Systems pharmacology and blood-brain barrier functionality in Parkinson's disease

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Chapter 2

Understanding Drug Response in Parkinson's Disease

The Role of the Blood-Brain Barrier

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Abstract

Symptomatic treatment of the later stages of Parkinson's disease is far from optimal, while drugs that halt disease progression are not, yet, available. To improve drug treatment and drug development, detailed information on the interrelationship between disease, pharmacokinetics and drug response is needed. This review aims at the understanding of the mechanisms that govern drug response in Parkinson's disease. Special emphasis is on the impact of the BBB on drug response, specifically under diseased conditions like Parkinson's disease. It is concluded that systems pharmacology approaches are needed to investigate and understand the various mechanisms that determine the drug response as well as their interplay.

1. Introduction

Parkinson's disease is characterised by the loss of dopamine producing neurons in the striatum and substantia nigra pars compacta (SNc), resulting in clinical symptoms like bradykinesia, resting tremors, rigidity and postural instabilities (Thomas and Beal, 2007). Treatment of these symptoms of Parkinson's disease is still by replacing the lost brain dopamine. L-3,4-dihydroxyphenylalanine (L-DOPA) in combination with an aromatic amino acid decarboxylase (AAAD) inhibitor is currently still the golden standard. At later stages of the disease, when L-DOPA-related motor complications arise, dopamine agonists may be added to the treatment (Mercuri and Bernardi, 2005). The future for new drug therapies in Parkinson's disease is heading towards neuroprotection or neurorescue (Bonuccelli and Del Dotto, 2006; Chen and Le, 2006; Schapira, 2008b) as L-DOPA or dopamine agonists merely give relief to symptoms thereby increasing the quality of life, but are not halting or reversing the progress of Parkinson's disease. In most neuropharmacological studies the effects of drugs in the CNS are still related to the dose (Girgin *et al.*, 2008; Homma *et al.*, 2008; Kinon *et al.*, 2008; LeWitt *et al.*, 2008; Mischoulon *et al.*, 2008; Stern *et al.*, 2004; Walsh *et al.*, 2008). However, a dose-effect relationship is not informative as there are many mechanisms (e.g. plasma protein binding, blood-brain barrier (BBB) transport, within brain distribution, target interaction) that affect the brain distribution kinetics of the CNS drug and ultimately the drug response. Differences in factors such as genetics, species, gender, age, environmental and pathological conditions can influence these individual mechanisms and should therefore also be taken into consideration when comparing or making predictions on drug response. This

review gives a summary of the mechanisms involved in CNS drug response and the factors that may influence them and thereby the drug response. Special emphasis is on the BBB as a keyplayer in CNS drug response, and how the BBB functionality may change under diseased conditions like Parkinson's disease.

2. Parkinson's Disease

Disease characteristics

Parkinson's disease is the second most common progressive movement disorder (Dauer and Przedborski, 2003). The disorder affects several regions of the brain. One of these areas is the SNc that controls balance and movement. Clinical manifestations are a result of the loss of 50-80% of neuromelanin containing dopaminergic neurons in the SNc (and striatum) and include motor impairments such as resting tremor, bradykinesia, rigidity, gait difficulty and postural instability as well as non-motor symptoms (Dauer and Przedborski, 2003; Forno, 1996; Thomas and Beal, 2007). Non-motor symptoms include depression, constipation, pain, genitourinary problems, and sleep disorders and dominate the clinical picture of advanced Parkinson's disease. They contribute to severe disability, impaired quality of life, and shortened life expectancy (Chaudhuri *et al*., 2006). Residual nigral neurones show characteristic, eosinophilic inclusions called Lewy Bodies (LB) that are made up of neurofilaments and show ubiquitin immunoreactivity (McNaught *et al*., 2001). Other affected brain areas include regions of the brain that regulate involuntary functions such as blood pressure and heart activity. A schematic overview of the neuronal interconnections in Parkinson's disease as compared to the "healthy" situation is depicted in Figure 1. Dopamine is the predominant catecholamine neurotransmitter in the mammalian brain, where it controls a variety of functions including locomotor activity, cognition, emotion, positive reinforcement, food intake, and endocrine regulation. Pathological conditions such as Parkinson's disease are linked to a dysregulation of dopaminergic transmission. (Missale *et al*., 1998).

Dopamine is the primary endogenous ligand for the G-protein-coupled dopamine receptors. These dopamine receptors can be divided into D1-like receptors (D1 and D5) and D2-like receptors (D2, D3 and D4). Through their different G-protein coupling, D1-like and D2-like receptors have opposing effects on adenylyl cyclase activity and cyclic AMP concentration. All five subtypes of dopamine receptors are found in the striatum (Smith and Kieval, 2000). The highest expression of both the D1 and D2 mRNAs are localised to the dorsal and ventral striatum as measured in normal human post mortem brains. Cortical expression is moderate

for D1 mRNA and very low for D2 mRNA. The SNc expresses D2 but not D1 mRNA. The D3 receptor has a specific distribution to limbic-related ventral striatum (Hurd *et al.*, 2001; Smith and Kieval, 2000). Low levels of the D4 receptor mRNA have been found in the basal ganglia. In contrast, this receptor appears to be highly expressed in the frontal cortex, amygdala, hippocampus, hypothalamus, and mesencephalon. The expression of the D5 receptor compared to the D1 receptor as measured in rat brain is very poor. The D5 receptor can be found in the cerebral cortex, lateral thalamus, diagonal band area, striatum, and, to a lesser extent, SNc, medial thalamus, and hippocampus (Hurd *et al.*, 2001).

The degree of forward locomotion is primarily controlled by the ventral striatum through activation of D1, D2, and D3 receptors. Activation of D2 autoreceptors, which results in decreased dopamine release, has been shown to decrease locomotor activity, whereas activation of postsynaptic D2 receptors slightly increases locomotion. Activation of D1 receptors has little or no effect on locomotor activity. However, there is synergistic interaction between D1 and D2 receptors in determining forward locomotion so that concomitant stimulation of D1 receptors is essential for D2 agonists to produce maximal locomotor stimulation. The D3 receptor, which has been shown to be mainly postsynaptically located in the nucleus accumbens, seems to play an inhibitory role on locomotion (Missale *et al.*, 1998). The synergistic interaction between D1 and D2 receptors is due to two main pathways in the nigrostratial tract. Both pathways receive a glutamatergic corticostriatal input (Gerfen, 2003). One pathway leads directly from the putamen to the Gpi (Figure 1, left panel). It has dopamine D1 receptors, co-express the peptides substance-P, and dynorphin and establish a monosynaptic inhibitory connection with GPi neurons. Neurons in the indirect pathway express the dopamine D2 receptor, which is coupled to the inhibitory Gi G-protein, as well as the A2A adenosine receptor, which is coupled to the stimulatory Golf G-protein. These neurons also co-express enkephalin (Gerfen, 2003). They project to the GPe which in turn influence the GPi by a monosynaptic inhibitory connection and indirectly through the GPe-STN-GPi projection (Obeso *et al.*, 2008). Thus, the direct and indirect pathways have opposing effects on the output function of the basal ganglia. Dopamine modulates glutamatergic effects on corticostriatal inputs by exerting a dual effect on striatal neurons: exciting Dl-receptor-expressing neurons in the direct pathway and inhibiting D2-receptor-expressing neurons in the indirect pathway (Obeso *et al.*, 2008).

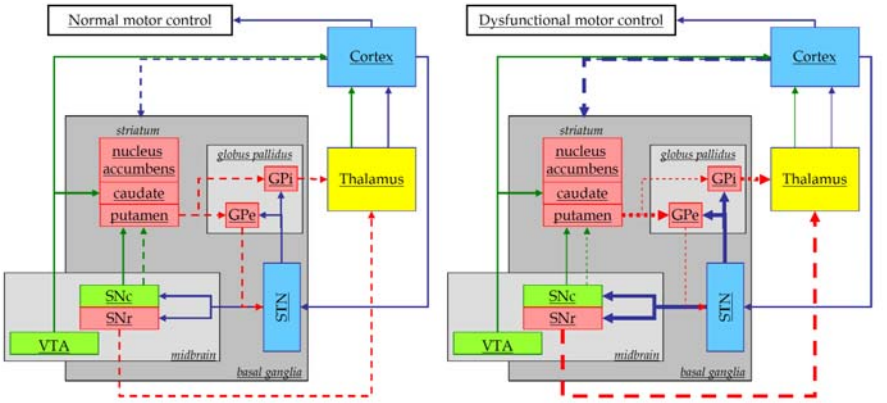

Figure 1: *Schematic overview of the neuronal interconnections involved in Parkinson's disease; (left) is the case of normal motor function and (right) is in the case of a dysfunctional motor control as seen in Parkinson's disease. The solid arrows depict excitatory projections and the dotted arrows depict inhibitory projections. The thickness of the arrows indicates the degree of activation of each projection (right). The red boxes and projections are GABAergic, the green boxes and projections are dopaminergic and the blue boxes and projections are glutamatergic. SNc: substantia nigra pars compacta; SNr: substantia nigra pars reticulata; VTA: ventral tegmental area; STN: subthalamic nucleus; GPe:globus pallidus, external segment; GPi: globus pallidus, internal segment. For the purpose of clarity, the neuroanatomy and interconnections are incomplete (Obeso et al., 2008; Smith et al., 2000; Smith et al., 2008)*

In Parkinson's disease, the direct striatal output tract from the striatum to the SNc is less active, as a result of decreased dopaminergic inhibitory tone. A study in MPTP (1-methyl-4-phenyl-1,2,3,6-tertahydropyridine)-lesioned mice also indicated a hyperactivity of the glutamatergic cortico-striatal pathway as a consequence of dopaminergic denervation resulting in an increase of striatal GABA levels (Chassain *et al.*, 2008). This leads to overactivity of the indirect striatal GABAergic output from the striatum to the GPe (Chassain *et al.*, 2008), diminishing inhibitory signals from the GPe to the STN and consequently resulting in excitatory projections to the GPi and SNc (Figure 1). These two basal ganglia nuclei, in turn, send inhibitory projections to the thalamus. Inhibition of the thalamus leads to decreased excitatory projections to the motor cortex, resulting in Parkinsonism (Del Tredici *et al.*, 2002). An increase in striatal glutamine concentrations was also seen in MPTP-lesioned mice, which might be a

strategy to protect neurons from glutamate excitotoxic injury after striatal dopamine depletion (Chassain *et al.*, 2008), although these results could not be confirmed in a study in 6-hydroxydopamine-lesioned (6-OHDA) rats (Kickler *et al.*, 2009). Excitotoxicity will be discussed in more detail later in this review.
Degeneration of the dopamine input to the striatum results in opposing affects in the direct and indirect pathways. The resulting functional imbalance is thought to be responsible for the bradykinesia of Parkinson's disease, which may be temporarily normalised by dopamine replacement therapy. However, direct striatal projection neurons become irreversibly supersensitive to D1 dopamine receptor activation, despite the fact that there is an actual decrease in receptor number. Recent studies show that this D1-supersensitive response results from a switch in D1-receptor-mediated regulation of protein kinase systems responsible for neuronal plasticity and is suggested to underlie dyskinesia produced by L DOPA treatment of Parkinson's disease (Gerfen, 2003).

