancing effects on this pathway. In Parkinson's disease, the regulatory function of dopamine is removed and the balance is therefore disturbed, resulting in unopposed adenosine signalling which presents in the form of Parkinsonian motor symptoms (Figure 2.12B) (Xu *et al.*, 2005).

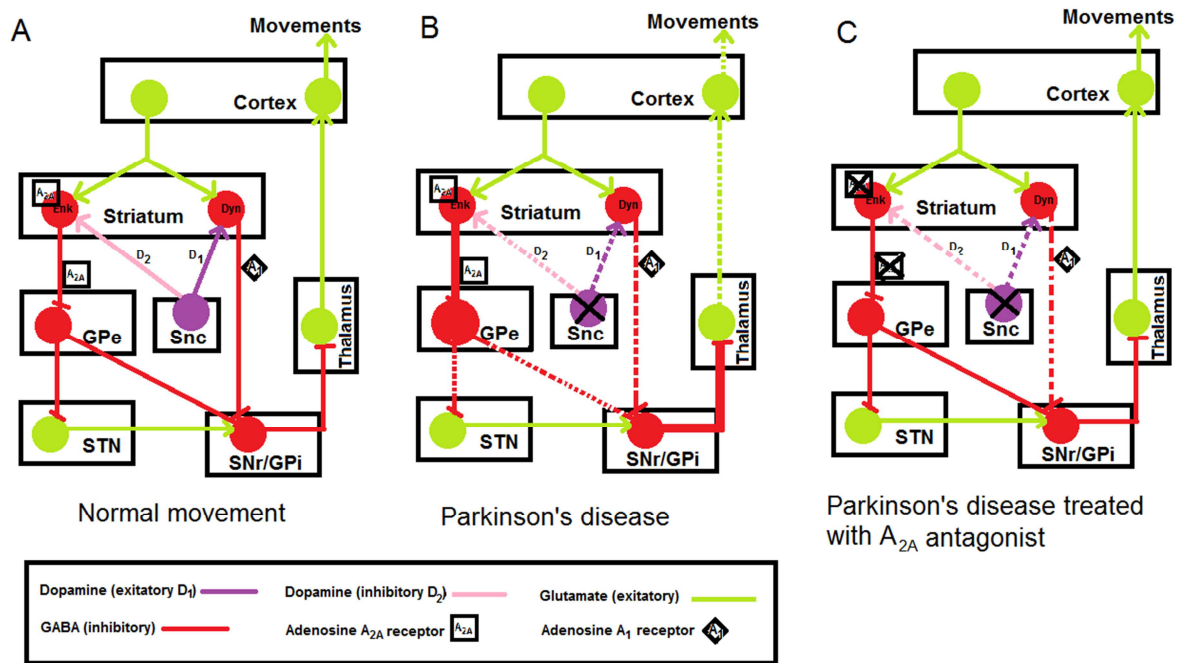


Figure 2.12: Mechanism of action of adenosine A_{2A} receptor antagonists in motor function (Xu *et al.*, 2005).

Antagonism of adenosine A_{2A} receptors will thus alleviate some of the excessive inhibitory drive from the indirect pathway and restore some balance between the direct and indirect pathways (Figure 2.12C). Unfortunately, it is impossible to completely normalize the reduced activity of the direct pathway by adenosine A_{2A} receptor inhibition alone (Xu *et al.*, 2005).

2.3.6. Adenosine A_{2A} receptor's interaction with other neurotransmitter receptors

Neurotransmission at the adenosine A_{2A} receptor affects other neurotransmitter receptors in the brain. This has advantages when considering its application in the treatment of PD. The most prominent examples are A_1 , D_2 , glutamatergic, cholinergic and opioid (Xu *et al.*, 2005). These interactions will be discussed briefly:

a) The adenosine A_{2A} receptor and the adenosine A_1 receptor

Antagonism of the adenosine A_{2A} receptor modulates the inhibitory action of adenosine A_1 on the direct pathway and therefore promotes stimulation of movement (Xu *et al.*, 2005). See section 2.5.1 for a discussion on the dualistic action of adenosine A_{2A} and A_1 receptor antagonists.

