

## CHAPTER 2

### Literature study:

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#### 2.1 Parkinson's disease:

##### 2.1.1 Aetiology:

PD is the most common age-related neurological disorder after Alzheimer's disease. There are three types of cellular dysfunction that may be important in the pathogenesis of PD namely: oxidative stress, mitochondrial respiration defects and abnormal protein aggregation. In humans, exposure to MPTP, a neurotoxic compound that causes degeneration of the dopaminergic system, mimics the core neurological symptoms of PD. A valuable animal research model for PD has been mice treated with MPTP. In addition, monkeys treated with MPTP remain the golden standard for the preclinical testing of new drugs that are developed for the symptomatic relief of PD (Dauer & Przedborski, 2003; Langston & Ballard, 1984).

According to the Eidelberg & Pourfar (2011), the hallmark of PD is Lewy bodies, which consist of  $\alpha$ -synuclein, in the nigrostriatal system. Lewy bodies are intraneuronal proteinaceous inclusions that occur in the cytoplasm (Dauer & Przedborski, 2003).  $\alpha$ -Synuclein is a presynaptic protein that occurs in the cytosol, neurons and glial cells. This protein weighs 14-kDa and can form insoluble fibrils by 'natively unfolding' (Spillantini *et al.*, 1997).

Synucleinopathy can occur in numerous parts of the nervous system: including the dorsal motor nucleus of the vagus nerve, basal nucleus of Meynert, neocortex, hypothalamus, olfactory bulbs, sympathetic ganglia and myenteric plexus of the gastrointestinal tract. Lewy bodies occur in a temporal sequence and many experts believe that PD is a late manifestation of systemic synucleinopathy, and that patients with PD may eventually also suffer from Lewy body dementia. Synucleinopathy also plays a role in the pathogenesis of Alzheimer's disease and many patients suffer from synucleinopathy as a co-morbid disorder (Eidelberg & Pourfar, 2011; Ferrer, 2011).

According to Di Giovanni *et al.* (2010),  $\alpha$ -synuclein plays an important role in the development of PD, despite the fact that it predominantly occurs in the cytosol. Both  $\alpha$ -synuclein toxicity and Lewy body formation are primarily mediated by extracellular  $\alpha$ -synuclein. A role for  $\alpha$ -synuclein in the pathogenesis of PD is supported by evidence that some monomeric and/or soluble aggregated forms of  $\alpha$ -synuclein are secreted and

detectable in the blood plasma and cerebral spinal fluid of patients with PD. Exogenous aggregated forms of  $\alpha$ -synuclein have also been shown to induce microglial activation and increase the production of reactive oxygen species and pro-inflammatory factors that damage mammalian cells and neurons (Lee, 2008).

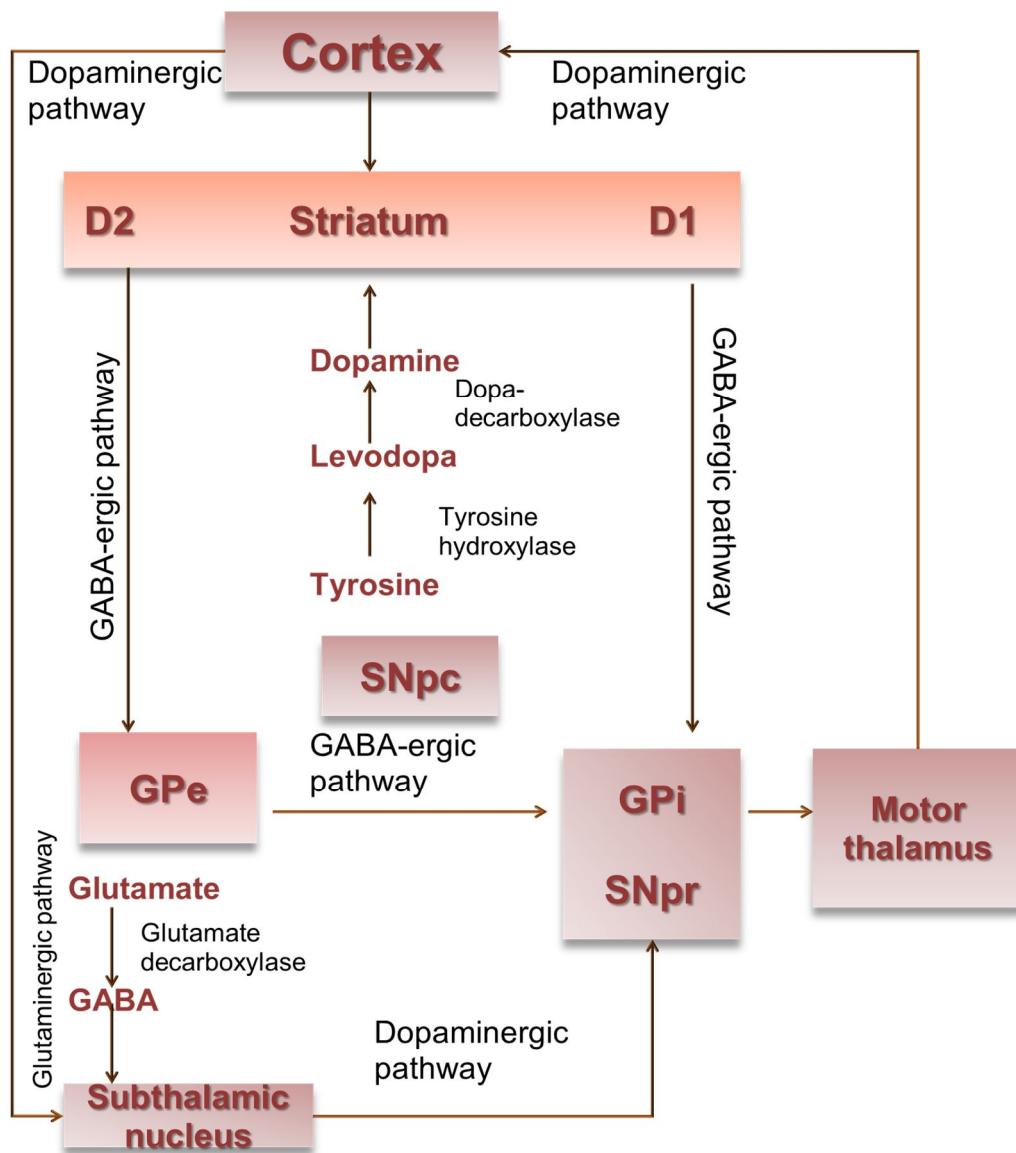
Extracellular  $\alpha$ -synuclein may either be removed by extracellular proteolytic enzymes or be taken up by neighbouring cells. The cellular uptake of extracellular  $\alpha$ -synuclein occurs by passive diffusion of the monomers and receptor-mediated endocytosis of the oligomers and protofibrils, depending on the aggregation state of the  $\alpha$ -synuclein (Lee, 2008). Only small amounts of extracellular  $\alpha$ -synuclein aggregates are needed to act as catalysts for the aggregation of intracellular  $\alpha$ -synuclein. The transmission of monomeric and aggregated  $\alpha$ -synuclein can occur through the neuron-to-neuron and neuron-to-glia routes. Therefore the release and subsequent uptake of monomeric and soluble aggregates of  $\alpha$ -synuclein, can lead to neuronal cell death and the spreading of further  $\alpha$ -synuclein pathology. Promising therapeutic strategies for the future treatment of PD include targeting the aggregation of extracellular  $\alpha$ -synuclein or promoting its clearance (Di Giovanni *et al.*, 2010).

### **2.1.2 Relevant normal brain anatomy and physiology:**

The basal ganglia of the human brain consist of: the neostriatum (that consists of the caudate and putamen); the external and internal globus pallidal segments (GPe and GPi), the subthalamic nucleus and the substantia nigra that consists of a *pars reticulata* (SNpr) and a *pars compacta* (SNpc). The basal ganglia are located in the subcortical section of the midbrain that is responsible for the integration of activity from the cortex in order to coordinate movement. Information passes through the basal ganglia to the thalamus, from where it returns to the supplementary motor area of the cortex through the dopaminergic pathway (Goole & Amighi, 2009).

Under normal physiological conditions, the striatum and the subthalamic nucleus receive information from afferent neurons to specific areas of the cerebral cortex or the thalamus and they transfer the information to the basal ganglia output nuclei, the GPi and the SNpr. The projections between the GPi and the SNpr are divided into a direct and an indirect connection pathway through the GPe and the subthalamic nucleus. In the direct pathway, output from the GPi and the SNpr goes to the motor thalamus that transfers it back to the cerebral cortex and then to the striatum. The striatum also receives a noticeable amount of dopaminergic input directly from the SNpc. This anatomical arrangement allows the dopaminergic input to regulate the corticostriatal transmission. Dopamine D1 receptors are

involved in the direct pathway, while D2 receptors play a role in the indirect pathway (Goole & Amighi, 2009; Obeso *et al.*, 2000).



**Figure 2.1.1** Schematic activity of the basal ganglia-thalamocortical motor circuit (Adapted from Goole & Amighi, 2009).

These pathways have antagonistic effects. Activation of the direct pathway may inhibit the activity of the GPi and SNpr and so undo the inhibition of the thalamocortical interactions. Activation of the indirect pathway has the opposite effect. Therefore, since physiological dopaminergic stimulation may increase activity in the thalamocortical projection neurons by inhibiting the GPi and the SNpr pathways, it may lead to greater activation of the cerebral cortex that can play a role in the facilitation of movement (Goole & Amighi, 2009; Obeso *et al.*, 2000).

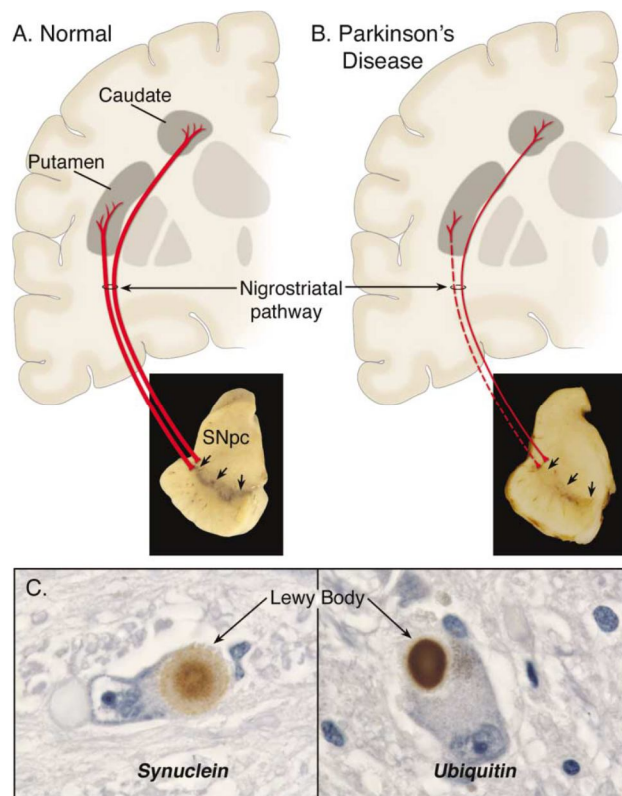
### **2.1.3 Pathophysiology in Parkinson's disease:**

#### **2.1.3.1 Sites of neurodegeneration:**

In humans the SNpc contains approximately 450 000 dopaminergic neurons (Lang & Lozano, 1998). In PD the pigmented neurons of the SN, locus coeruleus and other brain stem dopaminergic neurons are lost. The neurons normally contain conspicuous amounts of neuromelanin, therefore their loss lead to the classical neurological finding of depigmentation in the SN (Dauer & Przedborski, 2003). The loss of the SN neurons, which project into the caudate nucleus and putamen, depletes dopamine in these areas (Eidelberg & Pourfar, 2011).

At the onset of symptoms, approximately 80% of the putamenal dopamine and 60% of dopaminergic neurons in the SN have already been lost. The mesolimbic dopaminergic neurons and cell bodies in the ventral tegmental area, next to the SN, are much less affected and there is thus significantly less depletion of dopamine levels in the caudate, where these neurons project (Dauer & Przedborski, 2003).

Neuropathological studies of PD related neurodegeneration show that the losses of dopaminergic neurons follow a characteristic topology that is distinct from the pattern seen in normal aging. In PD, the cell loss is concentrated in the ventrolateral and caudal parts of the SN, but normal aging affects the dorsomedial aspect. Therefore, even though age increases the risk for PD, the processes that produce age related death of dopaminergic neurons are different than those that cause PD. The degree of terminal loss in the striatum appears to be more pronounced than the degree of dopaminergic neuron loss in the SN (Dauer & Przedborski, 2003; Fearnley & Lees, 1991).



**Figure 2.1.2** Neuropathology of PD. (A) Schematic representation of the normal dopaminergic neurons of the nigrostriatal pathway (in red) and a photograph of the normal pigmentation of the SNpc due to neuromelanin. (B) The dopaminergic neurons of the diseased nigrostriatal pathway. The dashed line indicates a marked loss and the thin red solid line indicates a more modest loss and a photograph of the depigmentation of the SNpc. (C) Immunohistochemical labeling of Lewy bodies in a SNpc dopaminergic neuron. Immunostaining with an antibody against  $\alpha$ -synuclein reveals a Lewy body (black arrow) with an intensely immunoreactive central zone surrounded by a faintly immunoreactive peripheral zone (left photograph). Conversely, immunostaining with an antibody against ubiquitin yields more diffuse immunoreactivity within the Lewy body (right photograph) (Dauer & Przedborski, 2003).

In PD, the primary focus has been on the neurodegeneration of the dopaminergic neurons. However, the neurodegeneration and formation of Lewy bodies can also be found in noradrenergic neurons in the locus coeruleus, serotonergic neurons in the raphe, cholinergic neurons in the nucleus basalis of Meynert and the dorsal motor nucleus of the vagus system. In addition, it also affects the cerebral cortex, olfactory bulb and the autonomic nervous system. Degeneration of the hippocampal structures and cortical cholinergic neurons is a contributing factor to the development of dementia that is associated with PD (Dauer & Przedborski, 2003; Ferrer, 2011).

There are two major hypotheses on the pathogenesis of PD namely the misfolding and aggregation of proteins that lead to the death of dopaminergic neurons in the SN and mitochondrial dysfunction that leads to oxidative stress and the formation of free radicals.

The two hypotheses are interlinked and it has been found that oxidative stress can cause the misfolding of  $\alpha$ -synuclein which leads to neuron death (Dauer & Przedborski, 2003).

Other hypotheses are the actions of excitotoxins, deficient neurotrophic support and immune mechanisms. A critical question in the pathogenesis of PD is why specific neurons are selectively vulnerable in PD. The answer may lie in their ability to take up both endogenous and exogenous toxic compounds through selective carrier methods such as the dopamine transporter. Other explanations may be increased metabolic stress, the high physiological rates of protein oxidation, selective generation of potential toxins or failure to dispose of them due to the presence of neuromelanin (Lang & Lozano, 1998).

### **2.1.3.2 Mechanisms of neurodegeneration:**

#### **Misfolding and aggregation of proteins:**

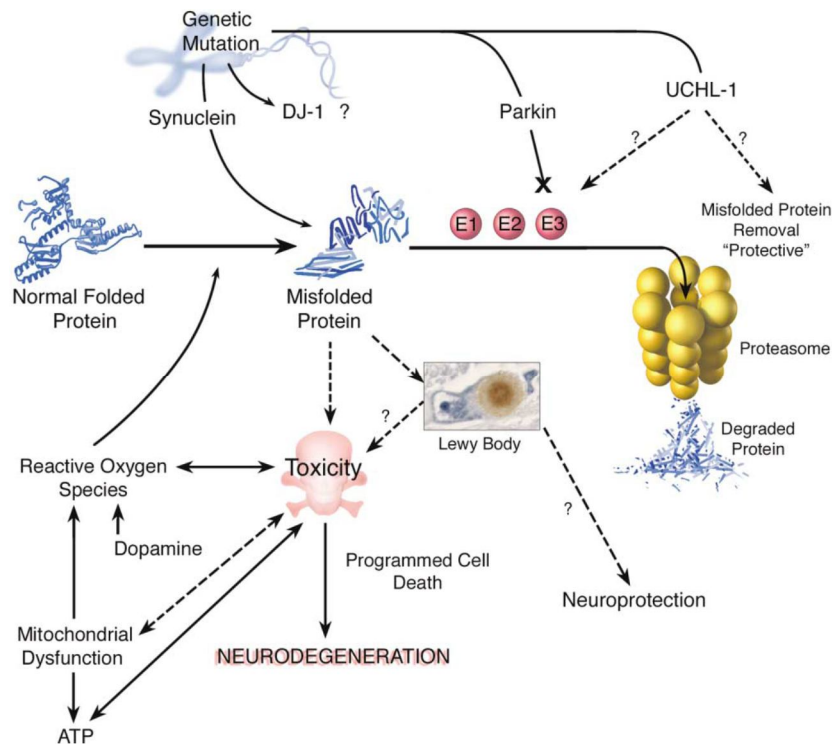
The deposition of abnormal proteins in brain tissue is a feature of several age-related diseases and it suggests that protein aggregates may be toxic for brain tissue (Ross & Poirier, 2004). The neurotoxicity can be caused by a variety of mechanisms including direct damage by protein inclusions by deformation of the cell, interference in the transport mechanisms in the cell or seizure of proteins that are important for cell survival. It could be assumed that there is a direct link between inclusion body formation and neurodegeneration, but studies in Huntington's disease have shown no correlation (Dauer & Przedborski, 2003).

