

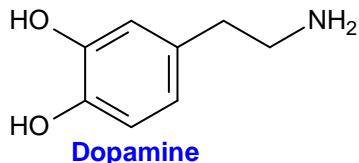
## Chapter 3

### Parkinson's disease and dopamine depletion

#### 3.1 Introduction

Various physiological processes are regulated by catecholamines in combination with other neuronal and hormonal systems (Kvetnansky *et al.*, 2009). Catecholamines function as neurotransmitters in the brain and as hormones in the circulatory system. These pathways operate independently due to the blood-brain barrier that only permits very hydrophobic species to cross over into the brain (Garrett & Grisham, 1997).

Dopamine is one of the catecholamine neurotransmitters in the brain and was first discovered in 1958 by Arvid Carlsson (Kvetnansky *et al.*, 2009). Depletion of this neurotransmitter is generally associated with PD. As mentioned before, PD is an age-related neurodegenerative disorder, characterized by the loss of dopaminergic neurons in the SNpc (Dauer & Przedborski, 2003). The resulting dopamine deficiency may lead to the development of parkinsonian associated motor symptoms (Chapter 2). To date the exact cause for the loss of dopaminergic neurons is still unknown (Jackson-Lewis & Smeynes, 2005; Beers *et al.*, 2006).



**Figure 3.1:** Chemical structure of dopamine.

Four major dopamine systems have been identified in the brain and include the nigrostriatal, mesolimbic, mesocortical and tuberoinfundibular pathways (Saklayen *et al.*, 2004). The nigrostriatal dopamine system consists of dopaminergic neurons, which project from the substantia nigra to the striatum (Cieślak *et al.*, 2008). This pathway plays a vital role in normal voluntary movement activity and its degeneration results in PD (Saklayen *et al.*, 2004). The importance of the nigrostriatal pathway is not only associated with regulation of motor activity (Vallone *et al.*, 2000), but this pathway also produces approximately 75% of brain dopamine (Cieślak *et al.*, 2008).

In this regard, the importance of the neurotransmitter, dopamine, is demonstrated in PD. Dopamine plays an essential role in the extrapyramidal system of the brain by regulating movement via a family of dopamine receptors (section 3.3). Various drug targets and strategies to enhance the depleted dopamine in the parkinsonian brain are based on the biosynthesis and

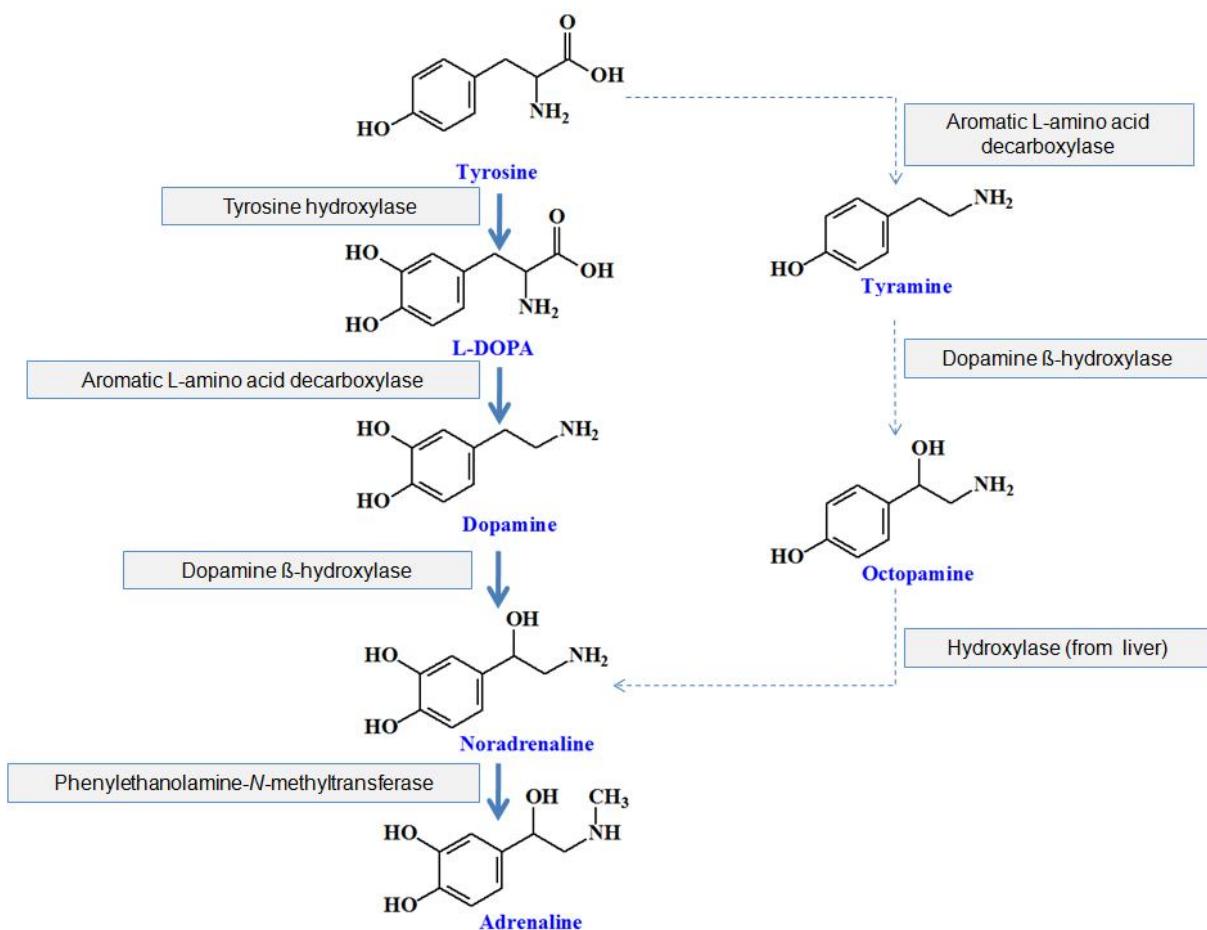
metabolic pathways of dopamine (section 3.2). Various drugs that modulate the biosynthesis and metabolic breakdown of dopamine are being used in the treatment of PD (section 3.5). In literature, several of the novel approaches to treat PD involve non-dopaminergic strategies. Antagonism of the adenosine A<sub>2A</sub> receptor is recognized as such a strategy. Section 3.4 will focus on the role of dopamine in PD, while the significance of monoamine oxidase inhibitors and the adenosine A<sub>2A</sub> receptor antagonists in PD are provided in Chapters 4 and 5, respectively.

