

CHAPTER 2

LITERATURE OVERVIEW

2.1 PARKINSON'S DISEASE

2.1.1 Clinical characteristics of Parkinson's disease

The onset of Parkinson's disease is gradual and the early symptoms can easily go unnoticed. The symptoms could manifest as a person who has a tendency to swim in circles, or finding it easier to walk on a pebbled beach than in a crowded street, or even just a trivial change in handwriting (Lees *et al.*, 2009). Inevitably, other motor symptoms soon follows...

The clinical characteristics of Parkinson's disease are described as tremor at rest, rigidity, bradykinesia (slowness or absence of voluntary movement) and postural instability (Dauer & Przedborski, 2003). Motor symptoms include a tendency to pass from a walking to running pace, a stooped posture, "freezing" at the initiation of a movement, reduced facial animation, reduced voice volume and diminution of handwritten letters or symbols (Wells *et al.*, 2009). Mental status changes include the experience of anxiety, apathy, slowness of thought processes, a state of bewilderment, dementia, depression, hallucinations, psychosis, and sleep disorders (Wells *et al.*, 2009).

In the late stages of Parkinson's disease the face of the patient is masked and expressionless, speech is slurred and monotonous, the posture is flexed, and there is a pill rolling motion of the hands. A freezing of gait may occur and overall movements are uncoordinated (Lees *et al.*, 2009). The risk of developing Parkinson's disease increases with age (McDonald *et al.*, 2003), and over time the symptoms will only worsen (Dauer & Przedborski, 2003).

2.1.2 Neurochemical and neuropathological features

The main neuropathological features of Parkinson's disease are the loss of dopaminergic neurons in the SNpc, and the presence of Lewy bodies (figure 2.1) (Lees *et al.*, 2009., Dauer & Przedborski, 2003). Lewy bodies are spherical eosinophilic cytoplasmic protein aggregates which are composed of different proteins, including α -synuclein, parkin, ubiquitin and neurofilaments, and are found in all affected brain areas (Spillantini *et al.*, 1998, Forno, 1996).

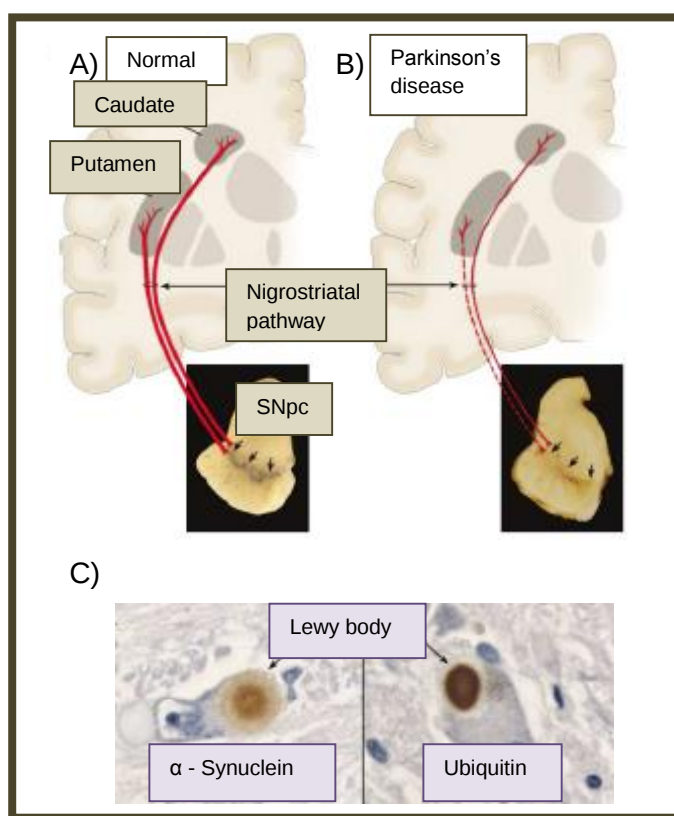


Figure 2.1: Neuropathology of Parkinson's disease (Dauer & Przedborski, 2003).

In the SNpc the cell bodies of nigrostriatal neurons project primarily to the putamen. The loss of these neurons (which contains melanin) produces SNpc depigmentation (figure 2.1 A versus B). This is consistent with the finding that depletion of DA is most prominent in the putamen (Bernheimer *et al.*, 1973), which is the main site of projection for these neurons. The dopaminergic neurons of the mesolimbic area, of which the cell bodies reside adjacent to the SNpc in the ventral tegmental area (VTA), are less affected (Dauer & Przedborski, 2003, Uhl *et al.*, 1985).

Apart from dopaminergic neuron loss, there is also cell loss in the locus coeruleus, dorsal nuclei of the vagus, raphe nuclei, and other brain stem areas (Damier *et al.*, 1999). According to Dauer & Przedborski (2003), neurodegeneration and Lewy body formation are found in the areas above, as well as the cerebral cortex, olfactory bulb, and the autonomic nervous system. The rate of dementia accompanying Parkinson's disease (especially in older patients), may partially be attributed to the degeneration of the hippocampus and cholinergic cortical inputs (Dauer & Przedborski, 2003).

There is a correlation between the degree of nigrostriatal DA loss and the motor symptom severity. Parkinson's disease is relatively asymptomatic until the depletion of 70-80% of the SNpc neurons has occurred (figure 2.1 B). The loss of nigrostriatal DA neurons leads to the inhibition of thalamus and motor cortex activity, as well as an increase of striatal cholinergic activity, which contributes to the tremor related to Parkinson's disease (Wells *et al.*, 2009).

2.1.3 Etiology

a) Ageing:

The major risk factor for developing Parkinson's disease is age (Lees *et al.*, 2009). Neuropathological studies of Parkinson's disease-related neurodegeneration revealed insights regarding the pathogenesis of the disease. Firstly, Parkinson's disease-associated loss of dopaminergic neurons has a characteristic topology, different from that of normal aging. In Parkinson's disease, cell loss is mainly in the ventrolateral and caudal areas (figure 2.1) of the SNpc, but during normal aging the dorsomedial area of the SNpc is mostly affected (Kubis *et al.*, 2000). Thus, even though age is an important risk factor for Parkinson's disease, the process of age-related dopaminergic neuron death is different from that of Parkinson's disease. Secondly, during normal aging the degree of striatum terminal loss is more prominent than the amount of SNpc neuron loss (Bernheimer *et al.*, 1973). It may therefore be assumed that striatal dopaminergic nerve terminals are the main target of the degenerative process, and that it is the result of a "dying back" process (Dauer & Przedborski, 2003).

The following factors may also influence the chance of developing Parkinson's disease:

b) Smoking:

Lifelong non-smokers are twice as likely to develop Parkinson's disease (Allam *et al.*, 2004, Hernán *et al.*, 2001). According to Quik and Jeyarasasingam (2000), nicotinic receptor activation protects nigral dopaminergic neurons from 1-methyl-4-phenylpyridinium (MPP⁺) toxicity *in vitro*. This data suggests that nicotinic agonist therapy could improve cognitive and locomotor deficits as well as be neuroprotective against the degenerative processes observed in Parkinson's disease.

c) Drinking coffee:

According to Ascherio *et al.* (2004), men who regularly drink caffeinated drinks have a lower risk of developing Parkinson's disease than non-drinkers. In women, that do not use postmenopausal hormones, caffeine reduces the risk of developing Parkinson's disease, but the risk is increased among hormone users. This may be related to DA's role in the reward pathways or low premorbid novelty seeking personality traits and not because of any neuroprotective effect of tobacco smoke, nicotine or caffeine (Evans *et al.*, 2006). Caffeine is also an adenosine A₂ receptor antagonist, a class of compounds which show anti-parkinsonian potential (Jankovic, 2008).

d) Living conditions:

Some association has been found between Parkinson's disease and head injury, rural living, middle-aged obesity, the lack of exercise, well-water ingestion, and herbicide and

insecticide exposure (paraquat, organophosphates, and rotenone) (Elbaz & Tranchant, 2007).

e) Environmental conditions:

According to Lees *et al.* (2009), some environmental toxins can also increase the risk of developing Parkinson's disease. Examples of neurotoxins which may induce Parkinson's disease are 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), cyanide, carbon disulphide and toluene (Tanner & Aston, 2000).

f) Medication:

There are also medication that can induce Parkinson's disease like symptoms. Examples of these are antipsychotics, phenothiazine antiemetics and metoclopramide (Wells *et al.*, 2009).

g) Genetic factors:

Results from genetic studies indicate that there are mutations in seven genes, which are linked with L-dopa-responsive parkinsonism. Six pathogenic mutations in leucine rich repeat kinase 2 (LRRK-2) have been reported, the most common of these, the Gly2019Ser mutation, has a worldwide frequency of 1% in sporadic cases and 4% in patients with hereditary parkinsonism (Healy *et al.*, 2008, Paisán-Ruíz *et al.*, 2004). A person inheriting the Gly2019Ser mutation has a 28% risk of developing parkinsonism (if the person is younger than 60 years of age), and 74% at 79 years of age (Healy *et al.*, 2008). Both point mutations and gene triplications of α -synuclein cause a parkinsonian syndrome that is difficult to distinguish from Parkinson's disease (Singleton *et al.*, 2003, Polymeropoulos *et al.*, 1997). Duplications of α -synuclein have rarely been found in sporadic Parkinson's disease (Lees *et al.*, 2009, Theuns & Van Broeckhoven, 2008).

When there is a loss of function due to mutations in the genes, parkin, DJ-1, PINK1, and ATP13A2, recessive early onset parkinsonism (age of onset < 40 years) may occur. Parkin mutations are the second most common genetic cause of L-dopa-responsive parkinsonism, whereas mutations in the other three genes are rare. All of these mutations lead to a disease that has a more benign course than Parkinson's disease, respond well to dopaminergic drugs, and frequently presents with gait disorder, rest tremor of the legs, and limb dystonia (Lees *et al.*, 2009).

2.1.4 Pathogenesis

According to Dauer & Przedborski (2003), there are two major hypotheses regarding the pathogenesis of Parkinson's disease. One hypothesis is that the misfolding and aggregation of proteins are instrumental in the death of SNpc dopaminergic neurons. The second proposes that mitochondrial dysfunction and the consequent oxidative stress, due to inter alia DA species, are key events in the pathogenesis of Parkinson's disease (figure 2.2) (Dauer & Przedborski, 2003). However, neurodegeneration can also be a result of apoptosis, neuroinflammation, a loss of trophic factors and excitotoxicity.

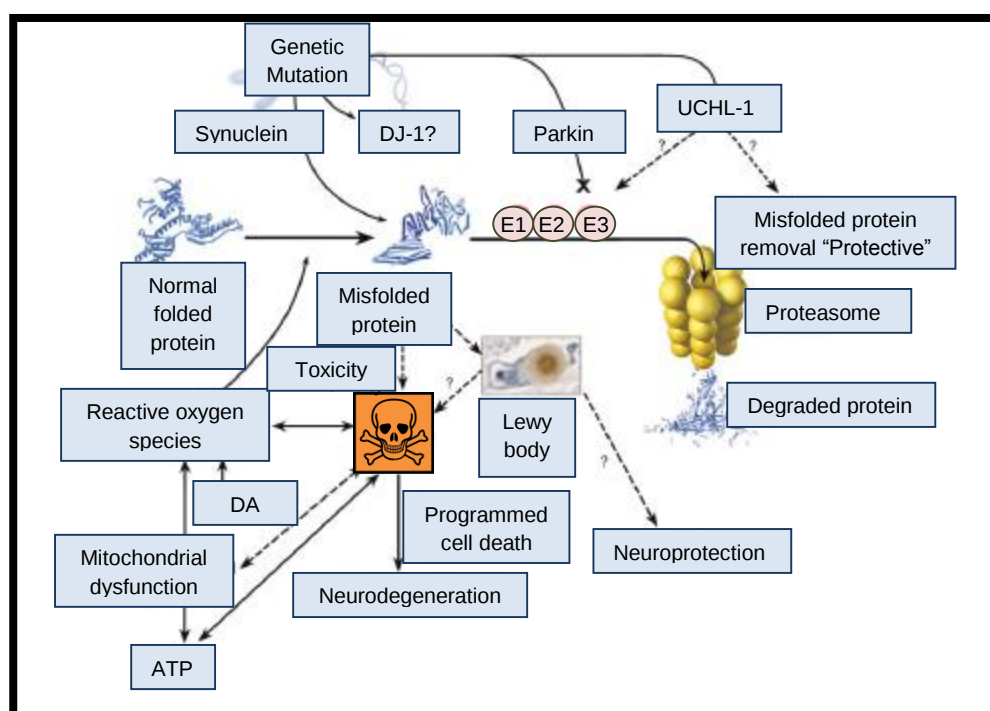


Figure 2.2: Mechanisms of neurodegeneration (Dauer & Przedborski, 2003).

2.1.4.1 Mechanisms of neurodegeneration

2.1.4.1.1 Oxidative stress and mitochondrial dysfunction

Oxidative damage, linked to mitochondrial dysfunction and abnormal DA metabolism, may also promote misfolding of proteins. Oxidative stress, an energy crisis (i.e., adenosine 5'-triphosphate (ATP) depletion) and the activation of programmed cell death machinery are also believed to be factors that trigger the death of dopaminergic neurons in Parkinson's disease (Dauer & Przedborski, 2003).

Oxidative stress is a result of an overabundance of reactive free radicals secondary to either an overproduction of reactive species or a failure of cell buffering mechanisms that would normally limit their accumulation. The excess reactive species can react with cellular macromolecules and

disrupt their normal functions. In Parkinson's disease, protein, lipid, and nucleic acid oxidative damage in the SNpc occurs (Alam *et al.*, 1997), while overproduction of reactive species and a failure of cellular protective mechanisms are also present (Yacoubian & Standaert, 2009).

Oxidative stress is promoted by DA metabolism through the production of quinones, peroxides, and other reactive oxygen species (ROS) (Sulzer & Zecca, 1999). ROS is also produced by mitochondrial dysfunction, which damages the mitochondria even further. In Parkinson's disease mitochondrial complex 1 activity is reduced in the SNpc (Schapira *et al.*, 1990). This corresponds with animal models where the complex 1 inhibitors MPP⁺ and rotenone, cause a parkinsonian syndrome (Betarbet *et al.*, 2000, Langston *et al.*, 1983). Inherited or acquired mutations in mitochondrial DNA can contribute to mitochondrial dysfunction in Parkinson's disease (Cantuti-Castelvetri *et al.*, 2005). Free radical damage, especially in the presence of neuromelanin, is heightened by increased iron levels found in the SNpc of Parkinson's disease patients (Yacoubian & Standaert, 2009, Riederer *et al.*, 1989).

There may also be an impairment of endogenous protective mechanisms in Parkinson's disease. GSH, an antioxidant protein, is reduced in the brains of postmortem Parkinson's disease patients (Sian *et al.*, 1994, Sofic *et al.*, 1992, Perry & Yong, 1986). Some genes that are involved in protection against oxidative stress have been linked to familial forms of Parkinson's disease. These include the genes PINK1 and DJ-1 (Yacoubian & Standaert, 2009, Clark *et al.*, 2006).

