Chapter 1

Introduction
1.1 HISTORICAL PERSPECTIVE

In the twentieth century a revolution took place in the understanding of the mechanisms involved in the human brain. Many new drugs were discovered that influenced the brain and the development of molecular biology led to the understanding of the processes involved on a cellular and molecular level. Drugs were developed that affected the brain in a previously unknown fashion. Changes in the molecular structure of drugs changed the selectivity of their actions. To this day, decoupling of pharmacological effects through chemical probing remains a valuable tool to increase our understanding of the brain.

This thesis will specifically deal with the role of the neurotransmitter dopamine (DA) in the brain. It reports on the design and development of synthetic compounds that by a remarkable bioactivation mechanism offers a potential treatment for Parkinson’s disease. Parkinson’s disease is a central nervous system (CNS) disorder that is the result to a progressive deterioration of dopaminergic cells in specific brain areas. In this chapter, an introduction is given into (disturbed) dopaminergic neuronal communication in the brain and how drugs affect this communication. The introduction of this thesis focuses on dopaminergic neurotransmission, the description of dopaminergic pathways associated with Parkinson’s disease, and the application of dopaminergic drugs intended to treat Parkinson’s disease. This provides a basis and a background for the research described in further chapters on attempts to improve the treatment of Parkinson’s disease.

1.2 THE BRAIN AND ITS MESSENGERS

1.2.1 Brain cells

Most drugs that are active in the brain exert their effects on certain a cell type called neurons.\textsuperscript{1,2} Neurons are distributed in the brain in a specific way connection certain brain areas. Through these connections, communication between neurons and thus between brain areas takes place. Only 15\% of the total brain matter is composed of neurons, the remaining 85\% is made up of an other cell type, called the glial cells. There is evidence that glial cells support the neurons by providing metabolic assistance and by taking care of neuronal waste material. The function of glial cells is not fully understood, and still is the subject of much research.
Introduction

Neurons, basically are like all other cells in the body but have long cellular extensions, called axons. At the end of an axon it may divide into up to 10,000 branches, each of which may connect with different neuron. Usually the ends of the branches, connect with several other neurons, providing a great diversity in interneuronal connections. These nerve terminals make contact with another neuron by connection with the cell body, or more frequently, to another kind of neuron extension called a dendrite. At these contact points, information from one neuron is transduced to another. Since a typical neuron has over 10,000 dendrites, an enormous amount of communication between neurons is possible.

Communication is initiated by an impulse that is generated in the neuronal cell body or dendrite. This impulse is propagated through the axon by an electrochemical process, in which either sodium or potassium ions are involved. Like a computer microchip, this is an all-or-nothing process. There is an impulse or there is no impulse. Propagation of the impulse may be very rapid, resembling the passage of an electrical current down a copper wire. This process is the same for all neurons, so drugs affecting the electrochemical propagation of the signal are not selective toward specific neuronal cells.

At the end of the branched axon, the nerve terminal, the electrical impulse triggers the release of neurotransmitters. Neurotransmitters then diffuse across a small gap separating the adjacent neurons. There they interact with the receiving neuron resulting in modulation of its activity. This interaction is very specific and is mediated through receptor proteins that are embedded in the cell membrane. The response of the receiving neuron differs for the type of neuron. Interaction of a neurotransmitter with its receptor may open or close ion channels on the receiving neuron. Another mechanism of receptor response is mediated through a second messenger system, which applies to the dopaminergic neurons.

Specific enzymes near the dopaminergic nerve terminal synthesize dopamine (DA). The common amino acid L-tyrosine is hydroxylated on the aromatic nucleus by the enzyme ‘tyrosine hydroxylase’ to give L-dopa (Scheme 1.1). The ‘L-aromatic amino acid decarboxylase’ enzyme specifically removes the carboxyl group yielding dopamine. Dopamine belongs to the chemical class of the catecholamines. Characteristics of catecholamines are the phenylethyl moiety, with two adjacent hydroxy groups attached to the aromatic nucleus and an amine functionality on the ethyl side chain. Other examples of catecholamines are noradrenaline and adrenaline. Noradrenaline is enzymatically synthesized by β-hydroxylation of DA. Adrenaline is formed by
N-methylation of noradrenaline and is relatively scarcely represented in the brain. Like DA, noradrenaline is abundant in many places in the brain but has a distinctly different function.\textsuperscript{3,4}

Scheme 1.1 Biosynthetic pathway of dopamine, noradrenaline and adrenaline out of tyrosine. TH, tyrosine hydroxylase; L-dopa, L-3,4-dihydroxyphenylalanine; L-AADC, L-aromatic amino acid decarboxylase; DA, dopamine; DABH, DA β-hydroxylase; PNMT, Phenylethanolamine-N-methyltransferase.

1.2.2 The role of DA in the brain

The dopaminergic neurons in the brain are divided into two major systems, the mesostriatal dopaminergic system, the dopaminergic mesolimbic systems and one minor group, the mesothalamic dopaminergic system.\textsuperscript{1} Part of the mesostriatal dopaminergic system is the nigrostriatal dopaminergic system that, together with a part of the mesothalamic dopaminergic system, is involved in the normal regulation of various motor abilities of the body. The mesolimbic dopaminergic system is thought to be involved in the regulation of mood, emotion, and memory. It is generally accepted that the mesostriatal dopaminergic system has an activating action on movement, and the mesothalamic dopaminergic system in this, has a regulating function. Another important function of the mesothalamic dopaminergic system is its involvement in the secretion of several hormones. In the pituitary gland, DA itself is released to inhibit the release of prolactin that is responsible for inducing lactation in mammals.

In a CNS disorder like Parkinson’s disease, in which dopaminergic neurons of the nigrostriatal system have deteriorated, voluntary movement of the body becomes difficult.\textsuperscript{4-6}
Reduced dopaminergic neurotransmission results in an hypokinetic disorder marked by characteristic symptoms. These symptoms can also be induced in non-Parkinson patients by administration of DA antagonists, that block the action of DA. For instance, in the treatment of psychotic disorders like schizophrenia, DA antagonists like haloperidol cause patients to suffer from parkinsonism as an adverse effect. These, so-called, extrapyramidal side effects are mainly the result from the inhibition of mesothalamic dopaminergic neurotransmission. Contrary to Parkinson’s disease is Huntington’s chorea that is the result of an excessive mesostriatal dopaminergic activity. This results in a hyperkinetic movement disorder characterized by abnormal involuntary movements. Artificially increasing DA neurotransmission to a normal person may induce a hyperkinetic condition and inhibit prolactin release.

1.3 SIGNAL TRANSDUCTION IN DOPAMINERGIC NEURONS

1.3.1 DA mediated signal transduction

In dopaminergic signal transduction, DA is released into a synaptic cleft, separating neurons, by an exocytic process at the nerve terminal, following an electrical pulse from a primary neuron (Figure 1.1). When DA diffuses to the postsynaptic neuron and binds to the DA receptor protein at a specific site inside the DA receptor, the receptor responds by triggering a postsynaptical cellular response. The ‘active site’ of the DA receptor that is responsible for binding DA, is a 3D space inside the receptor, accommodated for electrostatic interaction with the functional groups of DA. Actual binding is the result of the interaction of DA with specific amino acid residues, mainly through electrostatic and hydrophobic interactions.

Instead of binding to the postsynaptic neuron, DA may also diffuse to the presynaptic neuron and for instance interact with a different type of DA receptors called DA autoreceptors. These presynaptic autoreceptors function as a feedback mechanism, by modulating dopamine synthesis and release. When the neurotransmitters stimulate an autoreceptor, this autoreceptor signals the presynaptic neuron to slow down the synthesis and release of that neurotransmitter. Certain drugs have been discovered that act as selective autoreceptor agonists and decrease DA release without inducing a postsynaptic effect. Autoreceptors are also found on neuronal cell bodies where they are involved in the modulation DA release from dendrites and modulates the electrical activity of the neuron. The exact function of autoreceptors is unclear but they are thought protect the postsynaptic neuron from overstimulation.
Figure 1.1 Schematic representation of neurotransmission by neurotransmitter molecules. Adapted from ref 1.

Functional binding to the DA receptor is not restricted to DA specifically. Many natural and synthetic drugs act on DA receptors like DA itself (Figure 1.2). These DA agonists generally have a structure that mimics the structure of DA and thus are able to bind to the DA receptor. Optimising electrostatic interactions by structurally restricting the DA functional groups and by incorporation of additional substituents in synthetic molecules has led to the discovery of highly potent DA agonists. In the 70’s, this approach was the primary tool to understand the 3D space of the active site. Functional resemblance of DA for DA agonistic activity is not reserved for catecholamines only. Replacement of the catechol moiety by heterocyclic aromatic moieties that resembles the electron distribution of DA, can also give compounds that act as a DA agonist.

Binding of a neurotransmitter to a receptor is a dynamic process. There is a certain equilibrium in the association and dissociation of the neurotransmitters and the receptors. A cellular response will only take place when a certain threshold in receptor stimulation is exceeded. Successful stimulation of receptors depends on the concentration of the appropriate
neurotransmitter, the concentration of the receptor, the ability of the receptor to bind the neurotransmitter, and the intrinsic activity of the neurotransmitter. Strength of the electrostatic interactions of neurotransmitter and receptor, and the functional state of the receptor are very important factors in binding dynamics.