b) The adenosine A_{2A} receptor and the dopamine D₂ receptor

The adenosine A_{2A} receptor is co-localized with the dopamine D₂ receptor and has a very close interaction with this receptor. The deactivation of the adenosine A_{2A} receptor leads to a higher binding affinity of dopamine for the D₂ receptor as well as increased G-protein coupling of the D₂ receptor (Xu *et al.*, 2005).

c) The adenosine A_{2A} receptor and glutamate

Glutamate is involved in synaptic plasticity, memory and learning and repair of the central nervous system. It is also the major excitatory neurotransmitter in the central nervous system (Armentero *et al.*, 2011). Stimulation of the adenosine A_{2A} receptor facilitates glutamate release (Chen *et al.*, 2007), while antagonism of adenosine A_{2A} receptors inhibits glutamate release and therefore offers neuroprotection against glutamate mediated neurotoxicity (see section 2.1.5) (Cunha, 2005; Chen *et al.*, 2007).

d) The adenosine A_{2A} receptor and release of gamma-aminobutyric acid (GABA)

GABA has an inhibitory role in the central nervous system. Antagonism of the adenosine A_{2A} receptor leads to decreased extracellular GABA levels. It is therefore possible that adenosine A_{2A} receptor antagonism mediated motor activation may be in part due to the reduction of GABA release from striatopallidal axon terminals (Díaz-Cabiale *et al.*, 2002; Xu *et al.*, 2005).

e) The adenosine A_{2A} receptor and cholinergic system

There is an imbalance between cholinergic and dopaminergic activity in Parkinson's disease (hence the use of anticholinergic drugs in the disease). Antagonism of adenosine A_{2A} receptors leads to decreased levels of acetylcholine in the striatum thus restoring the balance between dopaminergic and cholinergic function which leads to a decrease in motor symptoms (Kirkpatrick and Richardson, 1993; Xu *et al.*, 2005).

f) The adenosine A_{2A} receptor and opioid receptors

Interestingly, adenosine A₁ and A_{2A} receptor agonists decrease incidence of withdrawal in opiate addiction. It is therefore believed that endogenous adenosine act as a modulator of withdrawal behaviour although the mechanism is yet to be determined (Salem and Hope, 1997).

2.3.7. Possible neuroprotective mechanisms of adenosine antagonism

It has been established that the incidence of Parkinson's disease is 30% less in coffee drinkers compared to non-coffee drinkers (Ross *et al.*, 2000; Hernan *et al.*, 2002). Caffeine has an inhibitory effect on both adenosine A_{2A} and A₁ receptors. Adenosine A_{2A} receptor antagonism appears to be linked to its neuroprotective effects, whereas adenosine A₁ receptor antagonism is linked to its effects on motor stimulation (Fredholm *et al.*, 1999; Shook *et al.*, 2012). The neuroprotective effect of caffeine (and other adenosine A_{2A} receptor antagonists for that matter) is not compromised by the development of tolerance (Popoli *et al.*, 2000).

Antagonism of the adenosine A_{2A} receptor results in the inhibition of glutamate release (see section 2.3.6c). Glutamate can mediate excitotoxic changes that underlie the motor symptoms of Parkinson's disease, by exerting its excitotoxic effects through a glutamate-triggered overload of intracellular calcium which contribute to, and sustain neurodegeneration (Armentero *et al.*, 2011).

Neuro-inflammation is considered as one of the most important factors in the development of Parkinson's disease (Hirsch and Hunot, 2009). Chronic inflammation can overwhelm normal mechanisms of control during constant stimulation and produce toxic factors that can increase underlying pathology (Armentero *et al.*, 2011). Inflammation further promotes dopaminergic cell death (Hirsch and Hunot, 2009). Adenosine A_{2A} receptors are expressed in cells associated with neuro-inflammation, namely astrocytes (Fiebich *et al.*, 1996; Brambilla *et al.*, 2003), microglia (Fiebich *et al.*, 1996) and oligodendrocytes (Stevens *et al.*, 2002). The administration of KW 6002, a known adenosine A_{2A} receptor antagonist, resulted in a reduction in neuro-inflammation and specifically a reduction in the number of large activated microglial cells (Yu *et al.*, 2008).