The cytoplasmic protein inclusions may be formed by an active process that is meant to seize misfolded proteins from the cell environment. The formation of inclusion bodies may therefore be a defensive measure for the removal of misfolded proteins. It may also serve as an indicator of an attack on the cell (Auluck *et al.*, 2002). Furthermore, soluble misfolded proteins may also be toxic, as seen by the ability of chaperones like Hsp-70 to protect against neurodegeneration caused by  $\alpha$ -synuclein mediated dopaminergic neuron loss and other disease-related proteins that cause neurodegeneration (Dauer & Przedborski, 2003; Muchowski, 2002).

In patients with inherited PD, the pathogenic mutations are thought to cause the disease directly by inducing abnormalities and toxic protein conformations, or indirectly by interference in processes that normally recognise or process misfolded proteins. This leads to an accumulation of the misfolded protein. Dysfunctional protein metabolism in sporadic PD may be caused by oxidative stress, but the causes are uncertain. The tissue content of abnormally oxidized proteins, which may misfold, increases with age and neurons may be

more susceptible because they do not undergo further mitosis. In PD, the Lewy bodies contain oxidatively modified  $\alpha$ -synuclein, which is more likely to aggregate than normal  $\alpha$ -synuclein. Misfolding or aggregation of  $\alpha$ -synuclein can also be caused by several herbicides and pesticides (Dauer & Przedborski, 2003).

The cells respond to the misfolded proteins by creating chaperones, but if the proteins cannot be properly refolded, they are targeted for proteosomal degradation by poly-ubiquitination. With aging, the ability to form chaperones and the proteosomal activity declines. The proteosomal dysfunction and the resulting accumulation of misfolded protein can cause a vicious cycle, wherein the excess misfolded proteins further inhibit the proteasomes. Thus aging and oxidative stress can converge and cause proteotoxic damage to the neurons and eventually cause PD (Dauer & Przedborski, 2003).



**Figure 2.1.3** Mechanisms of neurodegeneration in PD. Pathogenic mutations may directly induce abnormal protein conformations (such as  $\alpha$ -synuclein) or damage the ability of the cell to detect and degrade misfolded proteins. Oxidative damage, linked to mitochondrial dysfunction and abnormal dopamine metabolism, may also promote misfolded protein conformations. It is not certain whether misfolded proteins directly cause toxicity or damage cells via the formation of Lewy bodies. It is also unknown whether Lewy bodies promote toxicity or protect a cell from harmful effects of misfolded proteins by seizing them in an insoluble compartment away from cellular elements. Oxidative stress, energy crisis (such as ATP depletion) and the activation of apoptosis mechanisms are also believed to be factors that trigger the death of dopaminergic neurons in PD (Dauer & Przedborski, 2003).

### **Mitochondrial dysfunction and oxidative stress:**

According to Lipski *et al.* (2011), mitochondrial dysfunction is considered to be the main mechanism responsible for the degeneration of the nigrostriatal neurons in PD. SNpc neurons have a high metabolic rate and relatively high baseline  $\text{Ca}^{2+}$  levels. This, together with their dopamine and neuromelanin content, contribute to the neurons being in a 'pro-oxidant state' even under normal physiological conditions. Therefore, theoretically any additional pathological or genetic factor that may result in mitochondrial impairment can lead to: insufficient ATP production, additional generation of reactive oxygen species, oxidative damage of mitochondrial and cytosolic proteins, release of cytochrome-C and other pro-apoptotic factors, and intracellular  $\text{Ca}^{2+}$  overload that will eventually cause cell death. According to Ferrer (2011), several mutant proteins associated with familial PD are linked to the mitochondria.

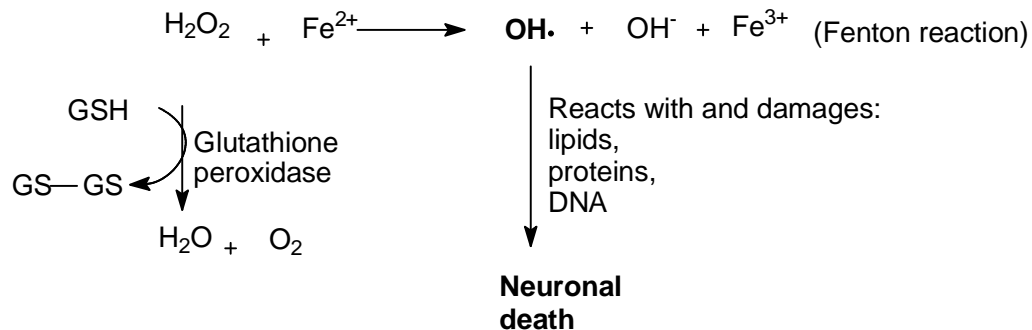
The discovery that MPTP treatment blocks the mitochondrial electron transport chain by inhibiting complex I led to studies that revealed abnormalities in complex I in PD (Ferrer, 2011). The defect in complex I may subject the cells to oxidative stress and energy failure. The abnormal functioning of complex I in patients with PD can be found outside the brain. It has been found in the platelets of PD patients and it may thus be inherited through mitochondrial DNA or caused by a mitochondrial toxin (Dauer & Przedborski, 2003). In PD there is a 30-40% decrease in complex I activity in the SNpc (Lang & Lozano, 1998).

As part of normal mitochondrial function, powerful oxidants are produced as by-products including superoxide radicals. Inhibition of complex I increases the production of the superoxide reactive oxygen species, which may form toxic hydroxyl free radicals or form peroxynitrite through a reaction with nitric oxide. These reactive products may interact with nucleic acids, proteins, lipids and the electron transport chain itself and therefore cause cellular damage. Interactions with the electron transport chain can lead to mitochondrial damage and the formation of further reactive oxygen species (Dauer & Przedborski, 2003; Ferrer, 2011).

There are elevated levels of iron in the SN of patients with PD and this is thought to be an important factor which results in oxidative stress in PD. The sites of neuronal death are also the sites where iron accumulates (Zecca *et al.*, 2004). In the Fenton reaction (figure 2.1.4) hydrogen peroxide reacts spontaneously with iron in the SNpc to form hydroxyl free radicals. The hydroxyl free radicals diminish cellular anti-oxidants and can damage lipids, proteins and DNA (Youdim & Bakhle, 2006). Normally hydrogen peroxide is inactivated by



glutathione (GSH) (via GSH peroxidase), but GSH levels are decreased in the brains of patients with PD. Under these conditions hydrogen peroxide is available for conversion to toxic reactive oxygen species (ROS) via the Fenton reaction (Lang & Lozano, 1998).



**Figure 2.1.4** The mechanism of neurotoxicity of hydrogen peroxide induced by the Fenton reaction (Youdim & Bakhle, 2006).

The presence of ROS can increase the misfolding of proteins and thus increase the strain on the ubiquitin-proteasome system to remove it. Normally the metabolism of dopamine generates hydrogen peroxide and superoxide. The auto-oxidation of dopamine produces dopamine-quinone that can damage proteins by reacting with the cysteine residues. Therefore dopaminergic neurons are a favourable environment for the generation of ROS (Dauer & Przedborski, 2003).

The metabolism of dopamine by mitochondrial MAO-A and MAO-B also produces ROS. Other pathways that can lead to enhanced production of ROS include the complexation of neuromelanin with iron. A higher than normal ratio of  $\text{Fe}^{3+} / \text{Fe}^{2+}$  in the SNpc of PD patients promote the formation of hydrogen peroxide and hydroxyl free radicals. ROS can also be generated when tyrosine hydroxylase oxidises levodopa (Lipski *et al.*, 2011).

### **Excitotoxins and calcium dysregulation:**

According to Lang & Lozano (1998), excitotoxicity may play a role in many neurodegenerative disorders, including PD. Persistent activation of N-methyl-D-aspartate (NMDA) glutaminergic receptors leads to increased levels of intracellular calcium ions which could lead to the activation of proteases, phospholipases, endonucleases and nitric oxide synthase. This may result in the formation of reactive nitric oxide free radicals, the release of iron from ferritin, lipid peroxidation and the inhibition of mitochondrial functioning. The role of increased intracellular calcium in the events that cause cell death in PD is supported by the observation that dopaminergic neurons that express calbindin (that binds calcium) may be

selectively preserved in PD. Also of interest is the observation that the subthalamic nucleus is overactive in PD and the resulting excessive glutaminergic activity can be a source of excitotoxicity in the SN.

### **Neurotrophic factors:**

Neurotrophic factors play an important role in neuronal survival during development and after an injury. Inadequate levels of support by the neurotrophic factors can lead to neuronal apoptosis in several systems. Glial cell line-derived neurotrophic factor (GDNF) and brain derived neurotrophic factor (BDNF) are important in the preservation of dopaminergic neurons due to their potent protective and regenerative effects. GDNF and BDNF are beneficial in PD models in animals. GDNF is in clinical trials for the treatment of PD in humans. A shortage of these factors or of other neurotrophic factors may contribute to the degeneration of dopaminergic cells (Gill *et al.*, 2003; Lang & Lozano, 1998).

### **Immune factors:**

Immune factors may be secondarily involved in progressive cell loss in the SN. There are HLA-DR-positive reactive microglia and increased levels of cytokines, such as tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) and interleukin-1 (IL-1) in the SNpc in later stages of PD (Lang & Lozano, 1998). Humoral immune reactivity, leading to microglial activation, has been associated with the degeneration and depigmentation of dopaminergic neurons in the substantia nigra. Additionally, anti-inflammatory medication appears to offer protection against PD. These findings suggest an immune involvement in PD (Orr *et al.*, 2005).

## 2.2 Epidemiology and genetics:

PD occurs around the world in all ethnic groups and it affects both sexes roughly equally with a slightly higher incidence in males. PD is a progressive disease with a mean age at onset of 55 years. The incidence increases markedly with age, increasing from 20/100 000 overall, to 120/100 000 at age 70 (Dauer & Przedborski, 2003). However, in 5-10% of patients, the symptoms start before the age of 40 years and this is known as young-onset PD (Lang & Lozano, 1998).

The highest incidence of PD is among whites and the lowest incidence is among Asians and African blacks. African blacks have a much lower incidence than American blacks but the prevalence of Lewy bodies in the brains of Nigerians are similar to those in the Western population. The pattern suggests that the tendency for the development of PD is universal, but that local environmental factors play a role. A lack of observation rather than elementary environmental variations can also explain this distribution (Lang & Lozano, 1998).

PD was first formally described at the time of the Industrial Revolution, but ancient *ayurvedic* Indian literature from 4500-1000 B.C. contains references of a disease called *kampavata* that consisted of tremor and akinesia. The discovery that MPTP selectively induced nigral cell death sparked research into toxins that could cause PD. There are various toxins that can produce the symptoms of PD. Rural environments have generally been associated with more cases of PD than urban environments. This could be due to the use of pesticides and exposure to well water. However, only about 10% of the population with PD had such an exposure (Lang & Lozano, 1998).

PD may have genetic causes in 15-20% of cases and can be carried by both autosomal dominant and autosomal recessive genes. Autosomal dominant PD may be caused by mutations of the  $\alpha$ -synuclein gene at 4q21 (PARK1/PARK4), the leucine-repeat rich kinase 2 (LRRK2 or PARK8) gene at 12p12 and the ubiquitin carboxylase L1 (UCHL1) gene (Aminoff, 2009; Bekris *et al.*, 2010). Most studies have found autosomal dominant inheritance of PD, even in families with few affected members. Most patients do not have a clear family history of autosomal dominant disease, probably because the causative genes have a low penetrance or the disease is multi-factorial (Lang & Lozano, 1998).

Mutations of the *parkin* gene (6q25.2-q27) may cause autosomal-recessive, early-onset, sporadic juvenile-onset and familial PD. Several other genes and chromosomal regions have also been associated with familial forms of the disease. Studies in twins show that the

genetic component plays a larger role in patients under the age of 50. In other cases it is an idiopathic disorder which may be related to exposure to some unrecognised neurotoxin or to oxidation reactions which generates free radicals (Aminoff, 2009; Bekris *et al.*, 2010). Epidemiologic studies have shown that apart from age, a family history of PD is the strongest predictor of an increased risk, although the shared environment by the same family has to be considered. However in most cases of familial PD, the disease has atypical characteristics such as early onset, a rapid course to death and frequent dementia (Bekris *et al.*, 2010; Lang & Lozano, 1998).

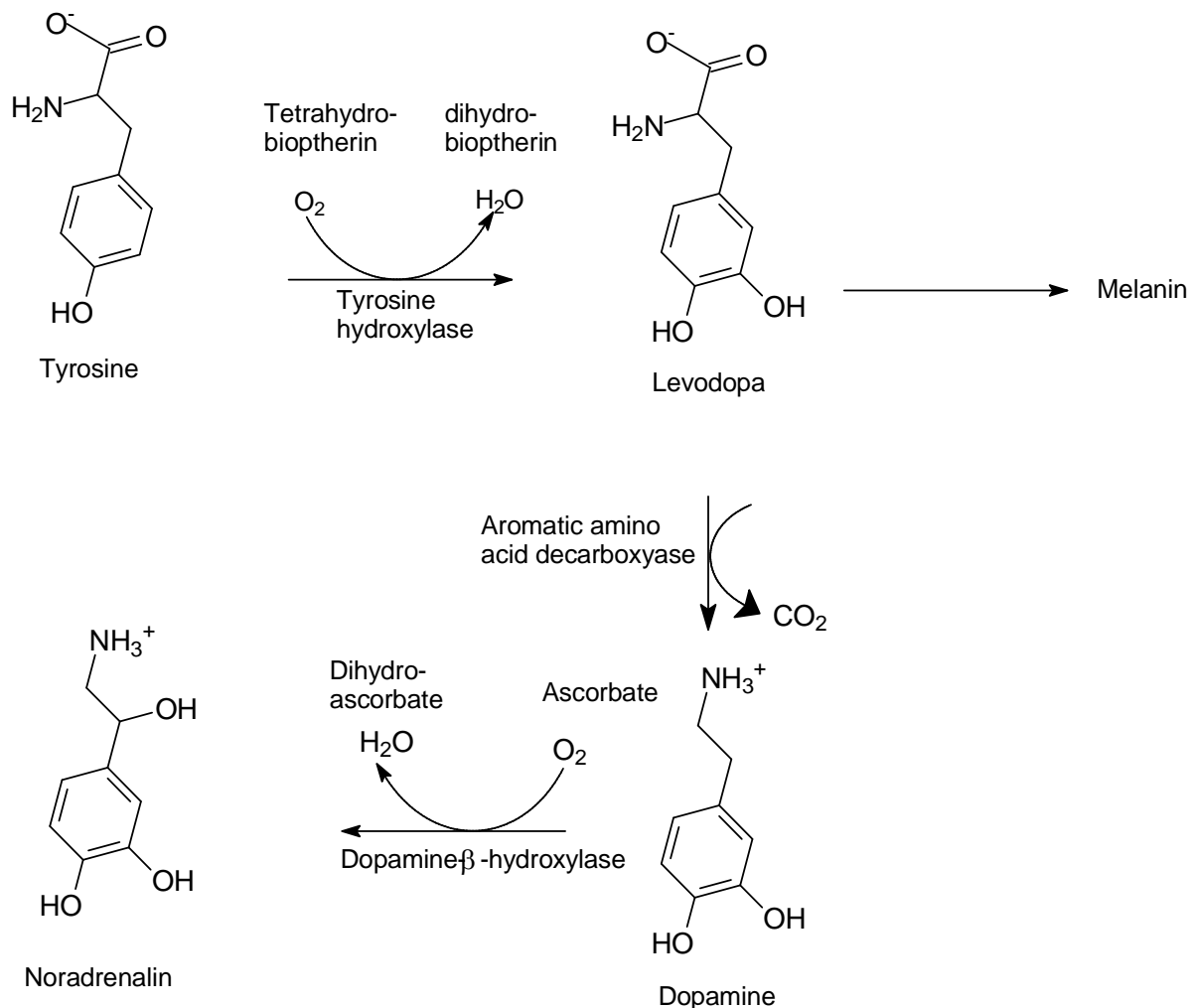
In some subjects, exposure to MPTP resulted in severe Parkinsonism, while in others it produced no, or very subtle, neurological defects. Follow-up studies with positron emission tomography (PET) using fluorinated levodopa (F-dopa) in those subjects whom had exhibited only subtle neurological defects, showed a slow progressive decline in F-dopa activity. This apparently progressive neurodegeneration after only a brief exposure to a neurotoxin earlier in life, combined with other cases where delayed-onset progressive Parkinsonism developed in patients with *encephalitis lethargica*, suggests that PD may be caused by a single event and not an ongoing process. The likeliness of a person to develop PD after such an event is believed to be related to a predetermined genetic susceptibility (Lang & Lozano, 1998).