![Chemical Structures](image)

**Figure 1.2** DA (1.1) and some examples of molecules incorporating the structural, electronic and functional characteristics of DA; apomorphine (1.2), dihydrexidine (1.3), quinpirole (1.4), quinelorane (1.5).

### 1.3.2 DA receptors are coupled to a G-protein

DA receptor proteins are part of what are called the G-protein coupled receptors. This type of receptor is a transmembrane protein characterized by the attachment of guanine nucleotide binding protein (G-protein) at the inside of the cell membrane (Figure 1.3). The G-protein is made up of $\alpha$, $\beta$, and $\gamma$ sub-units and interconverts between a guanosinediphosphate (GDP) and a guanosinetriphosphate (GTP) bound form. In the absence of neurotransmitters, nearly all the G-protein is in the inactive GDP bound form.

After the receptor has been stimulated by a neurotransmitter, the neurotransmitter-receptor complex triggers the exchange of GTP for GDP. When the G-protein has bound GTP, the $\alpha$ sub-unit bearing GTP, dissociates from the $\beta,\gamma$ sub-units. The $G_\alpha$-GTP complex then diffuses to the enzyme “adenylyl cyclase” and binds to it to give “adenylate cyclase”. Association of the $G_\alpha$-GTP complex with adenylyl cyclase stimulates or inhibits the activity of
the cyclase enzyme. The stimulation or inhibition of adenylate cyclase is switched off by the $G_\alpha$-protein catalyzed hydrolyses of $G_\alpha$-GTP to $G_\alpha$-GDP. The $G_\alpha$-GDP complex dissociates from adenylate cyclase and returns to the receptor to binds the $\beta,\gamma$ sub-units, thereby completing the neurotransmitter induced cycle.

**Figure 1.3** Schematic representation of neurotransmitters acting on a $G$-protein coupled receptor with a stimulatory acting $G$-protein. Adapted from ref 1.

Adenylate cyclase catalyzes the cyclization of adenosinetriphosphate (ATP) to cyclic adenosinemonophosphate (cAMP). Two distinct $G_\alpha$-protein families have been described to regulate cAMP formation: one is specific for stimulation ($G_s$), and the other is specific for its inhibition ($G_i$). Inside the cell, cAMP is part of what is called the second-messenger system, and is involved in regulation of one or more biochemical processes, ultimately resulting in cellular response. The whole sequence of first- and second-messenger events can be summarized by starting from an electrical impulse (first-messenger) leading to stimulation of a receptor, through a transducer ($G_\alpha$-GTP) and an amplifier (adenylate cyclase) to a second messenger (cAMP) that sets off or halts a cascade of second-messenger events followed by a cellular response.

The ability of a receptor to bind its neurotransmitter depends on whether a ‘$G$-protein’ is attached to the cytosolic side of the receptor. The associated form is considered the functional state of the receptor, and is called the ‘high affinity state’, the dissociated form is called the ‘low
High and low affinity states are in an equilibrium that can be influenced by for instance ‘inverse agonists’. An inverse agonist directs the equilibrium towards the low affinity state. This is distinctly different from the action of an antagonist that prevents receptor stimulation by neurotransmitters, but does not influence the equilibrium. Since the pharmacological effect of these different actions is the same, ‘inverse agonism’ was only recently discovered. Many compounds thought to be antagonist in fact are now being recognized as ‘inverse agonists’.  

1.3.3 Elimination of DA from the synaptic cleft

For a neuron to pass a second action potential, the synaptic cleft needs to be free of neurotransmitters. About 70 to 80% of DA is cleared from the synaptic cleft by a carrier mediated, active re-uptake mechanism that transports DA back into the presynaptic neuron. Drugs like cocaine, benztropine, nomifensine and also amphetamine potently inhibit the re-uptake mechanism, drastically increasing DA levels and dopaminergic neurotransmission. Cocaine seems to be most specific as a re-uptake inhibitor, the other drugs mentioned also enhance the release of DA.  

DA that escapes re-uptake is removed by passive diffusion or is metabolized by primarily three enzymes. The brain is also equipped with several omnipresent enzymes that rapidly chemically transform the functional groups of DA, rendering it inactive. Re-uptake and metabolism limits the passive diffusion (leaking) of neurotransmitters to other neurons, where they could interfere with signal transduction taking place there. Though a certain amount of leaking seems to be necessary for communication with parallel circuited neurons.  

The three enzymes that are primarily involved in the metabolism of catecholamines in the CNS are ‘monoamine oxidase’ (MAO), ‘aldehyde dehydrogenase’ (AH) and ‘catechol-O-methyltransferase’ (COMT). About 80% of the metabolic route proceeds by the oxidation of DA by MAO and AD to 3,4-dihydroxyphenylacetic acid (DOPAC) which can subsequently be O-methylated by COMT to homovanillic acid (HVA) (Scheme 1.2). Another metabolic route involves the action of the same enzymes in a different sequence. HVA and DOPAC are excreted from the brain as such or as a conjugate.  

COMT is an enzyme that is found in the cytoplasm of neurons and glial cells. MAO and AD are membrane-bound enzymes, which are found on the outer layer of the mitochondria in neurons and glial cells. Two types of MAO enzymes are recognized, yet both MAO-A and
MAO-B are able to oxidize DA. MAO-B predominantly exists in the human brain and can be selectively inhibited by Selegiline®, that is found useful as adjunct treatment to L-dopa therapy in the treatment of Parkinson’s disease.

\[
\text{O} \quad \text{NH}_2 \\
\text{HO} \\
\text{DA}
\]

\[
\text{O} \quad \text{NH}_2 \\
\text{HO} \\
3-\text{MT}
\]

\[
\text{O} \quad \text{CHO} \\
\text{HO} \\
\text{AD}
\]

\[
\text{O} \quad \text{CO}_2\text{H} \\
\text{HO} \\
\text{HVA}
\]

\[
\text{O} \quad \text{CO}_2\text{H} \\
\text{HO} \\
\text{DOPAC}
\]

**Scheme 1.2** Enzymatic metabolism of DA in the brain. Abbreviations: MAO, monoamine oxidase; AD, aldehyde dehydrogenase; COMT, catechol-O-methyltransferase; DOPAC, 3,4-dihydroxy-phenylacetic acid; HVA, homovanillic acid.

### 1.3.4 DA receptor subtypes

In 1978, two distinctly different DA receptors were identified. These were classified as D\(_1\) and D\(_2\) receptors based on their respective inhibition and stimulation of adenylate cyclase after receptor stimulation.\(^{10}\) Later, molecular biologist showed that D\(_1\) and D\(_2\) receptors had different structures and originated from the expression of different genes. When molecular biology techniques improved, at least five different DA receptors were identified (D\(_1\), D\(_2\), D\(_3\), D\(_4\), and D\(_5\)).\(^{11-19}\) These five so-called subtypes were designated to the original classification. The DA D\(_1\) and D\(_5\) receptors both act stimulatory on adenylate cyclase and are referred to as the D\(_1\)-like receptors. DA D\(_2\), D\(_3\), and D\(_4\) receptors act inhibitory on adenylate cyclase and are part of the D\(_2\)-like receptors. Homology of the protein sequence in human DA receptor subtypes varies considerably (40-82%).\(^{20-23}\) The largest differences are found between the D\(_1\)-like and D\(_2\)-like
receptors and within the D₂-like receptor family. Differences in the structures of DA receptor subtypes cause the binding affinity of DA to be different for each receptor subtype. Though the active sites of the receptor subtypes are located in the same vicinity, different amino acid residues involved in binding DA and each receptor subtype has its own equilibrium between the ‘high’ and ‘low affinity states’. The fact that the active sites are not identical means that DA needs to meet different spatial requirements to bind to different receptor subtypes.

The discovery of the DA receptor subtypes initiated research to find compounds that selectively would bind a single receptor subtype. Through pharmacological evaluation, subtype selective agonists and antagonists, could help unravelling the function of the receptor subtype and possibly lead to treatment of subtype related neurological disorders. Nowadays the development of subtype selective agonists is assisted by scientific data on receptor protein structure and computer assisted receptor modelling.

1.4 DA AGONISM; STRUCTURE, ACTIVITY AND SELECTIVITY

1.4.1 Rotameric conformation

Before advanced molecular biology was available, the spatial requirements of DA for DA receptor binding were investigated by the chemical synthesis of compounds incorporating different structural and functional characteristics of DA. Pharmacological evaluation of these compounds led to the development of several receptor models.

In 1975, Cannon proposed that DA might interact with its receptor in two trans coplanar conformations which he called the ‘α-’ and ‘β-rotameric’ conformation (Figure 1.4). In both rotamers the catechol, the ethyl chain, and the amine moiety display coplanarity in an extended conformation. Difference between the two rotameric forms is only the relative orientation of the meta-hydroxy group; the para-hydroxy group is stationary.