### 3.2 Biosynthesis and metabolism of dopamine

Adrenaline, noradrenaline, dopamine and L-DOPA are catecholamine neurotransmitters ([Garrett & Grisham, 1997](#)). In the brain, the catecholaminergic systems may be divided into four systems, namely the noradrenergic system, adrenergic system, dopaminergic system and L-DOPA neurons ([Kvetnansky et al., 2009](#)). Dopamine acts as a precursor to both adrenaline and noradrenaline in catecholamine biosynthesis ([Kvetnansky et al., 2009](#)) and constitutes approximately 80% of the brain catecholamine content ([Vallone et al., 2000](#)).

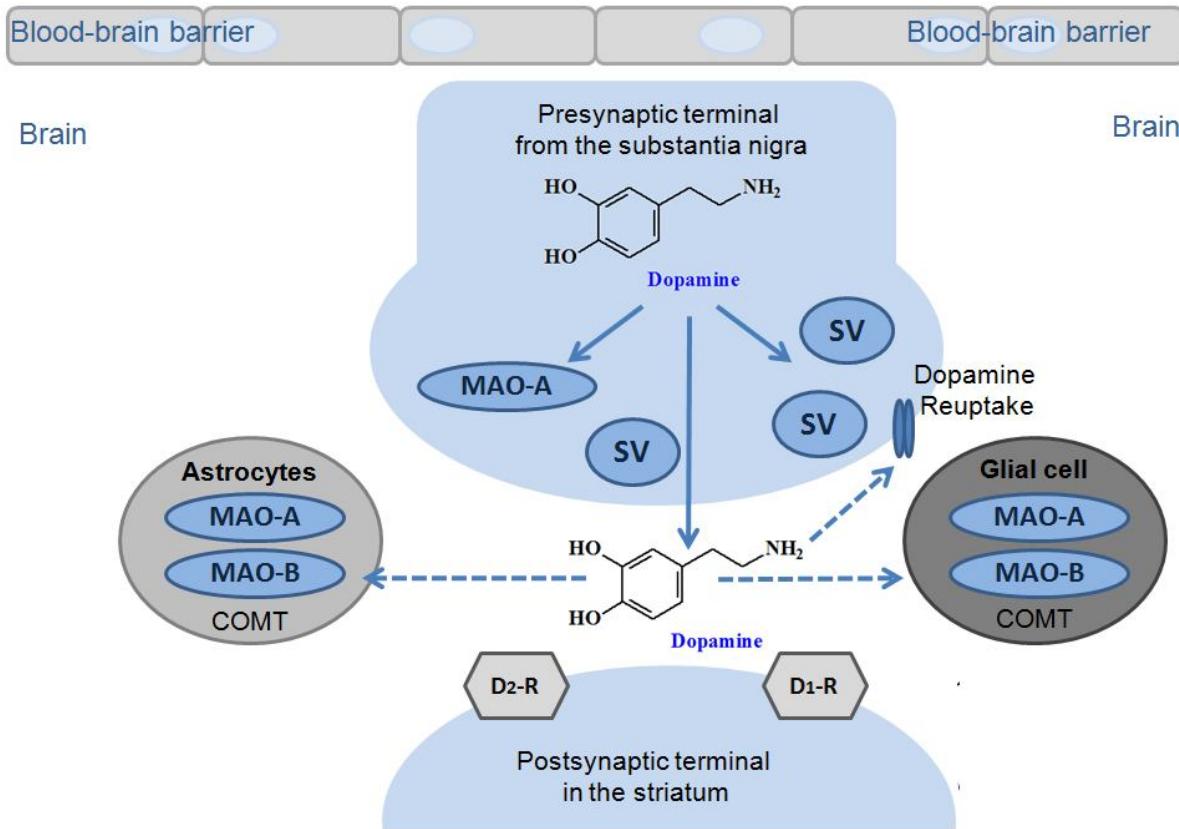
The synthetic pathway of the catecholamines (Figure 3.2) commence with the amino acid tyrosine passing through the blood-brain barrier ([Youdim & Bakhle, 2006](#)). Tyrosine is derived from two primary sources, namely the diet and hydroxylation of the amino acid, phenylalanine, in the liver ([Kvetnansky et al., 2009](#)). Subsequent hydroxylation of tyrosine via tyrosine hydroxylase yields the intermediate L-DOPA ([Standaert & Young, 2001](#); [Youdim & Bakhle, 2006](#); [Kvetnansky et al., 2009](#)). This is also the rate-limiting step in the synthesis of dopamine ([Standaert & Young, 2001](#)). L-DOPA is then decarboxylated rapidly via the nonspecific enzyme, aromatic L-amino acid decarboxylase (AAD), to yield dopamine ([Standaert & Young, 2001](#); [Youdim & Bakhle, 2006](#); [Kvetnansky et al., 2009](#)).

The hydroxylation of tyrosine to L-DOPA and the decarboxylation of L-DOPA to dopamine occur within neuronal terminals ([Standaert & Young, 2001](#)). Approximately half of the dopamine synthesized is then taken up from the cytoplasm into storage vesicles and is converted into noradrenaline by dopamine β-hydroxylase ([Hoffman & Taylor, 2001](#); [Kvetnansky et al., 2009](#)). Noradrenaline is then converted to adrenaline via the soluble cytoplasmic enzyme, phenylethanolamine N-methyltransferase ([Kvetnansky et al., 2009](#)).



**Figure 3.2:** Biosynthesis of catecholamines (see text for more detail). The dashed arrows indicate the normal by-product, tyramine, obtained from tyrosine metabolism in the body. Tyramine is also found in high concentrations in fermented foods, such as cheese. This by-product is readily metabolized by MAO in the liver and gut.