Smoking is a factor that has constantly been associated with a lower incidence of PD. A study of the negative correlation between smoking and PD has found that smoking lowered the risk by approximately 50%. Other studies found that the risk was mainly reduced in patients with young onset PD. Coffee drinking has also been linked to a lower risk of developing PD (Hemán *et al.*, 2002; Lang & Lozano, 1998). Studies assessing diet and nutritional deficiencies of PD patients, to explore the role of inadequate intake of anti-oxidants, have been inconclusive. However, a large community based study in the Netherlands showed that the intake of vitamin E by patients with PD was significantly lower than the intake by control groups (Lang & Lozano, 1998).

## 2.3 Chemical compounds of importance in PD:

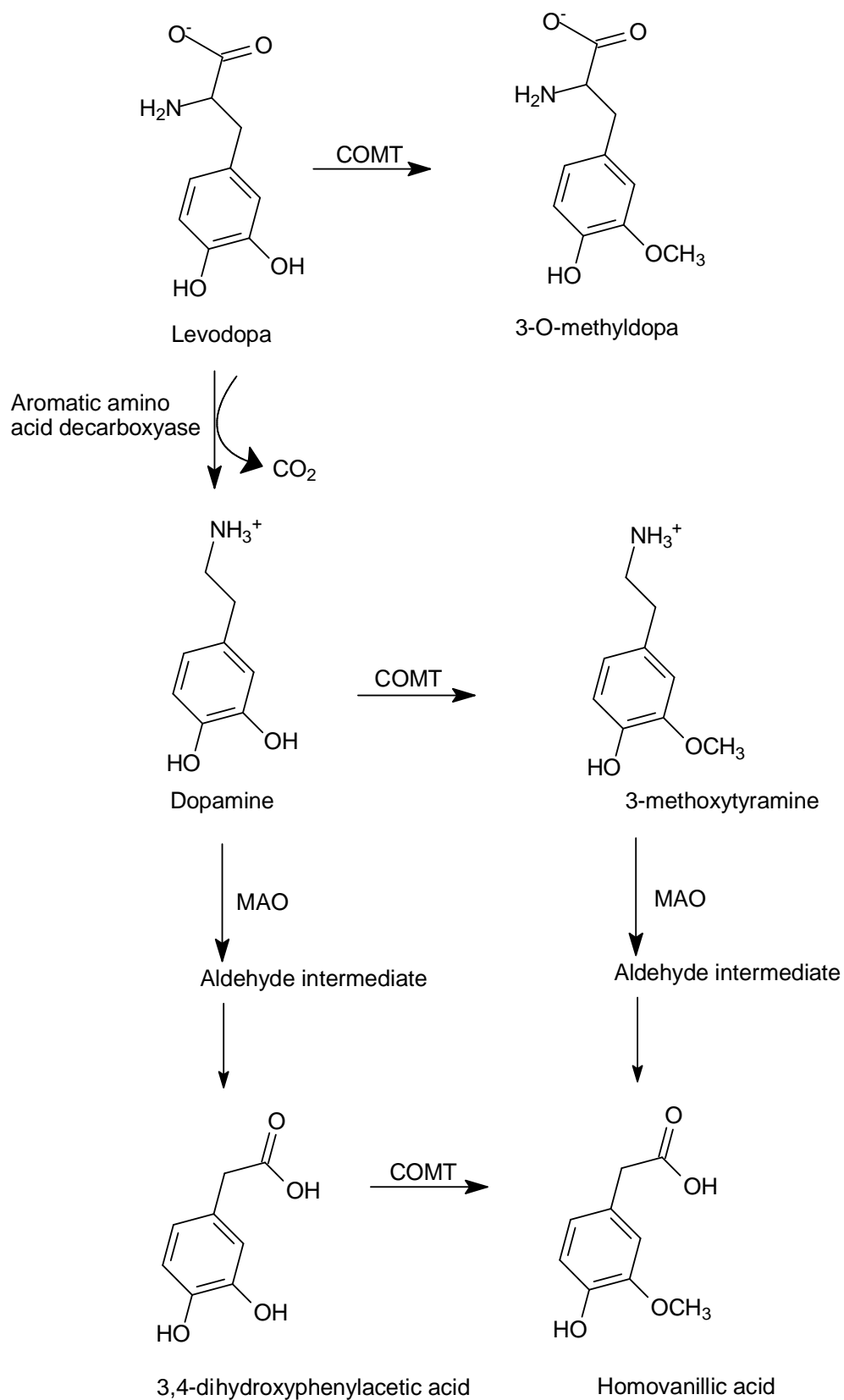
### 2.3.1 Dopamine:

According to the Eidelberg & Pourfar (2011), the dopamine synthesis pathway begins with *L*-tyrosine, which is taken up by dopaminergic neurons, and then converted by tyrosine hydroxylase to 3,4-dihydroxyphenylalanine (levodopa). Levodopa is converted by dopa decarboxylase (aromatic-*L*-amino-acid decarboxylase) to dopamine in the synthesis pathway illustrated in figure 2.3.1. After release and interaction with receptors, dopamine is actively pumped back (by reuptake) into the nerve terminal. Catechol-*O*-methyltransferase (COMT) and MAO metabolize dopamine and so regulate its levels in nerve terminals. Dopamine is a precursor of noradrenalin and adrenalin.



**Figure 2.3.1** The synthesis of dopamine.

In the brain, dopamine is metabolised by COMT and MAO in the pathways shown in figure 2.3.2.



**Figure 2.3.2** The main pathways of dopamine metabolism in the brain (Adapted from Okada *et al.*, 2011).

Dopamine interacts with both peripheral nerve fibres and central neurons in the SN, midbrain, ventral tegmental area and the hypothalamus. Dopaminergic receptors are classified as D<sub>1</sub>-D<sub>5</sub>. D<sub>3</sub> and D<sub>4</sub> receptors play a role in thought control. D<sub>2</sub> receptors control the extrapyramidal system. Receptor affinity does not predict the functional response as seen with ropinirole, a D<sub>3</sub> receptor agonist that has a functional response by activation of D<sub>2</sub> receptors (Eidelberg & Pourfar, 2011). D<sub>1</sub> receptors are located in the SNpc and presynaptically on striatal axons projecting from cortical neurons and from dopaminergic cells in the SN. D<sub>2</sub> receptors are located postsynaptically on striatal neurons and presynaptically on axons in the SN which belong to neurons in the basal ganglia. The benefits of dopaminergic anti-parkinsonism drugs are mainly seen through D<sub>2</sub> receptor stimulation, however D<sub>1</sub> stimulation may be required for maximal benefit. Drugs which block D<sub>2</sub> receptors can induce Parkinsonism (Aminoff, 2009).

### **2.3.2 Levodopa:**

#### **2.3.2.1 Rationale of levodopa therapy:**

Levodopa is a natural amino acid that acts as an intermediary product in several metabolic pathways. It is also the precursor of all catecholamine neurotransmitters and hormones, including dopamine, and melanin (Bonifati & Meco, 1999). Dopamine does not cross the blood-brain barrier and therefore has no central therapeutic effects if administered orally or in the peripheral circulation. However levodopa, the immediate precursor of dopamine, crosses the blood-brain barrier via a *L*-amino acid transporter, where it is decarboxylated to dopamine (Aminoff, 2009).

#### **2.3.2.2 Absorption and metabolism:**

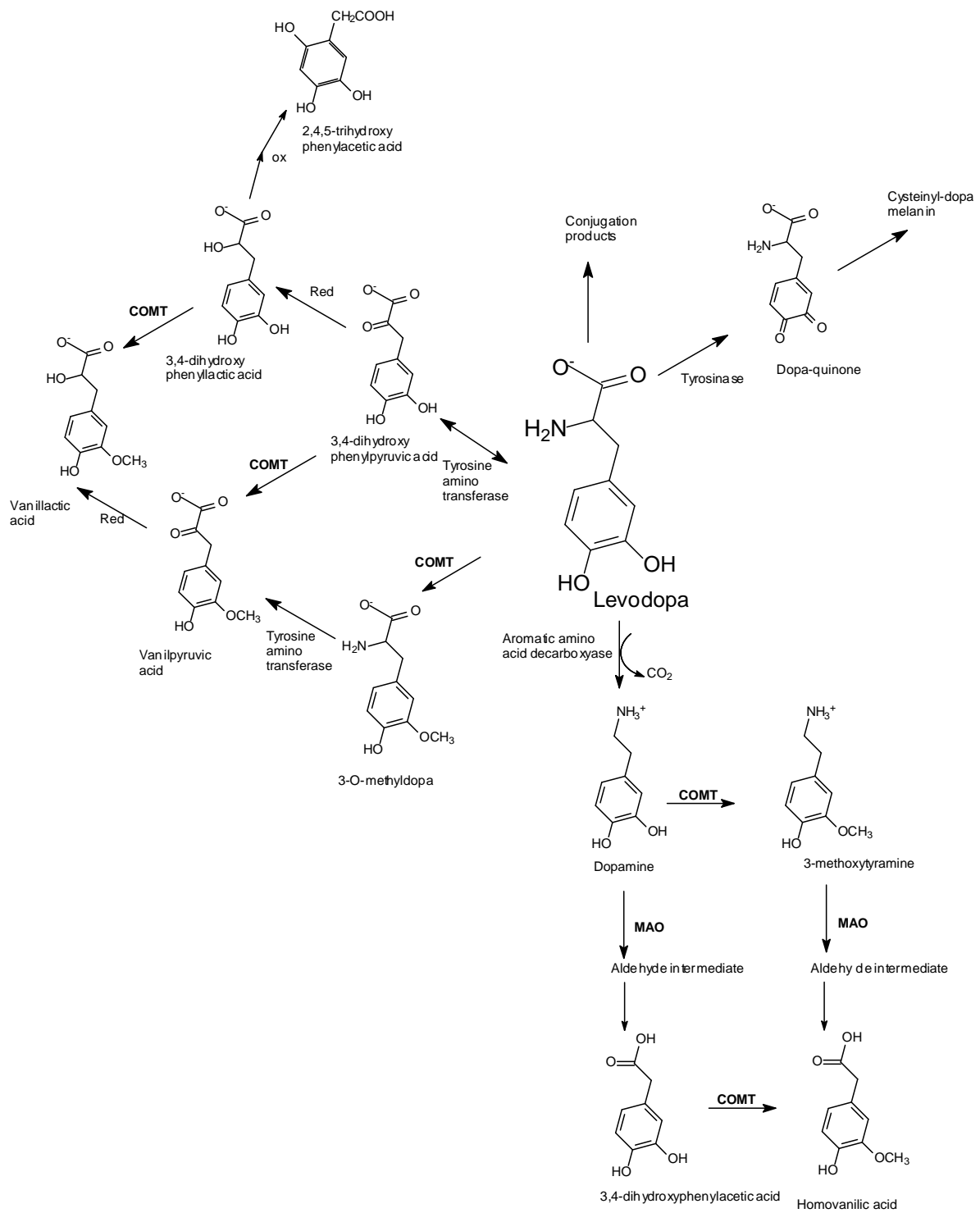
Levodopa is rapidly absorbed from the small intestine, but its absorption is influenced by gastric emptying time and the pH of the gastric contents. Certain amino acids can compete with levodopa for transport from the gut into the blood and from the blood into the brain (Aminoff, 2009).

The majority of orally administered levodopa is rapidly metabolized by four main routes namely decarboxylation, O-methylation, transamination and oxidation (Bonifati & Meco, 1999). In the first step, the majority of the levodopa is decarboxylated to dopamine in peripheral tissues (approximately 70%) and then it is further metabolized. The liver and the intestinal mucosa express high concentrations of dopa decarboxylase. The decarboxylation of levodopa already begins when it is absorbed from the gastrointestinal system. Further

decarboxylation occurs by intestinal bacteria. The other metabolic routes as illustrated in figure 2.3.3 are minor routes (Männistö & Kaakkola, 1990). About two thirds of the dose is excreted in the urine as metabolites within 8 hours of an oral dose, with its main metabolites being homovanillic acid and dihydroxyphenylacetic acid. Levodopa's plasma half-life is approximately 1-3 hours (Aminoff, 2009).

Unfortunately, only 1-3% of levodopa enters the brain intact due to extracerebral metabolism to dopamine, which does not cross the blood-brain barrier. Levodopa must therefore be given in large amounts when it is administered alone, but in combination with a peripheral decarboxylase inhibitor, such as carbidopa, the daily dose of levodopa can be reduced by as much as 75% (Aminoff, 2009). Since very little dopamine is formed outside of the brain with concurrent administration of a dopa decarboxylase inhibitor, the peripheral side effects of dopamine are largely eliminated (Männistö & Kaakkola, 1990).





**Figure 2.3.3** Schematic illustration of the metabolism of levodopa (Adapted from Bonifati & Meco, 1999).

### **2.3.2.3 Adverse effects of levodopa therapy:**

Levodopa treatment is most effective in the first few years of treatment. Sometimes a reduction of the daily dose that was well tolerated initially is necessary to avoid adverse effects. Some patients become less responsive to levodopa due to the loss of the dopaminergic nigrostriatal nerve terminals or loss of the dopamine receptors themselves. Levodopa does not stop the progression of PD, but the early initiation of therapy lowers the mortality rate. Long term use can lead to problems such as the 'on-off' phenomenon, which is a fluctuation in clinical response (Aminoff, 2009).

According to Lipski *et al.* (2011), after the so-called 'honeymoon period' ends, levodopa becomes less effective at ameliorating the various motor symptoms and side effects develop. These include dyskinesias, dystonias, longer 'off' periods when the drug is not working and shorter 'on' periods. Levodopa is not only the most potent drug available for the treatment of PD, but also the drug that is responsible for the most motor adverse effects. Other classes of drugs, such as COMT-inhibitors or MAO-inhibitors are often given in combination with levodopa to prolong its effects and reduce fluctuations.

## **2.4 Monoamine oxidase:**

### **2.4.1 Physiology of MAO:**

MAO is an enzyme that is present in the outer mitochondrial membrane of both neuronal and non-neuronal cells (Yamada & Yasuhara, 2004). There are two types of MAO in the nervous system, namely MAO-A; which is responsible for the deamination of noradrenalin, serotonin, dopamine, adrenalin and tyramine; and MAO-B that selectively deaminates dopamine, tyramine,  $\beta$ -phenylethylamine and benzylamine (Aminoff, 2009).