Diagnosis & Biomarkers
Currently, the diagnosis of Parkinson's disease is based on patient history and physical examination alone as there is no simple and effective biomarker available (Savitt *et al.*, 2006). Using the current diagnostic criteria, 90% may be the highest accuracy that can be expected (Hughes *et al.*, 2001). Biomarkers might be used to assist in the diagnosis of Parkinson's disease. The search for suitable biomarkers has led to various studies using imaging techniques such as positron emission tomography (PET) and single photon emission computed tomography (SPECT). However, such studies provide markers of nerve terminal function rather than cell density and thus, like clinical assessments, are potentially influenced by compensatory mechanisms and direct effects of medication (Brooks, 2004). In post-mortem investigations and in animal research, tyrosine hydroxylase (TH) immunostaining is used as a golden standard to determine the degree of lesion in the dopaminergic neurons in striatum or SNc. However, this analysis can only be performed post-mortem. Availability of relevant biomarkers which could be measured in vivo, could result in a more detailed and mechanistic description of Parkinson's disease progression and serve as useful tools in disease models. A combination of biomarkers might be needed to provide a complete characterisation of treatment effects (beneficial and harmful) or disease progression (Lesko and Atkinson, Jr., 2001).
Other biomarkers for Parkinson's disease include clinical tests, blood tests, cerebrospinal fluid (CSF) tests and genetic tests. An example of a clinical test is the

pharmacological challenge in which the response to L-DOPA or a dopamine agonists is examined to differentiate Parkinson's disease from other parkinsonian syndromes (Dorsey *et al.*, 2006). Blood and CSF tests mainly focus on markers of oxidative stress such as the mitochondrial complex I level in blood or 8-hydroxy-2'-deoxy-guanosine, which results from oxidised DNA among others in blood and CSF (Michell *et al.*, 2004). Alternative blood markers which are potential biomarkers for Parkinson's disease are platelet α-synuclein, platelet monoamine-oxidase-B (MAO-B) activity and dopamine transporter density on peripheral lymphocytes (Dorsey *et al.*, 2006; Michell *et al.*, 2004). For the purpose of early clinical evaluation and effective patient management, diagnostic strategies based on the usual screening panels for nigrostriatal somatomotor symptoms associated with Parkinson's disease might be supplemented for persons at risk by tests designed to elicit signs such as symptoms related to dysautonomia, signs indicating subtle disruptions within the gain setting nuclei of the lower brainstem or quantifiable deficits in olfactory acuity, differentiation, or memory of substances smelled (Del Tredici *et al.*, 2002).

For the majority of the patients with Parkinson's disease, genetic factors do not clearly play a role, however for individuals with a family history of the disease, genetic testing can indicate the risk. Genetic testing can also be helpful in diagnosis as many cases of 'genetic' Parkinson's disease have a different clinical course than sporadic Parkinson's disease (Dorsey *et al.*, 2006).

Etiopathogenesis

Parkinson's Diseases often begins after the age of 50 (late onset), but may also begin before the age of 50 (early onset) or even before the age of 20 (juvenile onset). Parkinson's disease occurs in 1-2 % of individuals over 50 and in more than 3% of individuals above 75 years of age (Pankratz and Foroud, 2007). The prevalence of Parkinson's disease is higher in men than women (Van Den Eeden *et al.*, 2003). Estrogen has an effect on dopamine neurotransmission i.e. inhibition of dopamine reuptake, effect on dopamine synthesis and release and estrogen has shown to protect nigrostriatal neurons from toxic influences (Shulman, 2007) which might explain the gender difference in prevalence. An advancing age is associated with a faster rate of motor progression, decreased L-DOPA responsiveness, more severe gait and postural impairment, and more severe cognitive impairment and the development of dementia in patients with PD (Levy, 2007).

Risk factors

For Parkinson's disease, no single causative factor has been identified. Instead, various mechanisms- including mitochondrial defects, oxidative stress, excitotoxicity, genetic factors and apoptosis- seem to play a role. This suggests that the etiopathogenesis of Parkinson's disease is most likely multi-factorial. Certain risk and protective factors have been identified for Parkinson's disease. Smoking and (moderate) consumption of coffee or tea (caffeine intake) have been associated with a decreased risk for Parkinson's disease (Ascherio *et al.*, 2001; Hong *et al.*, 2008; Kandinov *et al.*, 2008; Morozova *et al.*, 2008; Saaksjarvi *et al.*, 2008). Hydroquinone and nicotine, two components of cigarette smoke apparently inhibit α-synuclein fibrillation and stabilize soluble oligomeric forms (Hong *et al.*, 2008). Caffeine or its metabolite paraxanthine might be neuroprotective via antagonist action on the adenosine A2 (A2A) receptor (Guerreiro *et al.*, 2008). Animal studies suggest that caffeine may protect the BBB against experimental parkinsonism (Chen *et al.*, 2008). Animal studies (McGeer and McGeer, 2004b) also suggest that nonsteroidal anti-inflammatory drugs (NSAIDs) decrease the incidence of Parkinson's disease, but epidemiologic studies are inconclusive (Etminan and Suissa, 2006; Hancock *et al.*, 2007; Hernan *et al.*, 2006; Powers *et al.*, 2008; Ton *et al.*, 2006; Wahner *et al.*, 2007). Furthermore, rural living, farming, gardening and drinking well water have been identified as risk factors as these factors can be associated with exposure to pesticides (Chade *et al.*, 2006; Kamel *et al.*, 2007).

Toxic substances, in general, can cause neurological damage as was first demonstrated by MPTP which is associated with parkinsonism in young drug addicts (Langston *et al.*, 1983). MPTP, which is a pro-toxin, rapidly crosses the BBB and is converted to 1-methyl-4-phenylpyridinium (MPP+) which is transported into dopamine neurons where it impairs mitochondrial respiration by inhibiting complex I of the electron transport chain (Vila and Przedborski, 2003). Pesticides and metals are also named as neurotoxins involved in Parkinson's disease. Metals (eg iron and copper) have been investigated as potential risk factors on the basis of their accumulation in the SNc and their participation in harmful oxidative reactions such as the production of hydrogen peroxide during the oxidation of dopamine and the conversion of hydrogen peroxide to hydroxyl radicals which is catalysed by iron (Di Monte, 2003; Landrigan *et al.*, 2005). Manganese has been mentioned as potential risk factor (Landrigan *et al.*, 2005), however, it damages the globus pallidus and not the SNc. There is also an absence of nigral LBs and this is inconsistant with the neurological symptoms of Parkinson's disease (Perl and

Olanow, 2007). The widely used pesticide rotenone is, like MPTP, an inhibitor of complex I and able to induce the major features of Parkinson's disease in rats (Betarbet *et al.*, 2000; Ravenstijn *et al.*, 2008). Rotenone is able to cross cell membranes and is therefore likely to affect all cells but mainly targets dopaminergic neurons probably because dopamine metabolism is responsible for high basal levels of oxidative stress in the SNc (Giasson and Lee, 2000; Jenner, 2003; Mandemakers *et al.*, 2007). Epidemiological studies have revealed a relationship between pesticide exposure and a high risk for Parkinson's disease (Di Monte, 2003).

Mitochondrial dysfuntion & Oxidative stress

Molecular studies in neurotoxin based and genetic-based animal models suggest a major etiologic role for mitochondrial dysfunction in the pathogenesis of Parkinson's disease. Neurons in general appear to be more sensitive than other cells to mutations in genes, such as Opa1, Mfn1 and Dnm1l encoding mitochondrial proteins involved in the dynamic morphological alterations and subcellular trafficking of mitochondria (Mandemakers *et al.*, 2007). Post-mortem biochemical studies in patients have revealed mitochondrial defect (30-40% in complex I activity) in specifically the SNc of the brain and in platelets and muscle (Olanow and Tatton, 1999; Schapira, 2006) and animal studies in mice which have a knockout of mitochondrial transcription factor A in dopaminergic cells, have shown Parkinson-like symptoms (Onyango, 2008). Oxidative stress is linked to mitochondrial dysfunction as well as other components of the degenerative process such as excitotoxicity and inflammation (Schapira, 2008a). Oxidant stress and consequent cell death could develop in the SNc under circumstances in which there is (1) increased dopamine turnover, resulting in excess peroxide formation; (2) a deficiency in glutathione (GSH), thereby diminishing the brain's capacity to clear H_2O_2; or (3) an increase in active iron, which can promote OH· formation (Olanow and Tatton, 1999). The evidence for oxidative stress in Parkinson's disease has been summarised elsewhere (Jenner, 1991) and will not be repeated here. Complex I deficiencies in SNc mitochondria evoke free radical generation, which, in turn, impairs the function of the respiratory chain. Mitochondrial abnormalities decrease the activity of the ubiquitin proteasomal system (UPS), a process that is further exacerbated by the increased substrate load of oxidised protein from oxidative stress. Abnormalities in protein phosphorylation might influence the UPS and mitochondrial function.

Inflammation

Chronic inflammation may play an important role, if secondary, in the pathogenesis of Parkinson's disease (Hirsch *et al*., 2005; McGeer and McGeer, 2004a). The proinflammatory cytokine tumor necrosis factor-α (TNF-α) kills dopamine neurons *in vitro* and is elevated in the brains of patients with Parkinson's disease (Logroscino, 2005). The TNF-308A allele was found significantly more frequent in early onset patients compared to the controls and might influence the risk for the development and/or onset of Parkinson's disease (Bialecka *et al*., 2008a). Inflammation will enhance free-radical production including nitric oxide and peroxynitrite. It has recently been proposed that Parkinson's disease might be an autoimmune disease as a disruption of the BBB, as explained later, might result in the entry of immune cells leading to a progressive degenerative process (Monahan *et al.*, 2008).

Excitotoxicity

Free radical species will also be enhanced by excitotoxicty, which leads to nitric-oxide-mediated damage to the mitochondria (Schapira, 2008a). Excitotoxicity is an established cause of neurodegeneration that has been implicated in Parkinson's disease based on two possible mechanisms. The first is direct excitotoxicity resulting from increased glutamate formation. SNc dopaminergic neurons are rich in glutamate receptors and receive extensive glutamate innervation from the subthalamic nucleus (STN; Figure 1). Dopamine lesions disinhibit the STN which results in an increased firing rate of its output neurons leading to excessive calcium influx into the cell and nitric oxide (NO) formation (Figure 2). The second mechanism involves indirect excitotoxicity where a defect in mitochondrial function results in the loss of the ATP-dependent Mg-blockade of N-methyl-D-aspartate (NMDA) receptors leading to a calcium influx into the cell under physiological glutamate levels (Figure 2) (Olanow and Tatton, 1999). NO reacts with superoxide radical to form peroxynitrite and hydroxyl radical which are both powerful oxidizing agents (Beckman *et al.*, 1990). Also the mitochondrial respiratory chain might, in turn, be damaged by sustained exposure to NO (Bolanos *et al.*, 1996). Next to NO formation, the formation of L-ornithine decarboxylase (ODC) is increased due to excessive calcium influx. ODC is an enzyme involved in the synthesis of polyamines (spermidine, spermine and putrescine) (Williams, 1997). Polyamines may in turn stimulate the NMDA receptor and result in a 'run-away-process'. Cell death in Parkinson's disease occurs by way of apoptosis rather than necrosis (Olanow and Tatton, 1999).

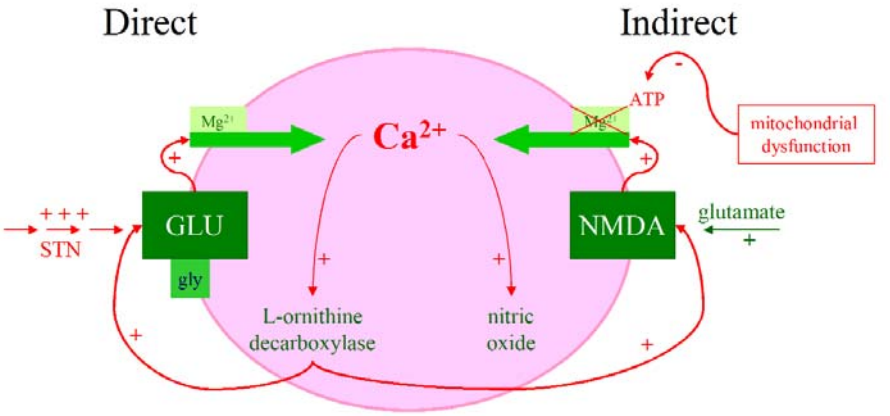

Figure 2: *Mechanisms of excitotoxicity STN: Subthalamic Nucleus; GLU: Glutamate; gly: glycine; NMDA: N-methyl-D-aspartate*

Neuronal apoptosis can be induced by low concentrations of toxins or substances such as L-DOPA, dopamine, iron, MPTP among others (Tatton *et al.*, 1997).

Genetics

Most cases of Parkinson's disease (>95%) are sporadic, although some genes (associated with the PARK loci) have been identified and linked to rare forms of Parkinson's disease. Among them are the SNCA or α-synuclein (PARK1), LRRK2 (PARK8), which result in autosomal dominant Parkinson's disease; PRKN or Parkin (PARK2), DJ-1 (PARK7), PINK1(PARK6), which result in autosomal recessive Parkinson's disease (Pankratz and Foroud, 2007); UCHL1 (PARK5), which has been implicated but not confirmed and a link with the locus PARK3 has been identified but no gene has been found. So there are only five clearly defined genetic causes of Parkinson's disease. A more detailed description of the consequences of these mutations are described elsewhere (Farrer, 2006; Pankratz and Foroud, 2007; Thomas and Beal, 2007).