The synthesized dopamine that is not transported into storage vesicles are either metabolized (Hoffman & Taylor, 2001; Kvetnansky *et al.*, 2009) by intraneuronal MAO-A (Youdim *et al.*, 2006) or released from the presynaptic terminal. In the normal state, dopamine release from the presynaptic neuron results in signaling in the postsynaptic neuron via D<sub>1</sub> and D<sub>2</sub> type dopamine receptors (Standaert & Young, 2001; Hoffman & Taylor, 2001). Termination of the action of the remaining extracellular dopamine in the synaptic cleft may be accomplished by several mechanisms (Garrett & Grisham, 1997). Dopamine may be metabolized via MAO-A, MAO-B or catechol-O-methyltransferase (COMT) found in either astrocytes or glial cells (Youdim *et al.*, 2006). Alternatively, the function of dopamine may be terminated via reuptake into the presynaptic terminal (Garrett & Grisham, 1997; Standaert & Young, 2001). These routes of dopamine metabolism and sequestration are depicted in Figure 3.3.



**Figure 3.3:** Termination of the action of dopamine (see text for more detail). In the presynaptic terminal, dopamine is either taken up into storage vesicles or metabolized via MAO-A. After dopamine is released from the presynaptic terminal, metabolism of the remaining extracellular dopamine occurs via sequential actions of the enzymes COMT and MAO, or alternatively dopamine is taken up into the presynaptic terminal. Abbreviations: COMT = catechol-O-methyltransferase; MAO-A = monoamine oxidase type A; MAO-B = monoamine oxidase type B; SV = Storage vesicles; D<sub>1</sub>-R = D<sub>1</sub> receptors; D<sub>2</sub>-R = D<sub>2</sub> receptors.

The metabolism of dopamine via the enzymes MAO and COMT yield the metabolic products 3,4-dihydroxyphenylacetic acid (DOPAC) and 3-methoxy-4-hydroxyphenylacetic acid, respectively (Standaert & Young, 2001; Kvetnansky *et al.*, 2009). As mentioned before, it is speculated that oxidative stress may be one of the contributing factors in PD (Chapter 2). Oxidative stress may result from the generation of H<sub>2</sub>O<sub>2</sub> in the catabolic pathway of dopamine by MAO. H<sub>2</sub>O<sub>2</sub> is spontaneously converted into the hydroxyl free radical in the presence of ferrous iron (the Fenton reaction) (Standaert & Young, 2001; Youdim & Bakhle, 2006).

### 3.3 Dopamine receptors

The action of dopamine is exerted via binding to guanine nucleotide-binding protein (G-protein) coupled receptors (Vallone *et al.*, 2000; Standaert & Young, 2001). Currently five dopamine receptors have been characterized (Vallone *et al.*, 2000; Cieślak *et al.*, 2008; Maggio & Millan, 2010) and these are subdivided into two subfamilies (Vallone *et al.*, 2000):

- D<sub>1</sub>-like receptors (D<sub>1</sub> and D<sub>5</sub>) and
- D<sub>2</sub>-like receptors (D<sub>2</sub>, D<sub>3</sub>, and D<sub>4</sub>)

Structurally dopaminergic receptors are similar by containing 7 α-helices. These receptors are also constructed of approximately 400 amino acids (Cieślak *et al.*, 2008; Missale *et al.*, 1998). The most prominent structural difference between the subfamilies is that D<sub>1</sub>-like receptors contain a long intracellular carboxy-terminal tail, whereas D<sub>2</sub>-like receptors share a large third intracellular loop (Standaert & Young, 2001).

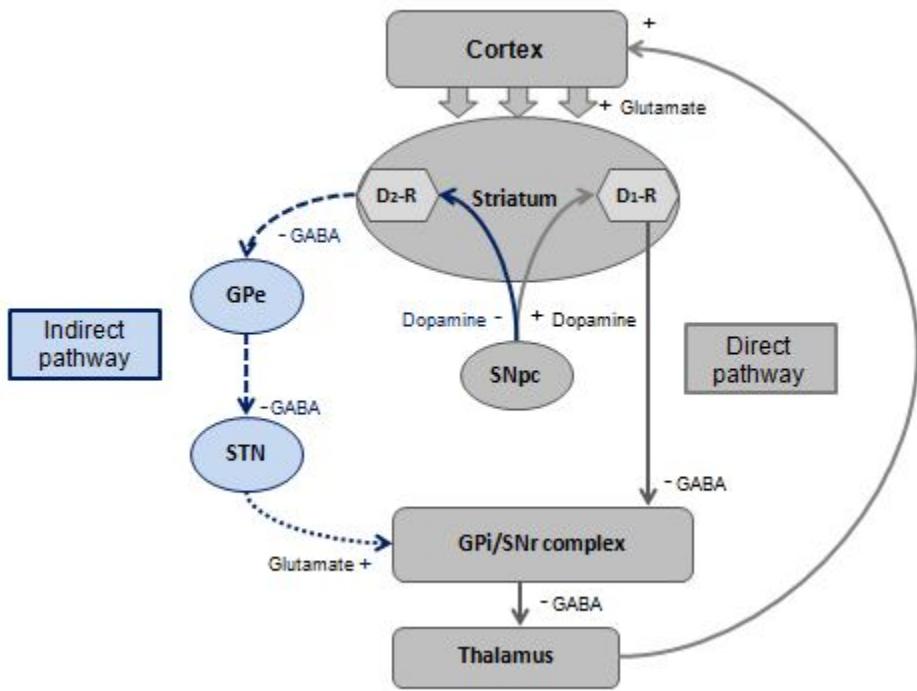
An additional difference between the two subfamilies of dopaminergic neurons are based on their pharmacological and biochemical properties (Vallone *et al.*, 2000; Standaert & Young, 2001). The regulation of cyclic adenosine monophosphate (cAMP) is seen as one of the major effects of dopamine receptors (Girault & Greengard, 2004). Stimulation of D<sub>1</sub> receptors through G<sub>s</sub>/G<sub>olf</sub> proteins increases cAMP levels, while stimulation of D<sub>2</sub> receptors through G<sub>i</sub> proteins decrease cAMP levels (Ferré *et al.*, 2001; Girault & Greengard, 2004; Pinna *et al.*, 2005; Maggio & Millan, 2010). It is also known that D<sub>3</sub> and D<sub>4</sub> receptors reduce endogenous cAMP levels. Alternatively, dopamine may also exert an effect through modulation of Ca<sup>2+</sup> signalling. Intracellular Ca<sup>2+</sup> concentrations may be modulated by D<sub>2</sub> receptors that are found both pre- and post-synaptically (Vallone *et al.*, 2000). The D<sub>2</sub> dopamine receptor is of particular importance in this thesis as it has been implicated in the pathophysiology of PD (Hoffman & Taylor, 2001).

