Translational pharmacology of dopamine receptor agonists and antagonists : prolactin and oxytocin as biomarkers

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Translational pharmacology of intranasal administration of dopamine receptor agonists and antagonists

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ABSTRACT

Many central nervous system (CNS) diseases related to dysfunction of the dopaminergic system are treated with dopaminergic drugs, but with varying degrees of success. Dopaminergic drugs often suffer from extensive and/or variable gastrointestinal- and hepatic first-pass elimination, as well as limited brain distribution. Therefore, the intranasal administration route is of interest as it may enhance brain target site distribution of these drugs, and may reduce side-effects, therewith improving the therapeutic possibilities of dopaminergic drugs.

The data obtained in investigations on intranasal administration to date, have not provided insight on the rate of brain distribution nor on the route; via the systemic circulation and/or direct from nose to brain. Most importantly, such data lacked information on brain target site pharmacokinetics (PK) that drives the pharmacodynamics (PD). Therefore these data are not suited to aid in quantitative prediction of PK–PD relationships in human.

It is anticipated that quantitative prediction of the time course of CNS drug effects of dopaminergic drugs in human is possible, using detailed PK and PD data following intranasal- as well as systemic administration and advanced mechanistic PK–PD modeling. To that end there is a need for refined animal models and biomarkers to provide insight into brain target site PK in relation to dopaminergic brain activity, to be extrapolated to the human situation using translational pharmacology approaches.

INTRODUCTION

The dopaminergic system is involved in many CNS functions, including behavior and cognition, voluntary movement, motivation and reward, inhibition of prolactin production, sleep, mood, attention, and learning. Many CNS diseases are related to dysfunction of this important system, such as Parkinson's disease, schizophrenia and sexual disorders (Marsden, 2006), and are symptomatically treated with dopaminergic drugs, with varying degrees of success (Kvernmo, et al., 2008). Unfortunately, many dopaminergic drugs often suffer from extensive and/or variable gastrointestinal- and hepatic first-pass elimination (Deleu, et al., 2002), while the blood-brain barrier (BBB) may also limit their brain distribution (El Ela, et al., 2004). This commenced, amongst others initiatives, the search for alternative routes of administration (Mahmood, et al., 1997; Degim, et al., 2003).

The intranasal administration route is of interest as it may increase brain target site pharmacokinetics (PK) of these drugs, and therewith may improve the effects (PD) of the dopaminergic drugs (Illum, 2004; Costantino, et al., 2007; Dhuria, et al., 2009). Intranasal administration is anticipated to rapidly target compounds into; 1) the systemic circulation by the respiratory epithelial pathway, and 2) into the CNS via olfactory epithelial- and olfactory nerve pathways. In a limited number of human studies, the merit of intranasal administration has been based on improvement of drug effects (Kapoor, et al., 1990).

Preclinical investigations to date have studied the potential added value of intranasal administration on the basis of descriptive analysis of concentrations (at best area under the curve (AUC) in brain or cerebrospinal fluid (CSF) compared to plasma (Dhuria, et al., 2009; Vyas, et al., 2005; Van den Berg, et al., 2005)). CSF, however, does not necessarily provide direct information on the target site concentration of dopaminergic drugs (De Lange and Danhof, 2002). The brain extracellular fluid (ECF) PK, is a much better sampling site as it represents most closely the site of action for most dopamine targets such as receptors, reuptake transporters and metabolizing enzymes (De Lange, et al., 2005; Jeffrey and Summerfield, 2010).

To quantitatively predict the time course of CNS drug concentrations and effects in human, insight on the rate of brain distribution and on the route of brain distribution either via the systemic circulation or direct from nose–to–brain is needed, as well as on the brain target site PK (De Lange, et al., 2005; Danhof, et al., 2007; Ploeger, et al., 2009), together with other bio-

markers on the causal chain between dose and effect. Having such information, translational PK–PD modeling approaches can be used for extrapolation of *in vivo* preclinical data to the human situation.

To that end, mechanism-based PK–PD modeling concepts are useful, as this allows explicit distinction between drug-specific- and biological-system (animal, human) specific parameters (Danhof, et al., 2008). Consequently, following intranasal administration studies, mechanism-based PK–PD modeling has the potential to provide quantitative information on the absorption pathways, target site distribution and effect.