To limit oxidative stress in Parkinson's disease, the following strategies may be used:

(a) Inhibition of MAO, which is a key enzyme involved in DA catabolism, as it will decrease the concentrations of reactive oxygen precursors such as hydrogen peroxide; (b) The use of DA receptor agonists; (c) Mitochondrial electron transport could be enhanced, by using coenzyme Q1 while compounds that can directly quench free radicals, such as the antioxidant vitamin E, or uric acid should lessen the accumulation of free radicals; (d) and lastly, the use of molecules that can enhance endogenous mechanisms to buffer free radicals, such as GSH promoters (Yacoubian & Standaert, 2009).

2.1.4.1.2 Protein aggregation and misfolding

It is suggested that the accumulation of misfolded proteins is likely to be a key event in Parkinson's disease neurodegeneration. However, it is still unclear whether misfolded proteins directly cause toxicity or damage cells via the formation of protein aggregates (Lewy bodies). Controversy further exists regarding whether Lewy bodies promote toxicity or protect a cell from harmful effects of misfolded proteins (by sequestering them in an insoluble compartment away from cellular elements) (Dauer & Przedborski, 2003).

The protein aggregates may directly cause damage by deforming the cell or by interfering with intracellular trafficking in neurons. In inherited Parkinson's disease, pathogenic mutations to

certain genes are assumed to cause disease directly by inducing abnormal toxic protein conformations (as believed to be the case with α -synuclein) (Bussell & Eliezer, 2001), or indirectly by interfering with the process that usually recognize or process misfolded proteins (parkin). In sporadic Parkinson's disease, direct protein-damaging modifications may lead to the misfolding of protein, or the dysfunction of the proteasome that may lead to the accumulation of misfolded proteins (Dauer & Przedborski, 2003).

In Parkinson's disease, the primary aggregating protein is α -synuclein, which is the major component of Lewy bodies as well as Lewy neurites (Lewy neurites are similar to Lewy bodies, and are protein type formations found in neurons of the diseased brain, comprising of abnormal α -synuclein filaments and granular material) (Irizarry *et al.*, 1998, Spillantini *et al.*, 1997). The role of α -synuclein in Parkinson's disease is further highlighted by the fact that the disease is also caused by gene duplication of the α -synuclein locus (Singleton *et al.*, 2003). Point mutations (El-Agnaf *et al.*, 1998), over expression (Masliah *et al.*, 2000), and α -synuclein oxidative damage (Souza *et al.*, 2000), can all enhance self-aggregation (Yacoubian & Standaert, 2009).

The mechanism by which α -synuclein causes neuronal injury is uncertain. Hypotheses include the following: firstly, the toxic effects of oligomers on cell membranes or proteasomal function; secondly, the effects of α -synuclein on gene regulation or transcription; thirdly, the interactions of α -synuclein with cell signaling and cell death cascades; fourthly, the alterations in DA storage and release caused by altered α -synuclein; and lastly, the activation of inflammation by α -synuclein (Yacoubian & Standaert, 2009, Kontopoulos *et al.*, 2006, Volles & Lansbury, 2003, Abeliovich *et al.*, 2000, Murphy *et al.*, 2000, Saha *et al.*, 2000).

A further trigger for dysfunctional protein metabolism may be oxidative stress, which may cause damage through ROS (Thorpe *et al.*, 2004). In Parkinson's disease, Lewy bodies contain oxidatively modified α -synuclein, which can aggregate more easily than unmodified α -synuclein (Giasson *et al.*, 2000). Some herbicides and pesticides may also induce misfolding or aggregation of α -synuclein (Uversky *et al.*, 2001)

These observations suggest that over production or impaired clearance of α -synuclein, which results in aggregation, may be a central mechanism for the development of Parkinson's disease (Yacoubian & Standaert, 2009). Some potential therapeutic strategies include the use of inhibitors of α -synuclein aggregation, the use molecules that promote protein clearance, for example enhancers of ubiquitin C-terminal hydrolase L1 (UCHL-1) function or parkin, or molecules that enhance proteasomal or lysosomal degradation pathways (Yacoubian & Standaert, 2009).

2.1.4.1.3 Neuroinflammation

Neuroinflammation may further contribute to the neurodegeneration as observed in Parkinson's disease (Tansey *et al.*, 2007, Orr *et al.*, 2005, McGeer *et al.*, 2003, Sherer *et al.*, 2003). In Parkinson's disease, the concentration of pro-inflammatory cytokines, such as interleukins (IL-1 β and IL-6) and the tumor necrosis factor-alpha gene (TNF- α), are high in the cerebrospinal fluid (CSF) and basal ganglia (Mogi *et al.*, 1994a, Mogi *et al.*, 1994b). Furthermore elevated serum levels of complement proteins and the presence of complement proteins in Lewy bodies have also been detected (Goldknopf *et al.*, 2006). *In vitro*, cytokines as well as aggregated and nitrated forms of α -synuclein can trigger a microglial response directly and release cytotoxic factors (Yacoubian & Standaert, 2009, Zhang *et al.*, 2005,).

Potential anti-inflammatory therapies may include the use of non-steroidal anti-inflammatory agents (NSAIDs), which reduces dopaminergic cell death (Esposito *et al.*, 2007). Minocycline is also a potential anti-inflammatory agent that is being investigated (Yacoubian & Standaert, 2009, NINDS NET-PD Investigators. 2006).

2.1.4.1.4 Excitotoxicity

Glutamate is a primary driver in the excitotoxic process and a primary excitatory transmitter in the mammalian central nervous system. In the substantia nigra (SN) there is a high expression of glutamate receptors which receive glutamatergic stimulation from the subthalamic nucleus and cortex. Glutamate causes excessive N-methyl-D-aspartate (NMDA) receptor activation that increases intracellular calcium levels, thereby activating cell death pathways (Yacoubian & Standaert, 2009, Dawson & Dawson, 1996, Mody & MacDonald, 1995).

Potential therapeutic strategies include the use of an NMDA or a calcium channel antagonists (Yacoubian & Standaert, 2009).

2.1.4.1.5 Apoptosis

Apoptosis is known as programmed cell death, and could further be a contributing factor to the neurodegeneration seen in Parkinson's disease. Physiological programmed cell death is a crucial part of normal development and a homeostatic mechanism of certain systems (such as the immune system), but the dysregulation of this pathway can contribute to neurodegeneration (Dauer & Przedborski, 2003). Both apoptotic and autophagic cell death pathways are hypothesized to be involved in Parkinson's disease by means of oxidative stress, protein aggregation, excitotoxicity or inflammatory processes. The activation of these cell death pathways probably represents end-stage processes in Parkinson's disease neurodegeneration. Potential therapies include inhibitors of cell death pathways (Yacoubian & Standaert, 2009).

2.1.4.1.6 Loss of trophic factors

The neurotrophic factors are responsible for the support and survival of the dopaminergic neurite outgrowth, and it is hypothesized that if there is a reduced expression of one or a combination of these neurotrophic factors, it could potentiate the degeneration of dopaminergic neurons (Appel, 1981). It has been demonstrated that brain-derived neurotrophic factor (BDNF), glial-derived neurotrophic factor (GDNF) and nerve growth factor (NGF) are reduced in the SN of patients with Parkinson's disease (Chauhan *et al.*, 2001, Howells *et al.*, 2000, Mogi *et al.*, 1999). Potential therapies include the administration of growth factors, as these stimulate growth and arborization (branching, of the dendrite of a nerve cell) of dopaminergic neurons. GDNF and neurturin are protective against neurodegeneration in animal models (Gasmi *et al.*, 2007, Eslamboli *et al.*, 2005). GDNF has been evaluated in human trials (Lang *et al.*, 2006), while neurturin is presently investigated in phase II trials (Yacoubian & Standaert, 2009).

2.2 ANIMAL MODELS OF PARKINSON'S DISEASE

The key to develop effective antiparkinsonian drugs is the availability of animal models that mimic the disease, as seen in humans, to such an extent that the results obtained could be extrapolated to humans. There are several models that are currently used and a few of these will be discussed.

2.2.1 6-Hydroxydopamine, rotenone and paraquat models

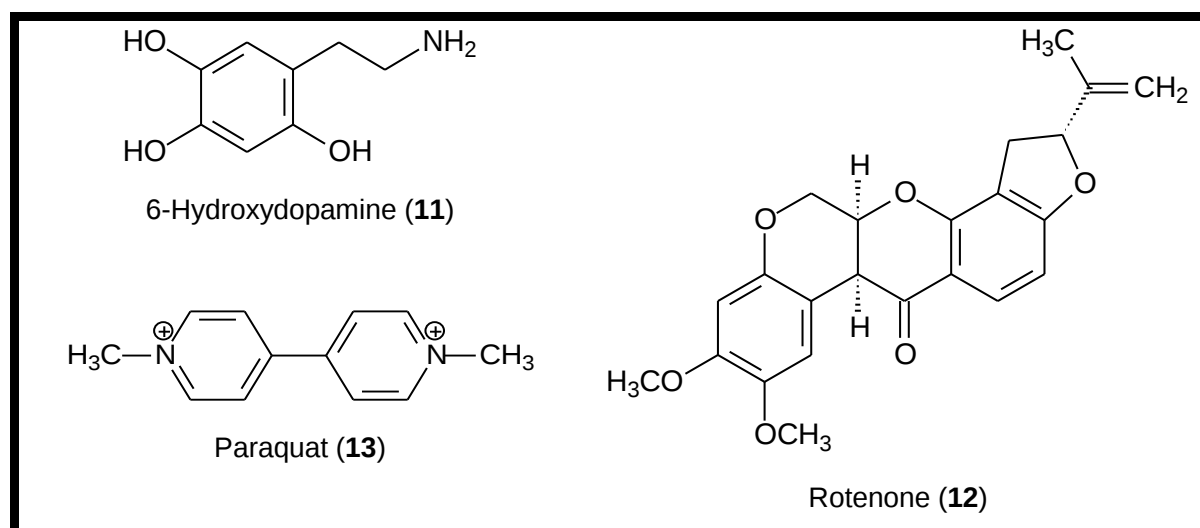


Figure 2.3: The structures of 6-hydroxydopamine, rotenone, and paraquat.

The administration of 6-hydroxydopamine (6-OHDA) (**11**) (figure 2.3) yielded the first animal model of Parkinson's disease associated with the loss of SNpc dopaminergic neurons. It is taken up into neuronal cells by DA and noradrenergic transporters (Luthman *et al.*, 1989) and its toxicity is

selective for monoaminergic neurons. 6-Hydroxydopamine accumulates inside the cytosol of neurons, where it generates quinones that attack nucleophilic groups, generate ROS and inactivate biological macromolecules (Choi *et al.*, 1999). As 6-OHDA cannot cross the BBB, it must be injected stereotaxically (into the SNpc / striatum) to be able to target the dopaminergic nigrostriatal pathway (Dauer & Przedborski, 2003).

Rotenone (**12**) (figure 2.3) is highly lipophilic and can gain access to most organs (Talpadé *et al.*, 2000). Rotenone inhibits complex 1 activity, similar to MPP⁺. In rats, rotenone produces selective neurodegeneration of dopaminergic neurons, which is accompanied by the presence of α -synuclein-positive Lewy body-like inclusions (Betarbet *et al.*, 2000). The characteristic of Lewy body-associated neurodegeneration of dopaminergic neurons, allows investigators to investigate the relationship between aggregate formation and neuronal death (Bové *et al.*, 2005, Dauer & Przedborski, 2003).

Paraquat (**13**) (figure 2.3) is structurally almost similar to MPP⁺, but unlike MPTP (**14**), paraquat (**13**) cannot penetrate the BBB. Its toxicity seems to be mediated by superoxide radical formation (Day *et al.*, 1999). Systemic administration of paraquat to mice leads to SNpc dopaminergic neuron degeneration, which is also accompanied by inclusions containing α -synuclein (Manning-Bog *et al.*, 2002). Because this model involves both dopaminergic neuronal loss as well as α -synuclein positive inclusions, it may be used to examine the role of α -synuclein in neurodegeneration (Bové *et al.*, 2005, Dauer & Przedborski, 2003).

2.2.2 MPTP model

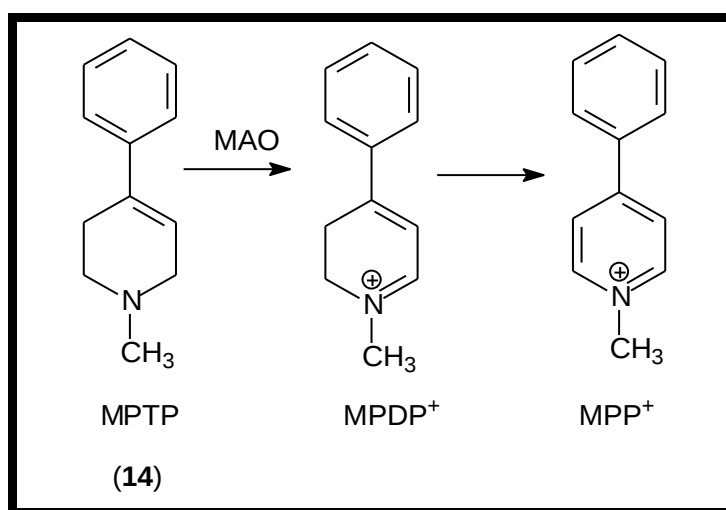


Figure 2.4: The formation of MPP⁺ (Singer & Ramsay, 1990).

MPTP (**14**) (figure 2.4) when administered to humans and monkeys produce an irreversible parkinsonian syndrome, displaying most characteristics of Parkinson's disease: tremor and rigidity,

slowness of movement, postural instability and freezing of gait. Studies on monkeys showed that, when treated with MPTP, there is preferential degeneration of putamen dopaminergic nerve terminals (Moratalla *et al.*, 1992), especially in the SNpc (Sirinathsinghji *et al.*, 1992). The monkey MPTP model is the golden standard used to assess novel strategies and agents to treat Parkinson's disease symptoms (Dauer & Przedborski, 2003).

MPTP (**14**) is administered systemically, because it is highly lipophilic and thus crosses the BBB (Dauer & Przedborski, 2003, Markey *et al.*, 1984). The first step in the development of neurotoxicity is the oxidation to the dihydropyridinium form (MPDP⁺) by MAO-B in the astrocytes of the brain. Afterwards MPDP⁺ is converted to the pyridinium form; 1-methyl-4-phenyl-pyridinium (MPP⁺) (figure 2.4). MPP⁺ is taken up by the DA reuptake system at dopaminergic synapses, and pumped into the soma of the neurons (Javitch *et al.*, 1985).