### 3.4 The basal ganglia: dopamine and motor function in PD

PD, Alzheimer's disease and Huntington's disease are examples of neurodegenerative diseases. This group of disorders are marked by the particular brain region involved in the progressive and irreversible loss of neurons. In Alzheimer's disease the loss of hippocampal and cortical neurons results in impairment of memory and cognitive ability, whereas neuronal loss from structures in the basal ganglia leads to abnormalities in control of movement in PD and Huntington's disease (Standaert & Young, 2001).

As mentioned, the neurotransmitter dopamine is essential in controlling normal movement and degeneration of dopaminergic neurons results in various motor symptoms (Vallone *et al.*, 2000). The following basal ganglia anatomical model (Figure 3.4) may be used to explain some of the

motor symptoms associated with PD. The basal ganglia are a group of subcortical nuclei in the brain that are important for certain processes which include motor, associative, cognitive and mnemonic functions (Bolam *et al.*, 2000; Obeso *et al.*, 2002).



**Figure 3.4:** Schematic of the basal ganglia model and neurotransmitters involved. In PD, degeneration of the SNpc is observed which causes overactivity of the indirect pathway (indicated in blue) and increased glutamatergic activity at the subthalamic nucleus. Abbreviations: SNpc = substantia nigra pars compacta; SNr = substantia nigra pars reticulata; GPi = globus pallidus internal; GPe = globus pallidus external; STN = subthalamic nucleus; D<sub>1</sub>-R = D<sub>1</sub> receptors; D<sub>2</sub>-R = D<sub>2</sub> receptors; GABA = gamma-aminobutyric acid.

The primary input of the basal ganglia is derived from the cortex, where cortical information is then provided to the basal ganglia via a main point of entry that is known as the striatum (Bolam *et al.*, 2000). The striatal neurons receive dopaminergic inputs from the SNpc (Morelli *et al.*, 2012) and the outflow of the striatum projects via two pathways known as the striatonigral pathway (direct pathway) and the striatopallidal pathway (indirect pathway) (Xu *et al.*, 2005). Under normal physiological conditions motor activation is obtained if a balance between the influence of the direct and indirect pathway exists (Standaert & Young, 2001). This results in a net effect of increased excitatory outflow to the thalamus and cortex from the complex of globus pallidus internal (GPi)/substantia nigra pars reticulata (SNr) to exert normal motor activation (Brown & Marsden, 1998; Standaert & Young, 2001; Xu *et al.*, 2005).

In the striatum endogenous dopamine activates neurons expressed in the direct pathway, which send GABAergic projections directly from the striatum to the GPi/SNr complex (Bolam *et al.*, 2000; Xu *et al.*, 2005; Morelli *et al.*, 2012). Stimulatory D<sub>1</sub> receptors and the neuropeptide, dynorphin, are mainly expressed by the striatonigral neurons (direct pathway) (Ferré *et al.*, 2001; Joyce, 2001; Pinna *et al.*, 2005; Müller & Ferré, 2007; Morelli *et al.*, 2012). At the same time dopamine depresses neurons in the indirect pathway, which send GABAergic projections indirectly to GPi/SNr complex via chronological synaptic connections from the external segment of globus pallidus (GPe) and then to the subthalamic nucleus (STN) (Bolam *et al.*, 2000; Xu *et al.*, 2005; Morelli *et al.*, 2012). Finally an excitatory glutamatergic projection from the STN to the GPi/SNr complex is observed (Bolam *et al.*, 2000; Xu *et al.*, 2005). Inhibitory D<sub>2</sub> receptors and the neuropeptide, enkephalin, are predominantly expressed by the striatopallidal neurons (indirect pathway) (Ferré *et al.*, 2001; Joyce, 2001; Pinna *et al.*, 2005; Müller & Ferré, 2007; Morelli *et al.*, 2012). Thus, dopaminergic projections from the SNpc affect both the direct and indirect pathway (Brown & Marsden, 1998).

Dopamine or a dopamine agonist will induce motor activation by activating the direct pathway (acting on stimulatory D<sub>1</sub> receptors) as well as depressing the indirect pathway (acting on inhibitory D<sub>2</sub> receptors) (Müller & Ferré, 2007), whilst the opposite effect is observed with PD (Standaert & Young, 2001). PD is characterized by hypokinesia that is associated with the loss of dopaminergic neurons in the SNpc (Dauer & Przedborski, 2003) and consequently a decrease in dopaminergic input to the striatum (Morelli *et al.*, 2012). The reduction of striatal dopamine leads to a decrease in activation of the direct pathway and simultaneously disinhibition of the indirect pathway (Morelli *et al.*, 2012). The subsequent impairment of basal ganglia circuits and output structures may be seen as the functional feature of PD (Müller & Ferré, 2007), exerting motor impairment.

Additionally, dopamine is not the only neurotransmitter affected by PD (Trevitt *et al.*, 2009). The adenosine A<sub>2A</sub> receptor is distributed between the striatum, nucleus accumbens and olfactory tubercle and is suggested to play a specific role in the neuronal communication in the basal ganglia (Shimada *et al.*, 1997). In the striatum the disinhibition of the indirect pathway, due to PD, is also associated with an increase of adenosine A<sub>2A</sub> receptor transmission (Morelli *et al.*, 2012). This consequently has implications for PD. The interactions of dopamine and adenosine in PD will be discussed in detail in Chapter 5 with the focus on identifying alternative targets for the treatment of PD.

Current treatments used for PD is based upon restoring dopaminergic function. The basal ganglia model (described in the text above) provides important suggestions for the design of pharmacological agents for the treatment of PD and specifically non-dopaminergic agents.