This review provides a short overview on the pharmacology of the dopaminergic system and its associated diseases, with subsequent description of the various anatomical and biological aspects of intranasal administration and currently used experimental approaches to investigate this route of administration. This is followed by a disquisition on the prospect of quantitative prediction of the time course of human dopaminergic CNS drug effects using detailed PK and PD data on intranasal as well as systemic administration, combined with advanced mechanistic PK–PD modeling.

Finally, as obtainment of such human data is highly restricted and extremely expensive, it is stated that important information should be derived from *in vivo* animal studies and translational pharmacology approaches. This indicates the need for refined animal models and biomarkers to gain insight into brain target site PK in relation to dopaminergic brain activity. It is concluded that preclinical mechanistic PK–PD models can provide the scientific basis for the translational pharmacology of dopaminergic drugs after intravenous and intranasal administration.

THE DOPAMINERGIC SYSTEM

Dopamine is the predominant catecholamine neurotransmitter in the mammalian brain, where it controls a variety of CNS functions including locomotor activity, cognition, emotion, positive reinforcement, food intake, and endocrine regulation. It also plays multiple roles in the periphery as a modulator of cardiovascular function, catecholamine release, hormone secretion, vascular tone, renal function, and gastrointestinal motility (Kandel, et al., 2000). The dopaminergic brain systems have been the focus of much research over the past 30 years, mainly because important pathological conditions have been linked to a dysregulation of dopaminergic transmission, such as Parkinson's disease, schizophrenia, sexual disorders, Tourette's syndrome, and hyperprolactinemia (Marsden, 2006).

■ Dopamine synthesis, release and metabolism

Dopamine is biosynthesized in the cell bodies of dopaminergic neurons in the brain. It is stored in neuronal vesicles and released into the synaptic cleft in response to presynaptic action potentials. In this extracellular space, dopamine may bind and activate dopamine receptors, which can be present at post- and presynaptic membranes (Carlson, 2001). The effect of dopamine is ended upon reuptake by reuptake-transporters (Chen and Reith, 2000), extracellular metabolism by catechol-O-methyl transferase (Bilder, et al., 2004) and/or outer mitochrondrial membrane associated monoamine oxidase B (Ben Jonathan and Hnasko, 2001) (Figure 1).

Figure 1 Synthesis and metabolism of dopamine (DA) and site of action of drugs that affect dopamine transmission (numbered stars). Drugs can affect the synthesis (1), storage (2) and release (3) of DA. Many drugs act on the DA receptors (DAR) in the synaptic cleft (4), while some interfere with DA reuptake (5) by DA transporter proteins (DAT) or on the metabolic pathways of catechol-O-methyl transferase (COMT, 6) or mitochondrial monoamine oxidase B (MAO-B, 7). 3M =, 3-methoxytyramine; HVA =, homovanillic acid; DOPAC = 3,4-dihydroxy-phenylacetic acid.
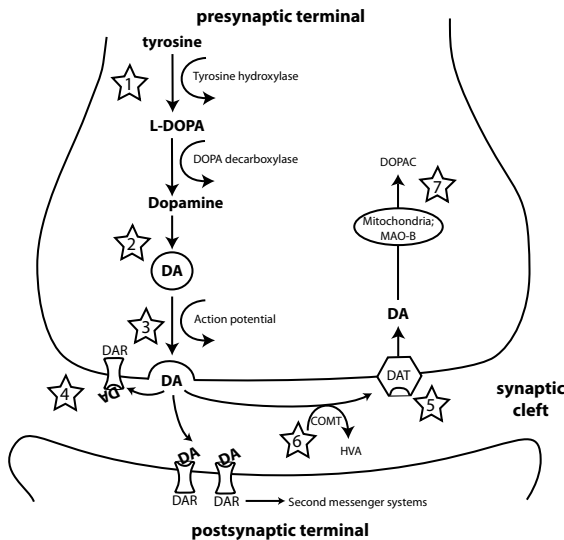

■ Dopaminergic pathways

Dopamine containing neurons are mainly located in the substantia nigra pars compacta, the ventral tegmental area and the hypothalamus, and pro-

ject their axons to large areas in the brain (Kandel, et al., 2000; Freeman, et al., 2000; Ben Jonathan and Hnasko, 2001; Ben Jonathan, et al., 2008). Dopamine is transmitted via three major pathways (Figure 2):

- the first, the nigrostriatal system, extends from the substantia nigra to the caudate nucleus-putamen (neostriatum) and is concerned with sensory stimuli and movement
- the second, the mesolimbocortical system, projects from the ventral tegmentum to the mesolimbic forebrain and is thought to be associated with cognitive, reward and emotional behavior
- and the third, the tuberoinfundibular system, is concerned with neuronal control of the hypothalamic-pituitary endocrine system.