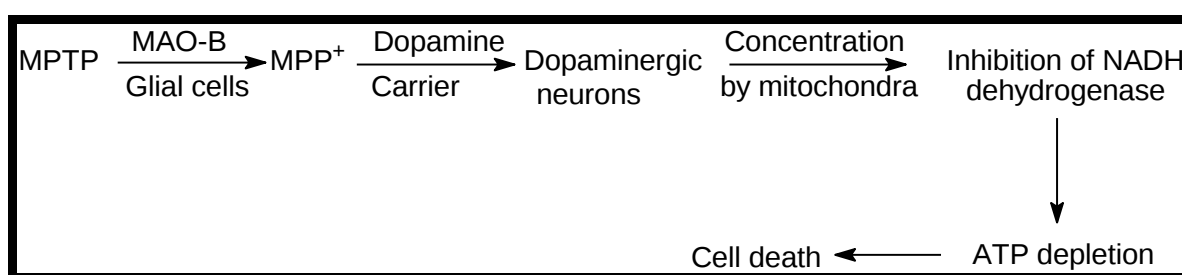


Figure 2.5: The steps in the expression of MPTP neurotoxicity (Singer & Ramsay, 1990).

Due to the electrochemical gradient of the inner membrane, MPP⁺ is concentrated into the mitochondrial matrix, where it combines with reduced nicotinamide adenine dinucleotide (NADH) dehydrogenase at the dehydrogenase-Q junction. This results in the inhibition of the function of the electron transport chain (Ramsay *et al.*, 1986a, Ramsay *et al.*, 1986b). This abolishes ATP synthesis and causes nigrostriatal cell death, as well as the neurological characteristics of Parkinson's disease (figure 2.5) (Ogunrombi *et al.*, 2007, Bové *et al.*, 2005, Singer & Ramsay, 1990).

2.3 SYMPTOMATIC TREATMENT OF PARKINSON'S DISEASE

As previously mentioned, idiopathic Parkinson's disease is associated with reduced levels of DA in the striatum, and to a lesser extent in the SNpc. Treatment would therefore be based on replenishing brain DA, either by administering the DA precursor amino acid, levodopa, or by inhibiting the enzymes, such as MAO, that catabolize DA in the brain (figure 2.6) (Brunton *et al.*, 2011).

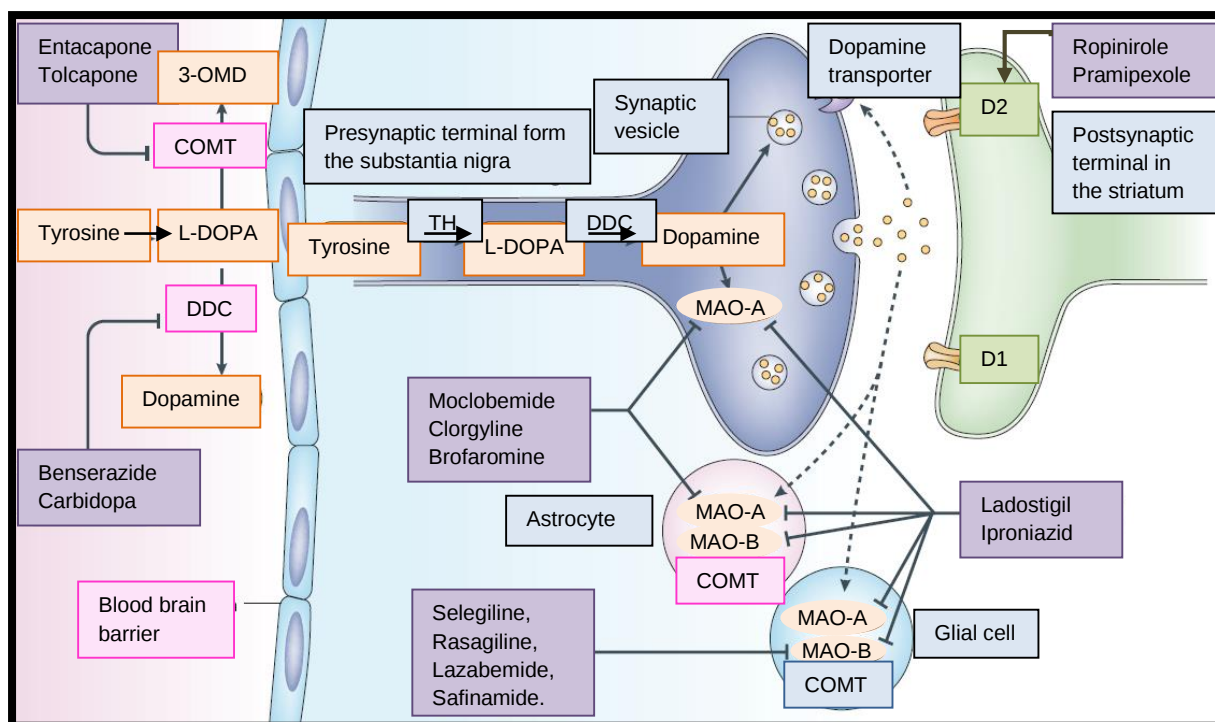


Figure 2.6: The synthesis and metabolism of DA (Youdim *et al.*, 2006, Aminoff, 2009).

2.3.1 Levodopa

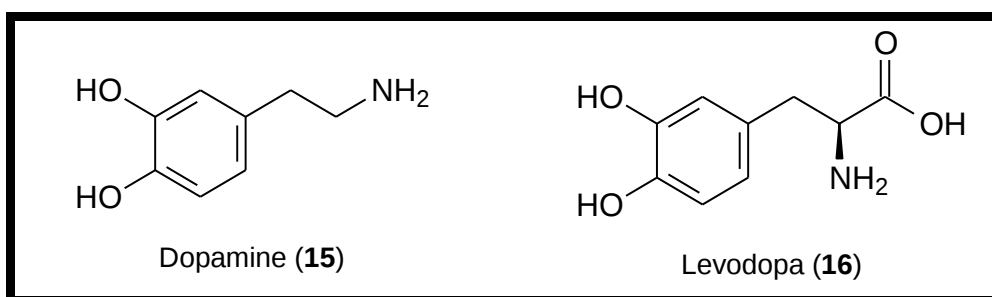


Figure 2.7: The structures of dopamine and levodopa.

Dopamine (15) does not cross the BBB, thus, if it administered to patients, it will not have a therapeutic effect in Parkinson's disease. However, levodopa [(-)-3-(3,4-dihydroxyphenyl)-L-alanine] (16), which is the immediate precursor of DA, enters the brain via a L-amino acid transporter (LAT), where it is decarboxylated to yield DA (figure 2.7) (Aminoff, 2009).

However, only 1-3% of the levodopa (16) administered enters the brain unaltered, the rest is metabolized extra-cerebrally, mainly by decarboxylation to DA (15), which cannot penetrate the BBB. Levodopa must thus be administered in high doses when used alone. Such high doses cause unpleasant side effects, especially nausea and vomiting as well as cardiovascular effects (Aminoff, 2009). Levodopa therefore is not used as monotherapy, but is combined with carbidopa (17) or

benserazide (**18**) (decarboxylase inhibitors), which allows for lower doses of levodopa, thus minimizing adverse effects of high doses of levodopa.

The risk of developing the “on-off” effect, associated with levodopa (**16**), increases with prolonged therapy (Abbot, 2010). The “on-off” effect is characterized by a marked fluctuation in mobility, with normal activity during the “on” period and akinesia during the “off” period. The slow-release combination formula of levodopa and carbidopa (**17**) reduces the peak plasma levels and increases the trough plasma levels of levodopa, thus diminishing the peak-trough fluctuations, and thus reducing the “on-off” effect (Nyholm *et al.*, 2013, Aminoff, 2009). According to Aminoff (2009), the use of levodopa should be reserved for the management of advanced idiopathic or postencephalitic Parkinson’s disease.

2.3.2 Carbidopa and benserazide

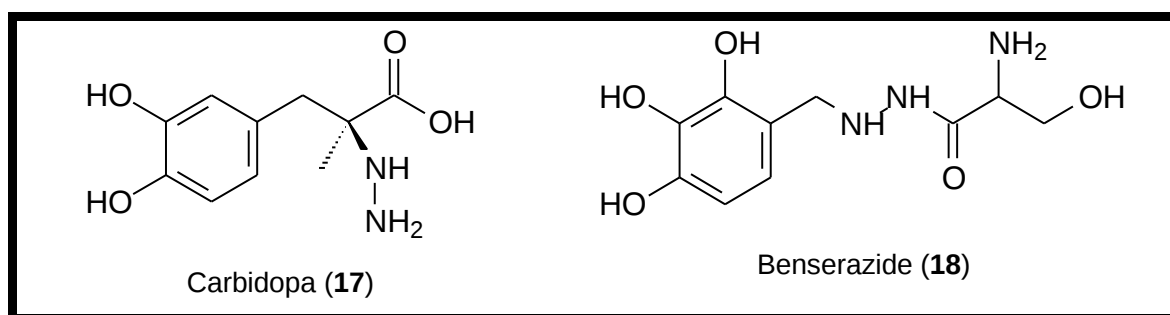


Figure 2.8: The structures of carbidopa and benserazide.

Carbidopa (**17**) and benserazide (**18**) are peripheral dopa decarboxylase inhibitors (figure 2.8). When levodopa (**16**) is given in combination with a dopa decarboxylase inhibitor that does not penetrate the BBB, it reduces the peripheral metabolism of levodopa (Nyholm *et al.*, 2013). Thus, the plasma levels of levodopa increases, the plasma half-life is longer, and more levodopa is available to enter the brain. The combination of levodopa and a peripheral dopa decarboxylase inhibitor may reduce the daily requirements of levodopa by 75%. This, as previously mentioned, is associated with a reduction in the side effects of high doses of levodopa (Nyholm *et al.*, 2013, Aminoff, 2009).

2.3.3 Catechol-O-methyltransferase inhibitors

When dopa-decarboxylase is inhibited, other pathways for the metabolism of levodopa (**16**) are activated, especially metabolism by catechol-O-methyltransferase (COMT). This increases the plasma levels of 3-O-methyldopa (3-OMD). Poor therapeutic response to levodopa is associated with elevated levels of 3-OMD, possibly because 3-OMD competes with levodopa for the active

carrier mechanism that mediates its transport across the intestinal mucosa and BBB (Nyholm *et al.*, 2013, Aminoff, 2009,).

Selective COMT inhibitors, namely entacapone (**19**) and tolcapone (**20**) (figure 2.9), prolong the action of levodopa (**16**) by decreasing its peripheral metabolism. COMT inhibitors are used together with levodopa in the late phases of Parkinson's disease, especially when motor fluctuations are problematic. When a COMT inhibitor is added, the daily dose of levodopa should be reduced since over dosage may lead to dyskinesia (Lew *et al.*, 2011, Aminoff, 2009).

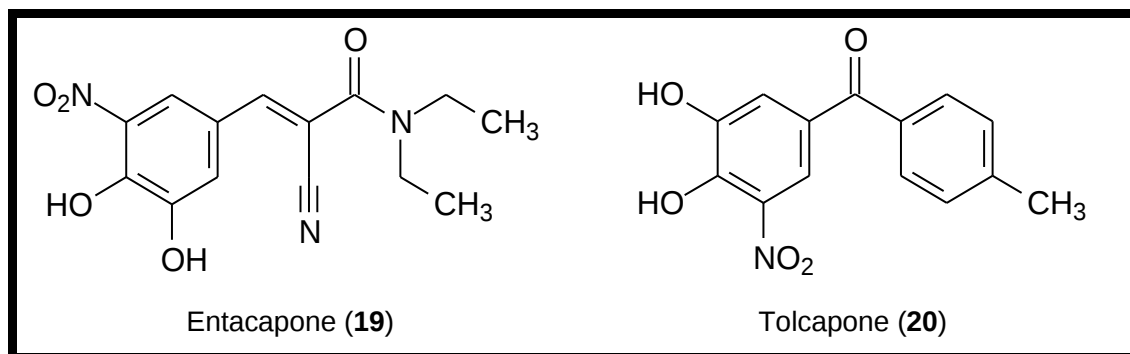


Figure 2.9: The structures of entacapone and tolcapone.

Entacapone (**19**) is preferred over tolcapone (**20**), because entacapone's effects and side effects are only peripheral, while tolcapone's effects are both central and peripheral (Aminoff, 2009, Nissinen, 1992).

2.3.4 Dopamine agonists

These drugs are direct agonists of striatal DA receptors, and a good alternative to levodopa treatment (Santiago & Olivier, 2010). This approach offers several potential advantages. Enzymatic conversion of these drugs is not required for activity and they do not depend on the functional capacities of the nigrostriatal neurons (Brunton *et al.*, 2011, Deleu, *et al.*, 2002). The DA receptor agonists have longer durations of action than levodopa. They are often used in the management of dose-related fluctuations of the motor state, as well as to prevent motor complications. It has been suggested that DA receptor agonists reduces the endogenous release of DA as well as the need for exogenous levodopa and thereby reduces free radical formation (Brunton *et al.*, 2011, Iida *et al.*, 1999, Zou *et al.*, 1999, Lange *et al.*, 1994).

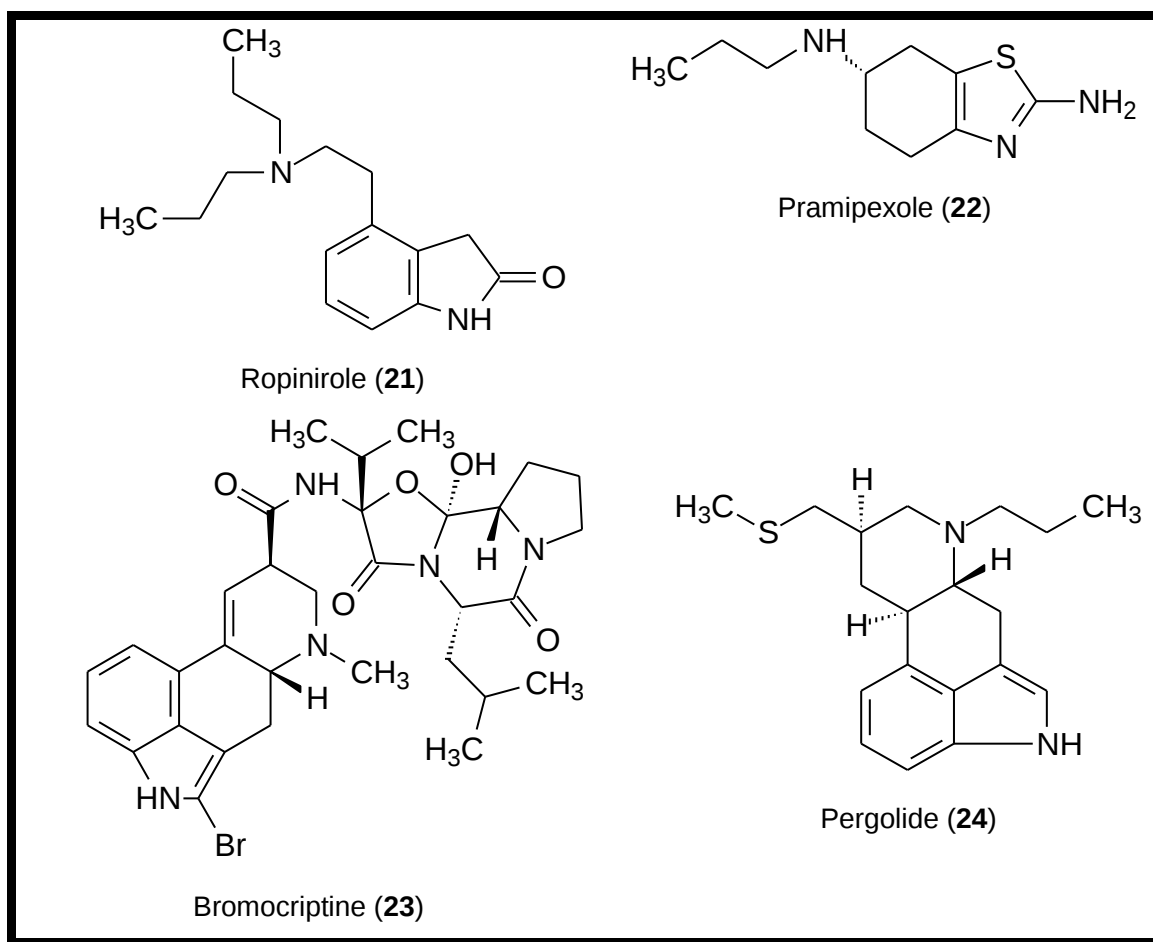


Figure 2.10: The structures of ropinirole, pramipexole, bromocriptine, and pergolide.