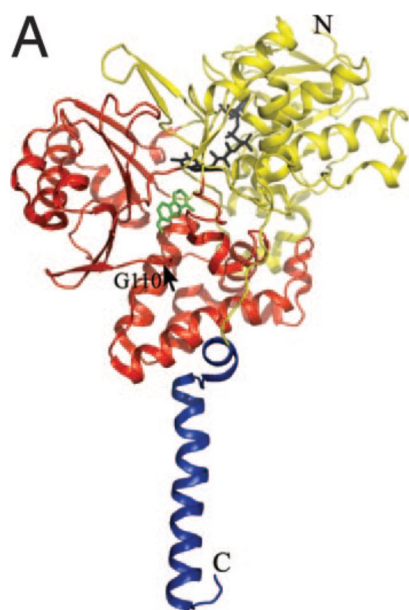
Most other human tissues also express both MAO-A and MAO-B, however in the placenta MAO-A is predominantly expressed, but human platelets and lymphocytes only express MAO-B. The concentration of MAO-B is higher in the adult brain than in the foetal brain and it increases with aging. The relative amounts of MAO-A in various tissues, arranged from the highest to the lowest is: small intestine, placenta, lung, muscle, kidney, brain, spinal cord, meninges, liver, spleen and adrenal gland. For MAO-B the distribution, from the highest to the lowest concentration is: small intestine, kidney, liver, spleen, adrenal gland, heart, spinal cord and lung. The brain distribution of MAO-A and MAO-B from highest to lowest is: frontal cortex, locus coeruleus, temporal cortex, posterior pensylvanian cortex-supramarginal gyri, anterior pensylvanian cortex-supramarginal gyri, hippocampus and thalamus (Nagatsu, 2004).

### **2.4.2 Composition and structure of MAO:**

#### **The sequences of MAO-A and MAO-B:**

The human MAO-A and MAO-B isoforms, respectively, consist of 527 and 520 amino acids each. MAO-A and MAO-B have subunit molecular weights of 59,700 and 58,000 Da, respectively. There is a 70% amino acid sequence identity between MAO-A and MAO-B (Nagatsu, 2004) and a 87% sequence identity between human MAO-A and rat MAO-A. Furthermore, the backbone structure of rat MAO-A is nearly identical to that of human MAO-B (Son *et al.*, 2008).

### 2.4.2.1 The structure of MAO-A:



**Figure 2.4.1** The overall structure of MAO-A drawn in ribbon mode. The structure consists of a N-terminus and a C-terminus and can further be divided into a membrane binding domain (in blue) and an extra-membrane domain which is further divided into a FAD binding region (in yellow) and a substrate/inhibitor binding region (in red). Also in the model are FAD (in black) and harmine (in green) (Son *et al.*, 2008).

Human MAO-A is a monomeric enzyme, while rat MAO-A is dimeric. The membrane binding domain consists of a C-terminal trans-membrane helix in both MAO-A and MAO-B. This structural element consists of 35-40 residues and it is of biological importance because it is responsible for targeting and anchoring the MAO proteins to the outer mitochondrial membrane. A decrease in the length of the C-terminal decreases the catalytic activity of MAO-A and MAO-B. Membrane anchoring is important in the functioning of the MAO enzymes (Son *et al.*, 2008).

The FAD cofactor is covalently bound through a 8 $\alpha$ -(S-cysteinyl)-riboflavin linkage between the cysteine of the pentapeptide Ser-Gly-Gly-Cys-Tyr, that occurs in the extra-membrane regions of both MAO-A and MAO-B, and the 8- $\alpha$  methylene of the isoalloxazine ring of FAD. In MAO-A the FAD is bound to Cys-406. The N(5) and the C(4a) of the flavin moiety are the most likely to form adducts with irreversible MAO inhibitors. Different inhibitors will form either a N(5) or a C(4a) adduct, depending on their mechanism of inactivation which is discussed later. The structure of the flavin ring is bent at a 30° angle along the N(5)-N(10) axis upon binding to the MAO enzyme, whereas the flavin ring of free FAD has a planar structure. The flavin side chains are in their extended conformations in MAO-A and MAO-B (Nagatsu, 2004; Edmondson *et al.*, 2004; Edmondson *et al.*, 2009).

The FAD lies at the end of a long tunnel from the outside of the protein close to the membrane surface. The tunnel is mainly hydrophobic, but it ends in an aromatic cage near the FAD where two tyrosines align the substrate towards the C4(a)-N5 region of the FAD. The substrate and the FAD are orientated at right angles by the tyrosines, perpendicular to the N(5) on the *re* face of the isoalloxazine ring, so that it is in the optimal position for catalysis. The tyrosines also exert a dipole effect on the substrate that can make the amine more susceptible for oxidation (Ramsay, 2012).

The substrate/inhibitor cavity of MAO-A is a single cavity with a volume of approximately 400 Å<sup>3</sup>. There are 16 residues that surround the substrate/inhibitor cavity of MAO and these residues are identical between human MAO-A and rat MAO-A. Studies of the structure of the active site of human MAO-A, bound with the selective inhibitor harmine, show that the inhibitor is located in the active centre cavity of the enzyme where it interacts with Tyr-69, Asn-181, Phe-208, Val-210, Gln-215, Cys-323, Ile-325, Ile-335, Leu-337, Phe-352, Tyr-407, Tyr-444, and FAD. There are seven water molecules that occupy the space between harmine and the residues. Harmine and FAD are bridged by hydrogen bonds with two water molecules. The amide group in the Gln-215 side chain interacts tightly with the harmine via pi-pi interactions (Son *et al.*, 2008).

The size and shape of the substrate/inhibitor cavity of MAO-A is restricted by Phe-208 and Ile-335. The side chain of Ile-335 can change its conformation to accommodate different inhibitors through an induced fit mechanism. A comparison of the structures of harmine bound to human MAO-A and clorgyline bound to rat MAO-A illustrates the different side chain conformations (Son *et al.*, 2008).

According to Son *et al.* (2008), the X-ray structure of human MAO-A differs from the structure of rat MAO-A and human MAO-B, with respect to the loop conformations of the amino acid residues 108-118 and 210-216. Both of these sets of residues form important components of the active site and can be used to explain why some compounds are specific for human MAO-A. In MAO-A, the three loops lying between the substrate/inhibitor cavity, formed by residues 93-95, 109-112 and 208-212, respectively, make the entrance to the cavity too narrow for harmine to pass through. Therefore it is necessary for the loops to undergo structural fluctuations to accommodate the entry of substrates into the active site of MAO-A.

#### **2.4.2.2 The structure of MAO-B:**

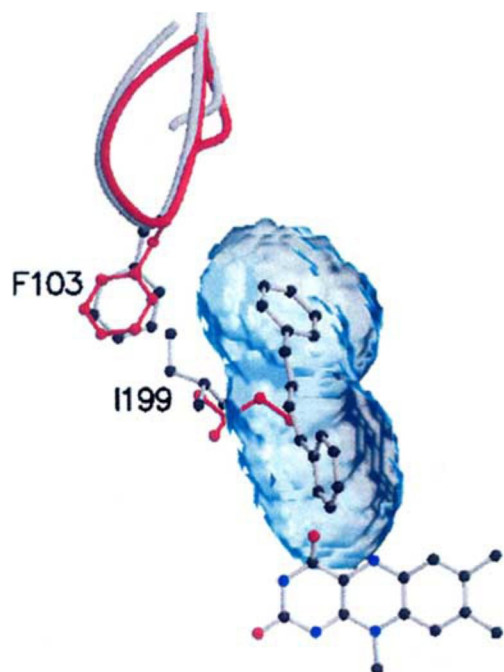
In contrast to human MAO-A, human MAO-B is dimeric. Each monomer of MAO-B consists of a globular domain anchored to the mitochondrial membrane through a C-terminal helix, similar to MAO-A. The FAD-binding region of the extra-membrane region is also similar to that of MAO-A, with FAD binding to the Cys-397 of MAO-B (Edmondson *et al.*, 2004; Hubalék *et al.*, 2005).

The residues surrounding the active site of MAO-B are very similar to those surrounding the active site of MAO-A, with a difference in only 6 of the 16 residues. A comparison of the binding mode of harmine to MAO-A with that of other reversible inhibitors to MAO-B shows that the positions of the aromatic rings of the different inhibitors are highly similar and that the inhibitors all undergo coplanar interactions with the Gln-206 of MAO-B (equivalent to Gln-215 of MAO-A) at one side of the ring and Phe-343 and Tyr-398 (equivalent to Phe-352 and Tyr-407 in MAO-A) on the opposite side. As in MAO-A, the size and shape of the MAO-B binding cavity is mainly restricted by two amino acid residues, namely Tyr-326 and Ile-199 (equivalent to Ile-335 and Phe-208 in MAO-A respectively) (Son *et al.*, 2008).

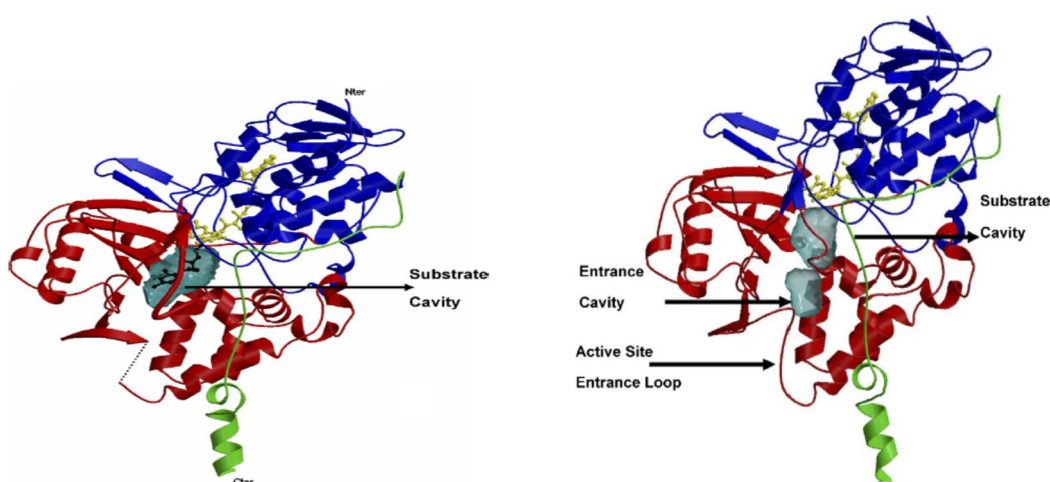
The amino acid residues that line the substrate/inhibitor cavity of MAO-B are mostly aromatic and aliphatic and this creates a hydrophobic environment. There are no amino acid residues that can act as acids or bases near the active site. The part of the cavity near the flavin site is more polar than the rest of the cavity and substrates/ inhibitors containing an amine group would preferentially bind there (Edmondson *et al.*, 2004).

In MAO-B, there are two cavities where molecules can bind: namely the substrate- and entrance cavities, separated from each other by the Ile-199 residue. The entrance cavity of MAO-B is closed off by the loop formed by residues 99-112. The cavities have a combined size of approximately 700 Å<sup>3</sup>. Inhibitors that are selective for human MAO-B and do not bind to MAO-A include 8-(3-chlorostyryl)caffeine, 1,4-diphenyl-2-butene and *trans,trans*-farnesol. The structures of these inhibitors are shown in a following section. All of these inhibitors have long extended structures that span over both the substrate and entrance cavities in MAO-B. In order for these substrates to bind, the Ile-199 has to rotate out of its normal conformation and this residue thus acts as a gatekeeper of the entrance cavity of MAO-B. When it is in a closed conformation, Ile-199 separates the substrate and entrance cavities, and when it is opened the two cavities are fused. The Phe-208 in the equivalent position of MAO-A has a larger side-chain compared to that of Ile-199 and this obstructs the binding of

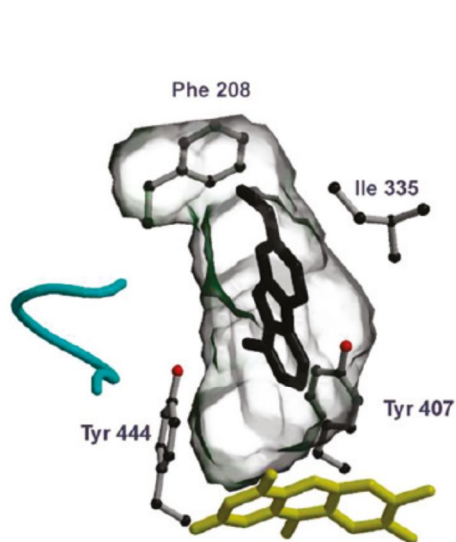
larger cavity-spanning inhibitors to MAO-A. Due to the fact that the Ile-199 residue is not conserved in MAO-B of all species, a conclusion can be drawn that it is important to use the human enzyme when conducting studies on substrate and inhibitor binding (Hubalék *et al.*, 2005).



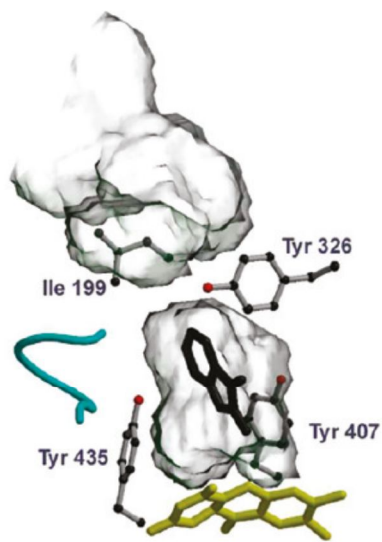
**Figure 2.4.2** The structure of 1,4-diphenyl-2-butene bound to the active site of MAO-B. The Ile-199 (in grey) is in an “open” conformation allowing the substrate/inhibitor and the entrance cavities to fuse. The “closed” position of this residue is indicated in red, like when isatin is bound to MAO-B (Edmondson *et al.*, 2004).



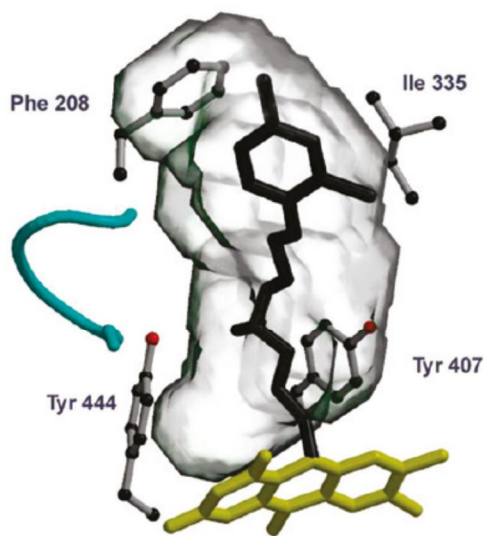
**Figure 2.4.3** Ribbon diagrams of the structures of human MAO-A (left) and human MAO-B (right) (Edmondson *et al.*, 2007).