### 3.5 Treatment of PD and the role of dopamine

Treatment of PD is currently symptomatic and mainly focused on restoring dopaminergic function (Brooks, 2000). Various strategies have been employed to increase dopamine levels in the brain and these mainly consist of altering dopamine's metabolism or increasing its production (Fung *et al.*, 2001). Therefore, the different treatment options for PD are numerous and include pharmacological treatments such as L-DOPA, dopamine agonists, COMT inhibitors, MAO inhibitors and the use of amantadine.

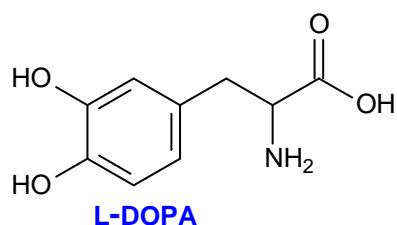
Non-motor symptoms such as depression (Youdim & Bakhle, 2006) and anxiety (Prediger *et al.*, 2012) are also an underlying problem with PD. However, evidence shows that the non-motor symptoms related to PD does not respond to dopaminergic replacement therapy (Prediger *et al.*, 2012). Some of the novel non-dopaminergic drug treatments include adenosine A<sub>2A</sub> receptor antagonists, α-2 noradrenergic receptor antagonists and GABAergic agents (Leung & Mok, 2005; Onofrj *et al.*, 2008).

Nonpharmacologic treatments are also used as therapy for PD. These types of therapies include specific food and vitamin additives as well as exercise and speech therapy (Suchowersky *et al.*, 2006). These nonpharmacologic treatments do not improve symptoms of PD patients, but are helpful to maintain the well-being of patients (Rao *et al.*, 2006).

In this section (3.5) a brief overview of some of the current pharmacological treatments used in PD will be outlined. The strategies to restore dopamine either directly (dopamine agonists) or via the metabolic precursor (L-DOPA) of dopamine will be addressed. Treatment with MAO inhibitors and adenosine A<sub>2A</sub> antagonists will be discussed in detail in Chapters 4 and 5, respectively.

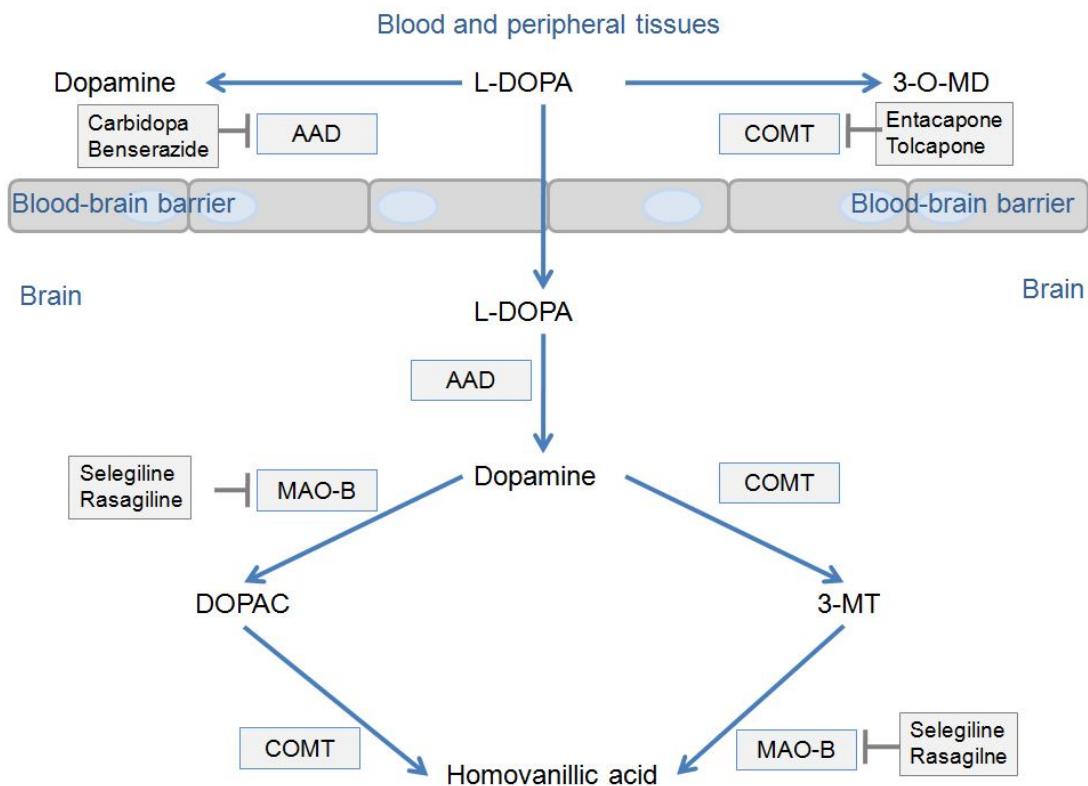
#### 3.5.1 L-DOPA

As previously mentioned, dopamine depletion in the brain is the hallmark feature in PD. Unfortunately, dopamine does not cross the blood-brain barrier (LeWitt, 2008), while the immediate metabolic precursor of dopamine (Nakagawa *et al.*, 2004), L-DOPA, does cross the blood-brain barrier when administered orally (LeWitt, 2008). Therefore, one of the strategies to replenish dopamine in the brain is via L-DOPA treatment (Onofrj *et al.*, 2008; LeWitt, 2008).



**Figure 3.5:** Chemical structure of L-DOPA.

When L-DOPA is administered orally it is transported from the small intestine into the brain (Fung *et al.*, 2001; Standaert & Young, 2001). However, in the periphery, L-DOPA is subjected to metabolism by AAD and COMT (Leung & Mok, 2005), consequently only a small quantity of the orally administered L-DOPA reaches the brain (LeWitt, 2008). Once in the brain, L-DOPA is rapidly decarboxylated to dopamine by AAD (LeWitt, 2008) mainly within the presynaptic terminals of dopaminergic neurons in the striatum (Standaert & Young, 2001). Figure 3.6 depicts the metabolism of orally administered L-DOPA with the relevant metabolites obtained.



**Figure 3.6:** Schematic presentation of L-DOPA metabolism, after oral administration, with the relevant metabolites obtained. Additionally, sites of action for inhibitory drugs used with L-DOPA are also provided. Abbreviations: L-DOPA = levodopa; 3-O-MD = 3-O-methyldopa; AAD = aromatic L-amino acid decarboxylase; COMT = catechol-O-methyltransferase; MAO-B = monoamine oxidase type B; DOPAC = 3,4-dihydroxyphenylacetic acid; 3-MT = 3-methoxytyramine.