The two DA receptor agonists most commonly used in the treatment of Parkinson's disease are ropinirole (**21**) and pramipexole (**22**) (figure 2.10). These agents are better tolerated and have mostly replaced the older agents, such as bromocriptine (**23**) and pergolide (**24**). Ropinirole and pramipexole have activity at D₂ receptors and little or no activity at D₁ receptors, while pramipexole also has affinity for D₃ receptors. Having similar therapeutic activities, these drugs can both relieve the clinical symptoms of Parkinson's disease and is thus used as monotherapy for mild Parkinson's disease, as well as in advanced Parkinson's disease (Santiago *et al.*, 2010). Pramipexole also has possible neuroprotective properties since it scavenges hydrogen peroxide and enhances neurotrophic activity in mesencephalic dopaminergic cell cultures (Aminoff, 2009).

Side effects of DA agonists include drowsiness, narcolepsy, hallucinations, confusion, postural hypotension and cardiac valvular fibrosis, cardiovascular effects and dyskinesia (Santiago *et al.*, 2010, Aminoff, 2009). In 2007, pergolide (**24**) was withdrawn from the U.S. market because its use was associated with the development and higher incidence of cardiac valve fibrosis (Aminoff, 2009).

2.3.5 Apomorphine

Apomorphine (**25**) (figure 2.11) is a dopaminergic agonist, having high affinity for D₄ receptors, moderate affinities for D₂, D₃, D₅ and adrenergic α_{1D} , α_{2B} , and α_{2C} receptors, and a low affinity for D₁ receptors.

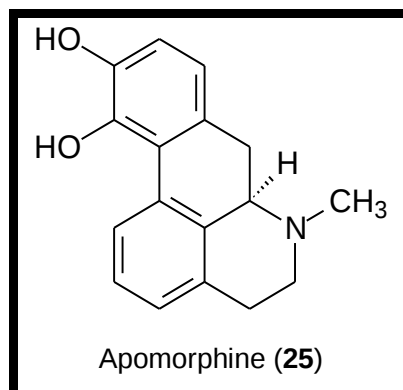


Figure 2.11: The structure of apomorphine.

In Europe, apomorphine is approved as "rescue therapy" for the acute intermittent treatment of "off" episodes in patients with a fluctuating response to dopaminergic therapy. The use of apomorphine (**25**) results in a levodopa-sparing effect (Brunton *et al.*, 2011, Deleu *et al.*, 2004)

2.3.6 Amantadine

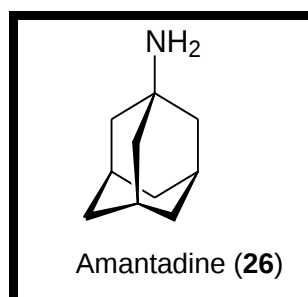


Figure 2.12: The structure of amantadine.

Amantadine (**26**) (figure 2.12), is an antiviral agent, which also possesses antiparkinsonian activity (Deleu *et al.*, 2002). Amantadine alters DA release in the striatum and has anticholinergic properties. Amantadine also blocks NMDA glutamate receptors.

Amantadine's pharmacological effects in Parkinson's disease are modest and it is therefore used in mild Parkinson's disease as initial therapy. It can be useful as an adjunct in patients treated with levodopa, as is thought to reduce dose related fluctuations and dyskinesia. Amantadine's antidyskinetic properties may be attributed to its action on NMDA receptors (Brunton *et al.*, 2011, Stoof, 1992).

2.3.7 Anticholinergic drugs

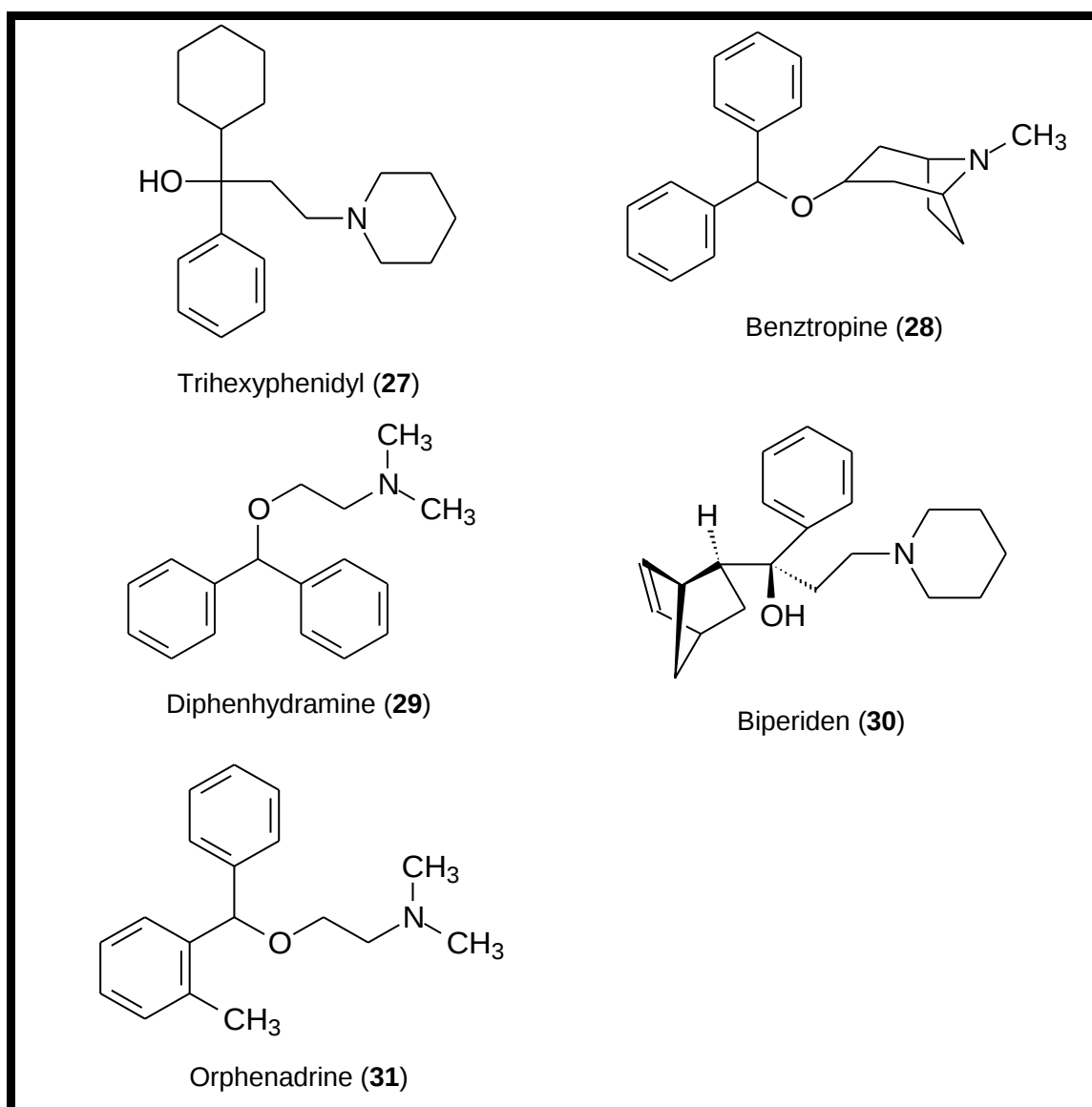


Figure 2.13: The structures of trihexyphenidyl, benztropine, diphenhydramine, biperiden, and orphenadrine.

Muscarinic acetylcholine receptor antagonists were widely used for the treatment of Parkinson's disease before levodopa was discovered. The therapeutic actions are not completely understood (Brunton *et al.*, 2011). It has been indicated that, except for the blocking of the muscarinic receptors in the striatum, some of these compounds also inhibit the presynaptic carrier-mediated DA transport mechanism (Krueger, 1990).

Some of the compounds such as biperiden, trihexyphenidyl and orphenadrine have shown antagonistic activity at the NMDA, resulting in the reversal of akinesia and the potentiation of levodopa effectivity in animal models of Parkinson's disease (Deleu *et al.*, 2002, McDonough & Shih, 1995). These drugs have modest antiparkinsonian activity and are used in the treatment of

early Parkinson's disease or as an adjunct to dopaminergic therapy such as levodopa (Brunton *et al.*, 2011, Brown & Laiken, 2011, Deleu *et al.*, 2002).

According to Brunton and co-workers (2011) the anticholinergic drugs (figure 2.13) most often used for Parkinson's disease treatment includes trihexyphenidyl (**27**), benztropine (**28**) and diphenhydramine (**29**). Biperiden (**30**) and orphenadrine (**31**) are also included as Parkinson's disease treatment options (Brunton *et al.*, 2011).

Benztropine (**28**) and trihexyphenidyl (**27**) are used as an adjunctive treatment for Parkinson's disease to treat drug-induced extra pyramidal symptoms. Benztropine may also inhibit the reuptake and storage of DA, thereby prolonging DA's action (Brunton *et al.*, 2011, Deleu *et al.*, 2002).

Biperiden (**30**) possesses both central and peripheral anticholinergic activity, which is used in the management of drug-induced acute dystonic reactions (Brown & Laiken, 2011). Orphenadrine (**31**) is an indirect skeletal muscle relaxant used to treat muscle spasm associated with acute painful musculoskeletal conditions. Its therapeutic properties may be attributed to its central atropine-like effects. Orphenadrine also has euphorigenic and analgesic properties (Brunton *et al.*, 2011). According to Deleu *et al.*, (2002), orphenadrine reduces tremor in Parkinson's disease and has almost no sedative effects.

2.3.8 Monoamine oxidase inhibitors

As mentioned previously, there are two isoforms of MAO enzymes (Collins *et al.*, 1970). The selective inhibition of the MAO-B isoform reduces the symptoms of Parkinson's disease, and may even have neuroprotective properties. The focus of current research is thus to develop reversible MAO-B inhibitors for the treatment of Parkinson' disease (Youdim & Bakhle, 2006, Youdim *et al.*, 2006). As this enzyme and its inhibitors are of particular importance to this study, the MAO enzyme and MAO-A and MAO-B inhibitors will be discussed in greater detail in the following sections.

2.4 MONOAMINE OXIDASE

2.4.1 Introduction

MAO is an enzyme responsible for the metabolism of amines such as DA, adrenaline and serotonin. As inhibition of the MAO enzyme enhances DA levels, MAO inhibition relieves the symptoms of Parkinson's disease. Since several studies have shown that MAO inhibitors have neuroprotective properties, these agents may have additional advantages as antiparkinsonian agents.

2.4.2 General background

In 1928, Mary Hare-Bernheim described an enzyme which catalyzed the oxidative deamination of tyramine, and called it tyramine oxidase. A few years later Hugh Blaschko realized that tyramine oxidase, noradrenaline oxidase and aliphatic amine oxidase was the same enzyme, which was capable of metabolizing primary, secondary and tertiary amines. Zeller named this enzyme mitochondrial MAO (Youdim & Bakhle, 2006, Youdim & Buccafusco, 2005).

During experiments on patients undergoing tuberculosis treatment, it was noticed that the tuberculosis drug isoniazid was also an MAO inhibitor. Iproniazid, a related compound, was the first drug to be used successfully as an antidepressive drug. In 1960 iproniazid and other MAO inhibitors showed antidepressive potential, but they had serious side effects, one of which was the cheese reaction. These side effects led to the development of other antidepressant drugs, such as serotonin and noradrenaline uptake inhibitors, the tricyclic antidepressants, and the serotonin selective re-uptake inhibitors (Youdim & Bakhle, 2006).

In 1960 it was discovered that MAO exists in two isoforms, namely MAO-A and MAO-B. These isoforms have different pH optima and sensitivity to heat inactivation. They also differ in their substrate and inhibitor specificity. It was discovered that the MAO isoforms are distributed differently in the mammalian brain. For example MAO-B has greater activity in the basal ganglia (Collins *et al.*, 1970). By mapping the distribution of human brain MAO isoforms, and combining this with isoform selective inhibitors, the possibility of developing treatments for depression and other neuropsychiatric diseases, such as Parkinson's disease, based on specific MAO inhibition emerged (Youdim & Bakhle, 2006, Youdim *et al.*, 1972).

Although MAO-A and MAO-B are two separate enzymes, they share a 70% sequence similarity. Both enzymes also have the same flavin adenine dinucleotide (FAD) co-factor (Edmondson *et al.*, 2004). However, the MAO isoforms also differ in some aspects. For example, they are encoded by separate genes and there are also differences in their core promoter regions (Youdim *et al.*, 2006, Wong *et al.*, 2002, Shih *et al.*, 1999, Zhu *et al.*, 1994). The two enzymes have separate but overlapping biological functions, and the ultimate goal is to develop inhibitors with a high level of specificity, and little or no side effects (Edmondson *et al.*, 2004).

The realization, in 1970, that irreversible selective and non-selective MAO-A inhibitors would always cause the cheese reaction, shifted the focus to developing reversible MAO-A and MAO-B inhibitors. Selective inhibitors of MAO-B possess anti-parkinsonian activity, while reversible inhibitors of MAO-A have anti-depressive activity, without the serious side effects of previously developed MAO-A inhibitors. MAO inhibitors further have neuroprotective potential and thus they may slow down, halt and even reverse neurodegeneration in Parkinson's disease (Youdim & Bakhle, 2006, Youdim *et al.*, 2006).