![Figure 1.4 α- and β-rotameric conformations of DA.](image)
This is illustrated by the prototypical mixed DA D₁/D₂ agonists (R)-apomorphine (1.2) and (R)-N-n-propyl-apomorphine (1.6) that are relatively flat molecules, incorporating the extended α-rotamer of DA.²⁴ (R)-N-n-propyl-apomorphine acts as a full agonist at both receptors, however, apomorphine acts as a full agonist at the DA D₂ receptor and as a partial agonist at the DA D₁ receptor.²⁵ The monophenolic analogs of both compounds (1.7 and 1.8), also possess dopaminergic properties, and have a considerable increased selectivity for the DA D₂ receptor over the DA D₁ receptor.²⁶,²⁷ 11-Hydroxy-aporphine 1.7 acts as antagonists at the DA D₁ receptor.²⁸ The aporphine family incorporates the most potent DA agonists known.

![Figure 1.5 Structures of (R)-apomorphine (1.2), (R)-N-n-propyl-apomorphine (1.6), (R)-11-hydroxy-aporphine (1.7) and (R)-11-hydroxy-N-n-propyl-aporphine (1.8).](image)

The absolute configuration of the (R)-apomorphine is essential for its DA agonism. Its optical antipode, (S)-apomorphine (1.9) does not act as a DA agonist, instead, it was found a weak DA receptor antagonist (Figure 1.6).²⁹ Studies on the β-rotameric analog of apomorphine, called isoapomorphine, have shown both enantiomers of that compound to be inactive at the DA receptor.³⁰,³¹

![Figure 1.6 Structures of (S)-apomorphine (1.9), (R)-isoapomorphine (1.10), (S)-isoapomorphine (1.11).](image)
Another class of synthetic compounds that incorporates potent DA agonists, is the 2-aminotetralin family. They are somewhat less rigid compared to the aporphines, but share their ability to restrict the extended rotameric conformation.\textsuperscript{32-36} (S)-5,6-dihydroxy-2-(N,N-di-n-propylamino)tetralin (1.12) is fixed in the $\alpha$-rotameric conformation and acts as a very potent mixed DA D\textsubscript{1}/D\textsubscript{2} full agonist (Figure 1.7). The (R)-enantiomer of this structure is much less active. In contrast to that, the $\beta$-rotameric analog, (R)-6,7-dihydroxy-2-(N,N-di-n-propylamino)tetralin (1.13) is considerably more active as a DA agonist than its (S)-optical antipode.

![Figure 1.7](structures.png)

\textbf{Figure 1.7} Structures of (S)-5,6-dihydroxy-2-(N,N-di-n-propylamino)tetralin (1.12) and (R)-6,7-dihydroxy-2-(N,N-di-n-propylamino)tetralin (1.13).

McDermid and co-workers explained these findings by the suggesting that binding of the enantiomers compounds discussed primarily was controlled by the position of the \textit{meta}-hydroxy group (see Figure 1.4).\textsuperscript{37} They rationalized that, since binding to the same DA receptor for $\alpha$- and $\beta$-rotameric conformations evidently required an opposite absolute configuration, one of the rotameric forms must be in a flipped position in the active site. This view was supported by the results of pharmacological studies with (S)-5-hydroxy-2-(N,N-di-n-propylamino)tetralin (1.14) and (R)-7-hydroxy-2-(N,N-di-n-propylamino)tetralin (1.15) (Figure 1.8).\textsuperscript{36,38,39}

These monophenolic derivatives lack the \textit{para}-hydroxy group (see Figure 1.4) of the compounds 1.12 and 1.13 yet their enantiomers behaved similar as their catechol analogs. Both compounds are the most potent monophenolic DA agonists in this series. The loss of the \textit{para}-hydroxy group increased DA D\textsubscript{2}/D\textsubscript{1} receptor selectivity, similar to the case of the aporphines.\textsuperscript{37} Both 1.14 and 1.15 have considerable affinity for the DA D\textsubscript{3} receptor and 1.15 seems to have some selectivity for the DA autoreceptors.\textsuperscript{40-45} Surprisingly, incorporation of both hydroxy groups in one molecule considerably reduced the activity at the DA receptors.\textsuperscript{46}
McDermid and co-workers explained the inactivity of isoapomorphine at the DA receptor in their model by including a steric boundary in the DA receptor’s active site, not tolerating the steric bulk of the non-oxygenated aromatic ring.\textsuperscript{37} Grol and co-workers independently came forth with a similar model.\textsuperscript{47,48} Wikström and co-workers extended the DA receptor model by explaining N-alkyl substituent effects, Seiler and Markstein contributed understanding of steric barrier and accessory binding sites differentiating D\textsubscript{1}-like and D\textsubscript{2}-like receptors subtypes.\textsuperscript{42,49-51} It is now generally accepted that DA binds in different conformations to the various DA receptor subtypes.

1.4.2 DA D\textsubscript{1} and DA D\textsubscript{2} receptor selective agonists

Especially catecholic DA analogs seem to incorporate mixed DA D\textsubscript{1} and DA D\textsubscript{2} agonism. The N-alkyl substituent seems to somewhat influence the balance between the affinity for the receptor subtypes. In N-n-propyl substituted 1.6, DA D\textsubscript{2}/D\textsubscript{1} selectivity emphasized relative to N-methyl substituted 1.2.\textsuperscript{26,27} Loss of the para-hydroxy group (see figure 1.4) in the DA \(\alpha\)-rotameric conformation drastically increases DA D\textsubscript{2}/D\textsubscript{1} selectivity.\textsuperscript{52} Further examples of compounds with increased DA D\textsubscript{2}/D\textsubscript{1} selectivity are given in Figure 1.9.

The conformationally flexible phenylethylamine RU24213 (1.16) can assume both \(\alpha\)- and \(\beta\)-rotameric conformations and is a DA D\textsubscript{2} agonist. Analogous to 1.16, the 2-aminotetralin derivatives N-0434 (1.17) and N-0437 (1.18) were developed, of which 1.18 made it to phase 3 clinical trials as a potential anti-Parkinson agent.\textsuperscript{52-58} (+)-PHNO (1.19) also has a high selectivity.
for the DA D₂ receptor over the DA D₁ receptor. Like 1.4, 1.5, and 1.15, 1.19 displays a higher affinity for the DA D₃ receptor than for the DA D₂ receptor. ⁵⁹

Figure 1.9 Structures of compounds with increased DA D₂/D₃ receptor selectivity. RU-24213 (1.16), (S)-N-0434 (1.17), (S)-N-0437 (1.18), (+)-PHNO (1.19), quinpirole (1.4), quinelorane (1.5).

Typical selective DA D₁ receptor agonists usually are structurally related to the β-rotameric conformation of DA (Figure 1.10). The first class of selective DA D₁ agonists identified were the benzazepines.⁶⁰-⁶⁸ Investigation of structure-activity-relationships (SARs) led to the discovery of several other compounds that displayed similar pharmacological properties.⁶⁶,⁶⁹-⁷⁶

Through extensive work on SARs many other DA agonists with selectivity for other DA receptor subtypes were discovered. The scope of this thesis, however, mainly concerns DA D₁ and DA D₂ agonism and its potential clinical applications. Therefore, DA antagonists in general or DA agonists selective for other DA receptor subtypes will not be discussed. The interest we have in DA D₁ and DA D₂ agonism is related to the function of these receptors in the brain and their involvement in Parkinson’s disease.
Chapter 1

Figure 1.10 Structures of compounds with DA D₁ receptor selectivity. (R)-SKF-38393 (1.20), (R)-SKF-82958 (1.21), DPTI (1.22), SKF89626 (1.23), dinapsoline (1.24), dihydrexidine (1.3), A-86929 (1.25).

1.5 PARKINSON’S DISEASE

1.5.1 Pathology of Parkinson’s disease

Parkinson’s disease is a progressive neurodegenerative disorder associated with the deterioration of the dopaminergic neurons in the medial forebrain bundle that project from the substantia nigra to the striatum in the nigrostriatal dopaminergic system. Loss of these neurons results in a hypokineti disorder marked by akinesia (impaired initiation of movement), bradykinesia (reduction of voluntary movement), muscular rigidity and tremor. In a healthy person loss of these neurons happens gradually in time and does not result in Parkinson’s disease. There is a large reserve of neurons in the dopaminergic nigrostriatal system and it is not until the loss of about 70-80% of all neurons that parkinsonian symptoms become apparent. In case of people, suffering from Parkinson’s disease there is an increasingly rapid deterioration of nigrostriatal neurons. Yet, the reserve of neurons is that large that the disease generally only becomes apparent after the age of 55. From that age there is an increasing incidence in people developing parkinsonian symptoms and Parkinson’s disease. It is usually after the symptoms
appear that Parkinson’s disease is diagnosed. Early stage diagnostics are being developed that apply to the loss of smell in early stage parkinsonism. Sensory stimuli like smelling are associated with the dopaminergic system in the olfactory tubercle that also seems to be affected in the early stages of Parkinson’s disease.⁷⁹

Parkinson’s disease results in severe motor dysfunction in patients because it affects the so-called reinforcing basal ganglia-thalamocortical ‘motor circuit’.⁷⁸,⁸⁰⁻⁸⁶ A simplified model of a normally functioning motor circuit is depicted in Figure 1.11, indicating the brain areas and different neurotransmitters involved. The motor circuit arises from the cerebral cortex that sends excitatory projections to the striatum. In addition, the striatum receives projections from the prefrontal and somatosensory cortex and an indirect cortical input through some of the thalamic nuclei (Thalamus). Direct cortical input is also received through the subthalamic nucleus (STN). The two major output systems of the striatum, the substantia nigra pars reticulata (SNr) and the internal segment of the globus pallidus (GPi) are linked to the striatum. The GPi/SNr output system projects in an inhibitory fashion to the thalamus that subsequently provides the striatum with feedback and also projects to the cortex that further channels neuronal impulses to the muscles.