Figure 2 Major dopaminergic pathways in the brain (redrawn from CNS-Forum educational resources http://www.cnsforum.com/educationalresources/imagebank/).
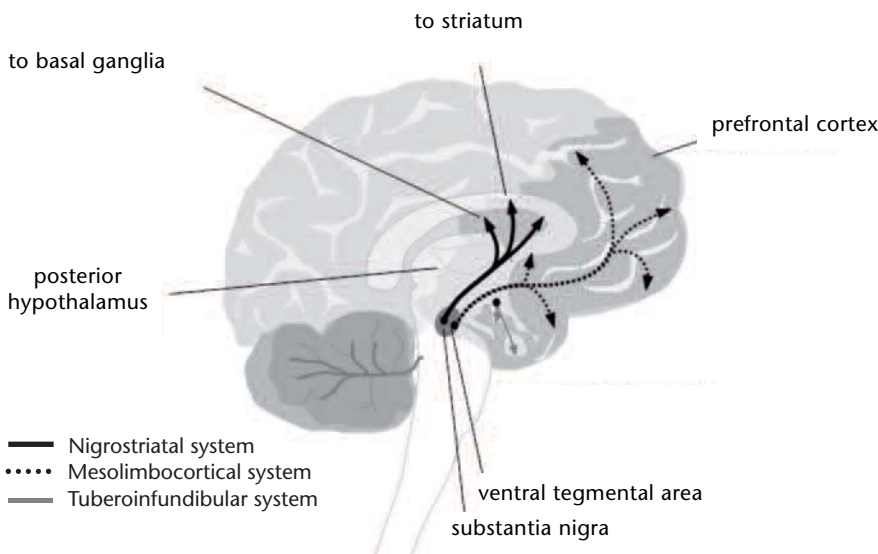
■ **Dopaminergic drugs**

Dopaminergic activity in the brain can be modulated by drugs via different targets (Figure 1).

- **Dopamine synthesis**
  Several drugs of clinical importance act indirectly on the synthesis of dopamine e.g. L-DOPA, which is converted into dopamine (Deleu, et al., 2002). Compounds like for example alpha-methyl-para-tyrosine can act directly on

dopamine synthesis by inhibiting the hydroxylation of tyrosine to dopamine (Ankenman and Salvatore, 2007).

- **Dopamine vesicles**
  Monoamine oxidase inhibitors like e.g. selegiline (Caslake, et al., 2009) may decrease intracellular metabolism and potentially increase vesicular dopamine.

- **Dopamine release**
  Other compounds influence the amount of dopamine reaching the synaptic cleft. For example reserpine, which depletes dopamine vesicles (Peter, et al., 1995), or amphetamine, which releases dopamine from terminal stores (Goodwin, et al., 2009).

- **Dopamine transmission**
  Many drugs affect dopamine transmission directly by either blocking or stimulating dopamine receptors in the synaptic cleft. Dopamine antagonists include antipsychotic drugs, whereas e.g. bromocriptine is a dopamine agonist, used to treat hyperprolactinemia and Parkinson's disease (Kvernmo, et al., 2006).

- **Dopamine elimination from synaptic cleft**
  Several drugs increase the synaptic concentration of dopamine by blocking the re-uptake or metabolism of dopamine. As an example, cocaine is a potent inhibitor of the dopamine re-uptake transporter (Schmitt and Reith, 2010), and OR-611 is a catechol-O-methyl transferase inhibitor (Kaakkola and Wurtman, 1992).

■ **Pharmacology of the dopamine receptors**

All dopamine receptor subtypes are expressed in the brain in distinct but overlapping areas (Defagot, et al., 1997; Missale, et al., 1998; Khan, et al., 2000; Marsden, 2006).

☐ **Receptor types and brain distributions**
There are five subtypes of dopamine receptors and these D1–D5 subtypes are widely distributed throughout both the cerebral cortex and the limbic system of the brain. Certain sub-types are also found in other specific areas of the brain, for example the D1- and D2-receptors are expressed in the striatum (Figure 3).