3. Current Drug Treatment of Parkinson's Disease

Symptomatic treatments

Treatment of the symptoms of Parkinson's disease is currently by replacing the lost brain dopamine. Unfortately these therapies only provide temporary relief from early symptoms and do not halt disease progression. Moreover, pathological changes outside of the motor system leading to cognitive, autonomic, and

psychiatric symptoms are not sufficiently treated by current therapies (Savitt *et al.*, 2006).

The most widely used drug in the treatment of Parkinson's disease is L-DOPA (Kostrzewa *et al.*, 2005; Mercuri and Bernardi, 2005). L-DOPA is metabolised by AAAD to dopamine. AAAD is present in low concentrations in most body tissues and in high concentrations in liver, kidney, intestinal mucosa and plasma (Leppert *et al.*, 1988). A second but less important biotransformation pathway is the O-methylation of L-DOPA to 3-O-methyldopa (3-OMD) by COMT (Nutt and Fellman, 1984). Carbidopa and benserazide are the two most commonly used decarboxylase inhibitors used in combination with L-DOPA, increasing the proportion of L-DOPA dose in plasma (increase of C_{max} and AUC, decrease of t_{max}) in such a manner that a 70 to 80% reduction in total daily dose of L-DOPA is possible in order to obtain the same clinical benefits (Cedarbaum, 1987). The rate of gastric emptying is the principle determinant in the disposition of L-DOPA. Absorption of L-DOPA in the small intestine occurs by means of an active, saturable, large neutral amino acid carrier system (Deleu *et al.*, 2002). L-DOPA reaches its effect site by crossing the BBB via an active transporter, the large amino acid transporter (LAT) (del Amo *et al.*, 2008). In brain tissue, L-DOPA is metabolised for about 69% by AAAD and for about 10% by COMT (Nutt and Fellman, 1984).

During early Parkinson's disease, the effect of L-DOPA is long lasting and stable. However, at later stage of the disease and after long-term L-DOPA treatment, motor complications may develop, starting with "wearing-off" which is the progressive shortening of the effect of L-DOPA to 4 hours or less after administration of the dose. In the more advanced stages of the disease, the "on-off" phenomenon appears, in which the effect of L-DOPA can suddenly and unpredictably disappear, resulting in a mobile ("on") patient to become immobile ("off") (Nyholm, 2006; Bhidayasiri and Truong, 2008). At the same time, patients may develop dyskinesias (involuntary movements), which are considered to be related to large and multiple doses of L-DOPA (Bhidayasiri and Truong, 2008; Obeso *et al.*, 2004). The underlying pathology of motor complications is believed to be due to the progressive loss of dopaminergic neurons that results in a decreased ability to buffer fluctuations in dopamine levels in the brain, coupled with the short half-life of L-DOPA of approximately 1 hour (Deogaonkar and Subramanian, 2005; Tse, 2006). Animal studies as well as a few human studies have revealed that motor complications developed after intermittent

administration of L-DOPA but did not develop when L-DOPA was given continuously (Nyholm, 2006; Olanow *et al.*, 2004; Stocchi, 2005). The high degree of interindividual variability in absorption after oral administration is significantly reduced when L-DOPA is given intraduodenally or intraperitoneally (Bredberg *et al.*, 1994). Consequently, to overcome these motor complications, L-DOPA can be given with a shorter dosing interval i.e. in a more continuous manner (Stocchi, 2005), but also by administration of L-DOPA in combination with a COMT inhibitor or a dopamine agonist. Also, L-DOPA may be administered together with a MAO-B inhibitor, such as selegiline or rasagiline, which inhibits dopamine metabolism (Bhidayasiri and Truong, 2008; Factor, 2008; Nyholm, 2006; Nyholm, 2006; Stacy and Galbreath, 2008). The symptomatic effects of selegiline were thought not only to relate to MAO inhibition but were also thought to be associated with an amphetamine effect (enhancing release of dopamine) as selegiline is metabolised to L-amphetamine via the first pass in the liver (Factor, 2008).

Concomitant medication with a COMT inhibitor, such as entacapone or tolcapone increases the amount of L-DOPA available for transport across the BBB as well as reduces the amount of 3-OMD which is a competitor to L-DOPA in the uptake by the LAT transporter at the BBB (Wade and Katzman, 1975). Entacapone is a mainly peripherally acting COMT inhibitor and has a low BBB penetration. Tolcapone, however, is able to cross the BBB and also act on central COMT (Ceravolo *et al.*, 2002; Kaakkola and Wurtman, 1992; Napolitano *et al.*, 2003; Russ *et al.*, 1999), although it has not been investigated whether this further enhances the efficacy of L-DOPA (Forsberg *et al.*, 2003). Tolcapone increases the AUC and C_{max} of L-DOPA but does not influence its PK-PD relationship, making it a suitable add-on to L-DOPA therapy (Baas *et al.*, 2001). L-DOPA treatment has been shown to increase plasma homocysteine levels in Parkinson's disease patients as it is metabolised via O-methylation by COMT using S-adenosyl-L-methionine (SAM) as the methyl donor which results in the subsequent formation of homocysteine (L-DOPA induced hyperhomocysteinaemia). Increased levels of homocysteine might lead to an increased risk for coronary arterial diseases. Based on studies using rats and confirmed in human studies, entacapone may reduce L-DOPA-induced hyperhomocysteinaemia in patients with Parkinson's disease (Nissinen *et al.*, 2005; Valkovic *et al.*, 2005) and thereby reducing the risk of pathologies probably linked to it.

Dopamine agonists, such as ropinerole, apomorphine, bromocriptine, pergolide, cabergoline, lisuride or pramipexole are less effective than L-DOPA in yielding

symptomatic relief and almost all patients will require L-DOPA at some point. Dopamine agonists are drugs acting directly to stimulate dopamine receptors, classified as D1-like (D1, D5) and D2-like (D2, D3, D4). Postsynaptic D2 receptor stimulation is closely associated with antiparkinsonian activity and presynaptic D2 receptor stimulation may have neuroprotective effects. Optimal therapeutic response is thought to require stimulation of both D1 and D2 receptors (Deleu *et al.*, 2002). Dopamine agonists have a reduced risk of the development of dyskinesias which is probably related to the longer $t_{1/2}$ relative to L-DOPA (Savitt *et al.*, 2006; Yamamoto and Schapira, 2008).

Future symptomatic therapies which are currently being investigated for the treatment of Parkinson's disease include non-dopaminergic agents such as the adenosine A2A antagonists which are related to caffeine. The A2A receptors are co localised with D2 receptors on striatal medium spiny GABAergic neurons of the striatopallidal pathway, and antagonists of A2A receptors have been found to enhance the effects of dopamine at the D2 receptors on striatopallidal neurons, thus suppressing inhibitory GABAergic output and improving parkinsonian symptoms (Petzer *et al.*, 2009). Another way to counter the excessive excitatory stimulation or to treat the glutamate excitotoxicity, as described earlier, is by glutamate antagonist drugs such as amantadine and riluzole which are currently under investigation for the treatment of patients with motor fluctuations and dyskinesias (Factor, 2008; Wu and Frucht, 2005). Finally, noradrenergic drugs are being examined, because this neurotransmitter appears to influence motor and affective symptoms.

Neuroprotective treatments

The most important goal for drug development in Parkinson's disease is neuroprotection or neurorescue (Bonuccelli and Del Dotto, 2006; Chen and Le, 2006; Schapira, 2008b). Some of the drugs already in use as treatment in Parkinson's disease or currently under investigation seem to possess neuroprotective properties

Dopamine agonists such as bromocriptine, pergolide, ropinirole and pramipexole have been shown to act as free radical scavengers against hydroxyl radicals, NO radicals and to have antioxidant effect (Deleu *et al.*, 2002). Pramipexole has been shown to reduce nigrostriatal cell death in MPTP-treated non-human primates (Iravani *et al.*, 2006). Furthermore, MAO-B inhibitors (selegiline, rasagiline) may also possess neuroprotective properties in part by reducing the damaging effect of dopamine turnover in the brain. These effects of MAO-B inhibitors are especially

relevant when considering that the brain shows an age-related increase in MAO-B activity (Petzer *et al.*, 2009). Next, as mentioned previously, caffeine consumption has been associated with a decreased risk for Parkinson's disease and it has been thought that it might be neuroprotective via antagonist action on the A2A receptor (Guerreiro *et al.*, 2008). This is further substantiated in a study in which caffeine was able to protect MPTP-induced BBB dysfunction in mice (Chen *et al.*, 2008). Another study revealed that caffeine and other A2A-antagonists were able to attenuate MPTP-induced loss of striatal dopamine and dopamine transporter binding sites in mice (Chen *et al.*, 2001).

Coenzyme Q10 is able to enhance mitochondrial function, increasing ATP production, and also functions as an antioxidant. A study in patients with early Parkinson's disease demonstrated that high doses of coenzyme Q10 were associated with a reduced rate of deterioration in motor function (Bonuccelli and Del Dotto, 2006). Diet supplement of creatine not only enhances mitochondrial function but also reduces oxidative stress through stabilisation of mitochondrial creatine kinase. Moreover, creatine supplementation may exert an anti-apoptotic effect because creatine kinase acts to inhibit opening of the mitochondrial transition pore and the consequent triggering of apoptosis. A few experimental studies suggest a neuroprotective role of dietary intake of creatine in Parkinson's disease models (Matthews *et al.*, 1999). In the MPTP model of Parkinson's disease, amantadine, a NMDA receptor antagonist, showed partial protection suggesting beneficial effects not only on the clinical features but also on disease progression (Rojas *et al.*, 1992). Memantine is another aminoadamantane derivative with weak NMDA receptor antagonistic properties. Preclinical data suggest that the potential neuroprotective effect of memantine might be mediated by the increase of endogenous production of brain cell-derived neurotropic factor (BDNF) in the brain (Bonuccelli and Del Dotto, 2006). Minocycline is an antimicrobial tetracycline compound which has shown in the MPTP and 6-OHDA rodent models of Parkinson's disease an enhanced survival of dopaminergic nigral neurons (McGeer and McGeer, 2007). This drug may act by inhibiting microglial activation. Other experimental data suggest that minocycline is able to block caspase 1 and 3 and to counteract apoptosis (Bonuccelli and Del Dotto, 2006). As mentioned above, animal studies (McGeer and McGeer, 2004b) suggest that NSAIDs decrease the incidence of Parkinson disease which gives it potential neuroprotective relevance. A more detailed understanding of neuroinflammatory mechanisms in Parkinson's disease will lead to new cellular and molecular targets. Future treatment may involve combination therapies with drugs directed

at both inflammatory and non-inflammatory mechanisms (Klegeris *et al.*, 2007).
In general, for major progress to be made in the treatment of Parkinson's disease, we need a paradigm shift away from focus on dopamine and dopaminergic neurons. With current medications and surgical options designed to restore brain dopaminergic function, we are close to going about as far as we can with this avenue of treatment and investigation. It has become increasingly clear that clues to the etiology of Parkinson's disease will not be found via studies of dopamine metabolism, and major treatment advances lie beyond the dopaminergic nigrostriatal system (Ahlskog, 2007). Understanding why and how susceptible cells in motor and non-motor regions of the brain die in Parkinson's disease is the first step toward preventing this cell death and curing or slowing the disease (Savitt *et al.*, 2006).

4. Mechanisms Involved in Target Site Distribution of CNS Drugs

In most neuropharmacological studies the effect of drugs in the CNS are still related to the dose (Girgin *et al.*, 2008; Homma *et al.*, 2008; Kinon *et al.*, 2008; LeWitt *et al.*, 2008; Mischoulon *et al.*, 2008; Stern *et al.*, 2004; Walsh *et al.*, 2008). However, a single unique dose-CNS response relationship does not exist. CNS target site distribution kinetics is determined by many mechanisms such as plasma protein binding, BBB transport and within brain distribution. The ultimate kinetics of the drug at the target will, combined with target interaction and signal transduction, account for the CNS drug response profile. Here we discuss the main mechanisms that play a role in target site distribution of CNS drugs, together with some examples of conditions that influence the particular mechanism.