At the start of this circuit in the striatum, DA D₁ and DA D₂ receptors are involved in neurotransmission. There is a ‘direct’, DA D₁ mediated pathway from the striatum that is inhibitory on the GPi/SNr. Inhibition of the GPi/SNr results in an excitatory signal to the thalamus that in turn sends excitatory signals to the cortex stimulating cortically generated movement. Striatal DA D₁ receptors activate striatal γ-butyric acid (GABA), substance P and dynorphine neurons that project to the GPi/SNR. There is also an ‘indirect’ pathway that originates in the striatum, mediated by DA D₂ receptors, that proceeds through the external segment of the globus pallidus (GPe) and the subthalamic nucleus (STN) to the SNr. The striatal DA D₂ receptors inhibit GABA and enkephalin neurons projecting to the GPe. The GPe is linked inhibitory to the STN through GABA neurons than upon inhibition activates GPi/SNR via glutaminergic neurons. DA D₁ and DA D₂ receptors are also linked to striatal acetylcholine release in an excitatory and inhibitory fashion respectively.⁸⁷,⁸⁸ Contrary to the direct pathway, the indirect pathway eventually acts stimulatory on the GPi/SNr, inhibiting cortically generation of movement. DA D₂ receptors in the striatum inhibit the indirect pathway and the DA D₁ receptors stimulate the direct pathway. Although DA D₁ and DA D₂ receptors are involved in opposing pathways, their ultimate effect on the output of the motor circuit is the same. Where
DA D₁ receptors throttle up the direct pathway, DA D₂ receptors function as a break on the indirect pathway.

**Figure 1.11** Schematic representation of the ‘motor circuit’ in the brain under normal circumstances. Abbreviations: DA, dopamine; D₁, DA D₁ receptor (■); D₂, DA D₂ receptor (▲); STN, subthalamic nucleus; SNr, substantia nigra pars reticulata; GPi, internal segment of the globus pallidus; GPe, external segment of the globus pallidus; SNc, substantia nigra pars compacta; Th, thalamus; GABA, γ-butyric acid; ENK, enkephalin; DYN, dynorphin, GLU, glutamate; SP, substance P; ACh, acetylcholine. + = stimulatory action, – = inhibitory action. Adapted from ref 89.

It is generally believed that a certain balance between the direct and indirect pathway needs to exist for movement to be expressed properly. Since the body continuously moves, under normal circumstances, the indirect pathway must be favored. An additional modulating role is played by the substantia nigra pars compacta (SNc). This nucleus releases DA in the striatum favoring the direct, inhibitory pathway. In addition it also projects to the SNr, where released DA interacts with the present DA D₁ receptors, further favoring the direct pathway. In preparing for and during movement, tonically released DA alters the balance between the two pathways in favor of the direct pathway. Striatal acetylcholine release favours the indirect pathway and is inhibited by the SNc through DA release in the striatum that stimulates the DA D₂ receptors.

In a movement disorder like Parkinson’s disease, neurons in the substantia nigra have deteriorated. Absence of DA, disables the direct pathway, favoring the indirect pathway,
reducing cortically generated movement as illustrated by parkinsonian symptoms. In a hyperkinetic disorder like Huntington’s chorea, the balance is thought to have flipped the other way. Overemphasizing, the direct pathway results in the increased motor activity as observed in Huntington’s chorea.

1.5.2 DA D1/D2 receptor interactions

Two types of DA D1/D2 interactions have been described, co-operative/synergistic interactions and opposing interactions. Opposing interactions were first demonstrated on a biochemical level, by the observation that a selective DA D2 agonist, reduced DA D1 agonist induced stimulation of cAMP in the rat striatum. Both receptor subtypes are involved in balancing the activity of adenylyl cyclase. Also on the level of interstriatal acetylcholine neurons the two receptor subtypes have opposing effects.

Synergistic interactions between the two receptor subtypes were reported in the regulation of the enzyme (Na+/K+)ATPase. This transmembrane enzyme couples the hydrolysis of ATP to the transmembrane exchange of Na+ and K+ ions, maintaining the ionic gradient involved in the generation of neuronal action potentials. The molecular basis of the synergistic interaction is that both DA receptor subtypes stimulate the release of arachidonic acid that potently inhibits (Na+/K+)ATPase. Therefore, stimulation of one receptor subtype selectively, will influence also affect the (Na+/K+)ATPase activity connected to the other receptor subtype.

The functional interaction of DA D1 and DA D2 receptors still is under debate. It should be noted that the terms DA D1 and DA D2 receptor subtypes commonly are used under the old classification meaning DA D1-like or DA D2-like receptors. Many of the formerly considered ‘selective’ compounds used in the exploration of the motor circuit, in recent years have proved to be non-selective within their own subtype family. With the availability of more selective dopaminergic agents also the potential involvement of DA D3 and DA D4 receptors in were investigated. It has recently been shown that DA D3 and DA D4 receptors do not synergize with DA D1 receptors while DA D1 and DA D2 receptors seem to synergize without interaction through action potentials.

Under conditions of impaired dopaminergic neurotransmission, DA D1/D2 interactions changes occur in DA receptor subtype density and sensitivity. Nigrostriatal DA D2 receptor mRNA is increased while nigrostriatal DA D1 receptor mRNA is decreased. Changes
Chapter 1

that can be reversed by the administration of DA D_{2} and DA D_{1} agonists, respectively.\textsuperscript{81,105} On a theoretical level the motor circuit, under normal and impaired conditions, DA D_{1} and DA D_{2} receptor interaction remains much of an enigma. Functional interactions of DA D_{1} and DA D_{2} receptors under both conditions actually are best observed by studies in behavioral models.

1.5.3 Behavioral consequences of D_{1}/D_{2} receptor interactions

In normal rats, subtype selective DA agonists are able to induce specific abnormal behavior. Yet, behavioral effects induced by selective DA D_{2} agonists or mixed DA D_{1}/D_{2} agonists could be partially reversed by selective DA D_{1} antagonists.\textsuperscript{106-111} For instance, licking behavior induced by the DA D_{2} agonist (S)-5-hydroxy-2-(N,N-di-n-propylamino)tetralin (1.14) can be fully counteracted by the DA D_{1} antagonist SKF-23390, demonstrating opposing DA D_{1}/D_{2} receptor interaction. Furthermore, selective DA D_{1} antagonists are able to counteract akinesia induced by selective DA D_{2} antagonists or mixed DA D_{1}/D_{2} antagonists. This shows that compounds may selectively bind to a DA receptor subtype yet the induced behavioral effect appears to be non-selective demonstrating a functional interaction between the two subtypes.

It is interesting that administration of a mixed DA D_{1}/D_{2} agonist or co-administration of a selective DA D_{1} and D_{2} agonist induces an increase in abnormal behavior. This additional behavior is characterized by compulsive rearing behavior (i.e. standing on the hind limbs) and by gnawing behavior (not to be confused with chewing). None of this behavior is observed for the subtype selective agonists and, therefore, must be the consequence of a synergistic DA D_{1}/D_{2} receptor interaction. The efficacy at the DA D_{1} receptor is important for the induction of gnawing behavior. Apomorphine (1.2), that is a partial agonist at the DA D_{1} receptor and a full agonist at the DA D_{2} receptor, is less able to induce gnawing than (S)-5,6-dihydroxy-2-(N,N-di-n-propylamino)tetralin (1.12), a full agonist on both receptor subtypes.\textsuperscript{25}

In animals with impaired dopaminergic neurotransmission and normally sensitive DA receptors a synergistic interaction is observed between the receptor subtypes. For instance in rats with a unilateral striatal lesion elicited by the excitotoxin quinolinic acid, only stimulation of the DA D_{2} receptors induces rotational locomotor behavior. However, simultaneous stimulation of the DA D_{1} and DA D_{2} receptors dramatically increases rotational behavior up to 300\%.\textsuperscript{112} Rotational behavior is mediated by the intact hemisphere and is directed towards the lesioned side (ipsilateral). A similar result was observed after systemic administration of the DA depletor reserpine to mice, rendering them akinetic, followed by DA agonists treatment 3 to 4h later.
Maximal possible locomotor activity then was only observed after simultaneous stimulation of both DA D_1 and DA D_2 receptors.^{113-116}