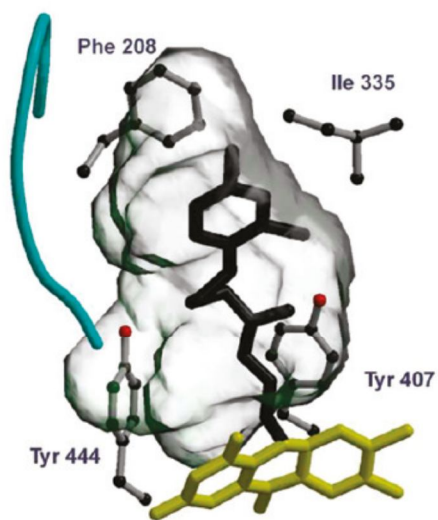
**Human MAO A**  
in complex with harmine  
at 2.2 Å resolution (2z5x)



**Human MAO B**  
in complex with isatin  
at 1.7 Å resolution (1oja)



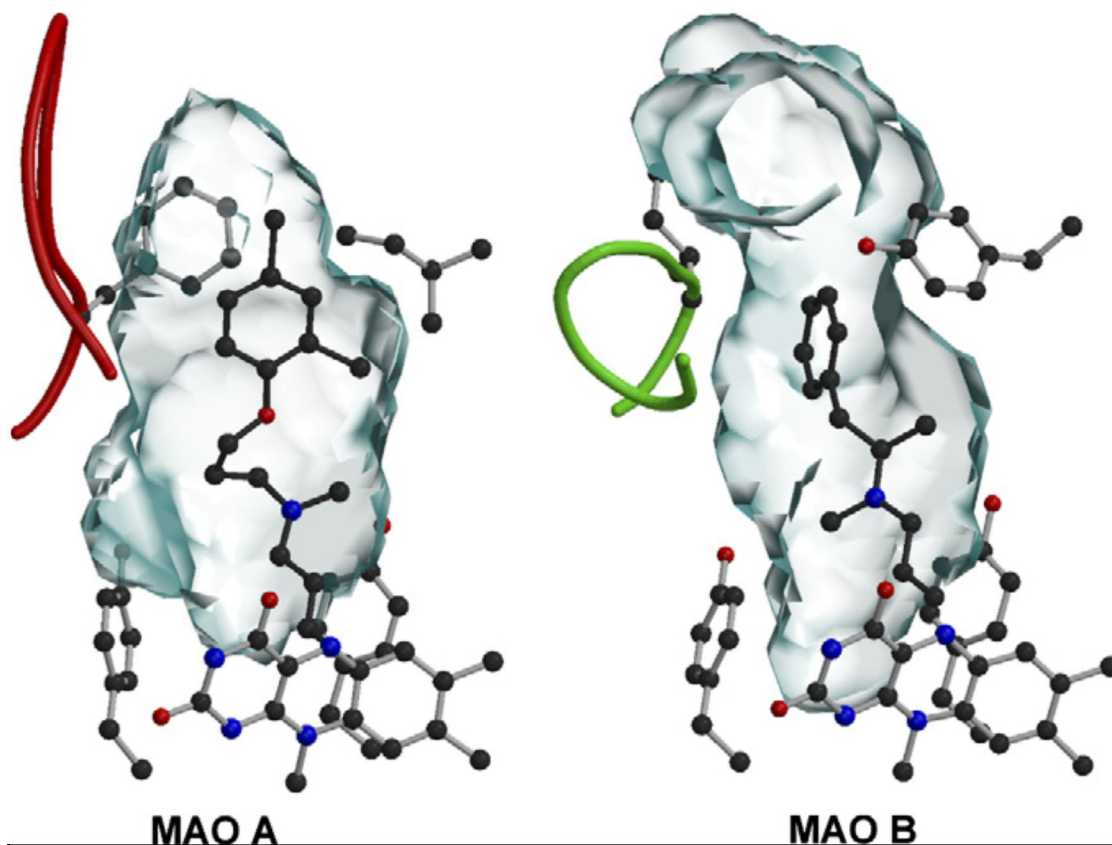
**Rat MAO A**  
in complex with clorgyline  
at 3.2 Å resolution (1o5w)



**Human MAO A**  
in complex with clorgyline  
at 3.1 Å resolution (2bxs)

**Figure 2.4.4** A comparison of the active sites of human MAO-A, human MAO-B and rat MAO-A in complex with selected inhibitors. Harmine and isatin both bind non-covalently, while clorgyline is a covalent inhibitor. The FADs are shown in yellow and the inhibitor molecules are shown in black. The cavity shaping loop is shown in cyan. The Protein Data Bank entries of the molecules are shown in parentheses (Edmondson *et al.*, 2009).





**Figure 2.4.5** Comparison of the active site cavities of human MAO-A (left) and MAO-B (right). Clorgyline is complexed with MAO-A and (*R*)-deprenyl with MAO-B. Both inhibitors form covalent N(5) flavocyanyne adducts with the FADs. The active site shaping loop is shown in red for MAO-A and in green for MAO-B (Edmondson *et al.*, 2007).

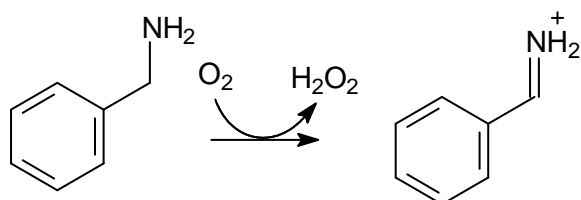
### 2.4.2.3 MAO as an imidazoline binding site:

It has been discovered that ligands that bind to the imidazoline 2 ( $I_2$ ) receptor also bind to MAO and that the  $I_2$  sites are lost in MAO-B knock-out mice. The  $I_2$  ligands were shown to inhibit MAO at millimolar concentrations, while they bind to the  $I_2$  site at nanomolar concentrations. They have a higher affinity for MAO-B than for MAO-A. Studies done with the irreversible  $I_2$  ligand, 5-isothiocyanato-2-benzofuranyl-imidazoline (2-BFI), show that it blocked the  $I_2$  site without affecting MAO activity or the binding of ligands to MAO, which lead to the conclusion that the  $I_2$  site is distinct from the active site (Ramsay, 2012).

The studies have also shown that normal MAO-B only bound small amounts of 2-BFI, but when the entrance cavity of MAO-B was altered by the irreversible binding of tranylcypromine, the residues surrounding the region where the entrance and substrate cavities join rearranged to form a new binding site for the 2-BFI, and this increased its binding. Therefore the  $I_2$  binding site is located at the gate between the entrance and the substrate/inhibitor cavities of MAO (Ramsay, 2012).

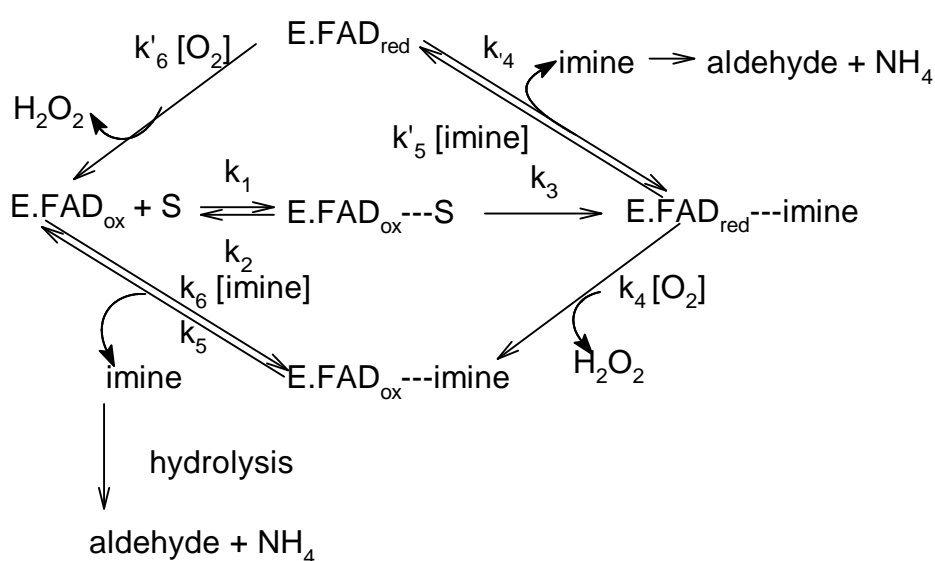
### 2.4.3 The MAO catalytic cycle:

Both MAO-A and MAO-B catalyze the oxidative deamination of primary, secondary and some tertiary amines as shown in the figure below. During the oxidation reaction there is a hydrogen transfer from the  $\alpha$ -CH<sub>2</sub>. This reaction is stereospecific and it is strictly pro-R for both MAO-A and MAO-B (Edmondson *et al.*, 2004).



**Figure 2.4.6** The catalytic pathway of the MAO-enzymes (Adapted from Edmondson *et al.*, 2004).

#### 2.4.3.1 The ternary complex mechanism:



**Figure 2.4.7** Reaction pathway for MAO catalysis (Adapted from Edmondson *et al.*, 2004).

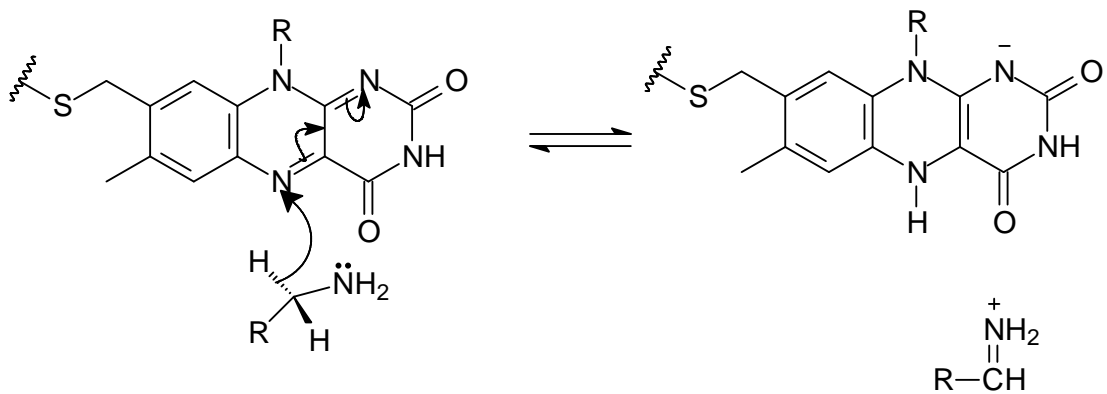
The pathway above illustrates the two general catalytic pathways of MAO-A and MAO-B using a ternary complex mechanism. For most substrates, catalysis by MAO-A and MAO-B follows the pathway illustrated in the lower loop of the figure. In this pathway oxygen reacts with the enzyme-product complex, before the product dissociates. The deprotonated amine, rather than the protonated form of the substrate binds to the active site of the enzyme where it is oxidized to the corresponding imine with the covalent FAD cofactor being reduced to its hydroquinone form. To complete the catalytic cycle, the reduced FAD cofactor reacts with

oxygen to generate the oxidized flavin and hydrogen peroxide. The dissociated protonated imine is released from the enzyme after which it undergoes non-catalyzed hydrolysis to form ammonium. MAO-A has a  $K_m(O_2)$  value of approximately 6  $\mu\text{M}$  while for MAO-B this value is approximately 250  $\mu\text{M}$  – the concentration of  $O_2$  in air saturated solutions. Therefore at a saturating concentration of the amine substrate, MAO-A is operating at maximal velocity, while MAO-B is only operating at half maximal velocity (Edmondson *et al.*, 2004; Edmondson *et al.*, 2009).

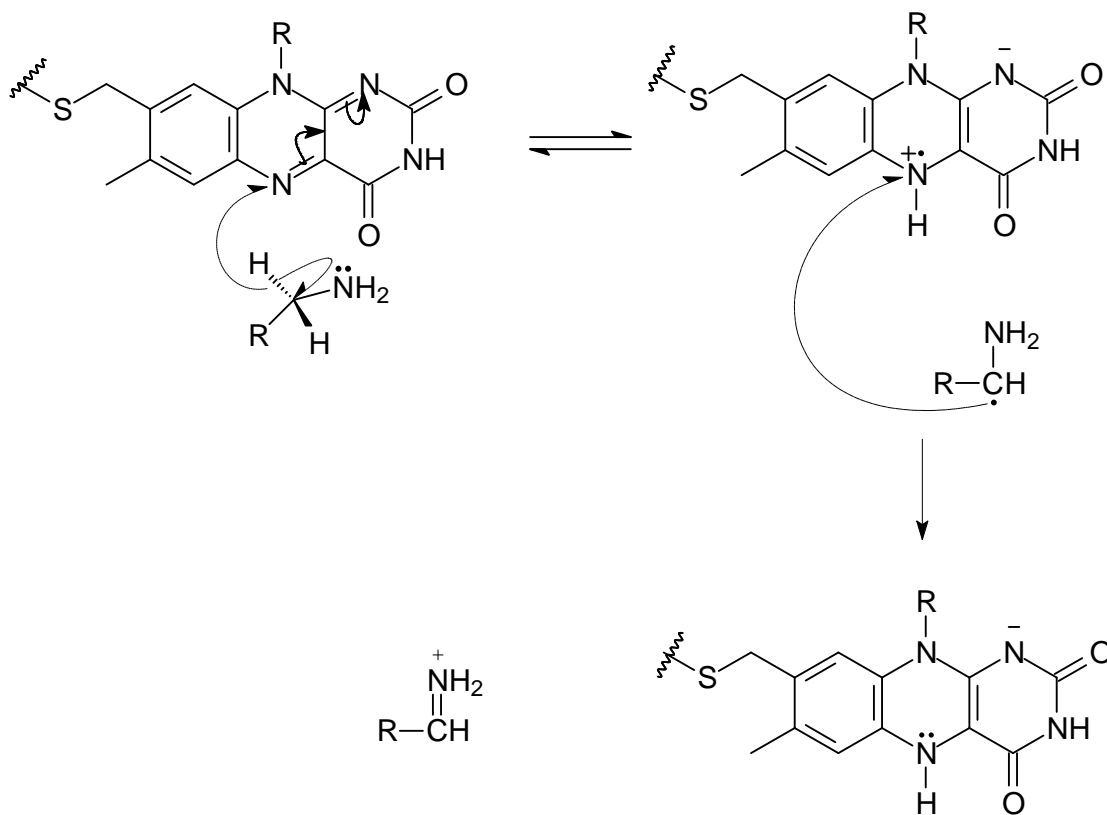
#### **2.4.3.2 Mechanisms for the cleavage of C-H bonds:**

According to Edmondson *et al.* (2009), the cleavage of C-H bonds can occur by three possible mechanisms, namely heterolytic hydride transfer, heterolytic proton abstraction (including the single electron transfer mechanism and the polar nucleophilic mechanism) or homolytic hydrogen atom abstraction. There are arguments for and against most of these mechanisms.

In the hydride mechanism, the hydrogen is abstracted as a hydride ion and both bonding electrons follow the hydrogen to its acceptor. It is favoured for the flavin-dependent amino acid oxidases. In the homolytic hydrogen atom abstraction mechanism, the C-H bond is homolytically cleaved to form a carbon based radical and a hydrogen atom. These reactions are usually favoured by radical generating systems like ribonucleotide reductase. In the proton abstraction reactions (discussed in detail below) a proton and a carbanionic substrate intermediate are formed (Edmondson *et al.*, 2007).



Hydride mechanism

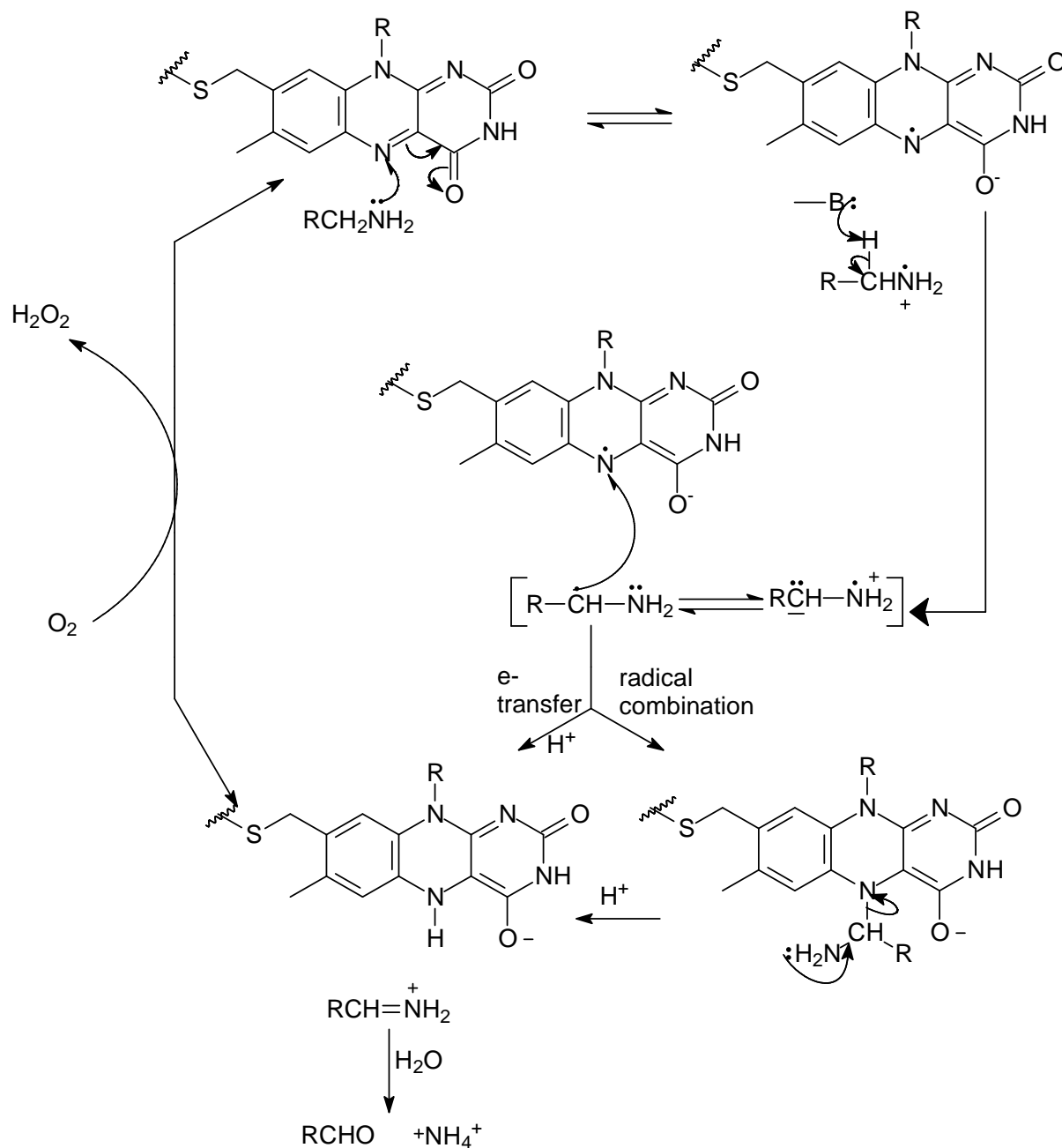


Hydrogen atom transfer mechanism.