Plasma protein binding

After administration, drugs will enter the plasma compartment and may circulate either in the free form or associated with one or more binding sites, such as on plasma proteins. Plasma has two major proteins: albumin and α_1-acid glycoprotein, with different capacities and characteristics for drug binding (Kremer *et al.*, 1988), and other plasma constituent like lipoproteins, erythrocytes and α-, β-, γ-globulins (Wright *et al.*, 1996). Albumin is the most important drug-binding protein due to its high concentration in plasma. Albumin has several high- and low-affinity binding sites and is mainly involved in the binding of acidic drugs (Day and Myszka, 2003; Murai-Kushiya *et al.*, 1993; Notarianni, 1990; Piafsky, 1980). In contrast α_1-acid glycoprotein is mainly involved in the binding

of neutral and basic drugs (Kopecky, Jr. *et al.*, 2003; Kremer *et al.*, 1988). Especially α_1-acid glycoprotein is of interest as its levels are susceptible to changes.

It is well-known that the extent and strength to which drugs are bound to plasma components is important for the distribution of the drug over the body compartments (Rowland and Tozer, 1995) and will determine the time-dependent fraction of a drug that is available for transport into the brain (Fenstermacher *et al.*, 1995; Filippi and Rovaris, 2000). It has been suggested that only the free (unbound, dissociated) drug in plasma (Robinson and Rapoport, 1986; Rowley *et al.*, 1997) is available for transport across the blood to brain barriers and determines the intensity of the response for drugs such as benzodiazepines, opiates and steroids (Cox *et al.*, 1998; Kim *et al.*, 1997; Mandema and Danhof, 1992; Visser *et al.*, 2003a). However, some plasma protein bound drugs seem to cross the BBB (Cornford *et al.*, 1992; Jolliet *et al.*, 1997; Lin *et al.*, 1987; Lolin *et al.*, 1994; Urien *et al.*, 1987). For propanolol (Pardridge *et al.*, 1983; Pardridge, 1988b) it has been reported that the total rather than the free concentration determines the response, indicating that the bound drug is available for transport into the brain. Furthermore, it has been shown that drugs which bind fairly selectively to one of the main binding sites of albumin, Sudlow II (e.g., benzodiazepines and tryptophan) showed enhanced dissociation and a greater uptake into the brain than could be accounted for by the Kety-Crone-Renkin equation which is commonly used to analyse capillary transport (Fenerty and Lindup, 1989; Jones *et al.*, 1988; Lin and Lin, 1990; Tanaka and Mizojiri, 1999). However, other studies indicated no enhanced dissociation for drugs bound to Sudlow I (warfarin) or Sudlow II (ibuprofen) or both (tolbutamide and valproate) as measured in human, bovine and rat (Mandula *et al.*, 2006).

Although albumin levels are generally decreased in the elderly, changes in plasma protein binding in the elderly are generally not attributed to age, but rather to physiological and pathophysiological changes or disease states that may occur more frequently in the elderly and which most often affect the binding affinity (Grandison and Boudinot, 2000). Furthermore, pathological conditions may alter the plasma protein concentrations, thereby affecting the binding capacity. The α_1-acid glycoprotein is a major acute phase protein of which the concentration may rise in response to systemic tissue injury, inflammation or infection (Fournier *et al.*, 2000). Surgical procedures such as instrumenting rats with permanent blood cannulas will increase serum α_1-acid glycoprotein levels and binding (van Steeg *et al.*, 2007). Also, genetic variation can influence plasma protein concentrations,

as shown in a study on differences in the pharmacokinetics of indinavir and lopinavir for the various phenotypes of the α_1-acid glycoprotein (Colombo *et al.*, 2006). The impact of genetic variation, age and (patho)-physiological changes on drug response will be discussed elsewhere in this paper.

Cerebral blood flow

The cerebral blood flow determines the maximal rate at which the drug can be delivered to the brain, if not restricted by the transport across the blood brain barriers itself. In flow-limited transport conditions, alteration in blood flow thus has an impact on drug delivery to the brain. Alteration in cerebral blood flow may be the result of changes in two parameters: 1) changes in the linear velocity of blood flow through the perfused capillaries and 2) variations in the total number of the perfused capillaries in the brain ("effective perfusion") (Fenstermacher *et al.*, 1995). When the linear velocity of blood flow is increased, the diffusional influxes of highly permeable drugs across the BBB will increase (and vice versa), while BBB transport of slightly or virtually impermeable drugs will essentially be unchanged. Increase in linear velocity of blood flow may result from situations like hypercapnia, hypoxia, or may be induced by drugs such as nicotine. Also a decrease in local cerebral blood flow may be drug induced as has been reported for pentobarbital. Changes in the number of effectively perfused brain capillaries will change the surface accesible for transport across BBB, and therefore potentially affect blood-brain transport of all drugs. A small increase in the number of perfused capillaries has been reported for hypercapnia and upon administration of nicotine (Fenstermacher *et al.*, 1995).

The blood-brain barrier (BBB) and blood-CSF-barrier (BCSFB)

The brain is separated from direct contact with blood by two barriers. The first and largest barrier is the BBB. The BBB is mainly formed by brain capillary endothelial cells (BCEC) which distinguish themselves from peripheral endothelial cells by the presence of tight junctions, the lack of fenestrations, an increased mitochondrial content and a very low pinocytotic activity (Hawkins and Davis, 2005). Tight junctions prevent the transport of large hydrophylic compounds between blood and brain (Brightman and Reese, 1969; Huber *et al.*, 2001). The BBB is a highly dynamic barrier with its functionality being regulated by surrounding astrocytes, neurons, perivascular microglial cells and microvascular pericytes (Abbott, 2002; Bodor and Brewster, 1982; Cornford, 1985; Davson and Oldendorf, 1967; Hawkins and Davis, 2005; Hori *et al.*, 2004; Kim *et*

al., 2006; Pardridge, 1988b; Rubin and Staddon, 1999; Vorbrodt, 1988). The morphology of the BBB restricts free flow of compounds between blood and the brain making diffusion difficult for the required nutrients such as oxygen and glucose and other essential substrates to penetrate into the brain. However, a number of mechanisms and highly selective transporters on the membranes of the BCEC are involved in the influx and efflux of the required substances (Abbott and Romero, 1996; Kusuhara and Sugiyama, 2005; Ohtsuki, 2004; Tsuji, 2005). A

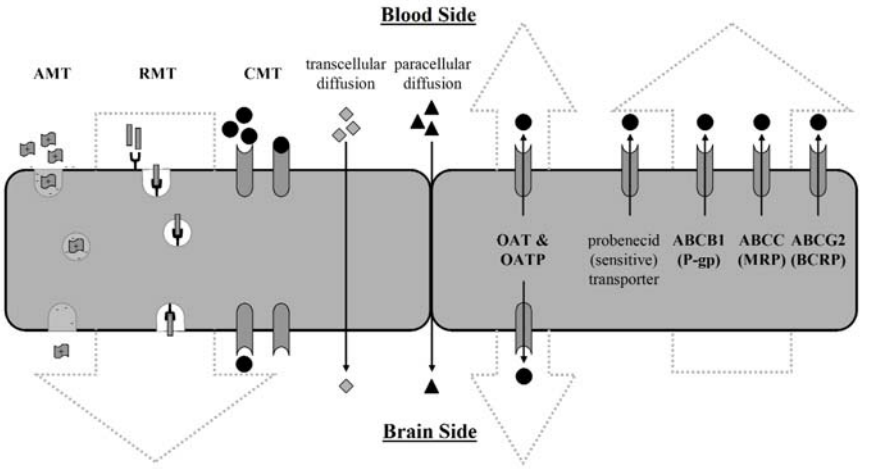

Figure 3: *Schematic overview of the influx (left side) and efflux (right side) transport mechanisms at the BBB. The OAT transporters are both inlfux and eflux transporters. This picture is adapted from Abbott and Romero (1996) and Kushuhara and Sugiyama (2005). AMT=absorptive mediated trancytosis; RMT=receptor mediated transport; CMT=carrier mediated transport; OAT= organic anion transporter; OATP= organic anion transporting polypeptide; P-gp=P-glycoprotein; MRP= multi-drug Resistance Protein; BCRP= Breast Cancer Related Protein.*

schematic overview of these transport mechanisms is depicted in Figure 3.

The second barrier is the BCSFB, which is a composite barrier made up of the choroid plexus epithelial cells, the arachnoid membrane and the circumventricular organs (such as the area postrema, median eminence, neurohypophysis and pinal gland) (de Lange, 2004). The choroid plexus is a leaf like structure that more or less floats in the brain ventricles. The BCSFB has fenestrated and, therefore, highly permeable capillaries. The barrier function of the BCSFB is provided by the tight junctions between the cells of the ependymal layer at the apical site, which contacts the CSF. These tight junctions are slightly more permeable than those of the BBB (Cserr, 1971; Meller, 1985; Milhorat, 1976;

Spector and Johanson, 1989). It should be noted, however, that in the circumventricular organs, the function and structure of the capillary endothelium is different (Gross *et al.*, 1986). In this small portion of the brain, the capillaries are fenestrated and permeable, for instance to serum proteins, thus in general have higher blood-to-tissue transport rates.

Passive transport

Solute molecules can cross membranes by the mechanisms of simple diffusion, either the paracellular or the transcellular route (Figure 3). The rate and extent of drug transport across the blood to brain barriers (Ghersi-Egea and Strazielle, 2001) is determined both by blood to brain barrier characteristics as well as by the physicochemical properties of the drug (Ghersi-Egea and Strazielle, 2001; Greig *et al.*, 1987; Groothuis and Levy, 1997; Gross *et al.*, 1986; Hammarlund-Udenaes *et al.*, 1997; Hammarlund-Udenaes, 2000; Hesselink *et al.*, 1999). Passive transport (diffusion) depends on size, charge at actual pH, and lipid solubility of the drug. Lipophilic, small, and non-charged drugs diffuse more easily across membrane (transcellularly) than hydrophilic, large and charged drugs (de Boer *et al.*, 2003). For drugs that easily cross the BBB, the cerebral blood flow may become the determinant in (mainly) the rate of transport across the barrier membranes. For the more hydrophilic drugs, paracellular diffusion becomes more important, which is restricted by the presence of the narrow tight junctions between the cells of the BBB and BCSFB. This makes that for paracellular diffusion the size of the drug relative to that of the space in the tight junctions is important (Groothuis and Levy, 1997; Levin, 1980; Oldendorf, 1970; Oldendorf, 1974).

Active transport

Transport across the BBB does not only occur on the basis of diffusion only. The brain endothelial cells and the choroid plexus epithelial cells express numerous influx and efflux transporters (Angeletti *et al.*, 1997; Bouw *et al.*, 2001b; Collins and Dedrick, 1983; Cordon-Cardo *et al.*, 1989; de Lange, 2004; Gao and Meier, 2001; Ghersi-Egea and Strazielle, 2001; Johnson *et al.*, 1993; Jolliet *et al.*, 1997; Loscher and Potschka, 2005; Nishino *et al.*, 1999; Ogawa *et al.*, 1994; Ooie *et al.*, 1997; Rao *et al.*, 1999; Schinkel *et al.*, 1994; Wijnholds *et al.*, 2000). Highly selective active transporters perform the influx of required nutrients and essential substrates and the efflux of waste and toxic product and can be divided into absorptive-mediated transcytosis (AMT), receptor-mediated transport (RMT) and carrier-mediated transport (CMT) (Figure 3). The AMT is based on the principle that polycations

interact specifically with negatively charged substances in the membrane of the endothelial cells. AMT mainly accounts for the transport of e.g. catonised albumins and histones (Alavijeh *et al.*, 2005; Bickel *et al.*, 2001). RMT transport large molecules like proteins and peptides which are required for metabolism processes in the brain. There are different types of RMT's at the BBB that have their own specific ligand such as the insulin receptor, the transferrin receptor or the leptin receptor. The RMT's transport the ligand into the cytosol through receptor mediated endocytosis (de Boer and Gaillard, 2007; Jones and Shusta, 2007). CMT's have the function to provide the brain with nutrients such as glucose (mainly by the GLUT-1 transporter (Pardridge *et al.*, 1990)), amino acids (e.g. Large Neutral Amino Acid -LNAA or LAT-transporter (Boado *et al.*, 1999)), purine bases, nucleosides, vitamins, hormones and others (Abbott and Romero, 1996). Other influx transporters include the nucleoside transporter system, the organic cation transport system (OCT), the organic anion transporter (OAT) and the organic anion transporting polypeptide (OATP) (Anzai *et al.*, 2006). The latter two systems (OAT and OATP) also act as efflux transporters. These transport systems may have a role in drug transport into the brain, although not for all transporters such a role has been clearly observed. For the LAT transport system, several amino-acid mimetic drugs like L-DOPA, α-methyl-dopa (Matsuo *et al.*, 2000; Wade and Katzman, 1975), α-methyl-trypsine, baclofen, gabapentin and phenylalanine mustard probably are substrates. Melphalan uptake into the brain is facilitated, by sharing the LAT system at the BBB (Greig *et al.*, 1987). Drugs bearing a monocarboxylic moiety such as simvastatin, lovastatin acid and pravastin, cross the BBB via the monocarboxylic acid transport systems (Tsuji, 2005). Pramipexole, a D2 receptor agonist used in the treatment of Parkinson's disease, is a cationic drug which not only crosses the BBB by diffusion but also via an (still to be identified) organic cation-sensitive transporter (Okura *et al.*, 2007).