The integrity of the blood brain barrier is necessary to maintain central nervous system functioning and any alteration leads to changes in the neuronal environment (Berislav, 2008). It has been reported that the blood brain barrier is disrupted in Parkinson's disease and that this may contribute to disease progression (Carvey *et al.*, 2005; Stolp and Dziegielewska, 2009; Weiss *et al.*, 2009). Adenosine A_{2A} receptors are strongly expressed by brain endothelial cells (Schaddelee *et al.*, 2003). Antagonism of the adenosine A_{2A} receptor may therefore play a valuable role in altering disease progression through modulation of the lipid and/or cholesterol metabolism in the blood brain barrier resulting in protection of this important defence mechanism (Brochard *et al.*, 2009; Armentero *et al.*, 2011).

Some xanthine adenosine A_{2A} receptor antagonists have shown reversible inhibition of MAO-B (Vlok *et al.*, 2006; Pretorius *et al.*, 2008; Petzer *et al.*, 2009) and these results indicate that the neuroprotective properties of these particular adenosine A_{2A} receptor antagonists may incorporate this neuroprotective mechanism. Oxidation of dopamine by MAO leads to production of H₂O₂ which reacts with free iron (II) ions which can worsen neurodegeneration. The inhibition of MAO-B therefore leads to a decrease in the generation of hazardous metabolic by-products in the brain (Armentero *et al.*, 2011).

Neuroprotection by adenosine A_{2A} receptor antagonists in Parkinson's disease is complex and involves a great variety of mechanisms. These agents are therefore actively researched at present, and possess great promise as therapy for Parkinson's disease.

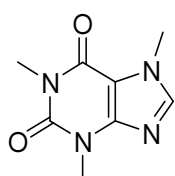
2.4. Current development of adenosine A_{2A} receptor antagonists

Adenosine A_{2A} receptor antagonists belong to two different chemical classes namely xanthine derivatives and amino-substituted heterocyclic compounds. Most of the heterocyclic compounds are derived from, or related to, adenine (Müller and Ferré, 2007). Although a myriad of compounds have been synthesised and published in literature, only a few will be discussed, with the focus on those that have reached clinical trials.

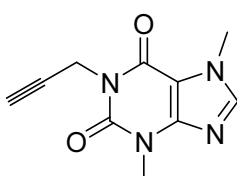
2.4.1. Xanthine derivatives

The alkaloids caffeine (**33**) and theophylline were the first identified adenosine antagonists and both have weak affinities for adenosine A_{2A} and A₁ receptors (Shook and Jackson, 2011).

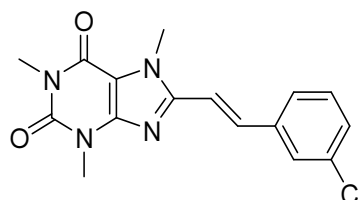
The first moderately selective receptor antagonist synthesised was DMPX (**34**), but it only exhibited low adenosine A_{2A} receptor affinity. The first potent and highly selective adenosine A_{2A} receptor antagonists synthesised, were the xanthine derivatives CSC (**35**) and istradefylline (**1**) (Müller and Ferré, 2007).



33
A_{2A}K_i = 48 000 nM
A₁K_i = 29 000 nM



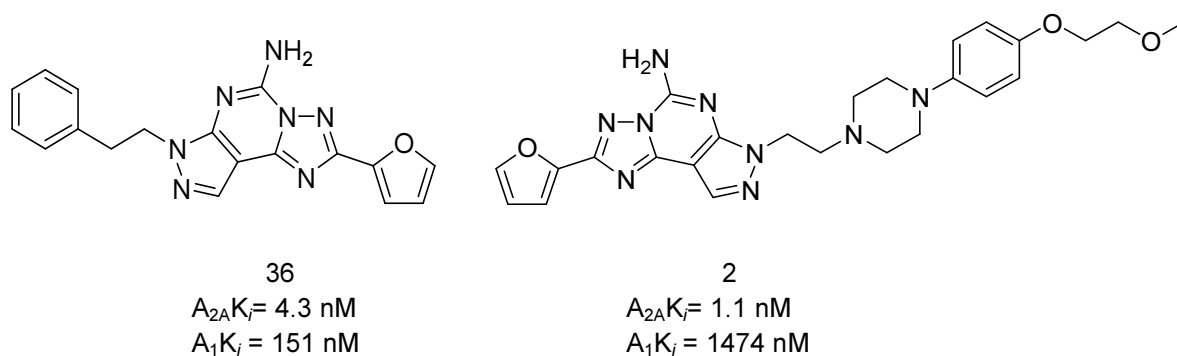
34
A_{2A}K_i = 1 600 nM
A₁K_i = 45 000 nM