2.4.3 Tissue distribution

MAO-A and MAO-B are associated with the mitochondrial outer membrane. During development MAO-A appears before MAO-B, but the level of MAO-B increases greatly after birth (Nicotra *et al.*, 2004). MAO is present in most mammalian tissues, but the isoform proportions vary from tissue to tissue (Youdim *et al.*, 2006, Westlund *et al.*, 1985).

MAO-B is the main form in the basal ganglia, and is thus of particular importance in movement disorders, such as Parkinson's disease (Mink, 2003). Immunohistochemical studies have also shown that MAO-B is the main form in serotonergic neurons and astrocytes, while MAO-A is mainly contained in the catecholaminergic neurons (Youdim *et al.*, 2006, Westlund *et al.*, 1985).

Immunohistochemical mapping revealed the MAO distribution in human tissues (Ramonet *et al.*, 2003). This distribution is summarized in table 2.1.

Table 2.1: The distribution of MAO in human tissues (Ramonet *et al.*, 2003).

Isoform	Tissue
Both MAO-A and MAO-B	Cardiomyocytes, hepatocytes, enterocytes, smooth muscle cells (duodenum, lung, blood vessels, and spleen), broblasts (blood vessels), proximal and distal renal tubules, pneumocytes, entroacinar cells (pancreas), and cortical secretory cells (adrenal gland)
Only MAO-A	Beta cells (islets of Langerhans), follicular and parafollicular cells (pancreas), podocytes (kidney). Endothelial cells (lymphatic vessels), collecting ducts (kidney), as well as acinar cells (pancreas)
Only MAO-B	Alpha cells (islets of Langerhans)

2.4.4 Biological function of MAO

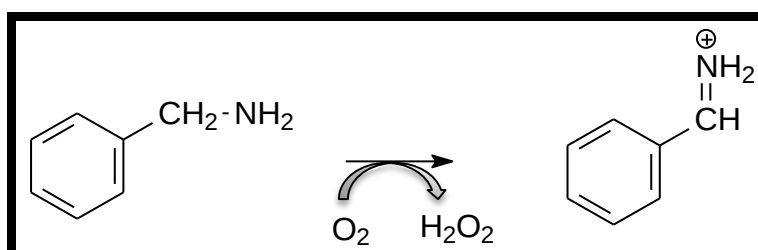


Figure 2.14: The oxidation reaction (Edmondson *et al.*, 2004).

MAO catalyzes the oxidation of amines to the aldehydes and amines (figure 2.14). It is therefore assumed that in the peripheral- and central nervous systems, intraneuronal MAO-A and MAO-B

protect neurons from exogenous amines, terminate the actions of neurotransmitters and regulate the contents of intracellular amine stores (Youdim *et al.*, 2006).

2.4.4.1 Substrate specificities

Although both isoenzymes are present in most mammalian tissues (Youdim *et al.*, 2006, Shih *et al.*, 1999), they have different sensitivities to the acetylenic inhibitors clorgyline and selegiline (*L*-deprenyl), as well as a difference in their substrate specificities.

Table 2.2: The substrate specificities of MAO in the cerebral cortex.

Substrate	MAO-A			MAO-B		
	K_m (μ M)	V_{max} (pmol min ⁻¹ mg protein ⁻¹)	V_{max}/K_m (μ mol M ⁻¹ min ⁻¹ mg protein ⁻¹)	K_m (μ M)	V_{max} (pmol min ⁻¹ mg protein ⁻¹)	V_{max}/K_m (μ mol M ⁻¹ min ⁻¹ mg protein ⁻¹)
Adrenaline	125	379	3.03	266	465	1.75
Dopamine	212	680	3.21	229	702	3.07
5-Hydroxy-tryptamine (Serotonin)	137	228	1.66	1093	6.6	0.006
Noradrenaline	284	561	1.98	238	321	1.35
2-Phenyl-ethylamine	140	20	0.14	4	309	77.3
Tryptamine	35	58	1.66	35	108	2.84
Tyramine	127	182	1.43	107	343	3.21
K_m , Michaelis-Menten constant; V_{max} , maximum rate.						

MAO-A is inhibited by clorgyline, and catalyzes the oxidation of noradrenaline and serotonin, while MAO-B is inhibited by selegiline, and catalyzes the metabolism of benzylamine and 2-phenylethylamine. Both forms of the isoenzyme oxidize DA, noradrenaline, adrenaline, tryptamine and tyramine in most species. Table 2.2 summarizes the substrate specificities of the MAOs (Youdim *et al.*, 2006).

2.4.5 *In vitro* measurements of MAO activity

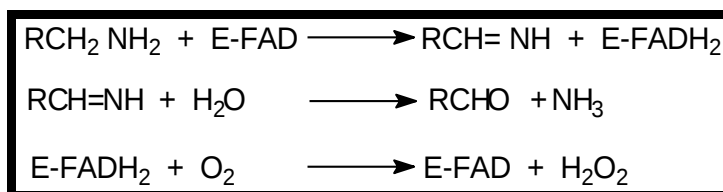


Figure 2.15: MAO-catalysed reactions.

MAO-A and MAO-B are flavoproteins of the outer mitochondrial membrane. In the central nervous system, they catalyze the oxidative deamination of biogenic amines (Nicotra & Parvez, 1999).

As shown in figure 2.15 the first step for a MAO substrate, is the deamination into the corresponding imine (RCH=NH). Secondly, the latter is hydrolyzed producing an aldehyde (RCHO) and ammonia (NH_3). The aldehyde is further metabolized, forming the corresponding acid or alcohol. The cofactor (E-FADH_2) is reoxidized by molecular oxygen, producing hydrogen peroxide. MAO activity is thus measured by monitoring the disappearance of the amine substrate, the consumption of oxygen, the formation of the aldehyde, or by monitoring the ammonia and hydrogen peroxide (Nicotra & Parvez, 1999).

Figure 2.15 is only applicable to primary monoamines, because secondary and tertiary monoamines do not form ammonia. It is also important to note that the metabolism of Parkinson-inducing MPTP does not result in the formation of ammonia or aldehydes (Nicotra & Parvez, 1999, Singer & Ramsay, 1995, Singer *et al.*, 1986).

There are different methods to estimate MAO activity; the reaction products or the disappearance of the reagent species can be measured. The methods may be continuous or discontinuous, including radiometric, fluorometric, luminometric, chromatographic, polarographic and spectrophotometric assays (Nicotra & Parvez, 1999).

2.4.6 The role of MAO in Parkinson's disease

2.4.6.1 MAO-A in depression

MAO-A inhibitors can be used to treat depression, as both noradrenaline and serotonin, the monoamines implicated in depressive illness, are substrates of MAO-A. Selective reversible inhibitors of MAO-A, such as moclobemide, have antidepressant activity, and also improves vigilance, psychomotor speed and long-term memory (Bonnet, 2003). In contrast to irreversible inhibitors, reversible MAO-A inhibitors do not cause the cheese reaction. A reversible MAO-A inhibitor, such as moclobemide, can also be used to treat therapy resistant depression. The antidepressant activity of MAO-A inhibitors can also be applied in Parkinson's disease patients, because 40-60% of these patients suffer from depression, as they exhibit reductions in

noradrenaline and serotonin concentrations in the locus coeruleus and raphe nucleus, respectively (Youdim *et al.*, 2006).

Care must be taken to not combine MAO inhibitors (especially MAO-A inhibitors) with uptake inhibitors, such as tricyclic antidepressants or serotonin-selective reuptake inhibitors, as these combinations will provoke the serotonin syndrome (Bonnet, 2003). It is important to note that the serotonin syndrome is not an idiopathic drug reaction, but the result of an overabundance of serotonergic agonism of the central nervous system receptors as well as the peripheral serotonergic receptors. Some of the symptoms of serotonin syndrome include tremor, diarrhea, delirium, neuromuscular rigidity and in life-threatening cases hyperthermia (Boyer & Shannon, 2005).

2.4.6.2 The role of MAO-B in Parkinson's disease

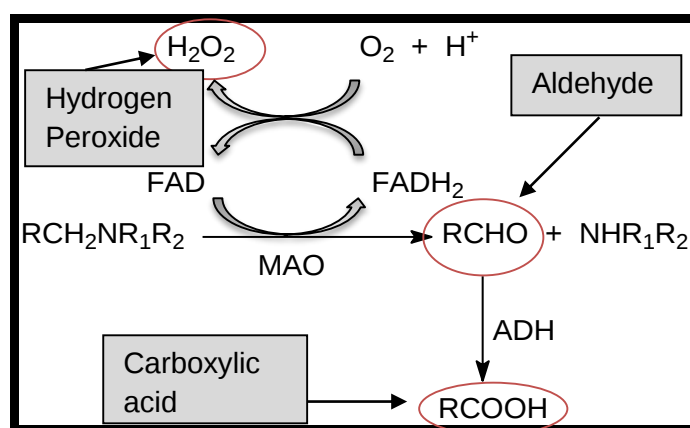


Figure 2.16: The reaction pathway of monoamine oxidative deamination by MAO.

As previously mentioned, MAO is responsible for the oxidative deamination of primary, secondary and tertiary amines to their corresponding aldehydes and amines (figure 2.16). The aldehyde is metabolized by aldehyde dehydrogenase to acidic metabolites. These are 5-hydroxyindole acetic acid (5-HIAA) from serotonin and dihydroxy-phenyl acetic acid (DOPAC) from DA. It is also important to note that during the FAD- $FADH_2$ cycle hydrogen peroxide is generated, which needs to be inactivated by catalase or GSH peroxidase. Gene profiling of post-mortem samples of the SNpc from Parkinson's disease patients show that they have a deficiency in the aldehyde dehydrogenase enzyme, thus resulting in the build-up of neurotoxic aldehydes (Youdim & Bakhle, 2006, Grünblatt *et al.*, 2004).

In Parkinson's disease, the sites of neuronal death in the brain are also the sites where iron accumulates (Mandel *et al.*, 2005, Zecca *et al.*, 2004). The link between MAO, iron and neuronal damage appears to be oxidative stress. Hydrogen peroxide is a by-product of MAO oxidation of monoamines (figure 2.16) and is inactivated mainly in the brain by GSH. In Parkinson's disease,

central GSH levels are low resulting in the accumulation of hydrogen peroxide, which then becomes available for the Fenton reaction (figure 2.17). In the Fenton reaction, Fe^{2+} generates an active free radical, the hydroxyl radical, from hydrogen peroxide. This radical depletes cellular antioxidants, damage lipids, proteins and DNA. Thus, MAO inhibitors decrease the formation of H_2O_2 , thereby reducing the formation of hydroxyl radicals and oxidative stress. Thus MAO inhibitors may prevent the neuronal damage caused by MAO-derived oxidative stress and hydroxyl radicals (Youdim & Bakhle, 2006).

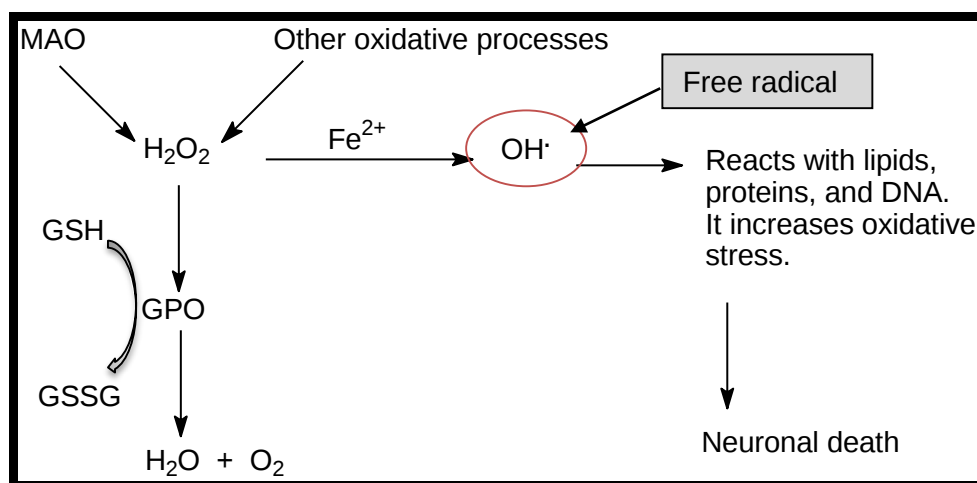


Figure 2.17: The mechanism of neurotoxicity induced by iron and hydrogen peroxide, via the Fenton reaction.

With aging, brain MAO and iron levels increase, thus both components of the Fenton reaction are elevated. This potentiates the production of hydroxyl radical formation. This observation provides a further rationale for the inhibition of MAO in the aged Parkinsonian brain (Youdim & Bakhle, 2006, Mandel *et al.*, 2005).

2.4.7 Known inhibitors of MAO-B

2.4.7.1 Irreversible inhibitors of MAO-B

The two MAO-B inhibitors that are approved for use are selegiline and rasagiline; these drugs can be used as monotherapy and adjunctive therapy to relieve parkinsonian symptoms. Selegiline and rasagiline also may have potential disease-modifying effects (Fernandez & Chen, 2007).

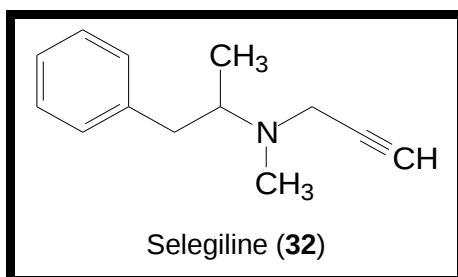
Selegiline (L-Deprenyl/R-Deprenyl)

Figure 2.18: The structure of selegiline.

Patients treated with selegiline (**32**) (figure 2.18) have shown a delay in the emergence of disability and in the development of symptoms and signs associated with Parkinson's disease (Olanow *et al.*, 1995). Selegiline also showed neuroprotective potential and reduces oxidative stress (*in vitro*) associated with MAO DA metabolism and glutamate-induced toxicity (Mytilineou *et al.*, 1997, Cohen & Spina, 1989).

Disadvantages of selegiline include the production of neurotoxic amphetamine-derived metabolites, such as L-amphetamine and L-methamphetamine. The most frequent adverse effects related to selegiline use are dizziness, dyskinesia and while its use has also been associated with cardiovascular, psychiatric and some motor adverse effects (Fernandez & Chen, 2007, Pålhagen, *et al.*, 2006, Montastruc *et al.*, 2000, Churchyard *et al.*, 1997).

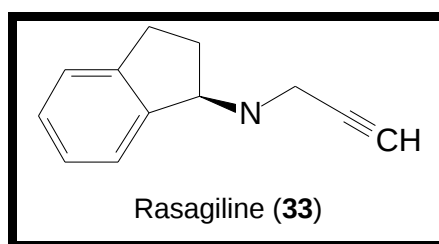
Rasagiline

Figure 2.19: The structure of rasagiline.