**Figure 2.4.8** General mechanism of C-H bond cleavage in the hydride mechanism and the homolytic hydrogen atom transfer mechanism (Adapted from Edmondson *et al.*, 2009).

### 2.4.3.3 Heterolytic proton abstraction mechanisms:

#### The single electron transfer mechanism:

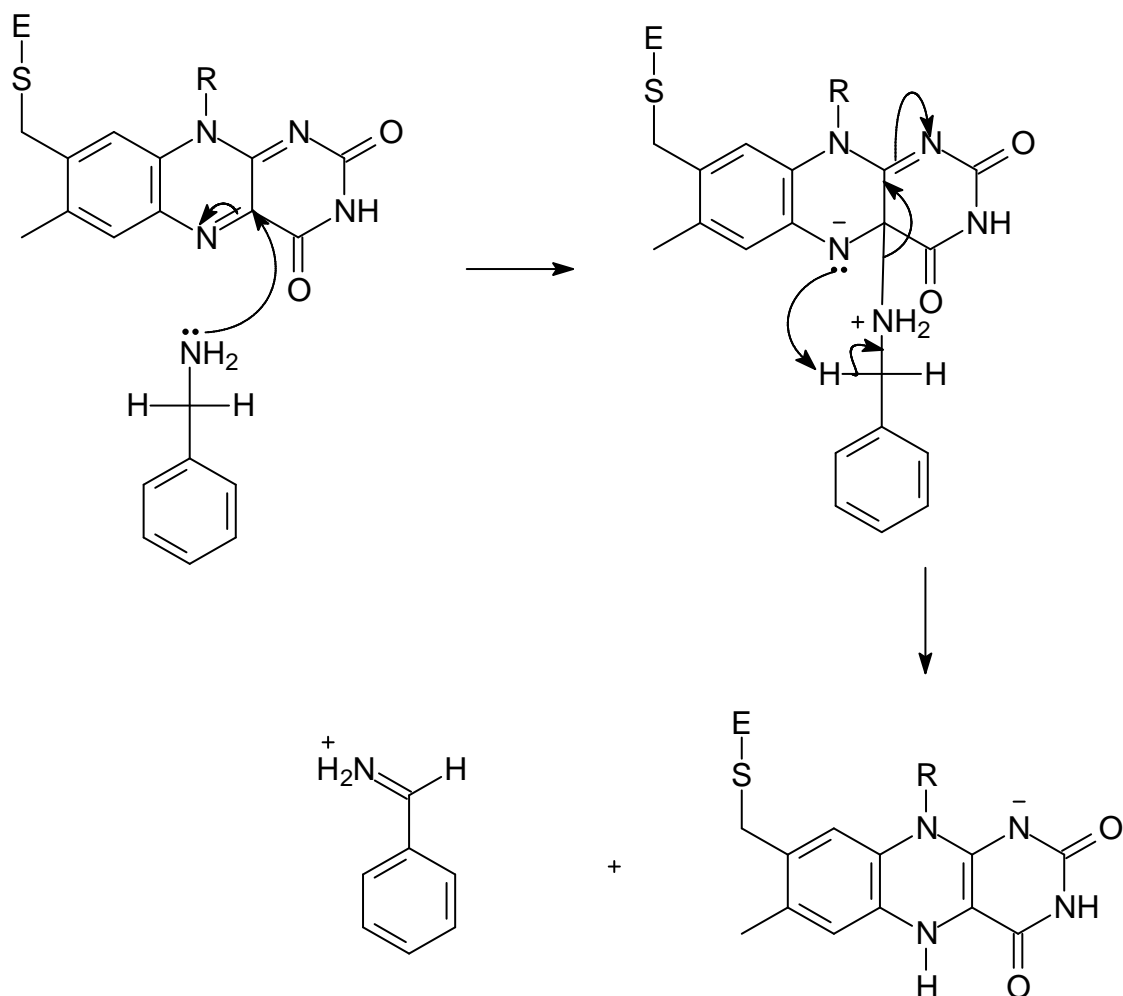


**Figure 2.4.9** Single electron transfer mechanism of MAO catalysis (Adapted from Silverman, 2004).

In the single electron transfer mechanism of amine oxidation proposed by Silverman (2004), the first step is the transfer of one of the electrons of the lone pair on the amine nitrogen to the FAD N(5) to form an aminium cation radical and a flavin radical. The formation of the aminium radical lowers the pKa of the  $-C-H$  which allows the abstraction of a proton by the

active site base in the catalytic site. However, according to Edmondson *et al.* (2004), there are no residues in the active site of MAO-B that can act as bases and other studies done to find a base in the active site failed to do so. Therefore the single electron transfer mechanism is improbable.

**The polar nucleophilic mechanism:**



**Figure 2.4.10** The polar nucleophilic mechanism of MAO catalysis (Adapted from Edmondson *et al.*, 2004).

A more probable mechanism than the single electron transfer mechanism is the polar nucleophilic mechanism proposed by Edmondson *et al.* (2004). In this mechanism, the deprotonated amine functionality of the substrate nucleophilically attacks the flavin at the C(4a) position to form a flavin C(4a)-nucleophile adduct, which rearranges itself to form the imine product.

## **2.4.4 MAO inhibitors:**

### **2.4.4.1 Introduction:**

It is interesting to note that MAO inhibitors are mainly used in psychiatric disorders such as depression and in neurological disorders such as PD and Alzheimer's disease. Non-selective MAO and selective MAO-A inhibitors mainly affect neurotransmitters that are important in depression and anxiety. These drugs increase the availability of the neurotransmitters at the nerve terminals. Reversible, selective MAO-A inhibitors have a markedly better safety profile than non-selective inhibitors (Yamada & Yasuhara, 2004). It is interesting to note that in the catecholamine biosynthesis pathway, certain intermediate products, such as levodopa, act as competitive, reversible inhibitors of MAO-A to a greater extent than of MAO-B. Thus the products in the catecholamine biosynthesis pathways could modulate the activity of MAO to regulate their metabolism. This may have clinical significance in patients receiving high doses of levodopa (Nagatsu, 2004).

In the human brain approximately 75% of all the MAO enzymes are the MAO-B subtype. Therapeutic MAO inhibitors used in PD are exclusively MAO-B selective. Among these inhibitors is (*R*)-deprenyl, a selective irreversible inhibitor of MAO-B. (*R*)-Deprenyl increases the basal dopamine levels in the nigrostriatal dopamine input pathway and prolongs the anti-parkinsonism effect of levodopa (Yamada & Yasuhara, 2004). (*R*)-Deprenyl may also be beneficial in patients experiencing a mild 'on-off' syndrome. (*R*)-Deprenyl is usually used as an adjuvant in levodopa therapy, because it exerts a small effect when used alone (Aminoff, 2009).

Another MAO-B inhibitor, rasagiline, is more potent than (*R*)-deprenyl as a MAO-B inhibitor and can be used for the early symptomatic treatment of PD. Non-selective inhibitors of MAO should not be used in PD, because in combination with levodopa such drugs may lead to a hypertensive crisis due to the peripheral accumulation of noradrenalin (Aminoff, 2009).

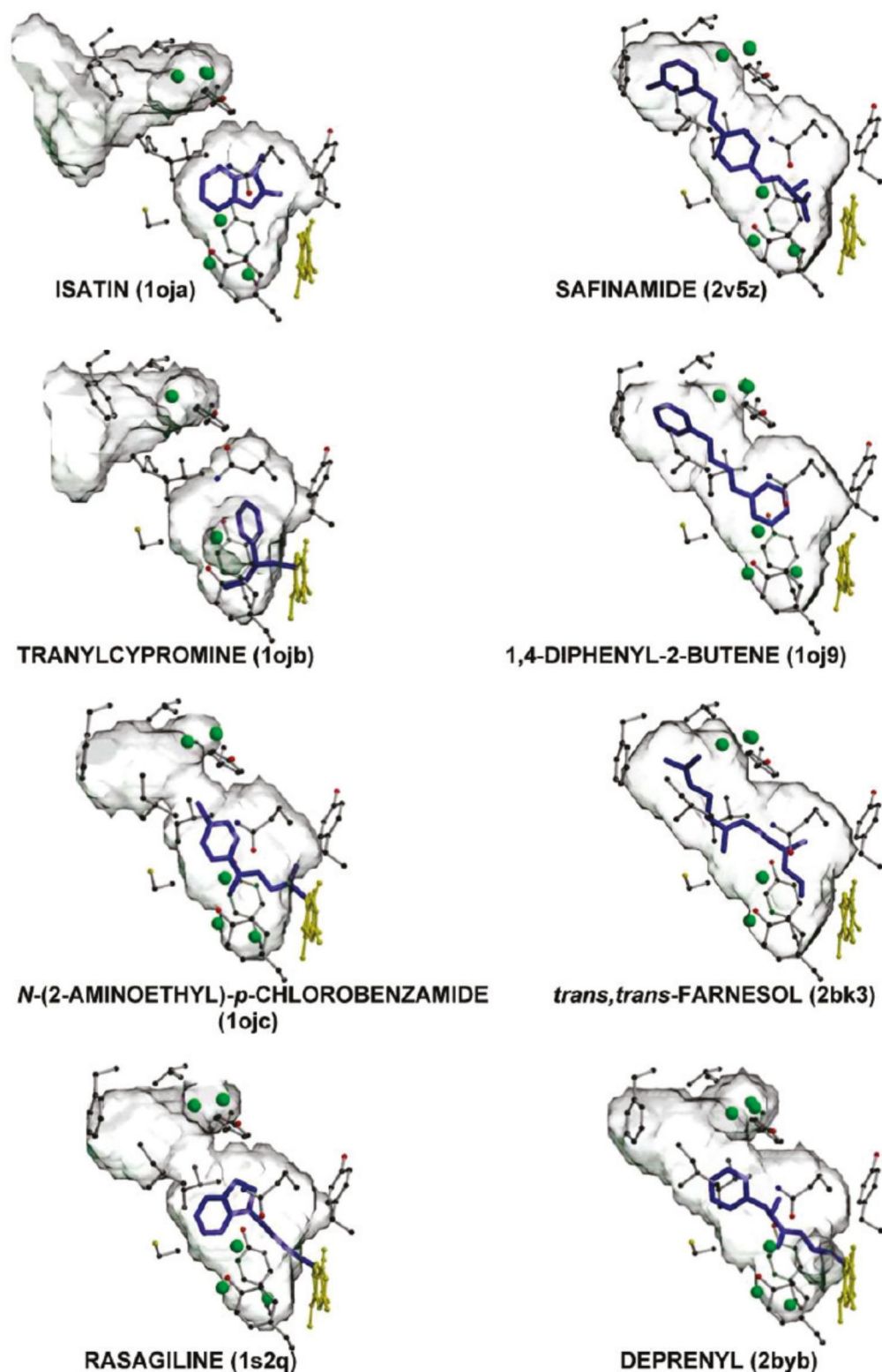
**Table 2.4.1** MAO inhibitors (adapted from Yamada & Yasuhara, 2004)

<b>MAO inhibitors</b>	<b>Selectivity</b>	<b>Reversibility</b>
<b>Tranlycypromine</b>	Non-selective	Irreversible
<b>Phenelzine</b>	Non-selective	Irreversible
<b>Nialamide</b>	Non-selective	Irreversible
<b>Isocarboxazid</b>	Non-selective	Irreversible
<b>Clorgyline</b>	MAO-A selective	Irreversible
<b>Moclobemide</b>	MAO-A selective	Reversible
<b>Brofaromine</b>	MAO-A selective	Reversible
<b>Toloxatone</b>	MAO-A selective	Reversible
<b>Cimoxatone</b>	MAO-A selective	Reversible
<b>Befloxatone</b>	MAO-A selective	Reversible
<b>Pargyline</b>	MAO-B selective	Irreversible
<b>(R)-deprenyl</b>	MAO-B selective	Irreversible
<b>Rasagiline</b>	MAO-B selective	Irreversible

**2.4.4.2 The mechanisms of inhibition of MAO by selected inhibitors:**

In this section, the binding of various inhibitors to MAO is discussed.

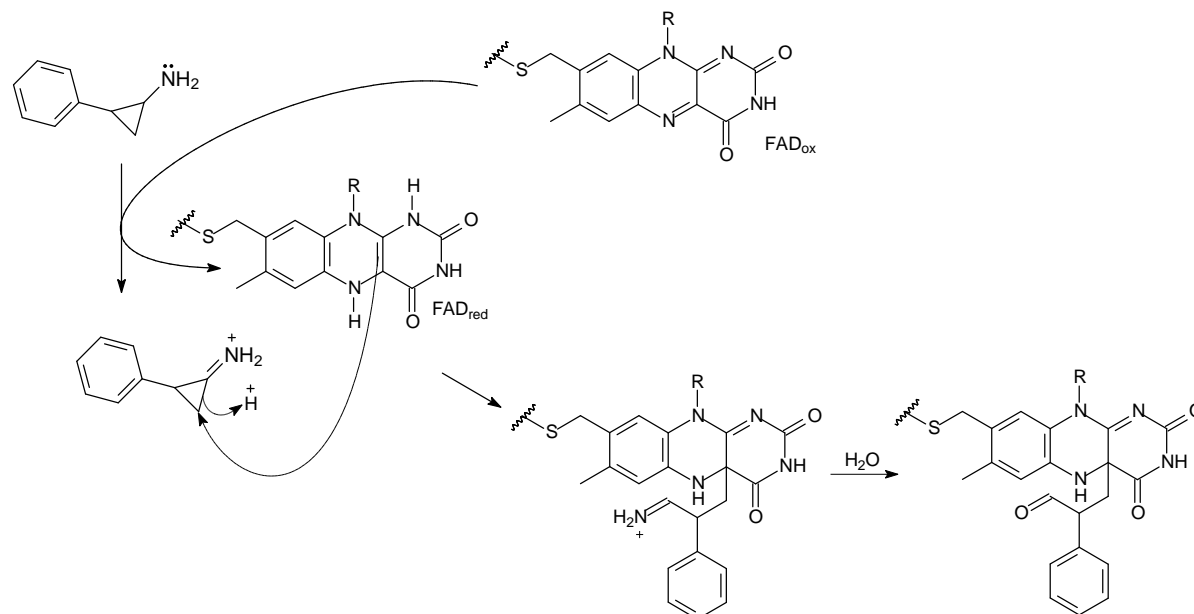




**Figure 2.4.11** The active site cavity structures of human MAO-B in combination with various inhibitors. The protein data bank structures for the various inhibitors are in brackets. The flavin is yellow, the inhibitor is blue and the water molecules are green spheres (Edmondson *et al.*, 2009).