35
A_{2A}K_i = 54 nM
A₁K_i = 28 000 nM

CSC (**35**) has shown potent inhibition of MAO-B and this may contribute to its efficacy in animal models (Chen *et al.*, 2002). KW 6002 (istradefylline (**1**)), has shown great promise as an orally active drug (Shimada *et al.*, 1997; Shiozaki *et al.*, 1999). For example, an istradefylline monotherapy trial (trial 6002-US-051) was undertaken in 2010 in the form of a 12-week double-blind study to evaluate the safety and efficacy of this drug as monotherapy. The results indicated that istradefylline is safe and well tolerated in untreated Parkinson's disease and that the adverse effect profile was comparable to the placebo group. However, the study failed to show a statistically significant improvement of symptoms in early Parkinson's disease and the FDA did not approve istradefylline for clinical use (Fernandez *et al.*, 2010; Armentero *et al.*, 2011).

2.4.2. Non-xanthine derivatives



Schering-Plough developed a series of non-xanthine based derivatives and SCH58261 (**36**) was found to be a potent adenosine A_{2A} receptor antagonist with moderate selectivity (Ongini, 1997). However, it showed no efficacy after oral dosing (Shook and Jackson, 2011). The aryl substituent was replaced with heterocycles such as pyridines and pyrazines. These compounds retained potent adenosine A_{2A} receptor antagonistic activity as well as selectivity, but suffered from poor solubility and poor bioavailability. Further optimization led to the methoxyethoxy analogue preladenant (**2**), developed by Merck & Co Incorporated. It has good oral bioavailability and *in vivo* activity in animal models against Parkinson's disease (Neustadt *et al.*, 2007). Results obtained in clinical trials (with patients remaining on their regular stabilised levodopa containing therapeutic schedules) indicated that preladenant was effective in reducing "off time", while increasing "on time" without a proportional increase in dyskinesia. A small increase in systolic and diastolic blood pressure was observed but blood pressure returned to normal after a 2-12 week period of administration. Adverse effects that were experienced were aggravation of Parkinson's symptoms, dyskinesia and somnolence (Hauser *et al.*, 2011; Armentero *et al.*, 2011).

Unfortunately, approval of preladenant was denied by the FDA after phase 3 clinical trials due to insufficient effectivity (Merck, 2013).

Although no adenosine antagonist has been FDA approved to date, the acceptable side-effect profiles together with the promising preclinical results obtained with several derivatives, serves as motivation for further research on these agents.

2.5. Possible adverse effects due to adenosine A_{2A} inhibition

When the physiological role of the adenosine A_{2A} receptor is considered, the potential for the development of adverse effects and toxicity exists. The main concern is weakening of the immune function and inflammatory changes in the body due to the involvement of adenosine A_{2A} receptors in this system. Clinical trials of known adenosine A_{2A} receptor antagonists have yet to substantiate this concern (Jenner *et al.*, 2009). During a 12-week double-blind randomized placebo-controlled exploratory study with istradefylline, the primary adverse-effect experienced was nausea in a mild degree, which dissipated after ten days. An increase in serum lipase was experienced in a few cases (Hauser *et al.*, 2003). Due to few available clinical studies on adenosine A_{2A} receptor antagonists, the data concerning adverse effects and toxicity are however limited.