Rasagiline (**33**) (figure 2.19) is a second generation MAO-B inhibitor, and it is recommended for adjunctive therapy to reduce the "off" time associated with motor fluctuation in Parkinson's disease patients. Rasagiline also has neuroprotective potential in MPTP-induced parkinsonism since it inhibited degeneration of dopaminergic nigral cells of nonhuman primates and MPTP-treated mice (Chen & Swope, 2005, Kupsch *et al.*, 2001, Youdim, *et al.*, 1999).

Rasagiline also possesses antiapoptotic and antioxidant potential. Rasagiline is reported safer to use than selegiline, as it is not metabolized to yield toxic amphetamine metabolites. In general

rasagiline is well tolerated, and it is not associated with vasoreactive or psychiatric adverse effects as is selegiline (Fernandez & Chen, 2007).

Pargyline

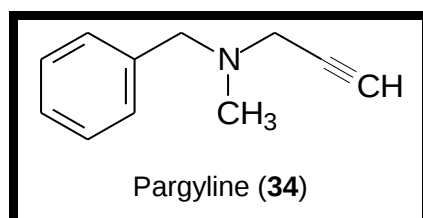


Figure 2.20: The structure of pargyline.

Pargyline (34) (figure 2.20) is a selective irreversible inhibitor of the MAO-B isoform (Yamada & Yasuharaz, 2004) and possesses neuroprotective activity in MPTP-treated mice (Kumagai *et al.*, 1999) as well as in 6-OHDA-treated rodents (Aristieta *et al.*, 2012). This drug may be used to reduce the symptoms of Parkinson's disease, although some side effects are expected as it is an irreversible inhibitor of the MAO-B isoform.

Ladostigil

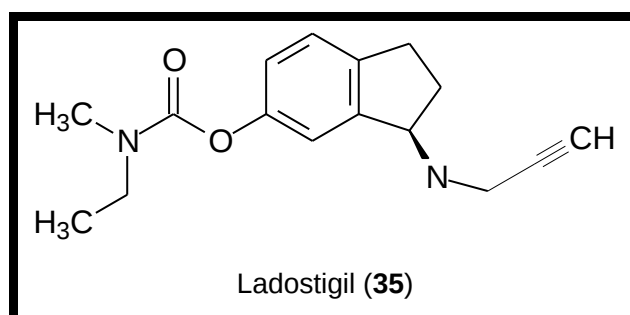


Figure 2.21: The structure of ladostigil.

Ladostigil (35) (figure 2.21) is currently in phase II clinical studies for the treatment of Parkinson's disease. While ladostigil is a cholinesterase inhibitor, it also inhibits MAO-A and MAO-B irreversibly. Studies have shown that MAO-A and MAO-B are inhibited in the brain by ladostigil, but not in the gut or liver, thus allowing tissue-selective irreversible inhibition in the brain (Mandel *et al.*, 2005, Sagi *et al.*, 2005). Ladostigil may have antidepressant activity because it increases noradrenalin, serotonin and DA in the hippocampus and striatum of rat and mouse animal models. Ladostigil also prevented striatal neurodegeneration and DA depletion induced by MPTP in a mouse model (Sagi *et al.*, 2005), and according to Mandel (2005), it also has anti-apoptotic properties (Youdim & Bakhle, 2006, Mandel *et al.*, 2005). This drug may be used to reduce the

symptoms of Parkinson's disease, although care should be taken not to consume tyramine containing foods while using the drug.

2.4.7.2 Reversible inhibitors of MAO-B

Lazabemide

Currently lazabemide (**36**) (figure 2.22) is the only reversible, selective MAO-B inhibitor on the market as treatment for Parkinson's disease (Youdim & Bakhle, 2006). It does not cause the cheese reaction, and should have less side effects than the irreversible and non-selective MAO inhibitors.

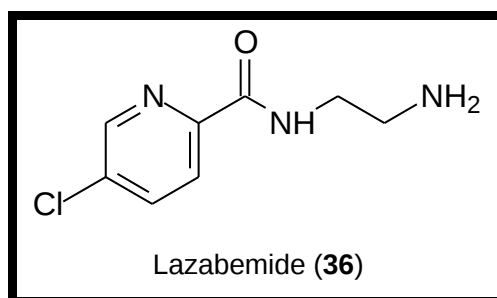


Figure 2.22: The structure of lazabemide.

Lazabemide can be used to treat therapy resistant depression when combined with a reversible MAO-A inhibitor. Lazabemide also prevents neurodegeneration and DA depletion induced by the neurotoxin, MPTP, in a mouse model of Parkinson's disease (Youdim & Bakhle, 2006).

Safinamide.

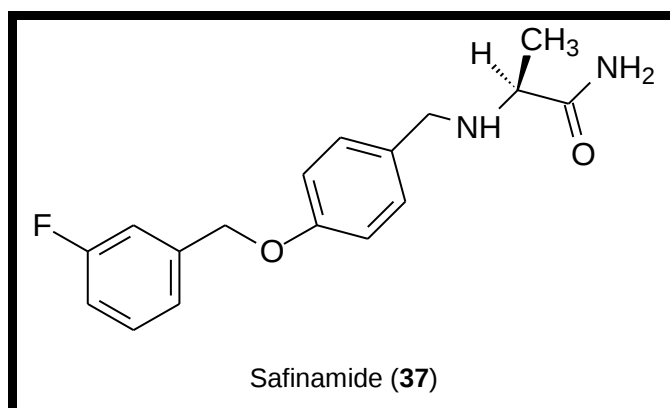


Figure 2.23: The structure of safinamide.

Safinamide (**37**) (figure 2.23) is currently in phase III development as an anti-parkinson drug. Safinamide it is a α -aminoamide derivative that shows potential as a selective, reversible MAO-B inhibitor and as well as a DA reuptake inhibitor.

Studies have shown that safinamide treatment improved motor scores in Parkinson's disease patients, and is also used in combination with DA replacement therapy to improved patients cognitive functions (Stocchi *et al.*, 2012, Binda *et al.*, 2007, Fernandez & Chen, 2007).

2.4.8 Known inhibitors of MAO-A

2.4.8.1 Irreversible inhibitors of MAO-A.

Clorgyline

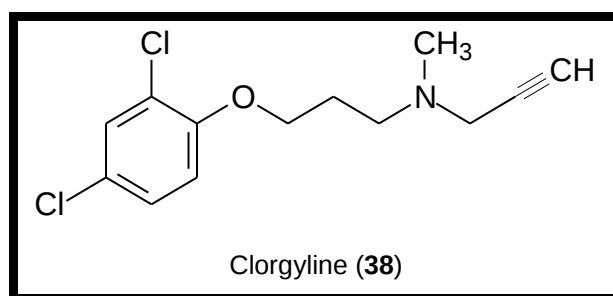


Figure 2.24: The structure of clorgyline.

Clorgyline (**38**) (figure 2.24) is an irreversible MAO-A selective inhibitor. It increases brain levels of noradrenaline and serotonin and as a result possesses antidepressant activity. Clorgyline, however, precipitates the cheese reaction, thus forcing its abandonment as an antidepressant (Youdim & Bakhle, 2006).

2.4.8.2 Irreversible inhibitors of both MAO-A and B.

Tranylcypromine and Phenelzine

Tranylcypromine (**39**) and phenelzine (**40**) are both irreversible MAO-A and MAO-B inhibitors (figure 2.25), which have antidepressant activity. Unfortunately, these drugs also cause the cheese reaction and is thus of limited clinical value (Youdim & Bakhle, 2006, Youdim *et al.*, 2006).

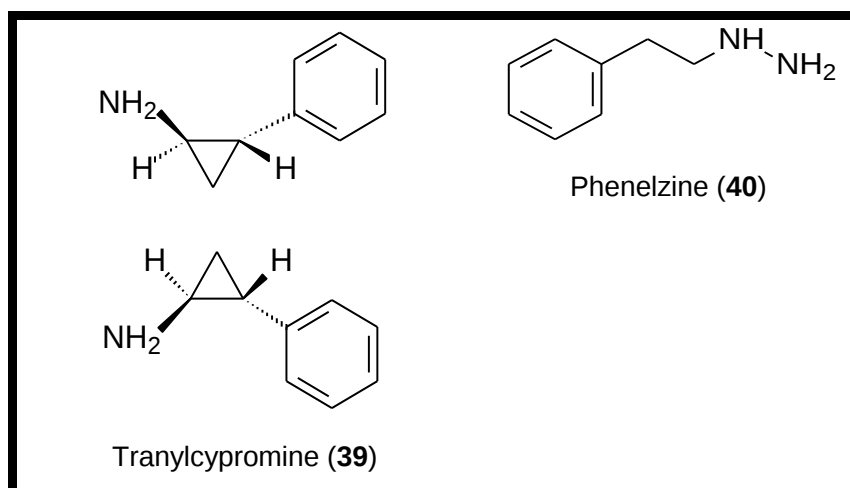


Figure 2.25: The structures of tranylcypromine and phenelzine.

Iproniazid

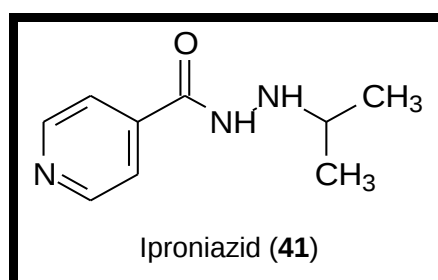


Figure 2.26: The structure of iproniazid.

Iproniazid (41) (figure 2.26) is an irreversible MAO-A and MAO-B inhibitor, and the first MAO inhibitor used successfully to treat depressive illness. Iproniazid, however, exhibits serious side effects such as the cheese reaction and liver toxicity. This led to the development of alternatives such as the non-hydrazine inhibitors, tranylcypromine and pargyline (Youdim & Bakhle, 2006, Youdim *et al.*, 2006).

2.4.8.3. Reversible inhibitors of MAO-A.

Moclobemide and Brofaromine

Both moclobemide and brofaromine are selective reversible MAO-A inhibitors (figure 2.27) with antidepressant activity. These drugs seem to be better tolerated and have less side-effects than the irreversible MAO-A inhibitors and do not cause the cheese reaction. These inhibitors also increase DA release, although it does not alter the steady state levels of DA in the brain. Studies have shown that, when moclobemide (42) was given in combination with levodopa, it had a mild symptomatic effect on motor functions (Youdim & Weinstock, 2004).

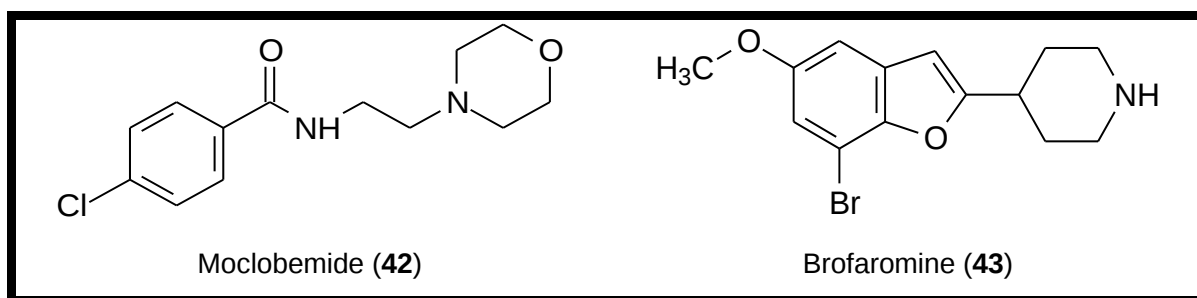


Figure 2.27: The structures of moclobemide and brofaromine.

Moclobemide also improves vigilance, psychomotor speed and long term memory (Bonnet, 2003). It has also been reported that moclobemide improves memory and choice reaction time in elderly patients. Moclobemide is used in therapy resistant depression. Moclobemide can also be used to treat depression in Parkinson's disease (Youdim & Bakhle, 2006, Youdim *et al.*, 2006).

2.4.9 Mechanism of action of MAO-B

2.4.9.1 The FAD cofactor and flavin adducts

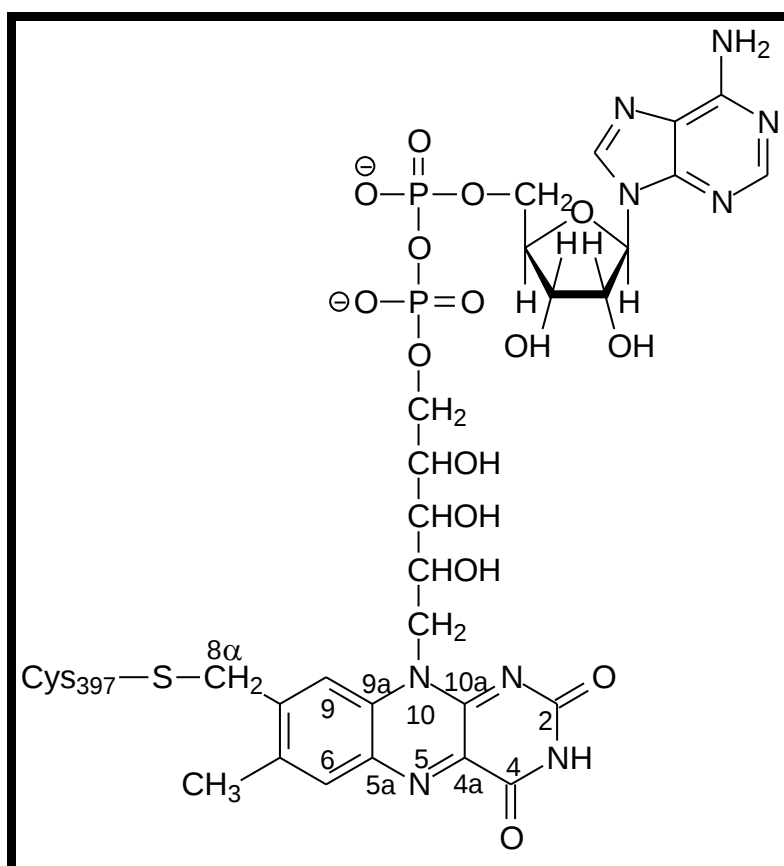


Figure 2.28: The structure of covalently bound FAD in MAO (Edmondson *et al.*, 2004).

MAO-A and MAO-B are mitochondrial outer membrane-bound flavoproteins and both contain covalently bound FAD as their only redox cofactor, which is important for catalysis. The site of covalent attachment of the flavin to the enzyme, is via a thioether linkage between the 8 α -methylene of the isoalloxazine ring and a cysteinyl residue (figure 2.28) (Kearney *et al.*, 1971). In MAO-B, the cysteine linkage is to Cys 397, but in MAO-A the linkage is to Cys 406 (Bach *et al.*, 1988), though in both enzymes the covalent linkage site is toward the C-terminal molecule portion (Edmondson *et al.*, 2004).