## Mechanisms of irreversible inhibitors:

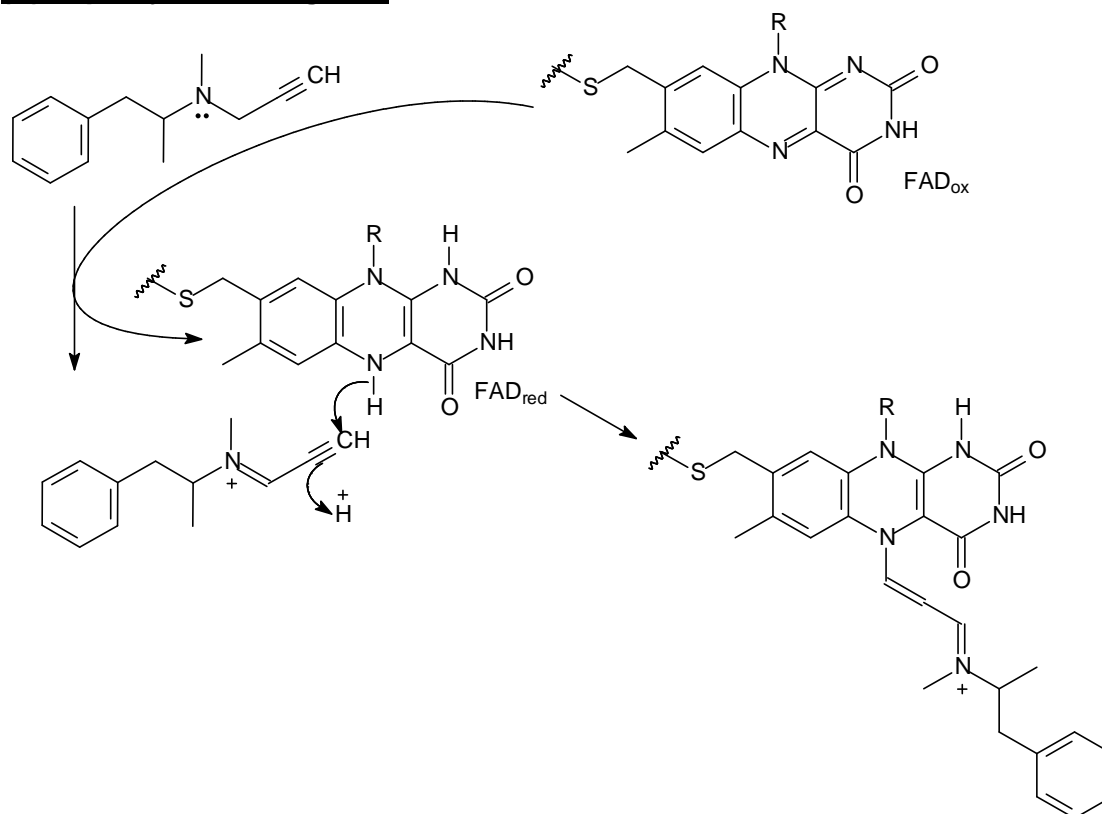
### Tranlycypromine:



**Figure 2.4.12** Hypothetical mechanism for the inactivation of MAO by tranlycypromine (Adapted from Edmondson *et al.*, 2004).

The strained cyclopropyl ring of tranlycypromine is opened by oxidation by MAO, where after it forms a flavin C(4a) adduct that decays slowly in normal enzyme conditions, but rapidly when the enzyme is denaturised. There has also been speculation about the involvement of an adduct to a cysteine residue, but since there are no cysteine residues in the active site, the adducts are formed when the oxidized product of tranlycypromine dissociates into the medium where it can react with a cysteine residue elsewhere in the enzyme. The phenyl ring of tranlycypromine is parallel to the flavin ring rather than perpendicular, like in most other complexes. The Ile-199 gate is in its 'closed' conformation (Binda *et al.*, 2003; Edmondson *et al.*, 2004).

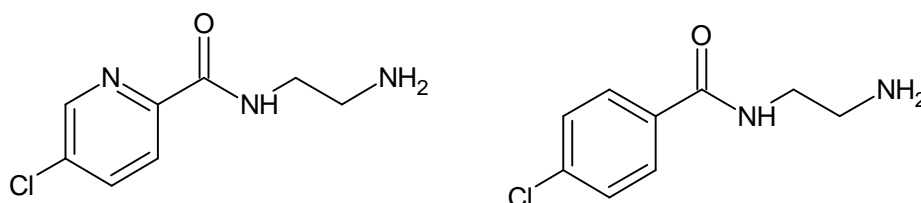
### **(R)-deprenyl and rasagiline:**



**Figure 2.4.13** Hypothetical mechanism for the inactivation of MAO by (R)-deprenyl (Adapted from Edmondson *et al.*, 2004).

(R)-deprenyl (previously known as L-deprenyl) and rasagiline are acetylenic compounds and act as selective, irreversible inhibitors of MAO-B. These inhibitors are mechanism-based inhibitors and, similar to tranylcypromine, act as substrates for the MAO-B enzyme. After oxidation, the product undergoes nucleophilic attack by the reduced FAD. The oxidized N-propargyl group thus reacts covalently with the N(5) of the isoalloxazine of FAD. One mole of the inhibitor binds to one mole of the enzyme (Binda *et al.*, 2007; Weinreb *et al.*, 2010). The Ile-199 gate is in its 'open' conformation upon binding of the inhibitor (Hubalék *et al.*, 2007).

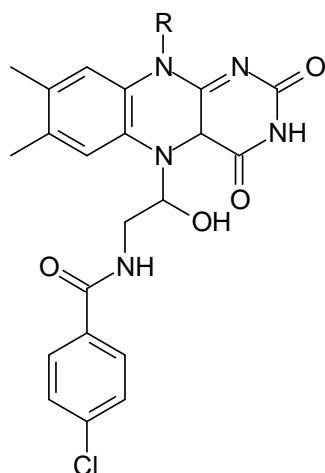
### **Lazabemide and N-(2-aminoethyl)-p-chlorobenzamide:**



**Figure 2.4.14** The structures of lazabemide (left) and N-(2-aminoethyl)-p-chlorobenzamide (right).

Lazabemide and N-(2-aminoethyl)-p-chlorobenzamide, are part of the N-(2-aminoethyl)arylcaboxamide class of MAO-B inhibitors. They are oxidized by MAO-B and

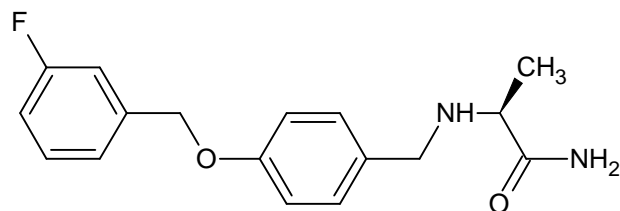
form adducts with the flavin N(5). The aromatic rings of the inhibitors adopt the same orientation and position as isatin. The amine nitrogen is released before the N(5) adduct is formed (Binda *et al.*, 2003).



**Figure 2.4.15** The structure of the flavin adduct with the MAO-B catalyzed oxidation product of N-(2-aminoethyl)-*p*-chlorobenzamide (adapted from Binda *et al.*, 2003)

### **Reversible inhibitors:**

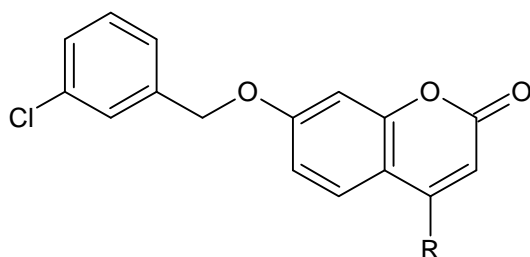
#### **Safinamide:**



**Figure 2.4.16** The structure of safinamide.

Safinamide is currently in phase III trials for the treatment of PD. It has a novel mode of action as a dopamine modulator, where it acts as both a selective, reversible inhibitor of MAO-B and it blocks the reuptake of dopamine with an additional effect on the glutamate pathway. Safinamide binds non-covalently to MAO-B in an extended conformation that occupies both cavities. The 3-fluorobenzoyloxy group is located in the entrance cavity, while the aromatic ring is in the substrate/inhibitor cavity with the amide group orientated towards the FAD. There are three ordered water molecules present in the active site upon safinamide binding. The amide group of safinamide interacts with Gln-206 and one of the ordered water molecules via hydrogen bonding (Binda *et al.*, 2007).

### Coumarin derivatives:

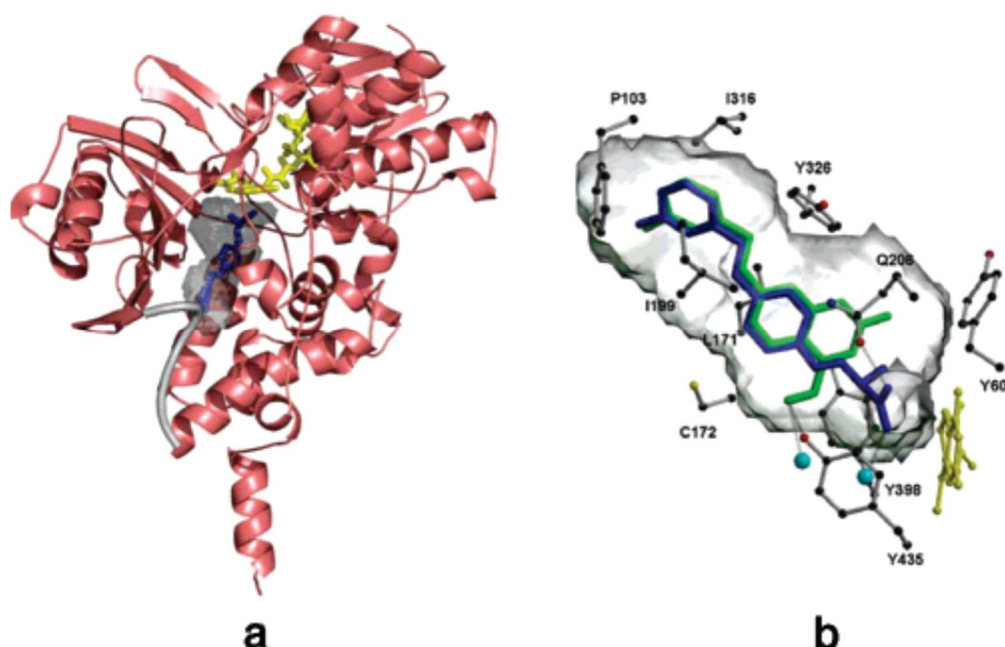


R= CH<sub>2</sub>NHCH<sub>3</sub>                    7-(3-chlorobenzyloxy)-4-(methylamino)methyl-coumarin

R= CHO                                7-(3-chlorobenzyloxy)-4-carboxaldehyde-coumarin

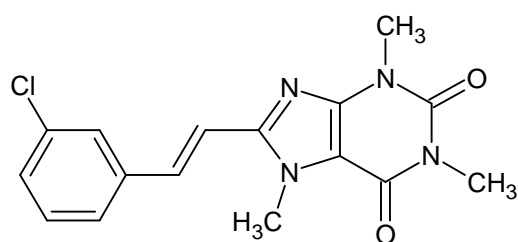
**Figure 2.4.17** The structure of coumarin derivatives.

Coumarins with a benzyloxy substituent on position 7 are potent, short acting and reversible MAO-B inhibitors both *in vitro* and *in vivo*. Similar to safinamide, they also bind non-covalently and they span the active site cavity in their entire length. The benzyloxy group occupies the entrance cavity while the coumarin moiety binds in the substrate cavity with the oxygen in the pyran ring pointing towards Tyr-326 at the top of the cavity in front of the FAD (Binda *et al.*, 2007).



**Figure 2.4.18** MAO-B in complex with safinamide (in blue) and 7-(3-chlorobenzyloxy)-4-carboxaldehyde-coumarin (green). (a) a ribbon diagram of the overall structure. (b) Close-up view of the inhibitors in the active site cavity. The FAD is shown in yellow and the active site residues in grey (Binda *et al.*, 2007).

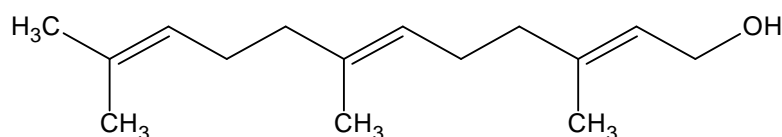
### Caffeine derivatives:



**Figure 2.4.19** The structure of 8-(3-chlorostyryl)caffeine.

8-(3-Chlorostyryl)caffeine is a potent and selective inhibitor of MAO-B but not of MAO-A. It is also an adenosine A<sub>2A</sub> receptor agonist. Although no direct evidence is available, it is thought to span both the entrance and the substrate cavities of MAO-B upon binding (Hubalék *et al.*, 2005).

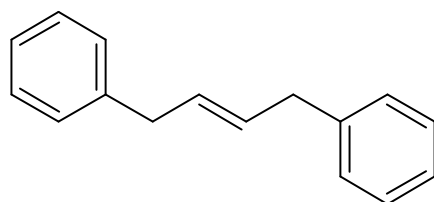
### Trans,trans-farnesol:



**Figure 2.4.20** The structure of *trans,trans*-farnesol.

*Trans,trans*-farnesol is a component of tobacco smoke. It acts as a potent, specific, reversible MAO-B inhibitor. It spans the both the entrance and substrate/inhibitor cavities with the OH-group located close to the C(4a) position of the flavin and the 1-methylene carbon close to the N(5) of the flavin. There are additional hydrophobic interactions between the isoprenoid chain and the amino acids of the active cavity (Hubalék *et al.*, 2005).

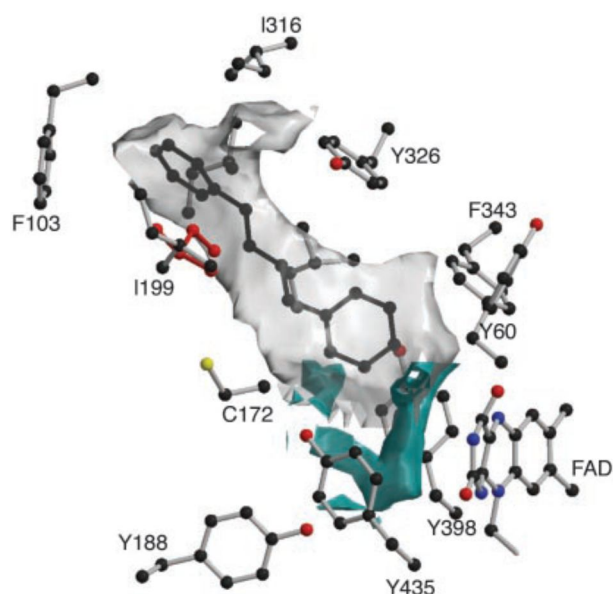
### 1,4-Diphenyl-2-butene:



**Figure 2.4.21** The structure of 1,4-diphenyl-2-butene.

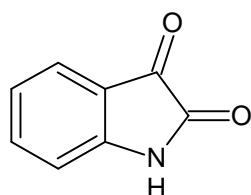
This compound is a potent, selective, reversible inhibitor of MAO-B. 1,4-Diphenyl-2-butene is a contaminant released by polystyrene micro-bridges used to crystallize MAO-B. It spans both the entrance and substrate/inhibitor cavities with the Ile-199 gate in an 'open'

conformation. The planes of the phenyl rings of the inhibitor are orthogonal to one another. Most of its interactions with MAO-B are hydrophobic, as shown in figure 2.4.22 (Binda *et al.*, 2003; Edmondson *et al.*, 2004; Hubalék *et al.*, 2005).



**Figure 2.4.22** The complex of MAO-B with 1,4-diphenyl-2-butene (in dark gray). The inhibitor ring in contact with Phe-103, Ile-199 and Ile-316 occupies the entrance cavity, while the inhibitor ring in contact with Tyr-398 and Tyr-435 occupies the substrate cavity. The conformation of Ile-199 in its 'closed' conformation is shown in red, while the 'open' conformation is shown in black and grey. The grey area represents the GRID molecular interaction fields computed with an aromatic sp<sup>2</sup> carbon probe, while the cyan area represents a neutral NH<sub>2</sub> probe (Binda *et al.*, 2003).

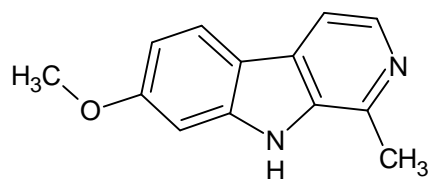
### **Isatin:**



**Figure 2.4.23** The structure of isatin.

Isatin is a non-selective, reversible inhibitor of both MAO-A and MAO-B (Hubalék *et al.*, 2005). The dioxindole ring is orientated perpendicular to the flavin ring in the substrate cavity, with the oxo groups on the pyrrole pointing towards the flavin. The 2-oxo and the pyrrole amine group have hydrogen bond interactions with the water molecules in the active site. The rest of the interactions are formed by Van der Waals contacts between the isatin ring and the amino acid residues of the substrate cavity (Binda *et al.*, 2003).

### **Harmine:**



**Figure 2.4.24** The structure of harmine.

Harmine is a reversible inhibitor of MAO-A. Its interactions with the active site are described in the section on the structure of MAO-A (Son *et al.*, 2008).