2.5.1. Selective and non-selective adenosine receptor antagonism

Both selective adenosine A_{2A} and A₁ receptor antagonists have demonstrated the ability to induce motor activation in animal models. Where A_{2A} receptor antagonism leads to changes in the second messenger system (lower cAMP in the indirect pathway), adenosine A₁ receptor antagonism leads to an increase in the response to dopamine (Nikodijević *et al.*, 1991; Ferré *et al.*, 2001). It is therefore proposed that the antagonism of both adenosine receptor subtypes will lead to a synergistic action on motor function (Shook *et al.*, 2012). Furthermore, it was demonstrated that antagonism of central adenosine A₁ receptors enhances cognitive function and therefore dualistic antagonism of both adenosine A_{2A} and A₁ receptors will potentially treat both the motor disability and cognitive dysfunction present in Parkinson's disease (Mihara *et al.*, 2007).

However, some caution is also advisable when this approach is taken. Dual antagonism of both adenosine A_{2A} and A₁ receptors can result in feelings of anxiety and agitation through an unknown mechanism (Yacoubi *et al.*, 2000). Adenosine A₁ receptor antagonism may further influence the cardiovascular system as adenosine has a regulatory function in this

system (Mihara *et al.*, 2007). These side effects are only theoretical as no such agent has been approved yet.

2.6. *In vitro* radioligand binding studies

During *in vitro* radioligand binding studies, the affinities of chemical entities for receptors (in this case the adenosine A_{2A} and A₁ receptors) are determined by measuring the displacement of a radiolabelled adenosine receptor ligand from the receptor. When the radioligand is displaced by the test compound, residual radioactivity will be lower; in other words, a lower counts per minute (CPM) value is indicative of high affinity, while a higher value indicates low affinity. Either cell cultures, or rat homogenates are routinely used as receptor sources (Baraldi *et al.*, 1995; Ongini and Fredholm, 1996).

One of the first high affinity radioligands for adenosine receptors was [³H]R-PIA which is an adenosine A₁ receptor selective probe. [³H]NECA is a non-selective ligand which labels adenosine A₁, A_{2A} and A₃ receptors with equivalent preference. [³H]-DPCPX is a radioligand with selective adenosine A₁ antagonistic characteristics (Klotz, 2000).

2.7. *In vivo* animal models for Parkinson's disease

a) The reserpine akinesia animal model

Systemic administration of reserpine in this animal model results in depletion of catecholamines at nerve terminals (Betarbet *et al.*, 2002). The storage capacity for dopamine in intracellular vesicles is thus lost and akinesia induced. Reserpine induced akinesia also commonly coincides with tremor (Hornykiewicz, 1966). The major drawbacks of this animal model are that provoked changes are short-lived and no morphologic changes in the SNc are observed. This model is further non-selective as the release of other catecholamines is also affected and may interfere with the effectiveness of the model (Hornykiewicz, 1966; Bertarbet *et al.*, 2002).

b) The 6-hydroxydopamine (6-OHDA) animal model

The administration of 6-hydroxydopamine, a neurotoxic analogue of dopamine, results in the permanent depletion of the catecholamines, dopamine and noradrenaline (Schober, 2004). This compound has specific neurotoxic actions on catecholaminergic neurons in the SNc, due to utilization of the same transport systems as dopamine and norepinephrine (Betarbet *et al.*, 2002; Schober, 2004). It has to be injected stereotactically due to an inability to cross the blood-brain barrier and neurodegeneration occurs 24 hours after injection. Normally the

injection is administered in only one hemisphere of the animal while the other hemisphere acts as internal control. Asymmetric circling behaviour commences after administration and the extent of this behaviour can be used to evaluate the effectiveness of therapy (Betarbet *et al.*, 2002; Schober, 2004). A weakness of the 6-OHDA animal model is that degeneration of only a few of the brain regions affected in Parkinson's disease occurs (Betarbet *et al.*, 2002) while the extra care required by animals with brain lesions is a further drawback (Schober, 2004).