2.4.9.2 The catalytic cycle of MAO-B

MAO-A and MAO-B both catalyze the reaction where primary, secondary and tertiary amines are oxidatively deaminated to imines (figure 2.29) (Milczek *et al.*, 2008, Edmondson *et al.*, 2004). The two compulsory factors needed for this reaction are the FAD covalently bound and oxygen (Edmondson *et al.*, 2004).

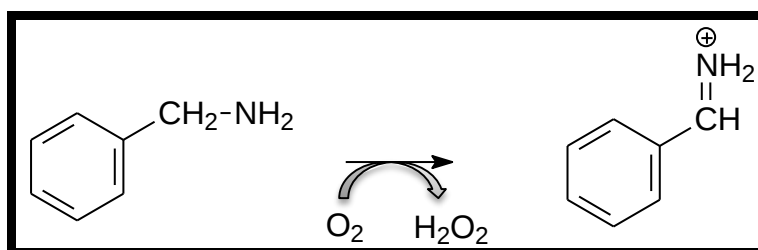


Figure 2.29: The oxidation reaction catalyzed by the MAOs (Edmondson *et al.*, 2004).

Oxygen is important because, during the catalytic reaction the amines are oxidized to form the imine products, and the FAD co-factor is reduced (Edmondson *et al.*, 2004). Oxygen reoxidizes the FAD co-factor and another amine molecule may thus be metabolized.

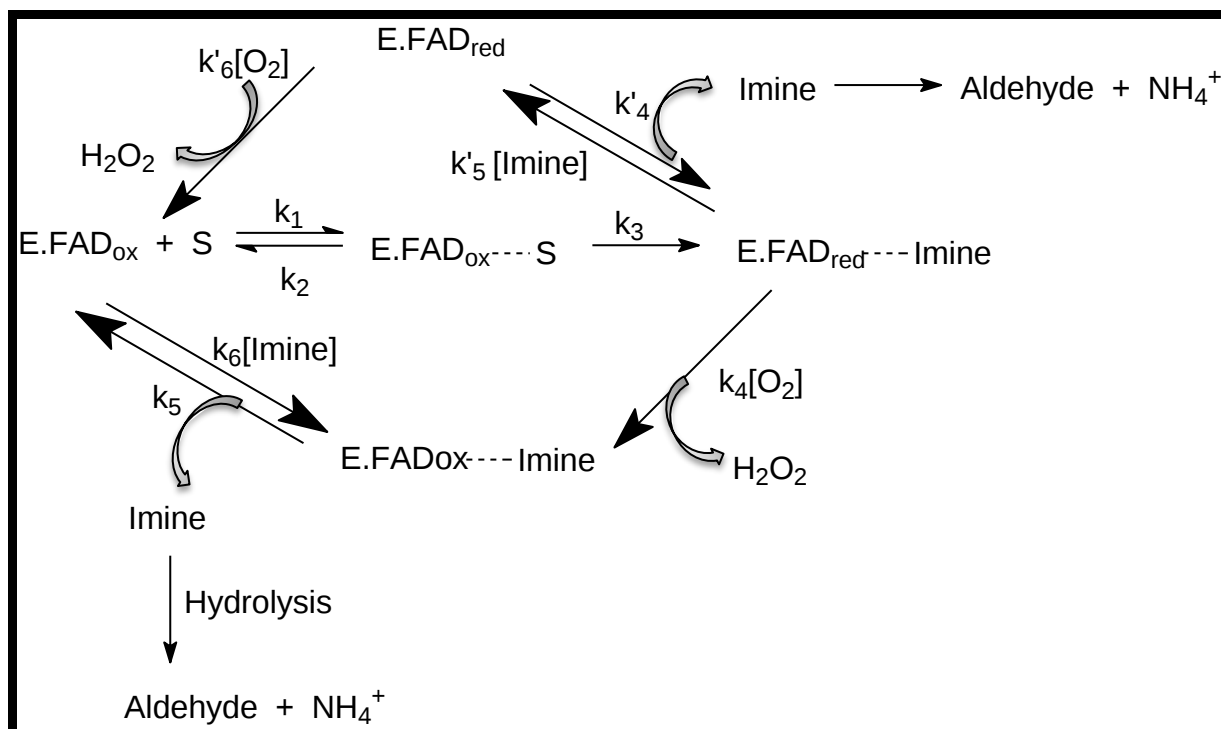


Figure 2.30: The reaction pathway for MAO catalysis (Edmondson *et al.*, 2004, Binda *et al.*, 2002).

The MAO-B catalytic pathway is dependent on the nature of the substrate (Ramsay, 1991). For benzylamine (**44**) (figure 2.31) and its analogs, the lower branch of the catalytic pathway in figure 2.30 is followed. On the other hand, when the substrate is phenethylamine (**45**), the imine product dissociates from the reduced enzyme. The free reduced enzyme reacts with O_2 (the rate limiting step) as shown in the top loop of the pathway in figure 2.30. In MAO-A the substrate catalysis occurs via the lower pathway of figure 2.30 (Ramsay, 1991).

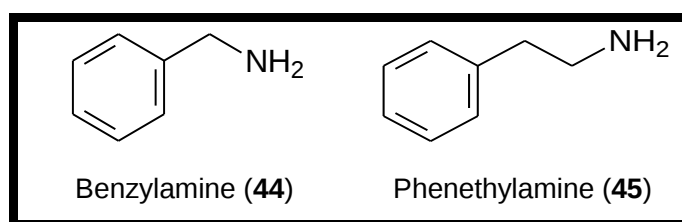


Figure 2.31: Structures of benzylamine and phenethylamine.

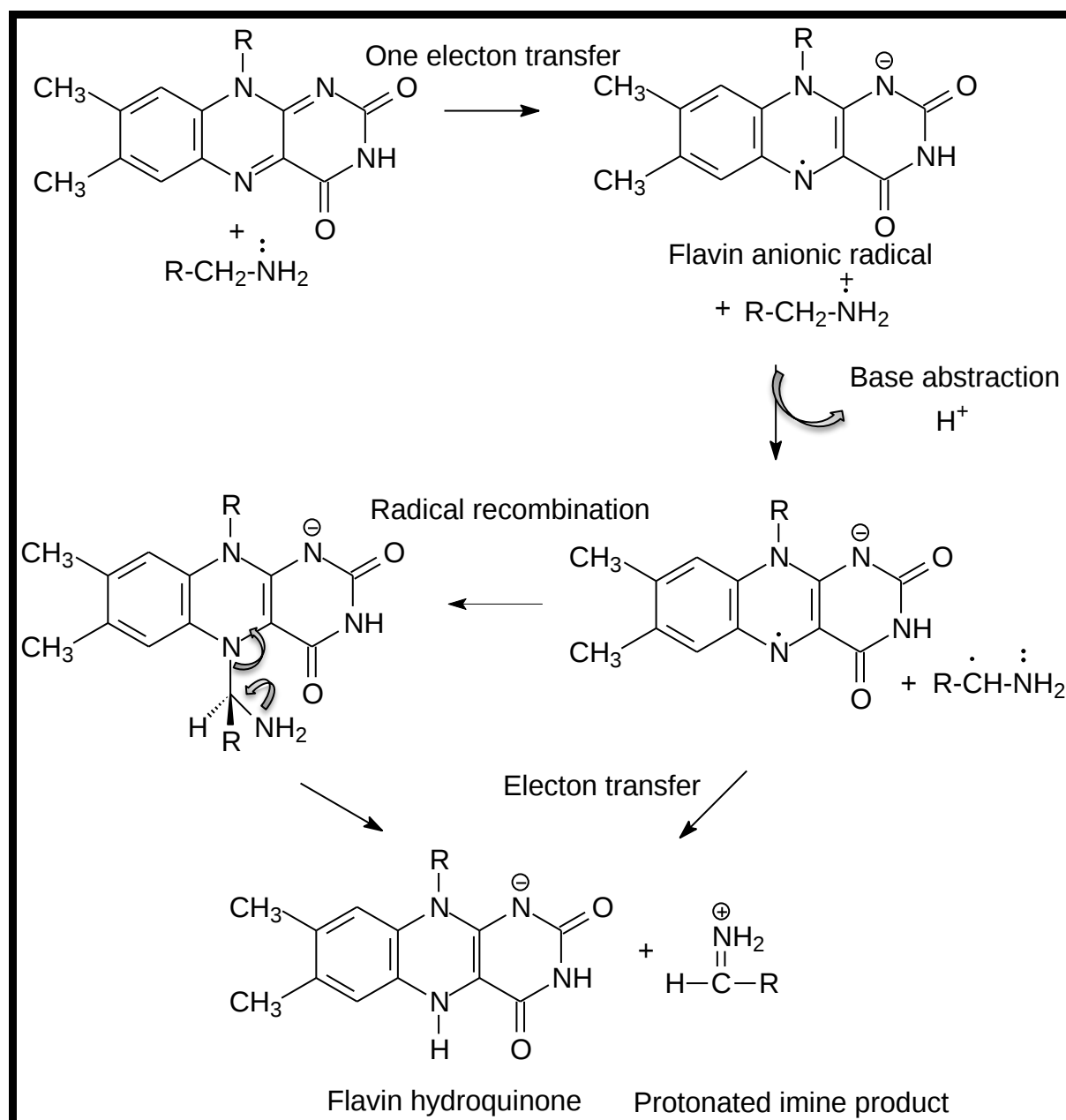


Figure 2.32: The SET mechanism for MAO catalysis (Edmondson *et al.*, 2004).

There is a major difference in the steady state catalytic properties between MAO-A and MAO-B when their respective K_m (O_2) values are compared. MAO-A has a K_m (O_2) of approximately 6 μM , whereas MAO-B has a K_m (O_2) of approximately 250 μM , thus MAO-A is operating at maximal velocity, while MAO-B is operating at half-maximal velocity, when the concentration of the amine substrate is saturated (Edmondson *et al.*, 2004, Yu *et al.*, 1986, Husain *et al.*, 1982, Silverman & Hoffman, 1980).

According to Edmondson *et al.* (2004), the exact mechanism for electron transfer from the amine to the flavin is unclear. There are at least two principal theories to explain the mechanism of substrate oxidation. The first is the single electron transfer (SET) mechanism and the second the polar-nucleophilic mechanism.

It is assumed that MAO-A and MAO-B operate via similar mechanisms regarding the C-H bond cleavage and flavin reduction. The SET mechanism is shown in figure 2.32 (Silverman, 1995, Jonson *et al.*, 1994). The first step is the one-electron oxidation of the amine nitrogen lone pair to form an aminium cation radical as well as a flavin radical. The aminium radical formation results in the reduction of the pKa of the α -C-H and this allows H^+ abstraction by an active site base in the catalytic site. However, detailed analysis shows that the SET mechanism for the oxidation of amines is kinetically and thermodynamically unlikely (Edmondson *et al.*, 2004, Miller *et al.*, 1995).

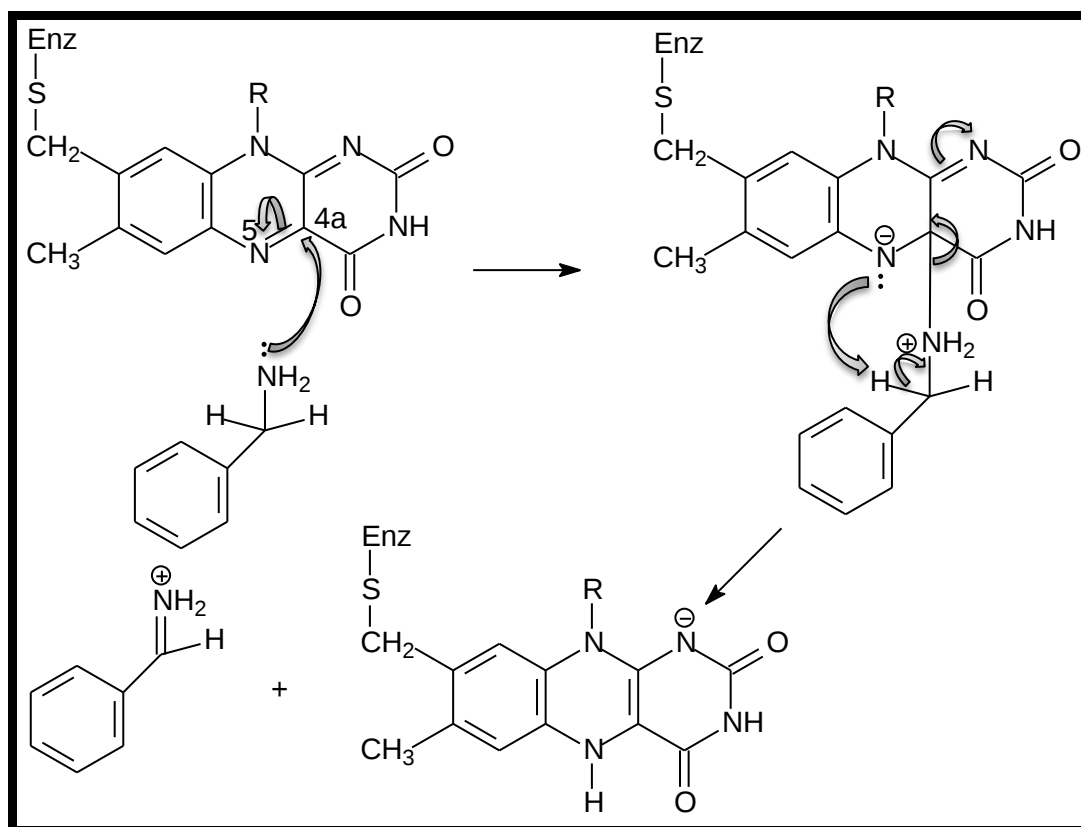


Figure 2.33: The polar nucleophilic mechanism proposed for MAO catalysis (Edmondson *et al.*, 2004).

The second theory is the polar nucleophilic mechanism, which is shown in figure 2.33 (Miller & Edmondson, 1999). In this mechanism, the deprotonated amine attacks the flavin nucleophilically at the C(4a) position. When the adduct is formed, the N(5) flavin position becomes a strong base. This would exhibit sufficient basicity to abstract the α -pro-R-H from the substrate (Edmondson *et al.*, 2004, Miller & Edmondson, 1999).

This mechanism is also consistent with current structural data of MAO-B. The flavin has a non-planar bent conformation, which may facilitate C(4a) adduct formation (Edmondson *et al.*, 2004).