However, in animals with supersensitive DA receptors the receptor subtype interaction has changed. When treatment by DA agonists follows >18h after treatment with reserpine, it suffices to administer a DA D_1 or a DA D_2 agonist alone, in order to achieve a maximum locomotor response. Dopaminergic effects induced >18h after treatment with reserpine, can only be counteracted by the corresponding subtype selective antagonist, thus the effects of supersensitized DA D_1 and DA D_2 receptors seem uncoupled.^{116,117} Likewise, a bilateral lesion in the rats medial forebrain bundle, using the neurotoxin 6-hydroxy-DA (6-OH-DA), also uncouples the functionality of DA D_1 or DA D_2 receptors.^{105,118} As this lesion is in place, the SNc no longer can modulate the balance between the direct and indirect pathway, and thus the indirect pathway is favored. The extent of the lesion is of great importance as was shown in the Ungerstedt rat rotation model for Parkinson’s disease. A unilateral lesion of the medial forebrain bundle renders the postsynaptic receptors on the lesioned side supersensitive. Introduction of both a DA D_1 and a DA D_2 agonist separately is expected to be able to induce a maximal effect in contralateral rotational behavior. Surprisingly, rats responded differently to the lesioning and two separate groups of could be identified. High rotators, that had functionally uncoupled DA D_1 and DA D_2 receptors and low rotators, that had less supersensitized receptors, an needed stimulation of both DA D_1 and DA D_2 receptors for a maximal effect. Biochemistry of the low rotator group indicated some form of adaptation to the introduced lesion.^{119,120}

Highly and little responsive animals were also observed in a primate model of Parkinson’s disease. In this model, MPTP treated monkeys have selectively lost their mesostriatal dopaminergic neurons by the neurotoxic activity of the MPTP metabolite MPP^+.^{121-124} In some studies treatment with a combination of DA D_1 and DA D_2 agonists was found be superior to treatment with separate DA D_1 or DA D_2 agonists.^{125-127} Other studies with different DA D_1 agonists showed that is was possible to treat parkinsonism by selective stimulation of the D_1 receptor.^{74,128} Possibly in the stages of Parkinson’s disease when DA D_1 and DA D_2 receptors are not supersensitized yet, stimulation of both DA receptor subtypes would result in the highest clinical efficacy. In the stage of supersensitization, treatment with either a DA D_1 agonist or a DA D_2 agonist independently also might give a good clinical efficacy. However, the downside of treatment with either a DA D_1 or a DA D_2 agonist, is that brain levels of NA and serotonin (5-HT) are lowered upon chronic treatment possibly leading to adverse events.^{129} In contrast,
chronic stimulation of both DA D$_1$ and DA D$_2$ receptors simultaneously have a mild opposite effect.

It is important to note that DA, that is increasingly depleted Parkinson’s disease, is an agonist at DA D$_1$-like and DA D$_2$-like receptors. Under normal conditions, DA is able to maintain a balance between all primary and secondary receptor interactions. Chronic treatment of Parkinson’s disease therefore is best aimed at reinstating the normal balance and sensitivity of all DA receptors. Treatment with subtype selective DA agonists will desensitize the corresponding DA receptor subtype, leaving the other supersensitized. Endogenous DA (while still present) will have an increasing effect on the remaining supersensitized receptor subtype, eventually resulting in its desensitization. As long as supersensitization is in state, functional uncoupling of DA D$_1$ and DA D$_2$ receptors probably remains. After desensitization of the non-targeted receptor, coupling of the receptor subtypes may be reinstated but neurotransmission of the targeted receptor subtype will prevail, disabling the normal balance in neurotransmission. If no more endogenous DA is present, as in advanced stages of the disease, the non-targeted receptor subtype remains supersensitive possibly maintaining functional uncoupling of DA D$_1$ and DA D$_2$ receptors.

Most likely, the best way to direct the whole system back to a normalized condition would be through simultaneous stimulation of both DA D$_1$ and DA D$_2$ receptors. Chronic treatment with a mixed DA D$_1$/D$_2$ agonist or a combination of a DA D$_1$ and a DA D$_2$ agonist will gradually desensitize both receptor subtypes. The balance between DA D$_1$ and DA D$_2$ agonism and desensitization will be a crucial factor in this. Results of clinical testing clearly show advantages of simultaneous stimulation of DA D$_1$ and DA D$_2$ receptors in patients suffering from Parkinson’s disease.$^{130}$

1.6 TREATMENT OF PARKINSON’S DISEASE

1.6.1 History of anti-parkinsonian agents

There is no known cure for Parkinson’s disease. Treatment is aimed at controlling the symptoms by controlling the imbalance of the neurotransmitter(s) involved. In 1817, James Parkinson described in a scientific essay for the first time the clinical picture of the disease.$^{131}$ He thoroughly described the nowadays well-known characteristic symptoms. Without any clear picture of the pathology of the disease, Charcot in 1860, prescribed Solanaceae plant to reduce
rigidity and tremor. It took until 1924 that the active compound in the plant, the anti-cholinergic atropine (1.26), was used in its pure form (Figure 1.12). Treatment with anti-cholinergics was only sufficient for patients that suffered from tremor and mild muscular rigidity.

In 1884, the semi-synthetic apomorphine (1.2) was first used to treat Parkinson’s disease patients (PD-patients). After its first synthesis, apomorphine became especially known for its emetic properties. Apomorphine was reported to have sedative and tranquilizing properties and was used to treat a variety of psychiatric disorders. It was also found to have a beneficial effect on Sydenham’s chorea, a movement disorder caused by acute rheumatic fever. The potential usefulness of apomorphine on Sydenham’s chorea inspired Weil and co-workers to use apomorphine PD-patients. In 1923 it was described by Amsler that apomorphine induced chewing behavior could be related to an influence of apomorphine on the striatum. In 1951, Schwab and co-workers found that subcutaneous injection of apomorphine could cause marked though short-lived improvement in PD-patients. With the indication of structural similarity of the molecular structures of apomorphine and DA by Ernst, a landmark was set.

![Figure 1.12 Structures of atropine (1.26), apomorphine (1.2) and L-dopa (1.27).](image)

However, with the growing availability of alternative drugs, apomorphine was hardly used in the clinical practice until a revival that started quite recently. Lack of oral availability of apomorphine and apomorphine induced adverse effects limited its application. This, despite the fact that apomorphine is a partial DA D_1 and full DA D_2 agonist, a combination which to this day provides the most powerful clinical efficacy. Some of the adverse effects could be reduced after in 1979, Agid and co-workers found that co-administration of domperidone with apomorphine, could prevent apomorphine induced nausea, drowsiness and arterial hypertension. Domperidone is a peripheral DA D_2 antagonist, meaning it does not
cross the blood brain barrier and therefore only counteracts apomorphine peripherally. Furthermore, in 1988, Stibbe and co-workers reported on the positive effects of apomorphine on PD-patients that had progressed to a state of the disease with “on-off” symptoms. The “on-off effect” is a condition marked by rapid fluctuation between mobility and immobility that arises after long-term treatment with the most efficacious anti-parkinsonian drug available, L-dopa (1.27).

1.6.2 The L-dopa era

The L-dopa era began when Carlsson and co-workers, in 1957, first demonstrated that reserpine induced akinesia in rabbits could be reversed by administration of L-dopa, the natural precursor of DA. Due to its inability to cross the blood-brain barrier, DA itself does not have this effect when administered systemically. The blood-brain barrier is a vital system enclosing most parts of the brain (Figure 1.13). Both L-dopa and DA cannot freely cross the blood-brain barrier because they lack sufficient lipophilicity but L-dopa does cross the blood-brain barrier because it is actively transported across.

**Figure 1.13** The blood brain barrier (Adapted from ref 1).
In 1960, striatal DA deficiency was diagnosed by post-mortem studies on the brains of people that had suffered from Parkinson’s disease. In 1961, L-dopa was first tried on PD-patients, but throughout the 1960’s inconsistent results were obtained. In 1967, the effectiveness of L-dopa was demonstrated by the dramatic improvement in the condition of PD-patients after oral administration of L-dopa in increasing doses over long periods. It was noticed that co-administration of aromatic amino acid decarboxylase (AADC) inhibitors like Benserazide (1.28), carbidopa (1.29), brocresine (1.30), and α-methyldopa (1.31) could potentiate the effect of L-dopa (Figure 1.14). Especially peripheral AADC inhibitors had that ability and at the same time reduced nausea and anorexia. Further advantages of adding AADC inhibitors to L-dopa treatment were discovered: rapid induction of treatment, reduced L-dopa dose requirement, and better diurnal symptomatic control.

![Figure 1.14](image)

*Figure 1.14 Structures of some AADC inhibitors. Benserazide (1.28), carbidopa (1.29), brocresine (1.30), and α-methyldopamine (1.31).*

Co-administration of L-dopa with catechol-O-methyl transferase (COMT) or monoamine oxidase (MAO-B) inhibitors may extend the action of L-dopa, allowing for lower doses thus reducing the L-dopa peak-dose induced complications. The new reversible peripheral COMT inhibitors entacapone (1.32), nitecapone (1.33) increase patients’ duration of response to L-dopa and reduce response fluctuations, however peak-dose dyskinesias are also prolonged though not increased (Figure 1.15).
Selegiline® (1.34) is a well-established MAO-B inhibitor used as add-on to L-dopa treatment (Figure 1.15). Early treatment with Selegiline® delays the need for initiation with L-dopa therapy. Next to its MAO-B inhibiting properties, Selegiline® also reduces DA re-uptake thereby further increasing extracellular DA levels. Other effects that are suggested to accompany treatment with Selegiline® are its neuroprotective actions but so far these effects have not been well established. It is hypothesized that Selegiline® is involved in both the reduction of free radical formation and as acts as a neurotrophic factor rescuing dopaminergic neurons.