☐ **Receptor subfamilies**
The dopamine receptor subtypes can be divided into two families, the D1-like and D2-like receptors. The D1-like receptors comprise D1- and D5-receptor subtypes that are associated with *stimulation* of adenylate cyclase. The D2-like receptors comprise D2-, D3- and D4-receptor subtypes and these are associated with *inhibition* of adenylate cyclase.

Figure 3 Brain distribution of dopamine receptor subtypes.
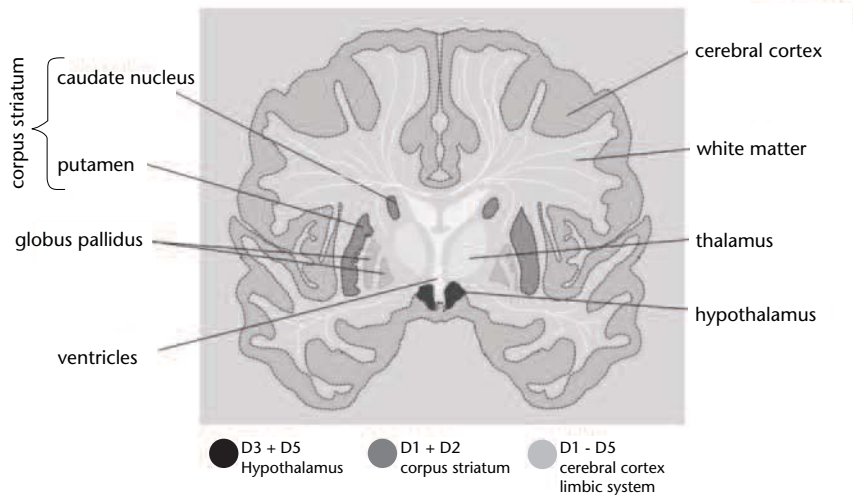
■ Dopaminergic dysfunctions and associated therapies

Dysfunctions of the dopaminergic system can result in disease states like e.g. Parkinson's disease, schizophrenia and sexual disorders. Other neurotransmitter systems may be involved as well, but are not further discussed here.

• Parkinson's Disease
Very low levels of dopamine in the motor areas of the brain that result from neural cell death in the substantia nigra pars compacta, and often also the formation of Lewy Bodies are known to produce Parkinson's Disease (Dauer and Przedborski, 2003). Symptoms include muscle rigidity and stiffness, stooped/unstable posture, loss of balance and coordination, gait (walking pattern) disturbance, slow movements and difficulty with voluntary movements, tremors and shaking, mask-like facial expression, and impairment in cognitive/intellectual ability.

Although research is directed toward prevention of dopamine neuron degeneration, all current therapies focus on (symptomatic) replacement of dopamine treatment with its precursor L-DOPA. This is combined with a peripheral decarboxylase inhibitor (like benserazide or carbidopa) as to prevent dopamine formation in the periphery, and a COMT inhibitor (like tolcapone or entacapone) to prolong the effect of L-DOPA.
In addition, elimination of dopamine is slowed down by MAO-B inhibitors (like rasagiline and selegiline).

However, unwanted effects of chronic L-DOPA administration are associated with end-of-dose deterioration of function, on/off oscillations, freezing during movement, and dyscoordination of movement (dyskinesias) at peak dose, accompanied by depression anxiety, pain, panic, and delusions.

Although L-DOPA is still accepted as "gold standard" in Parkinson's Disease treatment, D1- and D2-receptor stimulation by dopamine-agonists may improve therapeutic response (Deleu, et al., 2002; Kvernmo, et al, 2008). Dopamine agonists include ropinirole, cabergoline, bromocriptine, pergolide, pramipexole, rotigotine, and apomorphine.
In general, sole dopamine agonists treatment is less effective than L-DOPA treatment. However, dopamine agonists do not depend on neuronal uptake and release and have in general a longer duration of action. This reduces the risk of the development of dyskinesias (Savitt, et al., 2006). It has been suggested that several ergot derived dopamine agonists (pramipexole, ropinirole) reduce neuronal cell death, thereby slowing down disease progression relative to L-DOPA (Yamamoto and Schapira, 2008; Kvernmo, et al., 2008). It is however a major challenge to demonstrate a disease modifying effect in clinical trials (Ploeger and Holford, 2009).