2.4.10 Three-dimensional structure of MAO-B

2.4.10.1 The crystal structure

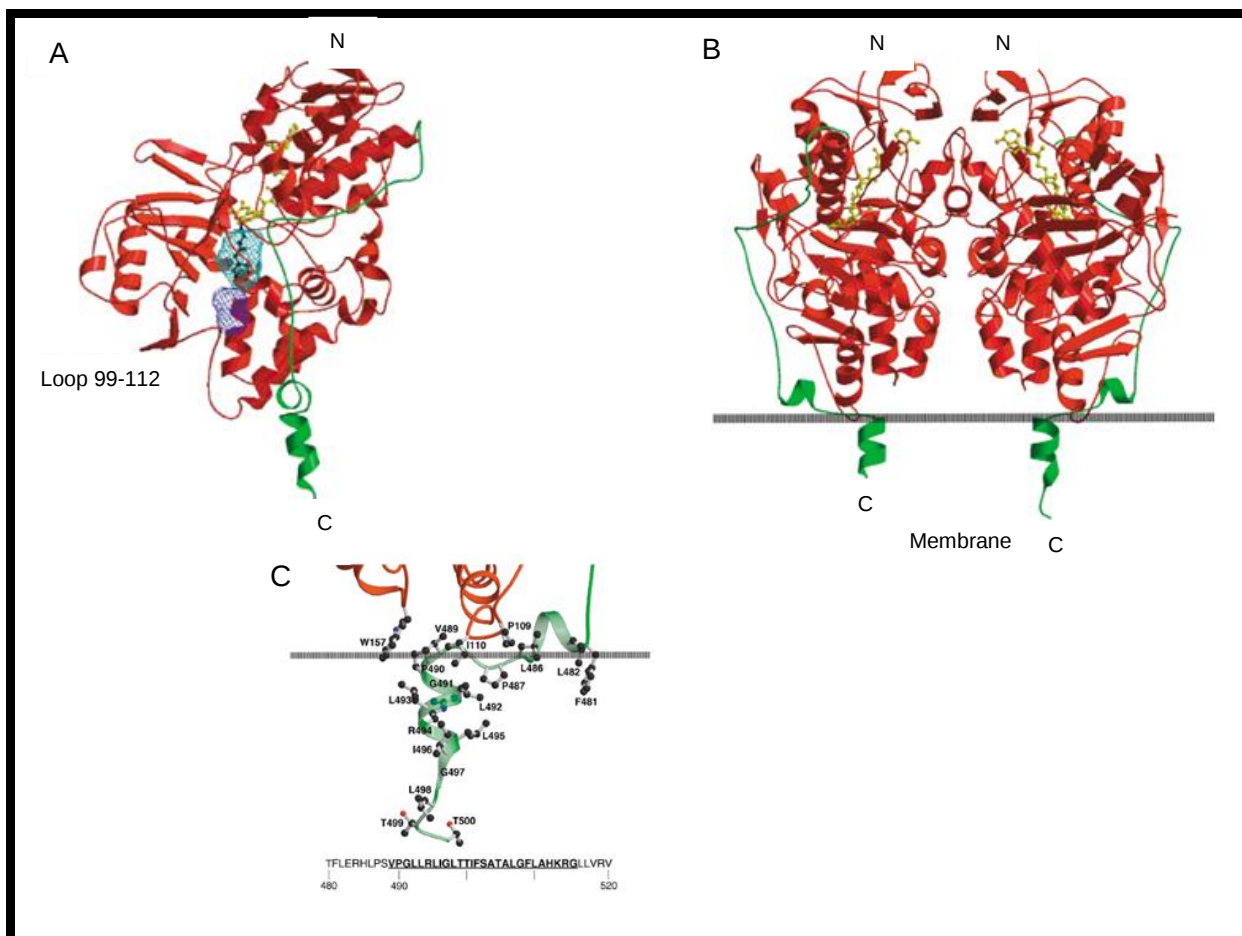


Figure 2.34: The structure of MAO-B. (A) The MAO-B monomer: Adjacent to the active site cavity (coloured in cyan) is the entrance cavity (coloured in blue). Residues 4-460 are coloured red, the C-terminal tail, residues 461-500, is coloured green. The FAD is coloured in yellow. The pargyline inhibitor is shown in black. (B) Shows a ribbon diagram of the MAO-B dimer, indicating the two monomers. (C) Shows a close up view of the membrane binding region in the human MAO-B structure. The C-terminal tail is in green (Binda *et al.*, 2002).

The crystal structures of MAO are used to understand the binding of inhibitors to the active sites of these enzymes. Modeling software can be used to visualize inhibitors in the active site of the enzyme and is used to predict how an inhibitor interact with the active site of the enzyme (Ramsay, 2013). Thus, different compounds can be evaluated in advance as potential MAO inhibitors, making it easier to select the most promising compounds for further research.

The crystal structure of MAO-B has shown that the enzyme crystallizes as a dimer. Each monomeric unit contains a membrane binding domain, a flavin-binding domain, and a substrate binding domain as shown in figure 2.34 (A) and (B) (Edmondson *et al.*, 2004).

2.4.10.2 The membrane binding region

According to Edmondson *et al.* (2004), the carboxyl terminal region, composed of residues 461 – 520, is possibly the membrane-binding domain via a 27-residue membrane α -helix (Rebrin *et al.*, 2001). Figure 2.34 (C) shows that the C-terminal helix has an apolar surface that would facilitate its insertion into the membrane. There are other predicted membrane interaction sites, including hydrophobic interactions from residues, such as Trp 157, the hydrophobic residue sequence 481-488 and probably Pro 109 and Ile 110 (Edmondson *et al.*, 2004, Rebrin *et al.*, 2001).

2.4.10.3 Active site structure

The MAO-B active site consists of two cavities which connect the opening to the active site and the site where the flavin binds (figure 2.34 A). The cavities are termed the “entrance cavity”, with a volume of 290 Å³, and a larger hydrophobic substrate cavity, with a volume of 420 Å³, resulting in a total volume of 710 Å³ (Binda *et al.*, 2002). The substrate cavity has a flat, elongated disc shape with its longer axis orientated perpendicular to the flavin ring. The amino acids lining the substrate cavity are mostly aromatic and aliphatic and provide a hydrophobic environment for competitive inhibitors or bound substrates. It is assumed that the entry point of the substrate occurs at the surface of the outer mitochondrial membrane. A flexible loop, which consists of residues 99-112, has to move for substrates to enter the entrance cavity (Binda *et al.*, 2003).

The negatively charged membrane surface electrostatically facilitates the attraction of the positively charged amine substrates to MAO-B. It has however been shown that the amine must be deprotonated for binding and catalysis to occur (Miller & Edmondson, 1999, McEwen *et al.*, 1969). The hMAO-B active site is narrower than the hMAO-A active site. The junction between the entrance and substrate cavities is narrow due to the presence of Ile 199 and Tyr 326 (Ramsay, 2013) (figure 2.36 B), which separates the two cavities. Crystallographic data of MAO-B in complex with reversible and irreversible inhibitors have shown that the entrance and substrate cavity separation is altered, depending on the chemical nature and size of the bound inhibitor (Binda *et al.*, 2003). This separation is controlled by the conformation of the side chain of Ile 199 that can exist in either an “open” or “closed” conformation. Residue Ile 199 thus serves as a “gate” between the two cavities (Edmondson *et al.*, 2007, Edmondson *et al.*, 2004). Thus for larger flexible inhibitors Ile 199 would be in a “open position, the cavities would become one, and the inhibitor would bind to both the substrate and entrance cavities of the MAO-B isoform (Ramsay, 2013), For a small inhibitor the Ile 199 would remain in the “closed” position, the cavities would exist as two separate cavities, allowing the inhibitor to bind to only one cavity.

2.4.10.4 Three-dimensional structure of MAO-A

The inhibitor selectivity between MAO-A and MAO-B is caused by structural differences, which arise from differences in the size and shape of the substrate/inhibitor cavities, for example the hMAO-A cavity is restricted by Ile 335 and Phe 208, while the cavities of hMAO-B is restricted by Tyr 326 and Ile 199.

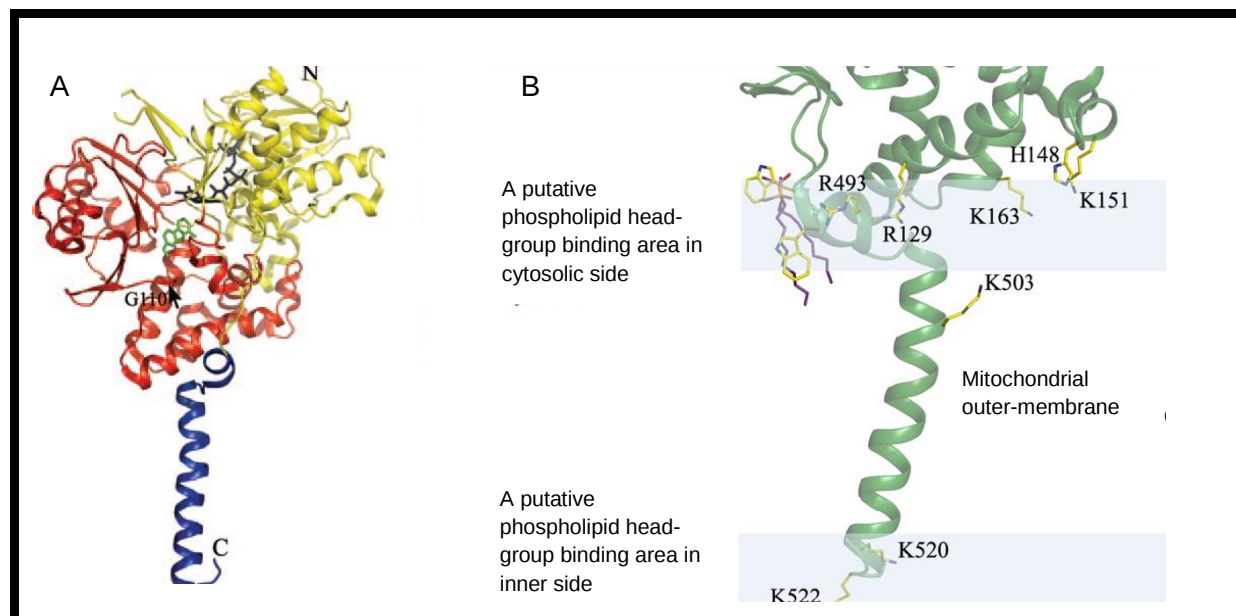


Figure 2.35: The structure of human MAO-A as well as a binding model of MAO-A to the mitochondrial outer membrane. (A) The MAO-A structure can be divided into two domains, the extra-membrane domain (yellow and red), and the membrane binding domain (blue). The FAD binding region (yellow), and the substrate/inhibitor binding region (red). FAD (black) harmine (green) (Son *et al.*, 2008). (B) Shows the positively charged residues Arg 129, His 148, Lys 151, Lys 163, Arg 493, Lys 503, Lys 520, and Lys 522. It is presumed that these residues interact with the phospholipid hydrophilic head group at the membrane surface, shown in the blue transparent areas. The upper area represents the cytosolic side (Son *et al.*, 2008).

There are also differences in the residues surrounding the cavities, the hMAO-A cavity contains Gln 215, Phe 352, Tyr 407, Ile 335 and Phe 208, while the hMAO-B cavity contains Gln 206, Phe 343, Tyr 398, Tyr 326, Ile 199, Cys 406 and Tyr 407 (Son *et al.*, 2008). In contrast to hMAO-B, hMAO-A crystallizes as a monomer and has only one cavity, the substrate cavity. The substrate cavity of hMAO-A, with a value of $550 \text{ \AA}^3$, is wider (Ramsay, 2013) when compared to the cavity of hMAO-B (Edmondson *et al.*, 2007).

According to Son *et al.* (2008), mutations on G110 of loop 108-118, causes a reduction in activity of the enzymes, unless the enzyme is anchored to the membrane. Thus the results suggest that the flexibility of loop 108-118, in accordance with the anchoring of the enzyme to the membrane,

is very important to allow the substrate access into the hMAO-A active site (Son *et al.*, 2008, Edmondson *et al.* 2007)

Similar to MAO-B, the C-terminal anchoring the enzyme is also important for its biological functions (figure 2.35), while only smaller inhibitors would bind in the substrate cavity of the hMAO-A substrate cavity, compared to the active site cavity of hMAO-B. Figure 2.36 compares the cavities of MAO-A and MAO-B, in both hMAO-A and hMAO-B the FAD is situated at the end of the tunnel from the surface of the protein, closest to the substrate cavity (Ramsay, 2013).

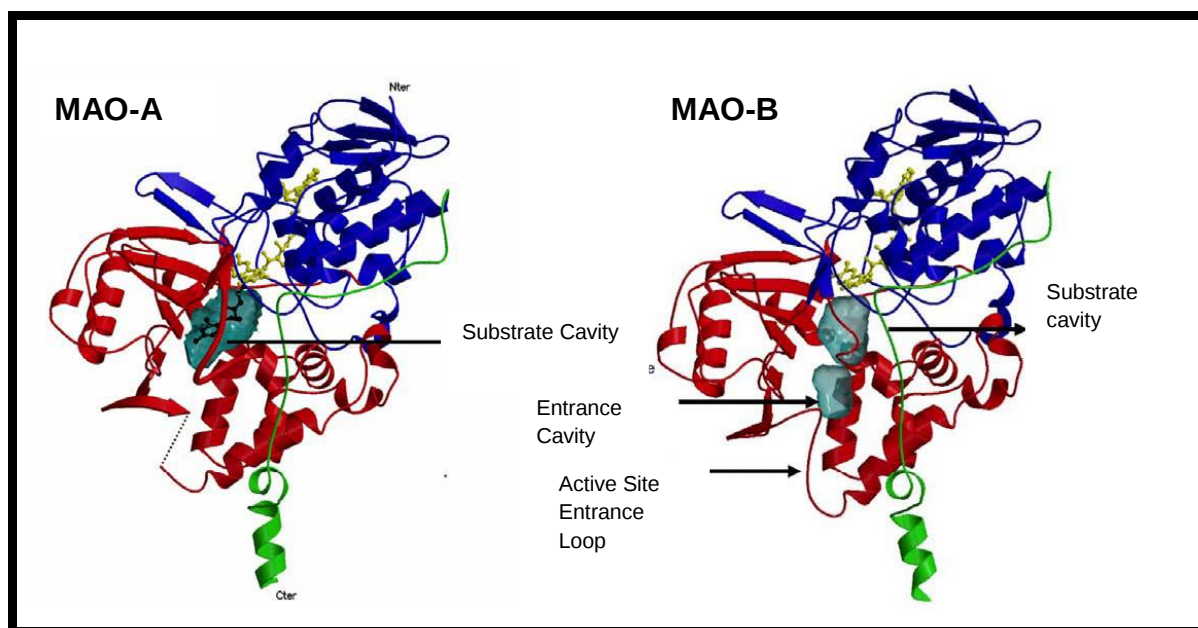


Figure 2.36: A) Comparing MAO-A and MAO-B substrate and entrance cavities (Ramsay, 2013, Binda *et al.*, 2011). B) Shows form and size of the MAO-A and MAO-B active sites, MAO-A is bound to clorgyline, while MAO-B is bound to deprenyl. The red and green ribbons indicate the active site loop structures for MAO-A and MAO-B, respectively (Edmondson *et al.*, 2007).