![Figure 1.15 Structures of COMT inhibitors entacapone (1.32), nitecapone (1.33), and the MAO-B inhibitor Selegiline® (1.34)]

Most PD-patients initially respond well to L-dopa treatment. To this day L-dopa is the most widely used and effective drug available for treatment of PD-disease and, regarding efficacy, its closest competitor is apomorphine. The most important adverse events of L-dopa treatment are hallucinations, confusion, orthostatic hypotension. Adverse events like nausea and vomiting can be suppressed by the peripheral DA D₂ antagonist domperidone, but also wear off after some time.

Within 5 years of L-dopa treatment about 50% of the PD-patients experience dose-response fluctuations and “on-off” effects. Patients require increasing doses of L-dopa to manage their symptoms as the efficacy of L-dopa “wears off” and experience “end-of-dose deterioration”. This is mostly blamed on the pharmacokinetics of L-dopa (the short half-life), and the progressiveness of the disease. The progressiveness of the disease may account for a less efficient turnover of L-dopa to DA and increasingly impaired neurotransmission. As L-dopa is decarboxylated to give DA in the deteriorating nigrostriatal dopaminergic neurons, the turnover is progressively limited. In addition, a reduction in the striatal uptake of L-dopa has been observed. Continuous intravenous infusion of L-dopa with an AADC to stabilize plasma
levels did not alleviate the “on-off” effects because DA receptors became desensitized. When the infusion time was reduced to 6h daily, the “on-off” effects were somewhat reduced in PD-patients. Long-acting L-dopa preparations have been developed for over 20 years. The first preparations gave inconsistent clinical result though recently a number of slow-release preparations were marketed. These preparations seems to have a positive effect on the “on-off” effects and “end-of-dose” deterioration.

The therapeutic efforts to control the long-term complications of L-dopa treatment are questionable. There is evidence that L-dopa actually acts as a double-edged sword, alleviating parkinsonism but hastening disease progression. The neurotoxicity of L-dopa, so far, has only been confirmed by in vitro studies, but could explain the progressively deteriorating condition of PD-patients. Neurotoxicity of L-dopa concerns the formation of peroxides, free radicals (oxidative stress), and formation of toxic metabolites by MAO-B. The peroxides oxidize glutathione (GSH), and react with iron-ions to produce the highly toxic free radicals. In post-mortem tissue of PD-patients, show increased levels of iron in the substantia nigra and reduced levels of GSH. Evidence of oxidative damage to lipids, proteins, and DNA was also found. When L-dopa therapy is given in combination with the anti-oxidant ascorbic acid further improvement in the condition of the PD-patient is reported. Only recently clinical trials were planned to examine the potential neurotoxicity of L-dopa in vivo.

Interestingly, recently evidence was produced that L-dopa along with DA and other catecholamines like apomorphine have a neurotrophic action. Induction of the brain derived neurotrophic growth factor (BDNF) envisages a neuroprotective role for catecholamines in general. Since this potential neuroprotective role probably can not prevent the long-term complications that are observed in Parkinson’s disease, its contribution to the treatment of Parkinson’s disease remains doubtful. Investigations on apomorphine or other potent mixed DA D1/D2 catecholamine based agonists could provide further insight. Since relatively low doses of these are required for treatment of PD-disease, this could possibly shift the balance from catecholamine induced neurotoxicity to catecholamine induced neuroprotection.

1.6.3 Dopamine agonists

Although L-dopa treatment is still regarded as the standard of anti-parkinsonian drug therapy, motor response oscillations and drug-induced, abnormal involuntary movements develop in PD-patients who undergo long-term L-dopa monotherapy. In addition to
these motor complications, other complications may develop like postural imbalance, gait disorder, freezing, speech impairment and neuropsychiatric disorders like depression, confusion and hallucinations.\textsuperscript{200} DA agonists have played a classical role as adjuncts to L-dopa therapy to smooth out motor response oscillations once they have developed. Today DA agonists are also used as add-on treatment in the early stages of L-dopa treatment and prior to starting L-dopa treatment.

DA agonists act directly on the pre- and postsynaptic DA receptors and do not rely on the same metabolic conversion of L-dopa to DA. By stimulating the autoreceptor, they decrease DA turnover thereby decreasing the formation of potentially neurotoxic peroxides and free radicals. Catecholamine based DA agonists may also induce the formation of these toxic compounds. Yet, a sufficiently potent agonist only needs to be administered at a low dose thereby, limiting potential harm. Furthermore, DA agonists may exert neuroprotective effects, which has indeed been shown in some animal models of Parkinson’s disease.\textsuperscript{201-203} DA receptor subtype selectivity was usually targeted at the DA D\textsubscript{2} receptor (Table 1.1). Recently also

\begin{table}[h]
\centering
\begin{tabular}{|l|l|l|l|}
\hline
Dopamine agonist & DA receptor interaction & NA & 5-HT \\
\hline
DA (through L-dopa)\textsuperscript{8,129,207} & D\textsubscript{1} > D\textsubscript{2} > D\textsubscript{3} & b & b \\
Apomorphine\textsuperscript{8,207} & D\textsubscript{2} > D\textsubscript{1} > D\textsubscript{3} & c & c \\
Bromocriptine\textsuperscript{155,196-199} & D\textsubscript{2} (D\textsubscript{3}) & + & + \\
Lisuride\textsuperscript{200-202} & D\textsubscript{2} (D\textsubscript{1}) & + & + \\
Pergolide\textsuperscript{8,141,203,208-211} & D\textsubscript{2} > D\textsubscript{1} > D\textsubscript{3} & + & + \\
Cabergoline\textsuperscript{169,212} & D\textsubscript{2} > D\textsubscript{1} & (+) & (+) \\
Ropinirole\textsuperscript{170,171,213} & D\textsubscript{3} > D\textsubscript{2} > D\textsubscript{4} & - & - \\
Pramipexole\textsuperscript{214,215} & D\textsubscript{3} > D\textsubscript{2} > D\textsubscript{4} & + & - \\
\hline
\end{tabular}
\caption{Receptor interactions of DA agonists (Adapted from ref. 206)}
\end{table}

\textsuperscript{a}NA = noradrenaline; 5-HT = serotonin, \textsuperscript{b}Indirectly decreases neurotransmitter level, \textsuperscript{c}Indirectly increases neurotransmitter level.
Selective DA D<sub>1</sub> receptor agonists proved efficacious in the treatment of Parkinson’s disease, and DA agonists with DA D<sub>3</sub> receptor selectivity and DA D<sub>4</sub> affinity receptor were developed. Whether there is a clinical advantage for DA D<sub>3</sub>/D<sub>2</sub> receptor selectivity remains unclear.

As stated, the first DA agonist used in the treatment of Parkinson’s disease was apomorphine. It was synthesized by a rearrangement of morphine under acidic conditions and upon subcutaneous injection it gave a marked but short-lived improvement in PD-patients. Given the poor *in vivo* kinetics of apomorphine and the potential application of DA D<sub>2</sub> agonists in the treatment of Parkinson’s disease in general, ergot based drug were developed in the 1960’s. The most important DA agonists in the treatment of Parkinson’s disease and their characteristics can be summarized as follows:

**Apomorphine (1.2).** A highly efficacious anti-parkinsonian drug that is limited by its low oral bioavailability because of its extensive first-pass metabolism. However, it is readily absorbed via the subcutaneous, sublingual, rectal, or intranasal route. When administered subcutaneous or intravenously it has a half-life of about 30 min, corresponding to about an hour of clinical efficacy.

**Bromocriptine (1.35).** The first oral available DA D<sub>2</sub> receptor agonist to be used as an add-on to L-dopa therapy. Bromocriptine has similar affinity for the DA D<sub>3</sub> receptor and acts as an antagonist on the DA D<sub>1</sub> receptor. Characteristic of ergot derived DA agonists, it directly interacts with NA and 5-HT receptors and has a half-life of approximately 4.5h.

**Lisuride (1.36).** Lisuride was considered a very promising drug because of its positive effect on motor oscillations. It has a shorter half-life than bromocriptine and was mainly administered through parental administration. Like bromocriptine it is also ergot derived and has predominant DA D<sub>2</sub> receptor activity, whereas it acts as a partial antagonist at the DA D<sub>1</sub> receptor. Long-term treatment however resulted many times in drug-induced psychosis, rendering it an unsuitable drug.