Efflux transport

With regard to active drug transport, more is known about drug transport out of the brain by efflux transporters (Figure 3). Examples of such transporters are P-glycoprotein (P-gp or ABCB), the Multi-drug Resistance Protein family (MRP's or ABCC's (de Lange, 2007)) and the more recently discovered Breast Cancer Related Protein (BCRP, also known as ABCG2) (Cisternino *et al.*, 2004; Eisenblatter *et al.*, 2003). P-gp, MRP and BCRP are energy-dependent efflux pumps and belong to the ATP-binding cassette (ABC) transporter family.

P-gp is a broad spectrum efflux pump which can recognize and transport basic

and uncharged substrates in the size of 250Da -1850Da, as well as zwitterionic and positively charged substrates. It has been shown to be present at the luminal face (blood side) of the BBB (Cordon-Cardo *et al.*, 1989) and is also expressed at the choroid plexus (Rao *et al.*, 1999), however at these epithelial cells at the abluminal face (CSF side) then acting as an influx transporter. Substrates for both P-gp and MRP1 (see below) actually are cleared from the choroidal epithelial cells and may therefore protect these cells, thereby contributing to the detoxification of the choroid plexus itself (de Lange, 2004). P-gp may be inducible (Tatsuta *et al.*, 1994) and has many structurally diverse substrates (Seelig, 1998). Especially the use of mdr1a(-/-) mice (Schinkel *et al.*, 1994) has clarified the impact of this efflux transporter on brain concentrations of many drugs including dexamethasone, domperidone, indinavir, digoxin, vinblastin, morphine, sparfloxacin, amitryptiline, and cyclosporin A (de Lange *et al.*, 1998; de Lange *et al.*, 2000; Desrayaud *et al.*, 1998; Kim *et al.*, 1998; Mayer *et al.*, 1997; Meijer *et al.*, 1998; Schinkel *et al.*, 1995; Schinkel *et al.*, 1996; Uhr *et al.*, 2000; Xie *et al.*, 1999).

The influence of MRP transporters on BBB and BCSFB transport is dependent on their localisation at these membranes. Using primary cultured bovine brain microvessel endothelial cells (BBMEC) as a model for the BBB, Western blot analysis for MRPs and confocal laser scanning microscopy to determine membrane localisation of MRPs in BBMEC indicated that MRP1 and MRP5 are predominantly present at the apical plasma membrane and an almost equal distribution of MRP4 on the apical and basolateral plasma membrane of BBMEC. These orientations are different from those observed in polarised epithelial cells as model of the BCSFB (Zhang *et al.*, 2004). The use of mrp1(-/-) and mdr1ab(-/-) mice have shown the importance of MRP1 efflux transport at the level of the BCSFB in lowering the CSF concentrations of etoposide at least in the absence of P-gp for which it is also a substrate besides for MRP1 (Wijnholds *et al.*, 2000).

BCRP is a half transporter that displays drug resistance. BCRP shows clear similarity to P-gp in tissue distribution and to which substrates it binds. BCRP can actively transport a wide range of substrates ranging from chemotherapeutic agents to organic anion conjugates and also fluorescent compounds such as rhodamine 123 and Hoechst 33342 as well as chemical toxicants. BCRP is involved in multi drug resistance in cancer, especially with regard to acute myeloid leukemia. Also, it plays a role in the survival of stem cells under hypoxic conditions and might play a role in regulating stem-cell differentiation. BCRP is highly expressed at the luminal surface of the endothelial cells of human brain microvessels (de Lange, 2007; Mao and Unadkat, 2005).

The probenecid (sensitive) transporter is another efflux transporter at the BBB and BCSFB, involved in elimination of probenecid and a variety of other drugs. Mainly brain elimination is increased for a number of anti(retro)viral drugs (Groothuis and Levy, 1997; Takasawa *et al.*, 1997; Wong *et al.*, 1993) while increased efflux from CSF has been reported for penicilline and to a smaller extent other ß-lactam antibacterials like ampicilline, cefodizime cefotaxime and ceftriaxone (Dacey and Sande, 1974; Ogawa *et al.*, 1994; Spector, 1986; Spector, 1990), and methotrexate (Balis *et al.*, 2000). Also, after coadministration of probenecid, brain ECF concentrations of a few NMDA-receptor antagonists increased, and a prolongation of their anticonvulsant activity was found (Hesselink *et al.*, 1999).

As mentioned before, OATP and OAT also belong to the efflux transporters present at the BBB. However, OATPs and OATs are driven by a drug or ion gradient and are not energy-consuming. For further information about the active efflux transporters please see Anzai *et al.*, 2006; Borst *et al.*, 2000; Dallas *et al.*, 2006; Ebinger and Uhr, 2006; Kusuhara and Sugiyama, 2005; Loscher and Potschka, 2005; Schinkel, 1999; Sun *et al.*, 2003. The active-efflux transporters are multidrug resistant and limit the penetration of drugs into the brain and, next to metabolic degradation (Lee *et al.*, 2001), this can restrict its effect (Ebinger and Uhr, 2006; Groenendaal *et al.*, 2007b; Schinkel *et al.*, 1996). Bromocriptine, a D2 agonist used in the treatment of Parkinson's disease, has shown to be a substrate for P-gp (Vautier *et al.*, 2006). Changes on drug distribution into the brain for compounds are expected upon coadministration of other substrates or inhibitors of the same transporter, i.e. P-gp substrates or P-gp reversal agents or inhibitors (Klopman *et al.*, 1997; Mayer *et al.*, 1997). Several overviews of drugs which are substrates for the various efflux transporters have been published, for example by (Loscher and Potschka, 2005) and by (Anzai *et al.*, 2006).

Within brain distribution

A generalised physiological compartment model (Collins and Dedrick, 1983) shows the main factors that determine the entry into and distribution of drugs between brain extracellular fluid (brain ECF), intracellular brain compartments, ventricular and lumbar CSF. Thus, once within the brain, the kinetics of drug exchange between brain ECF, brain intracellular space (ICS) and CSF, as well as elimination by bulk flow/CSF turnover and potential local metabolic enzymes determine the time course of the concentration in specific parts of the brain (Mayer *et al.*, 1997)

ECF-CSF exchange

Diffusion between brain ECF and CSF is governed by the concentration gradient across the layer of ependymal cells that separates the brain ECF from the CSF (de Lange and Danhof, 2002; Mayhan, 2001). This layer possesses structural and enzymatic characteristics necessary for the clearance of a wide variety of substances in the CSF, thus forming a metabolic barrier at the brain ECF-CSF interface (Bruni, 1998; Del Bigio, 1995). Apart from that, concentrations in brain ECF and CSF may influence each other by diffusion (Fenstermacher *et al.*, 1974; Malhotra *et al.*, 1994). Diffusive transport will be more important in case of larger concentration gradients. In relative terms, after systemic administration of a drug, only small concentration gradients occur between brain ECF and CSF when transcapillary passage is rapid compared with CSF turnover (de Lange and Danhof, 2002; Meijer *et al.*, 1998), whereas the situation is reversed for drugs with permeability-limited BBB transport. For these compounds, significant concentration gradients between brain ECF and CSF exist after systemic administration (Agon *et al.*, 1991). On the other hand, following administration of a drug directly into the CSF, rapid BBB passage creates a steep gradient between brain ECF and CSF, while for low permeability drugs there will be only small gradients between brain ECF and CSF (Aird, 1984; Blasberg *et al.*, 1975; de Lange *et al.*, 1994; Patlak and Fenstermacher, 1975). Differences in spatial distribution due to differences in BBB transport have also been shown following local administration of drugs in the brain (de Lange *et al.*, 1995). Furthermore, processes like brain metabolism and effective diffusion through brain tissue will respectively increase and decrease the concentration gradient between CSF and brain ECF following direct administration into the CSF.

Then, bulk flow or convection of brain ECF, potentially by perivascular channels of flow, into the direction of the CSF is another means by which drugs can be redistributed within the brain. Finally, CSF does not present one compartment. Clear differences in pharmacokinetics of drugs may exist at the CSF from lumbar and ventricle sites (the ones most often used for obtaining CSF), due to diffusion but also CSF dynamics (Baker *et al.*, 1996; Balis *et al.*, 2000; Blaney *et al.*, 1995; Freund *et al.*, 2001; Kawakami *et al.*, 1994; Marsala *et al.*, 1995; Morikawa *et al.*, 1998).

In analogy to the effect of plasma component binding, also binding to brain constituents may have a huge impact on within brain distribution (Goldbaum and Smith, 1954; Kalvass *et al.*, 2007; Kurz and Fichtl, 1983). Altogether, the

relationships of the different clearances of a drug between blood and CNS, and within the compartments of the CNS will ultimately determine the concentrations of a drug within a specific part of the CNS.

ECF-ICF exchange

After passage of the BBB, a drug enters the extracellular space of the brain and may thereafter distribute into brain cells. In general, this intracellular distribution is quantitatively more profound for the more lipophilic drugs. Extracellular concentrations will depend on the relation between BBB- and extra-intracellular clearances for the unbound drug and the fraction of the drug bound within the brain cells.

Drug metabolism

Drug metabolism may occur at the level of extracellular and intracellular sites of the brain (Kerr *et al.*, 1984), but also at the the BBB and BCSFB (Ghersi-Egea *et al.*, 1988). Several enzymes that are involved in hepatic drug metabolism have been found in the small microvessels of the brain and the choroid plexus. Enzymes like CYP haemoproteins, several CYP-dependent monooxygenases, NADPH-cytochrome P450 reductase, epoxide hydrolase, and also conjugating enzymes such as UDP-glucuronosyltransferase and α-class glutathion S-transferase have been detected in blood vessels of the brain or closely surrounding cell types obtained from both rat and human brain tissue (Ghersi-Egea *et al.*, 1988; Ghersi-Egea *et al.*, 1994; Ghersi-Egea and Strazielle, 2001; Johnson *et al.*, 1993). For the BCSFB very high activities (similar to those in the liver) have been found for UDP-glucuronosyltransferase and epoxide hydrolase, while also several CYP isoenzymes are relatively highly expressed in the choroid plexus. Then for both barriers, α and μ classes of gluthathion S-transferase and glutathion peroxidase in relatively high values have been found (Ghersi-Egea *et al.*, 1994; Johnson *et al.*, 1993). All these enzymes may serve as "enzymatic barriers" to drug influx into the brain, and even may be inducible (Volk *et al.*, 1991). In how far drug metabolism at this level will have an impact on drug concentrations in the brain compartments remains to be elucidated (Kurata *et al.*, 1995).

Target site distribution

Most drugs exert their effects not within the plasma compartment, but within the target containing tissue(s). The process of drug distribution to the active site is reflected by the need of the "link-bridge" in many PK-PD relationships (Lin, 2006).