L-DOPA is usually given in combination with an AAD inhibitor (Leung & Mok, 2005) to prevent the conversion of L-DOPA to dopamine in the peripheral tissues, and to enhance the amount of dopamine that reaches the brain. Carbidopa and benserazide are known peripheral AAD inhibitors (Leung & Mok, 2005; LeWitt, 2008) and do not cross the blood-brain barrier (Youdim *et al.*, 2006). Therefore, systemic side-effects such as nausea (Fung *et al.*, 2001) are limited (LeWitt, 2008). Nausea is possibly caused by the conversion of L-DOPA to dopamine outside

the blood-brain barrier leading to the subsequent stimulation of the dopamine receptors at the postrema or “vomiting centre” (Fung *et al.*, 2001).

Documented clinical benefits of L-DOPA treatment include improvement of bradykinesia, rigidity and tremor (Leung & Mok, 2005). L-DOPA treatment is most successful in the first years of treating PD. Long-term treatment with L-DOPA produces complications, such as motor fluctuations and dyskinesia (Leung & Mok, 2005; Onofrj *et al.*, 2008). Motor fluctuations may occur throughout the day, either because of inadequate dopaminergic stimulation, also known as “off-periods”, or due to excessive dopaminergic stimulation. Dyskinesia is the involuntary movements of the limbs or trunk and takes place when the maximal plasma levels of L-DOPA are reached, also known as peak dose dyskinesia (Fung *et al.*, 2001).

The management of peak dose dyskinesia may be achieved by reducing the individual L-DOPA dose. However, “off-periods” or inadequate dopaminergic stimulation may occur (Fung *et al.*, 2001). L-DOPA is often used with various adjuvants to ameliorate the side-effects associated with L-DOPA and to enhance its antiparkinsonian action. One of the alternative adjuncts identified to reduce the dose of L-DOPA include the use of a dopamine agonist (Fung *et al.*, 2001). It has also been documented that amantadine may be useful to reduce dyskinesia (Fung *et al.*, 2001; Onofrj *et al.*, 2008). MAO inhibitors (particularly MAO-B inhibitors) (Fernandez & Chen, 2007), COMT inhibitors (Rezak, 2007; Fung *et al.*, 2001) and adenosine A<sub>2A</sub> antagonists (Kalda *et al.*, 2006) have also been documented as possible adjunct therapy to L-DOPA and may lessen the side-effects associated with this drug.

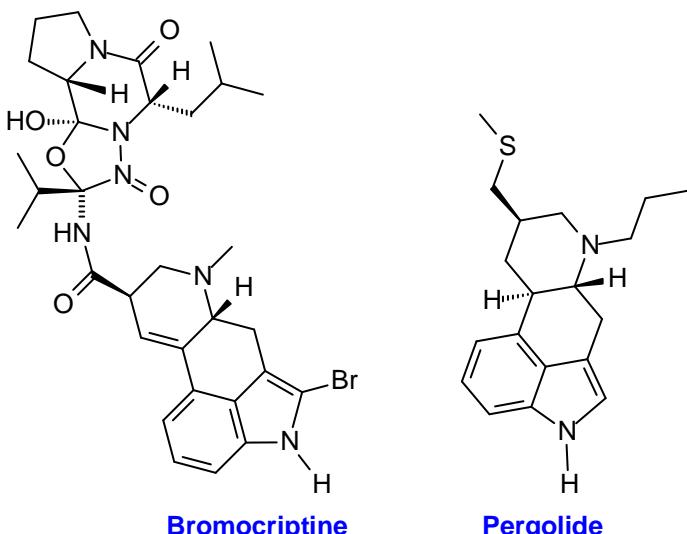
### 3.5.2 Dopamine agonists

Dopamine agonists are another group of dopamine restoring drugs used in the treatment of PD. The action of dopamine is mimicked by these drugs via the direct stimulation of striatal dopamine receptors (Onofrj *et al.*, 2008). The D<sub>2</sub>-receptor, in particular, is associated with the motor symptoms which characterises PD and dopamine agonists used to treat PD mainly stimulate the D<sub>2</sub>-receptor (Klopman & Sedykh, 2002).

Two major classes of dopamine agonists are being used, namely the older dopamine agonists or ergoline-derivatives (ergot-like structures) that include bromocriptine and pergolide and the newer dopamine agonists or non-ergoline derivatives that include pramipexole and ropinirole (Tintner *et al.*, 2005).

Generally, ergot derivatives act on D<sub>2</sub>-like (D<sub>2</sub>, D<sub>3</sub> and D<sub>4</sub>) dopamine receptors (Brooks, 2000). Bromocriptine stimulates the D<sub>2</sub> receptor (Montastruc *et al.*, 1989) and possesses antagonistic activity towards the D<sub>1</sub> receptor (Brooks, 2000). This dopamine agonist is used to treat PD (Montastruc *et al.*, 1989) and certain endocrinologic disorders (Spark & Dickstein, 1979). On

the other hand, pergolide directly stimulates both D<sub>1</sub> and D<sub>2</sub> receptors (Brooks, 2000; Brusa et al., 2005). Research has shown that this drug is more effective than bromocriptine to treat PD (Bowsher et al., 1992; Shulman, 1999; Leung & Mok, 2005). Furthermore, pergolide, as add-on therapy to L-DOPA, allows for a reduction in the dose of L-DOPA (Storch et al., 2005).