**Pergolide (1.37).** Pergolide is also a member of the ergot derived DA agonist family with mainly DA D<sub>2</sub> receptor activity but also has DA D<sub>3</sub> receptor affinity. Unlike bromocriptine and lisuride, pergolide acts a weak agonist on the DA D<sub>1</sub> receptor. Its half-life is substantially longer than that of bromocriptine making it a promising drug for controlling motor fluctuations in Parkinson’s disease.
Cabergoline (1.38). The ergot based DA D\textsubscript{2} receptor agonist cabergoline is now widely used as an add-on to L-dopa treatment of Parkinson’s disease. It has an even longer half-life than pergolide and is used for controlling motor fluctuations.\textsuperscript{212}

Ropinirole (1.39). A non-ergot based mixed DA D\textsubscript{3}/D\textsubscript{2} receptor agonist with some advantages over bromocriptine when given in early monotherapy. Ropinirole fits the ergot pharmacophore yet is a more flexible molecule.\textsuperscript{169-171}

Pramipexole (1.40). Pramipexole is another drug that is non-ergot based with selectivity for the DA D\textsubscript{3}, and similar affinity for the DA D\textsubscript{2} and DA D\textsubscript{4} receptors. In addition, it has some affinity for the $\alpha_2$-adrenoreceptor. Studies have confirmed the efficacy of pramipexole as add-on treatment to L-dopa in patients with fluctuating Parkinson’s disease.\textsuperscript{213}

In Figure 1.16, the molecular structures of DA and the DA agonists that are used in the treatment of Parkinson’s disease are depicted. Compounds 1.35-1.38 incorporate the ergot skeleton, ropinerole (1.39) is structurally related though less rigid. Keto-enol tautomerism of the amide in ropinerole renders a 2-hydroxy indole structure that possibly could be important for its functional properties. Although there is a growing interest in the role of DA agonists as primary monotherapy in Parkinson’s disease, their main indication is still as adjunctive treatment to L-dopa therapy once L-dopa treatment related long-term complications arise. For the ergot derived drugs it has been shown that addition to L-dopa therapy has a positive effect on “wearing off” and “on-off fluctuations”. Doses of L-dopa may be reduced contributing to the decrease of the severity of dyskinesias.\textsuperscript{214-216} The relatively high DA D\textsubscript{2} receptor potency and weak DA D\textsubscript{1} receptor agonism of pergolide combined with its long half-life makes it superior to other DA ergot based DA agonists.\textsuperscript{217} Pergolide proved to be beneficial in PD-patients that no longer respond to bromocriptine treatment. In addition, the non-ergot derived drugs with a long half-life, ropinirole and pramipexole have proved to be superior to bromocriptine, when added to L-dopa treatment. Ropinirole and pramipexole have shown to be able to smoothen out response oscillations.\textsuperscript{212} PD-patients that undergo L-dopa therapy with DA agonists as add-on treatment still may respond to additional treatment with apomorphine. Apomorphine proved highly effective and reliable in reversing “off” periods when administered subcutaneously within 15 min. The potency of pen injection systems, sublingual tablets, apomorphine and the fact that it is well absorbed allow for low doses of apomorphine. Special and intranasal sprays are available methods of
administration. Sublingual tablets have the disadvantage of a late onset of action and the intranasal sprays induce local inflammation upon long-term use.\textsuperscript{146,218-222} Recently, also portable minipumps have become available to provide a continuous subcutaneous administration of apomorphine.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{structures.png}
\caption{Structures of DA agonists used in the treatment of Parkinson's disease. DA (1.1), apomorphine (1.2), bromocriptine (1.35), lisuride (1.36), pergolide (1.37), cabergoline (1.38), ropinirole (1.39), pramipexole (1.40).}
\end{figure}
Especially for PD-patients suffering from complex, unpredictable and frequent motor fluctuations are likely to benefit from these systems. Long-term studies have shown a sustained benefit with little evidence of tolerance to treatment.223,224 Today, early monotherapy with DA agonists is recommended and rarely leads to the development of complications L-dopa induces. However, as the disease progresses, the need for L-dopa treatment increases as other treatment often loses efficacy. Adjunctive treatment with DA agonists may reduce the severity of the complications. A DA agonist suitable to completely replace L-dopa as golden standard in the treatment of Parkinson’s disease has yet to be developed.225-227

The efficacy of DA agonists in early monotherapy is believed to be related to DA D<sub>1</sub> and DA D<sub>2</sub> receptor interactions. In an early stage of the disease, as the receptor subtypes are not supersensitive and their actions are still coupled, DA D<sub>2</sub> agonists and mixed DA D<sub>1</sub>/D<sub>2</sub> agonists are least likely to induce dyskinesias.224,228-230 Despite the development of several new DA agonists for the treatment of Parkinson’s disease, the ideal DA agonist with long duration of action and efficacy equal to that of L-dopa is still lacking. Apomorphine has about equal efficacy to L-dopa but lacks suitable in vivo kinetics. The growing recent interest in apomorphine and the development of long duration preparations call for the development of mixed DA D<sub>1</sub>/D<sub>2</sub> agonists with increased oral bioavailability.

1.6.4 Regenerative approaches

In the central nervous system certain proteins cause neurons to grow and proliferate. These so-called neurotrophic growth factors (NGFs) are divided into brain-derived growth neurotrophic factors (BDNFs) and glial cell-derived neurotrophic growth factors (GDNFs); both promote differentiation and survival of existing neurons. All NGFs promote outgrow of neurites from neurons and rescue specific populations of neurons from apoptosis.

The ability of the NGFs to promote neuron survival has led to extensive investigation of their potential application in the treatment of neurodegenerative diseases, like Parkinson’s disease. GDNF exerts a powerful, trophic effect on dopaminergic neurons in the part of the brain involved in movement. It has been suggested that Parkinson’s disease develops as a result of malfunctioning glial cells and, therefore, this strategy is especially promising.231,232 However, disappointing results in the clinical application of these proteins have called for the development of small non-protein based molecules that have mimic the action of NGFs. Several promising
candidates have been discovered and surprisingly some immunosuppressant drugs proved to induce neurotrophic effects. 233-238

Regeneration and rescuing of neurons in the treatment of Parkinson’s disease opens up a new and promising approach in drug research. It is expected that in the near future clinical trials will start to investigate the clinical efficacy of a first generation of NGFs. It is to be expected that the regenerative approach will establish a balance in degeneration and regeneration of dopaminergic neurons but will not cure Parkinson’s disease. Altogether, in the near future there is still an increasing demand for drugs that alleviate parkinsonian symptoms in PD-patients. Based on clinical results the ideal drug for the treatment of Parkinson's disease that is able to compete with L-dopa, needs the characteristics of apomorphine with better in vivo profile. Therefore, an orally available catecholamine with balanced mixed DA D1/D2, seems to have the best chances. The development of such an orally active drug is propelled further by the hypothesized neurotrophic properties of catecholamines. 145

Many dopaminergic catecholamines with potential use in the treatment of Parkinson’s disease are known. Most of these compounds have been discussed in previous sections. Like DA, L-dopa and apomorphine they are subjected to extensive metabolism lowering their bioavailability. In the next section, chemical synthetic approaches are discussed that are targeted at the circumvention of extensive (first-pass) metabolism.

1.7 IMPROVING THE BIOAVAILABILITY OF CATECHOLAMINES

1.7.1 The fate of catecholamines in vivo

Catecholamines are prone to many different metabolic processes that severely limit their usefulness as drugs. Aside from the metabolism already discussed like O-methylation by COMT and oxidation by MAO, there are several other metabolic processes the body and, especially the liver, uses to rapidly dispose of catecholamines. Upon oral administration, the largest proportion of a compound first has to pass through the gastrointestinal tract and the liver before reaching the blood stream for transportation to the brain. When upon first pass through the liver most of a drug is rendered inactive it is said to have a large first-pass effect. For many catecholamines, this first-pass effect is considerable. Enzymatic conjugation like glucuronidation and sulfatation are mostly responsible but also auto-oxidation of the catechol moiety might be a factor provided it would preferentially occur in the liver (Figure 1.17). 239-241 Therefore, apomorphine is preferably
delivered through subcutaneous injection or by sublingual administration so that it bypasses the limitations presented by the poor oral absorption.