The pharmacological effect profile is determined by the concentration of drug at the target site or, more precisely, by the time course of target site drug concentration , (Danhof *et al.*, 2007; Eichler and Muller, 1998). For CNS compounds, an important question is whether the intra- or extracellular brain concentrations reflects the target concentration at best. For drugs such as corticosteroids (Falkenstein *et al.*, 2000), anticancer drugs and anti-HIV drugs (Fletcher, 1999; Peter and Gambertoglio, 1998), this is probably the intracellular concentration. However, most CNS active drugs have their target at extracellular recognition sites (membrane receptors), and for those drugs brain ECF concentrations may provide the most relevant information. Both BBB transport kinetics and intracellular distribution determine the concentration and time to equilibrium between concentrations in plasma and brain ECF (Liu *et al.*, 2005). In general, active transport out of the brain decreases and brain tissue binding increases the time to equilibrium in brain ECF. Apart from their role in BBB transport, active transporters may also play a role in ECF-ICF exchange of drugs because also at the brain parenchymal cells a number of transporters (P-gp and MRP) have been localised (Groenendaal, 2007; Lee *et al.*, 2001; Scism *et al.*, 2000).
Target site distribution may represent a rate-limiting step in the onset to the biological effect. This is reflected in a delay of the pharmacological effect relative to the drug concentration in plasma, which is often referred to as hysteresis (Danhof *et al.*, 2007). Intracerebral microdialysis is a useful tool in obtaining information on the kinetics of target site distribution of CNS compounds (de Lange *et al.*, 1997; Hammarlund-Udenaes *et al.*, 1997; Hammarlund-Udenaes, 2000; Xie *et al.*, 1999), which has been successfully applied to the characterisation of target site distribution kinetics especially in a number of investigations of the PK-PD correlation of morphine (Bouw *et al.*, 2001a; Groenendaal *et al.*, 2007a; Groenendaal *et al.*, 2008). Such knowledge is important for the progress in the development of novel mechanism-based target site distribution modeling concepts (Danhof *et al.*, 2007).

Target interaction

At the target-site in the brain, the relationship between the target site concentration of a drug and the response profile depends on several factors related to the drug and the biological system. Differences in such concentration-response relations are related to differences in receptor interaction kinetics (target affinity and intrinsic efficacy) but also on the biological system (i.e., the receptor density and the transducer function, relating receptor activation to

pharmacological response) (Danhof *et al.*, 2007; Kenakin, 1999). Therefore, the incorporation of receptor theory in PK-PD modelling for the prediction of *in vivo* drug concentration-response relationships is important as it enables a separation between drug-specific and biological system specific parameters (Van der Graaf and Danhof, 1997). Much of the conceptual framework regarding how to study receptor function evolved from the pharmacological investigation of drug action. A.V. Hill was the first to understand the relationship between drug concentration and response when he derived the Langmuir binding equation in the course of his studies on nicotine and curare (Hill, 1909). The occupancy theory evolved from then onwards and was refined to describe the effects of partial agonists (Ariens, 1954; Stephenson, 1956) and receptor reserve. The development of the occupancy theory has allowed the formulation of a series of mathematical models that describe the interaction of agonists and antagonists with their receptors, in terms of affinity and efficacy (Dougall, 2001). In the classical occupancy receptor theory, efficacy is a dimensionless proportionality constant denoting the ability of agonists to produce a pharmacological response. In theoretical terms, it is difficult to separate affinity and efficacy estimates of agonists for receptors (Kenakin, 1999). The operational model of agonism which describes the relationship between drug concentration, receptor interaction and response, was proposed by Black and Leff (Black and Leff, 1983). Concepts from receptor theory have been applied in the development of a PK-PD modeling strategy that constitutes a scientific basis for the prediction of *in vivo* drug concentration-effect relationships, for example in the PK-PD analysis of neuroactive steroids (Visser *et al.*, 2002), benzodiazepines (Tuk *et al.*, 1999; Tuk *et al.*, 2002; Visser *et al.*, 2003b), adenosine A1 receptor agonists (Van der Graaf *et al.*, 1997), 5-HT1A receptor agonists (Zuideveld *et al.*, 2004), opioids (Groenendaal, 2007; Yassen *et al.*, 2005) and β-blockers (van Steeg *et al.*, 2008). The incorporation of receptor theory in PK-PD modeling has recently been reviewed (Ploeger *et al.*, 2009)

5. Sources of Variation in Mechanisms Contributing to the Response Profile

The mechanisms on the causal path between dose and response of CNS drugs, as described above, can be viewed as being placed in series. Each mechanism may influence the CNS drug response. Here we will discuss sources of variation in individual mechanisms, showing that changed conditions may change the mechanism(s) which detremine the relationship between dose and response. For interpretation, comparison and extrapolation of research data, one should be able to identify the sources of variation in the individual mechanisms.

In most neuropharmacological studies the effect of drugs in the CNS are still related to the dose (Girgin *et al.*, 2008; Homma *et al.*, 2008; Kinon *et al.*, 2008; LeWitt *et al.*, 2008; Mischoulon *et al.*, 2008; Stern *et al.*, 2004; Walsh *et al.*, 2008).

However, a unique dose-effect relationship it is not generally applicable. This is because the above-mentioned mechanisms do not contribute equally in different conditions. The individual mechanisms may differ quantitatively due to differences in genetics, species, gender, age, environmental and pathological conditions etc. Thus, knowledge of the variability in these mechanisms under different circumstances, as well as their interplay is important in making comparisons between different conditions or for making predictions of drug response under certain conditions (Figure 4).

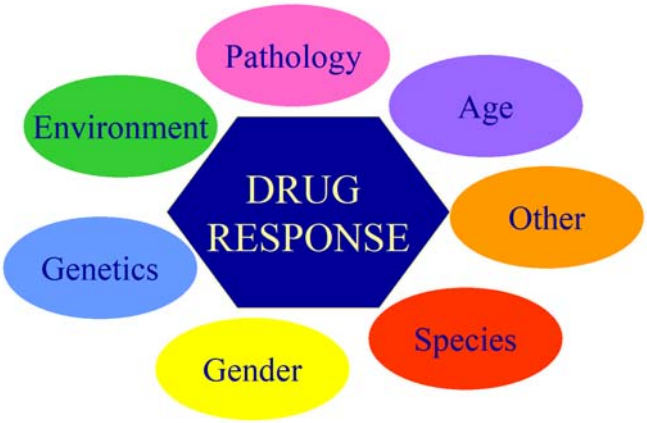

Figure 4: *Sources of variation in mechanisms between dose and response.*

Species

In drug development many pharmacological and toxicological studies are performed in small laboratory animals such as mice, rats, rabbits, dogs and monkeys. Species differences and therefore interspecies scaling is an important issue for the prediction of pharmacokinetic parameters from these animals to humans. The onset, intensity and duration of drug response depend on the absorption, distribution, metabolism and elimination that are inherent to the biological system (Hurst *et al.*, 2007). In addition to metabolic differences, the anatomical, physiological, and biochemical differences in the gastrointestinal tract (i.e. transit time, pH, microbial content, membrane transport) of the human and common laboratory animals can cause significant variation in drug absorption from the oral route (Kararli, 1995).

Serum albumins from mammalian species differ in amino acid sequence and protein structure. Consistent with this, binding-site affinity and selectivity vary from species to species as was observed for warfarin in rat, bovine, and human albumin. Because of these differences, albumins from separate species should not be considered interchangeable (Mandula *et al.*, 2006).

In general, small animals tend to eliminate drugs more rapidly than human beings when compared on a weight-normalised basis. There are relatively small differences in the primary amino acid sequences of the CYP enzymes across species, although these might have an impact on substrate specificity and catalytic activity and consequently on drug metabolism across species. Particularly CYP1A, CYP2C, CYP2D and CYP3A appear to show interspecies difference (Martignoni *et al.*, 2006).

With regard to the brain, species differences have been shown in brain microvessel MAO, with rat microvessels having one of the highest MAO activity among all tissues, whereas MAO activities in brain microvessels from humans, mice, and guinea pigs were very low (Kalaria and Harik, 1987). Species differences were also found in the brain and brain-to-plasma concentrations of of three P-gp substrates using PET technology showing higher brain distribution in humans, monkeys, and minipigs than in rats and guinea pigs. The species differences were still present after P-gp inhibition, specifically in rats. Differences in plasma protein binding or metabolism did not explain the species-related differences (Syvanen *et al.*, 2009).

Other physiological parameters, such as body temperature (36°C - 38°C), and haematocrit (40% - 45%) are relatively conserved among animals and are independent of animal size (Davies and Morris, 1993).

Genetics

Genetic variability in drug response occurs as a result of molecular alterations at the level of drug-metabolising enzymes (pharmacokinetics), drug transport molecules that mediate drug uptake into and efflux from intra- and or extra-cellular sites (pharmacokinetics) and drug targets/receptors (pharmacodynamics) (El Desoky *et al.*, 2006; Ensom *et al.*, 2001). The pharmacokinetic and/or pharmacodynamic consequences of gene polymorphisms may include increased risk of toxicity or a change in response (Shah, 2005).

Metabolising enzymes

The most widely known example in genetic polymorphisms of metabolising enzymes are in the cytochrome P450 enzymes (CYPs). Specifically CYP2D6, CYP2C19 and CYP2C9 are of interest as they metabolise a substantial portion of all the drugs used (Ensom *et al.*, 2001) with CYP2D6 being involved in the metabolism of more than 30% of CNS drugs such as cholinesterase inhibitors, antidepressants, neuroleptics, opioids and many others (Cacabelos, 2008a). CYP2D6 is also the main metabolising enzyme for many antiarrhythmic drugs. Cardiac patients who were phenotyped as being poor metabolisers for CYP2D6, have been found at high risk of severe side effects or drug toxicity when using propafenone or mexiletine (El Desoky *et al.*, 2006). Ultrarapid and extensive metabolisers for CYP2D6 are at risk of side effects caused by the active metabolite of the antiarrhythmic drug encainide. Phenytoin, used in the treatment of epilepsy, undergoes significant metabolism by CYP2C9 and CYP2C19 and is able to induce intoxication in patients with polymorphisms in these enzymes (Anderson, 2008b). Although the CYP enzymes are the most widely studied and best characterised metabolic enzymes, polymorphic variants can also occur in various dehydrogenases, esterases, NADPH-quinone oxidoreductase UDP-glucuronyltransferases (UGT), various methyltransferases and sulfotransferases (Cacabelos, 2008b). Differences in catechol-O-methyltransferase (COMT) activity (one of the metabolising enzymes for L-DOPA) and genotype may determine individual variations in the therapeutic response to L-DOPA or Parkinson's disease susceptibility (Bialecka *et al.*, 2008b).

Transporters

P-gp is a MDR-1 gene product and several polymorphisms have been reported, some of which might affect P-gp expression and function (Hoffmeyer *et al.*, 2000) and could lead to elevated drug levels in many tissues (Schinkel *et al.*, 1994). P-gp

polymorphisms have also been associated with increased risk for Parkinson's disease (Furuno *et al.*, 2002; Lee *et al.*, 2004). Bartels *et al* claimed a decreased P-gp function in patients with Parkinson's disease (Bartels *et al.,* 2008). Differences have been observed in pharmacokinetic parameters of digoxin between different genotypes of the MDR-1 gene (Johne *et al.*, 2002; Kurata *et al.*, 2002). Higher concentrations of rhodamine-123, dexamethasone, digoxin, cyclosporin A, ondansetron, loperamide and morphine were observed in brain tissue of mdr1a(-/-) mice compared to wild-type mdr1a(+/+) mice (de Lange *et al.*, 1998; Meijer *et al.*, 1998; Schinkel *et al.*, 1995; Schinkel *et al.*, 1996) incidating the consequence of the absence of functional P-gp.

Targets

Variant alleles are known to occur not only at the genes expressing target enzymes, channels and receptors but also at the genes responsible for intracellular signal transduction (Shah, 2005). There is evidence to indicate that the β_2-adrenergic receptor is polymorphically expressed and that this contributes to the interindividual variability in response of patients with asthma to certain drugs. Similar evidence has been found in the promotor region of the serotonin transporter with a consequence in the response to fluvoxamine (Shah, 2005). In a study involving twins, about 30% to 35% of variance in heart rate acceleration as induced by a 30-minute i.v. infusion of nicotine and cotinine was due to genetic sources (Swan *et al.*, 2007). The presence of APOE-4 allele in patients suffering from Alzheimer's Disease (AD), differentially affects the quality and extent of drug responsiveness when these patients were treated with cholinergic enhancers such as galantamine or with non-cholinergic drugs (Cacabelos, 2008a).