• **Schizophrenia**
Although direct evidence is lacking, it seems that when dopamine levels increase in the thinking areas of the brain (forebrain, hindbrain and limbic system), hallucinations start to occur in hearing, sensing, tasting and smell. At the more extreme, this results in schizophrenia, characterized by the loss of contact with reality by hallucinations, delusions, disordered thinking, unusual speech or behavior, and social dysfunction. The symptoms of schizophrenia are classified in categories as positive (delusions, hallucinations, thought disorder), negative (flat effect, poverty of thought, amotivation, social withdrawal), cognitive (distractibility, impaired working memory, impaired executive function), and mood (mania, depression) (Eon and Durnhaml, 2009).

Pharmacological studies indicate that the potency of antipsychotic drugs are strongly correlated to the ability to block D2-receptors (Kapur, et al., 2000; Seeman, 2010). Antagonizing D1-like receptors do not to lead to antipsychotic activity, but rather to aggravation of symptoms. In contrast, evidence is accumulating that administration of low doses of D1-receptor agonists can alleviate symptoms. The role of antagonizing or partially agonizing D3-receptors remains unclear (Miyamoto, et al., 2005).
In the treatment of schizophrenia, antipsychotic drugs are often very effective in treating certain symptoms of schizophrenia, particularly hallucina-

tions and delusions. Unfortunately, the drugs may not be as helpful with other symptoms, such as reduced motivation and emotional expressiveness. Especially, the older antipsychotics ("neuroleptics"), like haloperidol and chlorpromazine, may produce short-term side effects including drowsiness, restlessness, muscle spasms, dry mouth, tremor, or blurred vision, while one important long-term side effect is tardive dyskinesia. In such cases, a lower dose may help, or the use of newer antipsychotic drugs like olanzapine, quetiapine, and risperidone, which appear less likely to have this problem. Some-times when schizophrenic patients become depressed, other symptoms can appear to worsen. The severity of the symptoms may be reduced by the addition of an antidepressant drug.

- **Sexual dysfunction**
  Sexual dysfunction is often referred to as either disturbances in sexual desire or functioning. In the brain, sexual function is under control of inhibitory and stimulatory processes. For the treatment of erectile dysfunction, the use of the D1/D2-dopamine receptor agonist apomorphine provides a strong support in favor of a participation of the dopaminergic system in the control of sexual function. However, the exact involvement of dopamine in sexual motivation and in the control of genital arousal in humans is unknown (Giuliano and Allard, 2001; Kruger, et al., 2005). Pfaus (2009) reported on studies implicating D1-receptors being involved, while Baskerville and Douglas (2008) reported that merely D2-receptors mediate sexual behavior. This leaves the need for more investigations on specific dopamine receptor interactions in hypothalamic brain areas implicated in sexual behavior, being the medial preoptic area and periventricular nucleus.

■ **Problems and future perspectives in dopaminergic therapies**

Treatment of disorders associated with dopamine imbalance involves drug treatment in order to increase or decrease dopamine levels in the brain. Dopamine receptor antagonists have been developed for too high dopamine concentrations, to block hallucinations and delusions that occur in schizophrenic patients, whereas dopamine receptor agonists are effective in alleviating the hypokinesia of Parkinson's disease in which dopamine action is too low. However, blockade of dopamine receptors can induce effects similar to those resulting from dopamine depletion in Parkinson's disease, and high doses of dopamine agonists can cause psychoses. Also, the occurrence of sexual dysfunctions is high in Parkinson's disease patients, but dopamine replacement therapy can cause hyper sexuality and aberrant sexual behavior (Meco, et al., 2008). The therapies of disorders resulting from dopamine

imbalances are thus associated with severe side effects. Newer therapies are more specifically targeted to one of the dopamine receptor types, or focus on partial agonist in which in the absence of a full agonist the partial agonists will help to increase the response, while in the presence of a full agonist the partial agonist will reduce the response (Lieberman, 2004; Ohlsen and Pilowsky, 2005).