c) The MPTP animal model

As previously mentioned, MPTP is metabolised to MPP⁺ which induces mitochondrial dysfunction and subsequent nigrostriatal dopaminergic degeneration in a number of animals. The administration of MPTP in primates results in typical behavioural characteristics of Parkinson's disease including bradykinesia, rigidity and tremor. Formation of α -synuclein like inclusions has also been observed in primates. This model is the most extensively used and can be employed in the study of Parkinson's disease pathogenesis, evaluation of anti-parkinsonian drugs, assessment of cell transplantation therapies as well as testing of gene therapy (Betarbet *et al.*, 2002). The presentation of animals that are treated with a low dose of MPTP over long periods of time mimics the effects of chronic degeneration that is seen in human Parkinson's disease. The MPTP monkey model is also seen as the most effective model for the evaluation of neuroprotective therapies (Schober, 2004).

d) The haloperidol catalepsy animal model

Motor-related side effects are usually present in patients who receive antipsychotic drugs such as haloperidol. The administration of haloperidol to an animal induces catalepsy, which is characterized by long periods of immobility and failure to correct externally imposed postures (similar to akinesia observed in human Parkinson's disease). This animal model is therefore used to determine the ability of a test compound to reverse motor symptoms induced by the lack of dopaminergic transmission in the SNc (as simulated by haloperidol administration) (Andersen and Kilpatrick, 1996).

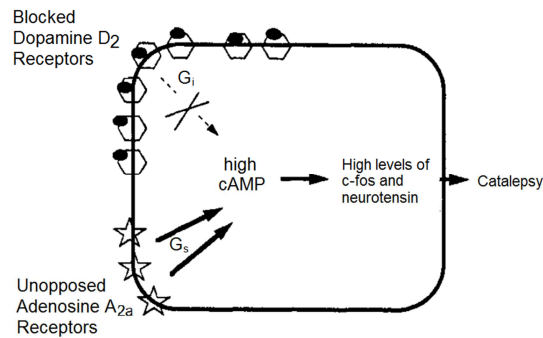


Figure 2.13: Effect of haloperidol on intracellular cAMP (Ward and Dorsa, 1999).

Haloperidol is a dopamine D₂ receptor antagonist which eliminates the inhibitory G_i-coupled input (Figure 2.13) that is normally created by endogenous dopamine. In the presence of haloperidol, adenosine A_{2A} receptor signalling is therefore unopposed, resulting in G_s stimulation, which leads to an increase in cAMP within the striatopallidal neuron. Transcription of genes responsive to cAMP is thus increased, resulting in catalepsy. (reduction of movement) (Ward and Dorsa, 1999).

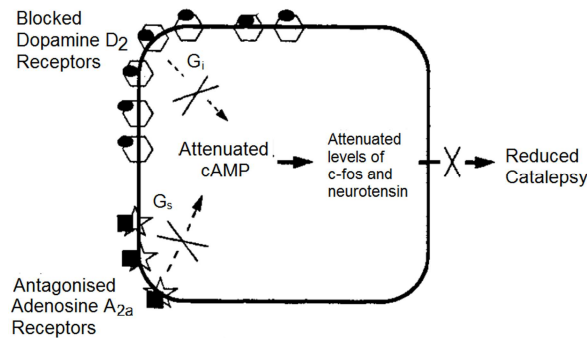


Figure 2.14: Antagonism of adenosine A_{2A} receptors leads to reversal of haloperidol induced catalepsy (Ward and Dorsa, 1999).

Antagonism of the adenosine A_{2A} receptor during haloperidol induced catalepsy (Figure 2.14) reduces the G_s-coupled input. Reversal of catalepsy occurs due to attenuated levels of cAMP, and cAMP responsive gene transcription is normalized (Ward and Dorsa, 1999).

Other models used less often is the paraquat-maneb animal model for decreased locomotor activity and the rotenone animal model for resting tremor, rigidity and stooped posture as well as the 3-nitrotyrosine animal model of oxidative stress (Betarbet *et al.*, 2002).

2.8. Summary

In this chapter an overview of current literature regarding Parkinson's disease was provided. This disease is a neurodegenerative disorder of the central nervous system and current therapy is focused on the alleviation of disease symptoms and does not address disease progression. An interesting development in research is dual adenosine A_{2A}/A_1 receptor antagonists. These agents have the potential to provide symptomatic relief as well as affording neuroprotection. In the following chapter the synthesis of novel 2-aminopyrimidines, as potential dual adenosine receptor antagonists will be discussed.