Gender

In general, females experience more adverse drug reactions (ADR) than males (Zopf *et al.*, 2008). Hormonal fluctuations in the menstrual cycle or pregnancy are considered to be the main reason for gender differences in the pharmacokinetics and pharmacodynamics of drugs. Evidence for physiological variation within the menstrual cycle is limited. Variations have been observed in the renal, gastrointestinal and cardiovascular systems as well as in plasma lipids and haematological and immune function (Kashuba and Nafziger, 1998). Also, variability during the menstrual cycle has been observed for α_1-acid glycoprotein. The influence of the menstrual cycle on the pharmacokinetics of drugs has been

shown for e.g. paracetamol (acetaminophen) and ranitidine (Flores *et al.*, 2003; Gugilla *et al.*, 2002) but was not seen for triazolam or caffeine (Kamimori *et al.*, 1999; Kamimori *et al.*, 2000).

Non-hormonal or reproductive-related differences between male and female subjects can be either physiological (body weight, body fat, glomerular filtration, organ size, gastric motility) or molecular (drug transporters such as the dopamine transporter (Dluzen and McDermott, 2008) or drug-metabolising enzymes).

In general, females weigh less than males, so they often receive higher doses which results in higher drug exposure. In the example of L-DOPA, used in the treatment of Parkinson's disease, this difference in bodyweight might be the reason for the difference in the proportion of men and women experiencing dyskinesias during the course of the disease (Martinelli *et al.*, 2003; Zappia *et al.*, 2002). Estrogen has been shown to inhibit COMT (Shulman, 2007) which would explain the higher exposure to L-DOPA in women. Furthermore, females have a higher percent body fat than males which can affect the volume of distribution of certain drugs. Differences in the activity of hepatic enzymes (specifically CYP3A4 (Greenblatt and von Moltke, 2008)), drug transporters and renal excretion between males and females could result in differences in elimination of a drug (Anderson, 2008a). P-gp has been mentioned as a drug transporter whose activity might be different in males and females but literature is contradicting on this point (Schuetz *et al.*, 1995; Wolbold *et al.*, 2003). Differences in response to drugs between men and women have been seen for various drugs, such as opiates (Dahan *et al.*, 2008), anti-HIV drugs (Floridia *et al.*, 2008), paroxetine (Gex-Fabry *et al.*, 2008), midazolam (Sun *et al.*, 2008) and citalopram (Young *et al.*, 2008).

Age

Roughly three age-groups can be distinguished among patients, namely paediatric, adult and geriatric patients. Both paediatric patients (0-18 years of age) and geriatric patients (>65 years) are considered special populations in drug discovery and development. Most drugs are first developed for the adult patient population (19-65 years) with studies subsequently performed in elderly and children. This review will not emphasize on drug disposition in the paediatric population, as this is irrelevant for Parkinson's disease and reviewed elsewhere (Kearns *et al.*, 2003).

In elderly patients drug response is not only dependent on physiological changes which are associated with age but the effects of disease and of comedications should also be taken into consideration. In general, drug absorption from the gut

is usually not diminished in elderly. However, compounds that cross the intestinal epithelium by carrier-mediated transport mechanisms might be absorbed at a lower rate in the elderly as well as for compounds which have been administered via the dermal, subcutaneous or intramuscular route, due to reduced tissue blood perfusion (Turnheim, 2003). Skeletal muscle mass and total body water content declines with age but total body fat increases with age which might have implications for the volume of distribution of a drug (for hydrophilic drugs it decreases and for lipophilic drugs it increases) and consequently on the half-life of a drug (Turnheim, 1998).

Despite significant research efforts, the effect of age on hepatic drug metabolism continues to be a controversial issue. Aging was found to be associated with a reduction in liver weight of about 25% - 35% (Le Couteur and McLean, 1998). Liver function, as measured via routine tests, however, does not change significantly with advancing age, although serum albumin can decrease slightly. Liver blood flow declines by about 40% with aging. Bile flow and bile salt formation are reduced by about 50% (Fu and Nair, 1998; Herrlinger and Klotz, 2001; Le Couteur and McLean, 1998). At all ages there is a wide genetic variability in the rates of drug clearance, specifically within the CYPs as described previously in this review. Interindividual variability in drug clearance accounts for the problems in detecting any changes due to aging (Zeeh and Platt, 2002)

The most pronounced pharmacokinetic change in elderly is the reduction in renal elimination of drugs due to reduced renal function. The glomerular filtration rate declines by 25% - 50% between the ages of 20 and 90. Because of these changes, an age-dependent decline of total clearance is to be expected for all drugs that are predominantly eliminated by the kidneys (Herrlinger and Klotz, 2001). Other physiological changes with age include altered structure of proteins (Gafni, 1997), altered pulmonary function, reduced cardiac output, decrease in serum levels of various hormones and decline in the immune system (Turnheim, 2003). Moreover, phase-contrast magnetic resonance imaging has shown a significant decrease of total cerebral blood flow in elderly individuals with a linear correlation with age (Stoquart-ElSankari *et al.*, 2007). Many studies have demonstrated substantial and important age-related changes in neurochemistry and neurobiology. Data on the decreases and alterations in the dopaminergic and serotonin neurotransmitter pathways have been relatively consistent in both animal and human studies (Adolfsson *et al.*, 1979; Bucht *et al.*, 1981; Wenk *et al.*, 1989). The numbers of dopamine D1 and D2 receptors, as well as serotonin 5HT1A and 5HT2A receptors are reduced in several brain regions with aging. Studies in other receptors show

that other neuronal systems and receptors are affected by age-related alterations as well (Schwartz and Abernethy, 2009). The changes in these neurotransmitter and receptor systems may account for, in addition to changes in drug reaction and metabolism, alterations in behaviour, cognitive abilities, and mood in the elderly. Other CNS signaling systems-the GABAergic (i.e., producing γ-aminobutyric acid) and enkephalin-endorphin system have not been well studied in older individuals; however, clinical studies of drugs acting on these systems have noted age-related changes in their pharmacodynamics (Schwartz and Abernethy, 2009).

Environmental factors

Cigarette smoking and other environmental factors have been shown to influence the metabolism of some drugs (Vestal and Wood, 1980; Wood *et al.*, 1979). Cigarette smoking reduces the therapeutic response to certain drugs such as theophylline through the induction of hepatic cytochrome P450 isoenzymes (Braganza *et al.*, 2008). Polycyclic aromatic hydrocarbons (PAHs) are some of the major lung carcinogens found in tobacco smoke. PAHs are potent inducers of the hepatic CYP1A1, CYP1A2, and, possibly, CYP2E1. Other compounds such as acetone, pyridine, heavy metals, benzene, carbon monoxide, and nicotine may also interact with hepatic enzymes but their effects appear to be less significant. The primary pharmacokinetic interactions with smoking occur with drugs that are CYP1A2 substrates, such as caffeine, clozapine, fluvoxamine, olanzapine, tacrine, and theophylline. The primary pharmacodynamics drug interactions with smoking are hormonal contraceptives and inhaled corticosteroids (Kroon, 2007). Cigarette smoking can affect the pharmacokinetic and pharmacodynamics properties of many psychotropic drugs as summarised elsewhere (Desai *et al.*, 2001).

Pharmacokinetic interactions between food and orally administered drugs involve (1) before and during gastrointestinal absorption; (2) during distribution; (3) during metabolism; and (4) during elimination (Singh, 1999). Absorption and metabolism are the phases where food has most effect and this may have clinical implications for drugs such as in the treatment of cancer (Singh and Malhotra, 2004). Food can decrease the rate of L-DOPA absorption, but has no effect on the systemic exposure to L-DOPA and the degree of 3-O-methyldopa formation (Crevoisier *et al.*, 2003).

Pathological conditions

Disease is defined as "any deviation from or interruption of the normal structure

or function of any body part, organ, or system that is manifested by a characteristic set of symptoms and signs" (The Free Dictionary, 2009). Given this, alteration(s) at any place in the body as a result of a pathological condition might influence the pharmacokinetics and/or the pharmacodynamics of a drug. Some general examples are given below. The distict example of Parkinson's disease is discussed later in this review.

Obesity is a condition in which excess body fat has accumulated to such an extent that health may be negatively affected. It is commonly defined as a body mass index (BMI) of 30 kg/m^2 or higher. This distinguishes it from being overweight as defined by a BMI of 25 kg/m^2 or higher. Cardiac performance and adipose tissue blood flow may be altered in obesity. There is uncertainty about the binding of drugs to α_1-acid glycoprotein in obese patients. Some data suggest that the activities of hepatic CYPs are altered, but no clear overview of drug hepatic metabolism in obesity is currently available. Pharmacokinetic studies provide differing data on renal function in obese patients (Cheymol, 2000).

Alterations of drug transporters, as well as metabolic enzymes, in patients with chronic renal failure (CRF) can be responsible for reduced drug clearance. CRF not only alters the renal elimination of drugs. Pharmacokinetic studies conducted in patients with CRF demonstrate that the nonrenal clearance of multiple drugs is reduced. CRF affects the metabolism of drugs by inhibiting key enzymatic systems in the liver, intestine and kidney. The down-regulation of CYPs has been reported next to a decrease in gene expression. Liver phase II metabolic reactions are also reduced in CRF. Moreover, intestinal drug disposition is affected in CRF. Increased bioavailability of several drugs has been reported in CRF, reflecting decrease in either intestinal first-pass metabolism or extrusion of drugs (mediated by P-glycoprotein). Decreased activity of P-gp was observed in CRF rats with no significant change of protein content, suggesting that uremic toxins may suppress P-gp function. With the development of CRF, the renal secretion of organic ions mediated by OAT and OCT is decreased. Organic anionic uremic toxins may directly inhibit the renal excretion of various drugs and endogenous organic acids by competitively inhibiting OAT. In addition, the expression of OAT1 and OCT2 was reduced in chronic CRF rats (Pichette and Leblond, 2003; Sun *et al.*, 2006).

The capacity of the liver to metabolise drugs depends on hepatic blood flow and liver enzyme activity, both of which can be affected by liver disease. Liver disease can modify the kinetics of drugs biotransformed by the liver. Studies on the effects of liver disease on specific isoenzymes of CYP have shown that some isoforms are

more susceptible than others to liver disease. In addition, liver failure can influence the binding of a drug to plasma proteins which in turn could influence the processes of distribution and elimination. Portal-systemic shunting, which is common in advanced liver cirrhosis, may substantially decrease the presystemic elimination (i.e., first-pass effect) of high extraction drugs following their oral administration, thus leading to a significant increase in the extent of absorption. Glucuronidation is often considered to be affected to a lesser extent than CYP-mediated reactions in mild to moderate cirrhosis but can also be substantially impaired in patients with advanced cirrhosis (Rodighiero, 1999; Verbeeck, 2008).

Other

Circadian rhythms can influence the pharmacokinetics of a drug which has been shown for over 100 drugs, among them is L-DOPA, for which a higher plasma clearance and lower area under concentration curve was observed in rats after the administration in the late evening (Andre *et al.*, 1996). Absorption may be influenced and most lipophilic drugs seem to be absorbed faster when the drug is taken in the morning compared with the evening; for water-soluble compounds, no circadian variation in the absorption of drugs has been found. Concerning drug distribution, the higher the blood flow fraction an organ receives, the higher the rate constant for transferring drugs out of the capillaries. This drug pharmacokinetic phase may be influenced by circadian variations in the protein binding of acidic and basic drugs. Drug metabolism may be influenced by daily modifications of blood flow. For drugs with a high extraction ratio, metabolism depends on hepatic blood flow, while that of drugs with a low extraction ratio depends on liver enzyme activity. Hepatic blood flow has been shown to be greatest at 8 am and metabolism seems to be reduced during the night. Finally, concerning drug elimination, the clearance of 'flow-limited' drugs that present a high extraction rate is affected by the blood flow delivered to the organ, independent of the cardiac output fraction supplied (Baraldo, 2008). As the cardiovascular system is the means of transport, blood flow fraction destined to each organ determines the relative mass of solute in plasma, which is constantly in contact with the tissue. Hence, not only the rate but also the extent of drug transfer would be increased when tissues are irrigated by a higher fraction of cardiac output. Aging and circadian rhythms present similar cardiac output distribution patterns when moving from young to aged adult and from nocturnal to diurnal hours. These two changes lead to an increased blood flow delivery to the renal region in the elderly and in the morning, but with a decreased cardiac

output in aged individuals and an increased one during the day. This scenario allows us to forecast substance concentrations outside the blood vessels, which are responsible for the extent of drug elimination and the intensity of drug effect (Fagiolino *et al.*, 2006).