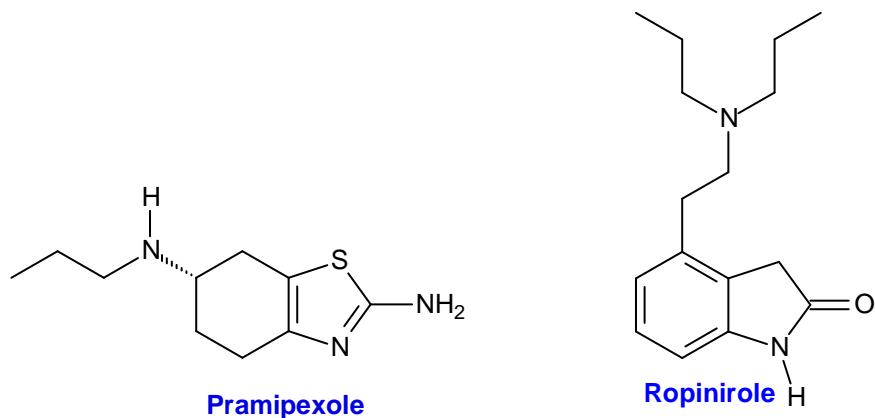


**Figure 3.7:** Chemical structures of the ergot-derivatives, bromocriptine and pergolide.

The side-effects associated with ergot-derived drugs are likely to be dopaminergic in origin and include nausea, vomiting, orthostatic hypotension, hallucinations and delusions (Brooks, 2000). Other side-effects specifically documented with the use of ergot-derivates include vasospasm, erythromelalgia, and pleuropulmonary or retroperitoneal fibrosis. However, these side-effects seem to be very rare (Brooks, 2000). Previous studies have also shown that ergot-derived pergolide may increase the risk of cardiac-valve regurgitation (Ohno et al., 2009).

The non-ergoline derivatives have selectivity for the D<sub>2</sub> receptors, lesser activity towards D<sub>3</sub> receptors and no activity towards D<sub>1</sub> receptors (Standaert & Young, 2001). Both ropinirole and pramipexole are used as monotherapy in patients with early PD (De Mey et al., 1991; Shulman, 1999; Brooks, 2000) and are also effective as adjunct therapy with L-DOPA. These drugs allow for the reduction of the L-DOPA dose and smoothing out response fluctuations in advanced PD (Brooks, 2000).

Ropinirole was the first orally available dopamine agonist. Additionally, as monotherapy, ropinirole was found to be associated with a considerable reduction in the occurrence of dyskinesia compared to monotherapy with L-DOPA (Brooks, 2000). Pramipexole stimulates both the D<sub>2</sub> and D<sub>3</sub> receptor (Shulman, 1999; Lorenc-Koci & Wolfarth, 1999) with the highest affinity for the D<sub>3</sub> receptors (Lorenc-Koci & Wolfarth, 1999; Brooks, 2000). The D<sub>3</sub> receptors are involved in behavioural symptoms such as mood, anxiety and apathy (Shulman, 1999).



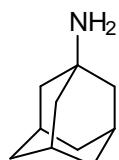
**Figure 3.8:** Chemical structures of the non-ergoline derivatives, pramipexole and ropinirole.

Even though the non-ergoline dopamine agonists are well tolerated they are still associated with the dopaminergic side-effects that include nausea, dizziness and hypotension (Brooks, 2000; Leung & Mok, 2005). Additionally, mental disturbances (Leung & Mok, 2005) and dyskinesias associated with L-DOPA treatment may also occur (Brooks, 2000).

### 3.5.3 Amantadine

Amantadine is generally known as an antiviral agent exerting dopaminergic and anticholinergic effects (Leung & Mok, 2005; Rezak, 2007). It has been documented that amantadine treatment may reduce dyskinesia (Fung *et al.*, 2001; Onofrj *et al.*, 2008) at high doses (Leung & Mok, 2005).

However, the exact mode of action of amantadine in PD is still unclear. Recent studies have proposed that amantadine may also exhibit neuroprotective properties (Onofrj *et al.*, 2008). It is speculated that these neuroprotective properties may be responsible for the reduction of dyskinesia in amantadine treated PD patients (Leung & Mok, 2005). The neuroprotective properties may be attributed to the ability of amantadine to block N-methyl-d-aspartate receptors (Leung & Mok, 2005; Onofrj *et al.*, 2008). Previous studies illustrated that these receptors may mediate excitotoxicity in the basal ganglia (Rezak, 2007; Onofrj *et al.*, 2008). The occurrence of dopaminergic neuronal cell death in PD has previously been linked to the latter process (Rezak, 2007).



**Amantadine**

**Figure 3.9:** Chemical structure of amantadine.

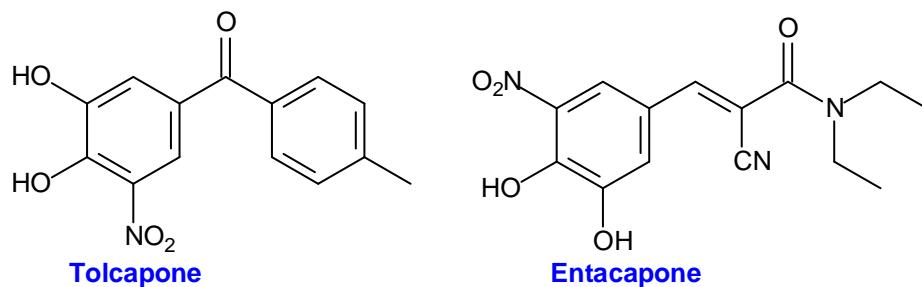
Side-effects of this drug include mental status changes, lower extremity oedema and livedo reticularis (Rezak, 2007; Onofrj *et al.*, 2008) as well as nightmares and anticholinergic side-effects (Fung *et al.*, 2001).

### 3.5.4 Catechol-o-methyltransferase inhibitors

In PD the dopamine producing cells are damaged and other treatment strategies need to be found to use the existing dopamine more effectively. Both the enzymes, COMT and MAO, participate in the catabolism of L-DOPA and dopamine (Fung *et al.*, 2001). Inhibition of these enzymes increases the levels of dopamine by inhibiting its metabolism or termination.

Selective COMT inhibitors include tolcapone and entacapone (Rezak, 2007) and these drugs are used in combination with L-DOPA in PD therapy (Fung *et al.*, 2001). This combination is used in order to reduce motor fluctuations (Fung *et al.*, 2001). It is believed that COMT inhibitors prevent the conversion of L-DOPA to 3-O-methyldopa (Onofrj *et al.*, 2008). It is speculated that COMT inhibitors prolong the action of L-DOPA by reducing its peripheral metabolism. This results in increasing levels of unmetabolised L-DOPA in the SNpc (Onofrj *et al.*, 2008).