#### **2.4.4.3 Interactions and adverse effects of MAO-inhibition:**

Drug interactions with MAO inhibitors can be both pharmacokinetic and pharmacodynamic interactions. Pharmacokinetic interactions occur when MAO inhibitors affect the metabolism or protein binding of another drug. Several MAO inhibitors are substrates of the cytochrome P450 enzymes. Interactions with other drugs that induce or inhibit those enzymes may occur. Tranylcypromine is a relatively potent inhibitor of CYP2C19. Moclobemide is an inhibitor of CYP2D6, CYP2C19 and CYP1A2. Many commonly prescribed drugs such as  $\beta$ -blockers, other antidepressants, etc. are substrates of CYP2D6. Other inhibitors of the CYP2D6 system can inhibit the clearance of moclobemide (Yamada & Yasuhara, 2004).

Pharmacodynamic interactions occur when MAO inhibitors potentiate or antagonize the effects of other drugs. An example is when MAO inhibitors are combined with selective serotonin reuptake inhibitors, the life-threatening serotonin syndrome can occur. Another example is the so-called 'cheese reaction' in combination with tyramine rich foods. Although tyramine is a substrate of both MAO-A and MAO-B, only traditional irreversible MAO-A inhibitors, such as tranylcypromine, can cause a hypertensive crisis. By inhibiting MAO-A, the first-pass metabolism of exogenous tyramine is inhibited, thus leading to an accumulation of tyramine that causes a hypertensive crisis. The time required for MAO function to be restored after treatment with tranylcypromine is several weeks. Tyramine is prevalent in most cheeses, yoghurt, wines, beer, sardines, herring, caviar, yeast extracts, liver, processed meats and ripe avocados, and these foods should be avoided in patients receiving irreversible MAO-A inhibitors. Dietary tyramine should be avoided for up to 4 weeks after the cessation of tranylcypromine therapy and for more than 11 weeks after the cessation of phenelzine therapy (Da Prada *et al.*, 1988; Yamada & Yasuhara, 2004; Youdim & Bakhle, 2006).



Side effects of the MAO-B inhibitors (*R*)-deprenyl and rasagiline, especially in combination with levodopa therapy, include: nausea and anorexia, dry mouth, dyskinesia and orthostatic hypotension in patients with PD. When used alone, they can cause nausea and anorexia, musculoskeletal injuries and cardiac arrhythmias. Elevated levels of the liver enzymes aspartate aminotransferase and alanine aminotransferase have also occurred (Yamada & Yasuhara, 2004).

#### **2.4.4.4 The clinical significance of MAO inhibition:**

##### **The role of MAO-inhibitors in the treatment of depression:**

Depressive disorders are characterized by sadness that is severe enough to interfere with normal functioning. It is related to the normal emotions of sadness and mourning but it does not disappear when the external cause of those emotions disperses. There is often a decreased interest in pleasure or activities, and the exact cause is unknown. The term depression is often used to refer to any of the depressive disorders, including major depressive disorder, dysthymia, depressive disorders not otherwise specified, substance-induced depressive disorder and depressive disorder due to a general physical condition (Belmaker & Agam, 2008; Coryell, 2011).

Depressive disorders can occur at any age, but they usually develop between the mid-teens and 30's. Although the exact cause is unknown, genetic and environmental factors are thought to contribute to the development of depressive disorders. Heredity accounts for approximately 37% of the aetiology and genetic factors probably influence the tendency to develop depressive responses to adverse effects. Other theories have investigated the link between the abnormal regulation of cholinergic, catecholaminergic and serotonergic neurotransmission and depression. In particular, neuro-endocrine dysregulation of the hypothalamic-pituitary-adrenal, hypothalamic-pituitary-thyroid and growth hormone axes are thought to be involved. Episodes of major depression are usually preceded by major life stresses but the episode is only long lasting in people that are predisposed to mood disorders. Women are at higher risk, but so far no definite theory can explain why. A possible risk factor is that women have higher levels of MAO, and therefore they have less neurotransmitters that are important for mood regulation (Belmaker & Agam, 2008; Coryell, 2011).

The signs and symptoms of depression are cognitive, psychomotor and other types of dysfunction such as poor concentration, fatigue, loss of pleasure and sexual desire, as well

as a depressed mood. Major depression is classified as episodes that include more than five mental or physical symptoms that last for more than two weeks. One of the symptoms must be deep sadness, despair or loss of interest in usual activities. Other mental symptoms include feelings of worthlessness and guilt, recurrent thoughts of death or suicide and a loss of concentration. Physical symptoms include changes in appetite and weight, loss of energy and fatigue, psychomotor agitation or retardation and sleep disorders (Belmaker & Agam, 2008; Coryell, 2011). Major depression is an important illness because it ranks fourth among causes of death or injury (Meyer *et al.*, 2006). Dysthymia is classified as low-level or sub threshold depressive symptoms that persists for more than 2 years (Coryell, 2011).

Depressive disorders are treated with pharmacotherapy in conjunction with psychotherapy. MAO is thought to have a close causative relation to depression due to its control over serotonin, dopamine and noradrenalin levels (Meyer *et al.*, 2006; Nagatsu, 2004). Although selective serotonin reuptake inhibitors and tricyclic antidepressants are the drugs of choice in the treatment of depression, the classical indication for MAO inhibitors is depression. The antidepressant effect of non-selective, irreversible MAO inhibitors, such as tranylcypromine, was discovered in the 1950's, but these drugs had considerable side effects. Patients had to follow a tyramine free diet and it was thus soon replaced by safer, more convenient antidepressants. More recently the reversible selective MAO-A inhibitor, moclobemide, has been proven to be effective in the wide spectrum treatment of major depression (Da Prada *et al.*, 1988; Yamada & Yasuhara, 2004).

Moclobemide's effective antidepressant action together with its absence of anticholinergic side effects and sedation has given it an advantage over the more commonly used tricyclic antidepressants. It is especially useful in the elderly population, because they are more susceptible to the side effects of the tricyclic antidepressants and moclobemide exhibits similar pharmacokinetics in both young and elderly patients. With normal aging noradrenergic neurotransmission may decrease and MAO activity may increase. This may lead to a change in the sensitivity to antidepressant drugs and a different clinical profile is needed to treat depression in the elderly (Yamada & Yasuhara, 2004; Youdim & Bakhle, 2006).

### **MAO inhibitors in the treatment of anxiety disorders:**

Irreversible, non-selective MAO inhibitors have demonstrated efficacy in the treatment of certain types of anxiety disorders, including social anxiety disorder, panic disorder, obsessive compulsive disorder and posttraumatic stress disorder. MAO inhibitors were the

first antidepressants shown to be effective in social anxiety disorders, but the dietary restrictions and side effect profile of MAO inhibitors often lead to other drugs being used. Moclobemide was approved for the treatment of social anxiety disorder in Europe in 1996. For panic disorders MAO inhibitors, selective serotonin reuptake inhibitors, tricyclic antidepressants or high-potency benzodiazepines may be used. MAO inhibitors are still the most effective class of antidepressants for the treatment of posttraumatic stress disorder and they have been shown to improve the re-experiencing and avoidance symptoms. For obsessive compulsive disorder, clomipramine and selective serotonin reuptake inhibitors are preferred to MAO inhibitor therapy (Yamada & Yasuhara, 2004).

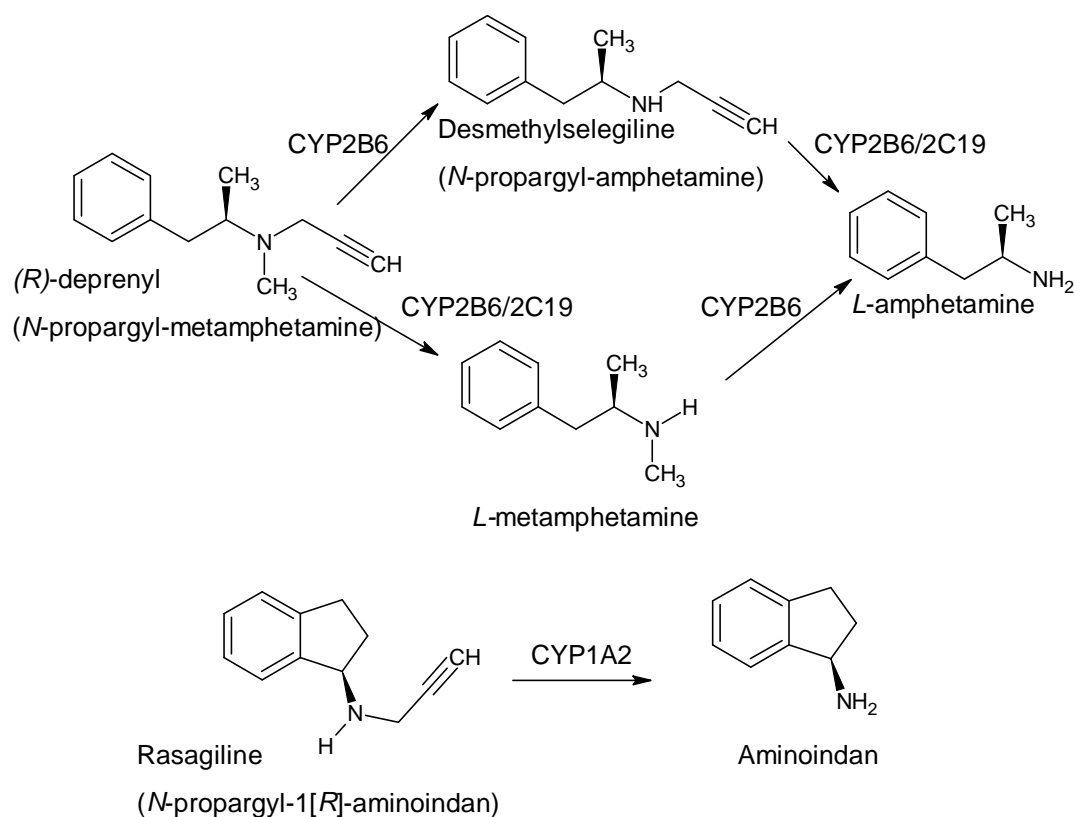
### **The role of MAO-B inhibitors ((R)-deprenyl and rasagiline) in the treatment of Parkinson's disease and their neuroprotective properties:**

Because dopamine is preferentially deaminated by MAO-B in the brain, MAO-B inhibitors should increase the basal dopamine levels of patients with PD. The effect of levodopa therapy should also be prolonged because MAO-B inhibitors increase the time that dopamine levels are elevated in the brain (Yamada & Yasuhara, 2004). (R)-Deprenyl and rasagiline prevent the metabolism of dopamine in the brain by their MAO-B inhibitory action (Magyar & Szende, 2004).

(R)-Deprenyl is a propargylamine derivative of *L*-amphetamine that is metabolized to metamphetamine by depropargylation. (R)-deprenyl is also demethylated to form desmethyl-deprenyl. Both metabolites are converted to *L*-amphetamine (as shown in figure 2.4.25). The methamphetamine and amphetamine, together with the parent drug, block the reuptake and increase the release of dopamine and noradrenalin. *p*-Hydroxylated metabolites also form and are conjugated with glucuronic acid. Liver cytochrome-P450 enzymes play a significant role in the metabolism of (R)-deprenyl, which undergoes significant 'first-pass' metabolism. The metabolites are excreted in the urine (Magyar & Szende, 2004).

Since both *L*-metamphetamine and *L*-amphetamine are powerful stimulants with amphetamine-like sympathomimetic actions that can result in increased blood pressure and heart rate, a new highly potent MAO-B inhibitor was required. Rasagiline was developed as an anti-Parkinsonian drug. It is stereo-specific, with the *R*-enantiomer being a thousand fold more potent than the *S*-enantiomer as an MAO-B inhibitor. Rasagiline is metabolized by the hepatic cytochrome P-450 isoenzyme 1A2, in a N-dealkylation process to form the nontoxic metabolite 1-(*R*)-aminoindan, which has weak MAO inhibitory properties. Rasagiline is tenfold more potent than (R)-deprenyl and it maintains its selectivity for MAO-B with chronic

therapy. Rasagiline also has a greater inhibitory potential on human platelet MAO-B (Weinreb *et al.*, 2010). Studies have also shown that both rasagiline and its aminoindan metabolite act as non-selective glutamate receptor antagonists *in vitro* (Gerlach *et al.*, 2012).



**Figure 2.4.25** The metabolism of (*R*)-deprenyl and rasagiline (Adapted from Gerlach *et al.*, 2012).

Early treatment of PD patients with (*R*)-deprenyl and rasagiline may delay the need for levodopa treatment. These drugs also decrease the dose of levodopa required and fluctuations in response. (*R*)-Deprenyl and rasagiline are reported to exert a long term beneficial effect on ‘on-off’ motor fluctuations and freezing of the gait, but may increase dyskinesias and orthostatic changes in blood pressure. They do not have an influence on the mortality rate of PD. MAO-B inhibitors potentiate the functioning of dopamine in other areas of the brain, besides the nigrostriatal pathway. They also increase dopamine in the mesolimbic/mesocortical pathways that are responsible for emotions and the drug-induced reward system. (*R*)-Deprenyl and rasagiline trigger the dopaminergic system in the chemoreceptor trigger zone, responsible for nausea and vomiting, and in the tuberohypophyseal pathway. These other dopaminergic effects might be responsible for adverse effects (Magyar & Szende, 2004).

MAO-B inhibition may also have a neuroprotective effect due to the fact that the oxidation step catalyzed by MAO-B forms hydrogen peroxide as a by-product. The generation of hydrogen peroxide and other ROS can cause neuronal damage by oxidative stress that may eventually lead to neuronal death (Yamada & Yasuhara, 2004). MAO-B inhibition therefore may protect against neuronal damage by reducing the MAO-catalyzed formation of hydrogen peroxide. Chronic treatment with (*R*)-deprenyl and rasagiline may also induce indirect antioxidant activity by the induction of scavenger functions. A (*R*)-deprenyl concentration lower than that needed to inhibit MAO-B, can decrease the damage due to oxidative shock. Long term treatment with (*R*)-deprenyl and rasagiline has been shown to increase the activity of the anti-oxidant enzymes, superoxide dismutase and catalase, in experimental animals (Magyar & Szende, 2004).

There are several toxins that selectively damage specific neurons. The selective toxicity occurs in nerves that inactivate their neurotransmitters by reuptake through membrane bound, high affinity, energy- and sodium-dependent monoamine transporters. Toxins can be taken up by the same reuptake mechanism. MPTP is a selective toxin that affects dopaminergic neurons. MPTP is a pre-toxin that is converted by MAO-B to the active toxin, 1-methyl-4-phenylpyridinium (MPP<sup>+</sup>). MPP<sup>+</sup> is actively taken up by dopaminergic neurons via the neurotransmitter reuptake process. Since MPTP has to be activated by MAO-B in order to become toxic, MAO-B inhibitors can prevent neurodegeneration caused by this toxin. (*R*)-Deprenyl can also prevent its uptake into dopaminergic neurons (Magyar & Szende, 2004). In addition to its neuroprotective properties, rasagiline also induced *in vivo* neurorestoration in the SNpc in test subjects exposed to MPTP. Recent phase III clinical studies in patients with PD, showed that rasagiline may be the first drug to slow disease progression (Weinreb *et al.*, 2010).

### **MAO inhibitors in the treatment of Alzheimer's disease:**

Because selective, irreversible MAO-B inhibitors, such as (*R*)-deprenyl and rasagiline, have neuroprotective properties, they may slow down the progression of Alzheimer's disease. MAO-B activity increases with aging and it is particularly high around the senile plaques in patients with Alzheimer's disease. There has also been evidence of cognitive enhancement with treatment with moclobemide, a reversible, selective MAO-A inhibitor (Magyar & Szende, 2004).

## **2.5 Summary:**

In this chapter PD was discussed as a neurodegenerative disorder with a largely unknown pathogenesis, although certain mechanisms of neurodegeneration are known. The symptoms of PD are the result of depleted dopamine levels in certain regions of the brain. Although PD is an incurable disease, symptomatic treatments are available. Of these treatments, dopamine replacement therapy by oral levodopa has been the most successful. Since only a small amount of levodopa reaches the brain and because its effects wear off, it is rarely used as monotherapy. MAO-inhibitors, in particular MAO-B inhibitors, decrease the degradation of dopamine in the brain and therefore may prolong the action of levodopa. Non-selective and selective MAO-A inhibitors are also used in the treatment of depressive disorders. In addition to the therapeutic value of MAO inhibition, the three dimensional structure of MAO, the mechanistic proposals for MAO catalysis and known inhibitors were explored in this chapter. This provides the necessary background information for the discovery of drugs that can be repurposed as MAO-inhibitors in the next chapters.