Body position may influence physiological characteristics, such as perfusion, gastrointestinal function and plasma volume. These characteristics may interact with key factors determining the pharmacokinetics of drugs. Postures which favor rapid gastric emptying accelerate the absorption of orally administered drugs. Consequently, these postures favour a shorter time to reach peak plasma drug concentration and a higher C_{max} and-in the case of transient saturation of first-pass metabolism-total exposure (area under the concentration-time curve, AUC) in comparison to recumbent left and supine. For highly protein-bound drugs (e.g. phenytoin, imipramine), the total plasma concentration has been found to be approximately 10% higher in standing than lying subjects due to changes in plasma volume (Queckenberg and Fuhr, 2008).

Research has shown ethnic differences in the clinical presentation, treatment, clinical response and outcome of mental illnesses. A number of ethnically specific variations have been found in the genetic and non-genetic mechanisms affecting pharmacokinetics and dynamics of psychotropic drugs, which might underlie the differences in drug use and response across ethnicities. Although some of these ethnic differences could be partially explained by genetic factors, a number of ethnically based variables like culture, diet and societal attitudes could potentially have a significant, but as yet unquantified influence as well (Chaudhry *et al.*, 2008). The pharmacokinetic factors which can be expected to potentially exhibit racial differences are (1) bioavailability for drugs which undergo gut or hepatic first-pass metabolism, (2) protein binding, (3) volume of distribution, (4) hepatic metabolism, and (5) renal tubular secretion. Absorption (unless active), filtration at the glomerulus, and passive tubular reabsorption would not be expected to exhibit racial differences (Johnson, 1997).

6. The BBB in Neurodegeneration: Implications for PK-PD Relationships of Antiparkinson Drugs

The BBB is a key role player in the relationship between plasma and brain pharmacokinetics as the rate of penetration into the brain tissue is limited by the diffusion of the drug across the BBB. Drug transport to the brain is dependent on physico-chemical properties of the drug suchs as lipophilicity, ionistation and pH in relation to membrane properties. The BBB behaves differently from most other membranes in the body due to the presence of tight junctions and active influx and efflux transporters in the membrane resulting in an unequal (unbound) drug concentration on both sides of the BBB at steady state. Other factors may also contribute to this phenomenon, such as metabolism within the brain, or drug transport to the CSF via interstitial fluid (ISF) bulk flow (Hammarlund-Udenaes *et al*., 2008). In fact, for many drugs (both lipophilic and hydrophilic) unbound brain concentrations are much lower than the corresponding blood concentrations (Hammarlund-Udenaes *et al.*, 1997). Hammarlund-Udenaes and collegues (Hammarlund-Udenaes *et al.*, 1997) have performed simulations using a model with one body compartment and one brain compartment to describe unbound brain concentration-time profiles in relation to unbound blood profiles to

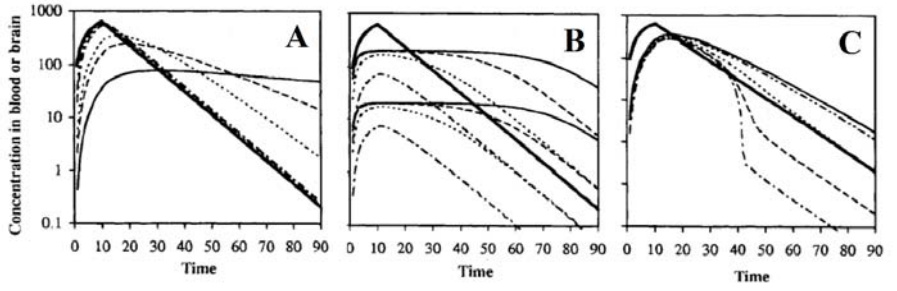

Figure 5: Simulations of brain concentrations. In all figures, the thick line depicts the blood concentration (*taken with permission from Hammarlund-Udenaes et al 1997;Pharm. Res. 14:128-134)*

A: Passive diffusion into and out of the brain.
Cl_{in} = Cl_{out} and decreases from 1 (-··-··-), 0.5 (-·-·-), 0.1 (·····), 0.05 (---) and 0.01 (—)
B: Active, saturable transport into the brain and passive transport out of the brain.
Cl_{out} = 0.5; $T_{m,in}/K_{m,in}$ varies from 100/1 & 10/1 (—), 100/10 & 10/10 (---), 100/100 & 10/100 (·····) and 100/1000 &10/1000 (-·-·-)
C: Active, saturable transport out of the brain and passive transport into the brain.
Cl_{in} = Cl_{out} = 0.1; $T_{m,out}/K_{m,out}$ varies from the top 10/1000 (-·-·-), 10/100 (·····), 10/10 (---) and 10/1 (-·-·-)

investigate the effect of changes in passive and active transport into and out of the brain. Figure 5A shows the simulation of passive diffusion across the BBB (Cl_{in}=Cl_{out}). Decreasing Cl_{in} and Cl_{out}, which simulates increasing hydrophilicity of a drug (or a decrease in BBB permeability), results in a higher t_{max} and longer half-life in the brain. Active, saturable transport into the brain and passive transport out of the brain is depicted in Figure 5B. Cl_{out} is fixed in this simulation and $T_{m,in}/K_{m,in}$ varies (T_m is the maximal transport rate and $K_{m,in}$ is the blood concentration at half-maximal transport). A higher T_m results immediately in higher brain concentrations, whereas a decrease in $K_{m,out}$ compared to blood concentration results in brain concentrations to remain at a constant level for a longer period of time. Active, saturable transport out of the brain and passive transport into the brain is depicted in Figure 5C. Here, Cl_{in} = Cl_{out} and is fixed and $K_{m,out}$ in $T_{m,out}/K_{m,out}$ varies ($K_{m,out}$ is the brain concentration at half-maximal transport). With decreasing $K_{m,out}$, the AUC_{brain}/AUC_{blood} is smaller. These simulations also give insight into altered brain concentrations of drugs due to possible disease-induced changes in BBB transport characteristics.

As already described above, BBB transport is related to BBB functionality and occurs by passive diffusion as well as by active transport. BBB functionality is dynamically controlled by blood components and the surrounding brain cells by direct contact or indirectly by their extracellular products. Thus, BBB functionality may vary among different physiological, pathological, and chronic drug treatment conditions. As BBB transport of a particular CNS drug into and out of the brain is the sum of all actual BBB transport mechanisms applicable to that particular drug, any changes in BBB transport mechanisms may therefore affect actual BBB transport as simulated in Figure 5 and therewith the effects of the drug. This may also apply for neurodegenerative diseases like Parkinson's disease. PK-PD relationships of symptomatic drugs such as L-DOPA in patients with different stages of Parkinson's disease have been described previously (Chan *et al.*, 2005; Harder and Baas, 1998) and indicate that Parkinson's disease progression is able to influence the PK of anti-Parkinsonian drugs in plasma. It has been known for many years that the BBB is less effective during aging. The transport systems involved in the transport of e.g. choline, glucose, lactate or peptides across the BBB have shown to be affected by age (Banks and Kastin, 1985; Mooradian, 1988; Mooradian, 1994). Common age-related changes of the BBB are reduced thickness of endothelial cells, vacuolar inclusions in pericytes and decreased release of amino acids in the cerebral parenchyma (Barcia *et al.*, 2004). It has also long been proposed that neurodegeneration is associated with changes

in the functional and biochemical integrity of the BBB (Mooradian, 1988; Pardridge, 1988a). Evidence has been found in the break-down of the BBB in patients with AD (Algotsson and Winblad, 2007; Bowman *et al.*, 2007; Desai *et al.*, 2007), more specifically in white matter (Tomimoto *et al.*, 1996) and in biopsy tissue from AD patients (Claudio, 1996). Moreover, age-related changes in the morphology of capillaries located in the white matter of dogs are thought to be associated with BBB dysfunction (Morita *et al.*, 2005) as well as the presence of micro-angiogenesis in the Parkinson's diseased brain (Barcia *et al.*, 2004). An increase of vascular endothelial growth factor and increase of blood vessels in the SNc of Parkinson's disease patients have been observed (Barcia *et al.*, 2005) and pathological changes in capillary microanatomy in patients with Parkinson's disease and AD have been shown (Farkas *et al.*, 2000). These studies all suggest that neovasculation is observed in the (Parkinson's) diseased brain, which could result in the disruption of the BBB. Monahan and colleagues propose that Parkinson's disease is, in part, an autoimmune disease as a disrupted BBB could result in the entry of immune cells leading to a progressive degenerative process (Monahan *et al.*, 2008).

Several studies have shown that oxidative stress in the SNc is associated with inflammatory processes such as increase of microglia activation (Hirsch *et al.*, 2005; McGeer and McGeer, 2004a) and increase levels of pro-inflammatory cytokines like tumor necrosis factor (TNF)-alpha, interleukin (IL)-1beta, and IL-6 (Teismann *et al.*, 2003). In meningitis and sepsis, inflammation can disrupt the function of the BBB, and this has also been shown in trauma, stroke, multiple sclerosis and epilepsy (Huber *et al.*, 2001). The activated microglia can stimulate the release of TNF-alpha, and this pro-inflammatory cytokine can disrupt the barrier function *in vitro* and *in vivo* (Lynch *et al.*, 2004; Mark and Miller, 1999; Tsao *et al.*, 2001). Carvey *et al* have suggested that activation of microglia released TNF-alpha leading to the breakdown of BBB (Carvey *et al.*, 2005). They have demonstrated the leakage of fluorescein isothiocynate (FITC)-labeled albumin and horseradish peroxidase in the SNc and striatum of 6-OHDA-lesioned rats and associated it with dopamine neuron loss, activated microglia, presence of neovascularisation and the increase entry of small molecules into the brain. Also, the areas of leakage in the BBB were associated with increased P-gp expression. At the same time, a reduced P-gp function in patients with Parkinson's disease was claimed using PET to measure brain uptake of [^{11}C]-verapamil (Bartels *et al.*, 2008; Kortekaas *et al.*, 2005). However, the elevated uptake of [^{11}C]-verapamil could also be related to an increase in paracellular transport of the compound

instead of a reduced P-gp function as Carvey *et al* demonstrated in the same study that domperidone, a dopamine antagonist which is normally not transported into the brain, was able to inhibit dopamine-mediated behaviour in the 6-OHDA lesioned rats (Carvey *et al.*, 2005). Futhermore, 6-OHDA rat models with L-DOPA induced dyskinesia have demonstrated that dyskinesias in animals were associated with increased entry of L-DOPA into the striatum (Carta *et al.*, 2006; Westin *et al.*, 2006) which was also seen using PET imaging in patients with peak-dose dyskinesias at 1 hour after L-DOPA administration (Fuente-Fernandez *et al.*, 2004). The dyskinetic 6-OHDA rats also exhibited a significant increase in total blood vessel length and a visible extravasation of serum albumin in the SNc (Westin *et al.*, 2006), indicating a role for the BBB in the altered transport of L-DOPA to the brain.

7. Summary and Concluding Remarks

Parkinson's disease is a progressive neurodegerative disease that lacks good treatment especially at later stages. Apart from plasma pharmacokinetics, mechanisms that govern CNS drug distribution and response include the rate and extent of BBB transport and the kinetics of distribution within the brain including the brain target distribution. For the development of new drugs as well as for the optimisation of therapy with the current drugs, the variability of these individual mechanisms and contribution in terms of rate and extent should be investigated. As the BBB is a key player in the relationship between plasma and brain pharmacokinetics, the influences of disease states on BBB functionality in the various stages of the disease is important in order to judge on drug effects. This warrants the application of a systems pharmacology approach in investigations on variability in drug response in Parkinson's disease.

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