However, the increased L-DOPA exposure generally leads to side-effects that include dyskinesias in susceptible patients (Onofrj *et al.*, 2008) and nausea (Leung & Mok, 2005). Tolcapone and entacapone display similar pharmacological effects (Rezak, 2007), though entacapone is preferred to tolcapone as the latter drug is associated with hepatotoxicity (Fung *et al.*, 2001).



**Figure 3.10:** Chemical structures of the COMT inhibitors, tolcapone and entacapone.

### 3.5.5 Monoamine oxidase inhibitors

MAO plays an essential role in the oxidative deamination of important neurotransmitters. The two identified isoforms, MAO-A and MAO-B, are attracting interest as drug targets in the therapy of neurodegenerative diseases (MAO-B) and depression (MAO-A).

MAO-B preferentially deaminates the neurotransmitter dopamine and inhibitors thereof are useful for the treatment of PD (Yamada & Yasuhara, 2004; Nagatsu & Sawada, 2006).

Furthermore, oxidative deamination via MAO-B may result in the formation of H<sub>2</sub>O<sub>2</sub> and the accumulation of toxic aldehyde metabolites of dopamine (Nagatsu & Sawada, 2006; Youdim & Bakhle, 2006). H<sub>2</sub>O<sub>2</sub> may produce highly reactive oxygen species via the Fenton reaction, which is catalyzed by iron and neuromelanin (Nagatsu & Sawada, 2006). Therefore, MAO-B inhibitors are not only important for the inhibition of dopamine metabolism (a symptomatic effect), but also for the reduction of the development of neurotoxic dopamine metabolites (Nagatsu & Sawada, 2006).

MAO-B selective inhibitors may also be co-administered with L-DOPA as PD drug therapy (Binda *et al.*, 2007). There is evidence that MAO-B inhibitors (such as selegiline) may prevent the progress of PD (LeWitt & Taylor, 2008; Youdim & Bakhle, 2006). Non-motor symptoms of depression (Youdim & Bakhle, 2006) and anxiety (Prediger *et al.*, 2012) are also an underlying problem with PD. It is speculated that MAO-B inhibitors may reduce anxiety in PD patients (Prediger *et al.*, 2012), while selective reversible MAO-A inhibitors (such as moclobemide) are a favourable treatment for PD associated depression (Youdim & Bakhle, 2006). As the optimal drug treatment for PD has not been achieved for both motor and non-motor symptoms, development of novel MAO inhibitors is attracting interest.

### 3.6 Conclusion

Current treatments indicated for use in PD are based on restoring dopaminergic function (Brooks, 2000). Various drugs aim to alter the metabolism of dopamine or to increase its production (Fung *et al.*, 2001). The basal ganglia model provides a rationale for the design of pharmacological agents in the treatment of PD. The dopaminergic projections from the SNpc affect both the direct and indirect pathway (Brown & Marsden, 1998) and degeneration of the nigrostriatal dopaminergic system leads to a reduction of striatal dopamine (Dauer & Przedborski, 2003). In PD, the impairment of the functioning of the basal ganglia circuits decreases the activation of the direct pathway and simultaneously causes disinhibition of the indirect pathway. The latter imbalance is also associated with an increase in adenosine A<sub>2A</sub> receptor transmission that may have consequences in PD treatment (Morelli *et al.*, 2012).

Different classes of drugs have been used to treat PD. The aim of most of these therapies is to relieve symptoms and long-term complications with treatment. Current symptomatic treatments are focused on restoring dopaminergic function. L-DOPA treatment is effective in early treatment of PD. Unfortunately, long-term treatment result in side-effects such as dyskinesias and motor fluctuations (Leung & Mok, 2005). Therefore, new treatment strategies are needed to either delay or minimise L-DOPA therapy (Onofrj *et al.*, 2008). COMT and/or AAD inhibitors may be used to increase the levels of unmetabolised L-DOPA for transport across the blood-brain barrier (Fung *et al.*, 2001; Onofrj *et al.*, 2008). The side-effect of dyskinesia is still a reality

with the use of a COMT inhibitor in susceptible patients (Onofrj *et al.*, 2008). Other adjunct therapies to L-DOPA treatment include dopamine agonists (Fung *et al.*, 2001), MAO-B inhibitors (Binda *et al.*, 2007) and amantadine (Onofrj *et al.*, 2008). Adenosine A<sub>2A</sub> receptor antagonists have also been identified as a possible PD treatment strategy (Kalda *et al.*, 2006).

As mentioned, the main focus of treatment is dopaminergic replacement in order to alleviate the motor symptoms associated with PD. However, non-motor symptoms of depression (Yousdim & Bakhle, 2006) and anxiety (Prediger *et al.*, 2012) are also an underlying problem with PD and these symptoms do not respond to dopaminergic replacement therapy (Prediger *et al.*, 2012). MAO-B inhibitors are used as monotherapy or adjunct therapy in treatment of early PD in order to reduce the incidence of motor fluctuations associated with L-DOPA (Simonson *et al.*, 2007), and may also be used to treat some of the non-motor symptoms associated with PD (Yousdim & Bakhle, 2006; Prediger *et al.*, 2012).

In summary, restoring dopaminergic function is mainly focused on the treatment with L-DOPA, the dopamine precursor, and with dopamine agonists. Unfortunately these treatment regimes are only effective in the early stages of PD and long-term treatment is associated with side-effects such as dyskinesia and motor fluctuations (Leung & Mok, 2005; Onofrj *et al.*, 2008). In order to compensate for the current inadequacies of dopamine replacement therapy, future research needs to identify neuroprotective agents for PD treatment and improve current symptomatic treatments. The improvement of non-motor symptoms must also be taken into consideration (Prediger *et al.*, 2012).

In this regard the development of novel compounds for MAO inhibition is of interest (Yamada & Yasuhara, 2004; Nagatsu & Sawada, 2006). A detailed discussion regarding MAO and its role in PD follows in Chapter 4. Another alternative target currently under investigation for the symptomatic treatment of PD, is the adenosine A<sub>2A</sub> receptor. In previous studies, antagonists of this receptor have shown potential for the treatment of PD (Müller & Ferré, 2007). The role of adenosine and in particular the A<sub>2A</sub> receptor in PD will be discussed in Chapter 5.

### 3.7 References

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