The rate and extent of these metabolic transformations depend on the molecular structure of a catecholamine. Whereas DA is a flexible molecule that is readily accessible for some enzymes, rigid and bulky analogous structures induce steric hindrance influencing conjugation. COMT methylates apomorphine to isoapocodeine (C-11 methylation) and apocodeine (C-10 methylation) but to a much lesser extent than it methylates DA.\textsuperscript{242} Methylation of apomorphine is regioselective for the hydroxy group at the C-10 position, most likely as a result of steric hindrance of the phenyl moiety towards the C-11 hydroxy group. Glucuronidation and sulfatation of catechols mainly takes place in the liver and the extent of these conjugations varies very much on the species of animal. In humans, the excretion of unchanged apomorphine and O-methylated apomorphine are negligible and about 10% is excreted as glucuronide or sulfate.\textsuperscript{130} Surprisingly only 10% of the clearance of the total amount of apomorphine administered is accounted for. It is suggested that N-dealkylation or auto-oxidation may be responsible for clearance of the remaining 90%.\textsuperscript{243}

\textbf{Figure 1.17} Metabolic transformation of a catechol moiety. Explanation: a, auto-oxidation; b, COMT O-methylation; c, O-sulfatation; d, O-glucuronidation (GLU = glucuronyld)
Auto-oxidation of a catechol moiety as major route of transformation is especially likely for a molecule like apomorphine. Unlike other structurally related dopaminergic catecholamines as 5,6-di-OH-DPAT (1.12) or 6,7-di-OH-N-n-propyl-1,2,3,4,4a,5,10,10a-octahydrobenzo[g]quinoline (1.41), that lack a second aromatic moiety, auto-oxidation of apomorphine is facilitated by a energetically favorable aromatization of the C-ring (Scheme 1.18).  N-dealkylation is known to be an important metabolic pathway for tertiary amines in rodents. N-dealkylation tends to be most favorable for an N-methyl substituent and is occurring to a lesser extent as the size to the N-substituent increases. An N-n-propyl substituent is also enzymatically removed and this is the major metabolic route for the phenolic DA agonist PHNO in rat liver microsomes in vitro. N-dealkylation of one N-substituent has proven to be a very important in rodents.  

![Chemical structure of apomorphine](image)

**Figure 1.18** Auto-oxidation of apomorphine is facilitated by aromatization of its C-ring.

It seems that an N-n-propyl substituted catecholamine, lacking a second aromatic nucleus, from the point of preventing auto-oxidation and N-dealkylation may be optimal. Of course, the lesser bulkiness in compounds like 1.12 and 1.41 may increase susceptibility for O-methylation, glucuronidation and sulfatation. An advantage of a second aromatic nucleus, as in apomorphine, is its contribution to a higher lipophilicity that gives rise to an excellent ability to penetrate the brain. On the other hand, although 1.12 and 1.41 have a somewhat decreased lipophilicity they also penetrate the brain well.
It is evident that the extensive metabolic transformations of catecholamines make it
difficult for them to become successful drugs. Slow-release preparations and continuous
infusions make catecholamine treatment more effective but the low oral bioavailability remains
the most limiting factor. Oral bioavailability can be improved by the bioisosteric replacement of
the catechol moiety. In this approach, the catechol moiety is exchanged for an aromatic nucleus
that is less prone to metabolism. In the case of pramipexole (1.40), the catechol moiety is
exchanged for an aminothiazole nucleus. Many more examples of bioisosteric replacement exist,
but the bottom line is that by changing the molecular structure, the pharmacological profile is
changed, and therefore also the efficacy in the treatment of Parkinson’s disease.

1.7.2 Dopaminergic prodrugs

Increasing oral bioavailability of a catecholamine while retaining the catecholamine
itself as the active component is achieved by protection of the hydroxy and amino groups. The
protecting groups are designed to be less susceptible to metabolism and to gradually dissociate
from the catecholamine molecule in vivo. This approach may be regarded as a molecular, slow-
release preparation and is generally referred to as the prodrug approach.

A prodrug is an, usually pharmacologically inactive, compound that is transformed in vivo into a pharmacologically active compound. The most important prodrug that is used in the
treatment of Parkinson’s disease is L-dopa. L-dopa is the biological precursor of DA and may be
considered a prodrug. However, from a kinetics point of view, it does not make a good prodrug.
L-dopa is a catecholamine and, therefore, prone to most of the metabolic transformations
discussed. There are also other prodrugs of DA and of analogous catecholamines, designed to
cross the blood brain barrier and centrally slowly hydrolyze to give of the active species (Figure
1.19). The identity of the protecting groups is chosen for an optimal balance between absorption
and hydrolysis. The hydroxy groups on the catechol ring are usually protected by preparation of
di-O-pivaloyl or di-O-benzoyl esters. Ester derivatives like di-O-pivaloyl-N,N-di-n-propyl-DA
(1.42), 6,7-di-O-benzoxyloxy-2-aminotetralin (1.43), and di-O-benzoyl-apomorphine (1.44) exert
central pharmacological effects and some are markedly more active than their parent
catechols.249-251 The di-O-acetyl prodrug ABT-431 (1.45), a selective DA D1 receptor agonist,
has proven to be efficacious in the treatment of PD-patients, however, only upon intravenous
administration. Esterase speed is that fast, that the half-life of the prodrug in the blood is about
60 sec, making it unsuitable for oral administration.74
Bodor and co-workers discovered an interesting prodrug approach. They attached a dihydropyridine derivative to di-O-pivaloyl-DA (1.46) allowing the compound to cross the blood brain barrier. Upon oxidation of the dihydropyridine ring, a pyridinium ion is formed that can not leave the brain, because ions can not readily migrate over the blood brain barrier. The ionic DA prodrug is locked inside the CNS where upon hydrolysis it yields DA. Oxidation of dihydropyridine unfortunately does not exclusively occur in the brain but throughout the periphery also.

Despite a vast amount of differently substituted esters an amides derivatives of dopaminergic catecholamines, to this day, L-dopa is the only prodrug used in the clinic for central delivery of a drug.
Examples of peripherally acting prodrugs of dopaminergic catecholamines are numerous.\textsuperscript{253} Essentially the catecholamines are, again, protected by ester and amide groups, though the nature of these protecting groups allow peripheral action only. Rapid hydrolysis of the protecting groups or inability to cross the blood brain barrier are their essential features. Introduction of amino acids as protecting groups as in gludopa (1.47) and $\gamma$-glutamyl-DA (1.48) was used to preferentially target DA to the kidney (Figure 1.20).\textsuperscript{254-259}

![Figure 1.20 Peripherally acting prodrugs of dopaminergic catecholamines. Gludopa (1.47) and $\gamma$-glutamyl-DA (1.48).](image)

The need for prodrugs that centrally deliver the active dopaminergic catecholamine is evident. None of the approaches investigated so far has been able to produce substantial improvement to make it to an effective drug. The increased efficacy of DA and apomorphine over non-catecholamine based DA agonists in the treatment of Parkinson’s disease and the neurotrophic properties ascribed to catecholamines warrant the development of fundamentally new prodrug approaches. An orally active prodrug that by efficacy can compete with L-dopa but does not induce long-term complications like L-dopa does needs to be developed. Prodrugs of apomorphine, aminotetralins, or benzo[g]quinolines have the potential of being excellent candidates for the treatment of Parkinson’s disease. Yet, development of a new prodrug concept is inevitable to obtain the necessary improvement in drug kinetics.

### 1.7.3 Scope of this thesis

This thesis describes the research that was initiated by the unexpected observation that an intermediate (PD148903) in the attempted synthesis of 5-hydroxy-N,N-di-n-propylaminotetralin (1.14) induced dopaminergic behavior in rats \textit{in vivo} whereas it had no binding affinity for the DA D\textsubscript{1} or DA D\textsubscript{2} receptors (Scheme 1.21).\textsuperscript{260} Since this type of compounds was not described in
literature, it was decided to investigate the pharmacology of this compound. Investigations were aimed at elucidating the course of events leading up to the pharmacological effect and describing the pharmacology of racemic PD148903 and of its separated enantiomers.

Another principal goal was to investigate whether the induction of a pharmacological effect was uniquely preserved for PD148903 or that analogous compounds and derivatives also could induce a similar pharmacological effect. By focussing on the synthesis of compounds that potentially induced dopaminergic behavior, we set out to develop an orally active compound with a potential application in the treatment of Parkinson’s disease. In the course of this research several analogues were prepared of which the pharmacology is not yet fully described. Among these compounds are analogs of PD148903 that potentially may be of use in treatment of other pathological conditions.

Figure 1.21 Attempted synthesis of 1.14 through the PD148903 intermediate. Reagents: a) \((CH_2O)_n\), \(Pr_2NH\), acetone; b) NaBH\(_3CN\); c) \(I_2\), MeOH; d) BBr\(_3\).

1.8 REFERENCES


23. Jarvie, K. R.; Tiberi, M.; Caron, M. G. Dopamine D_{1α} and D_{1β} receptors; In *Dopamine receptors and transporters-Pharmacology, Structure and Function*; Niznik, H. B., ed. Marcel Dekker Inc: New York, **1994**; pp 133-150.


102. LaHoste, G. J., Henry, B. L., Marshall, J. F. Dopamine D$_1$ receptors synergize with D$_2$, but not D$_3$ or D$_4$, receptors in the striatum without the involvement of action potentials. *J.Neurosci.* 2000, 20, 6666-6671.


111. Waddington, J. L. Functional interaction between D₁ and D₂ dopamine receptor systems: their role in the regulation of psychomotor behaviour, putative mechanisms, and clinical relevance. J. Psychopharmacol. 1989, 3, 554-63.


114. Jackson, D. M., Hashizume, M. Bromocriptine induces marked locomotor stimulation in dopamine-depleted mice when D₁ dopamine receptors are stimulated with SKF38393. Psychopharmacology Berl 1986, 90, 147-149.


142. Ernst, A. M. Relation between action of dopamine and apomorphine and the O-methylated derivatives upon the CNS. *Psychopharmacologia (Berlin)* **1965**, 7, 391-399.


Introduction


Chapter 1


Introduction