Another issue is the problem associated with target site distribution of the dopaminergic drugs, that drive their effects. Between dosing and target site distribution, a lot of factors are involved that may contribute significantly to intra-individual variability and therefore inadequate/unpredictable outcome. Thus, many dopaminergic drugs often suffer from extensive and/or variable gastrointestinal- and hepatic first-pass elimination (e.g. apomorphine, chlor-promazine, bromocryptine, pergolide, lisuride, and risperidone)
(Contin, et al., 2000; Deleu, et al., 2002; Blin, et al., 2003; Del Dotto and Bonuccelli 2003; Nyholm, et al., 2006; Menon and Stacy, 2007), while for a number of dopaminergic drugs (e.g. pramipexole, amisulpiride, fluphena-zine, olanzapine, and risperidone) active transport mechanisms are involved in BBB transport (El Ela et al., 2004; Wang, et al., 2004a, Wang, et al., 2004b; Zhu, et al., 2006; Okura, et al., 2007). This initiated, amongst others, the search for alternative routes of administration (Mahmood, et al., 1997; Contin, et al., 2000; Degim, et al., 2003). One of the options is intranasal administration.

INTRANASAL ADMINISTRATION

Intranasal administration has gained special interest as a strategy to circum-vent unfavorable absorption and/or extensive first-pass metabolism charac-teristics of orally administered compounds, as well as a potential means to circumvent the blood-brain barrier (BBB) when this barrier restricts brain distribution of a compound (Dhuria, et al., 2009). As the nasal cavity is co-vered by a thin mucosa and a well vascularised epithelium, a drug molecule can quickly be transferred across the single epithelial cell layer directly into the systemic blood circulation. On the other hand, direct nose–to–brain transport may result in brain distribution enhancement (Illum, 2004; Costantino, et al., 2007; Dhuria, et al., 2009; Pires, et al., 2009).

Thus, intranasal administration may offer a more rapid onset of action and an increased therapeutic window due to targeted drug delivery for com-pounds having a high clearance, low oral bioavailability and/or low BBB transport characteristics. Intranasal administration can therefore be used as an alternative to oral administration if a fast effect is desired or if the drug

is extensively degraded in the gut or liver. To identify its potential to circumvent the BBB, the different nasal transportation routes need to be considered.

■ **Nasal transport**
Part of the nasal cavity is covered by respiratory epithelium, across which systemic drug absorption can be achieved. The olfactory epithelium is located in the upper posterior part of the nasal cavity (Gross, et al., 1982). The nerve cells of the olfactory epithelium project into the olfactory bulb of the brain, which provides a direct connection between the brain and the external environment. Two major routes of transport can be distinguished in direct nose–to–brain transport (Figure 4). The respiratory and the olfactory epithelial pathway (Illum, 2000; Dhuria, et al., 2009).

Figure 4 Schematic presentation of direct nose-to-brain (dotted square) and systemic absorption and elimination routes following intranasal administration. CNS, central nervous system; BBB, blood brain barrier; GI-tract, gastrointestinal tract. Adjusted from Illum 2000.
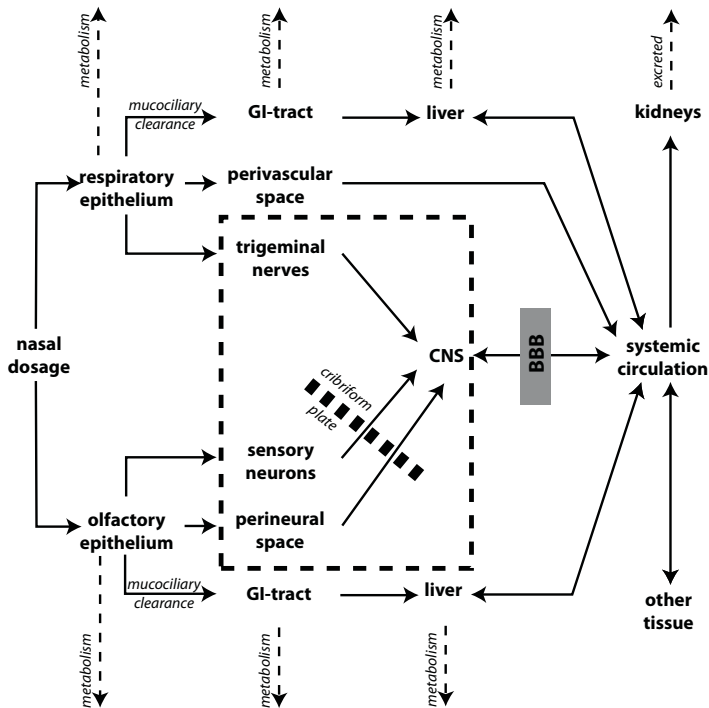
- **Respiratory epithelial pathway**
  Following absorption over the respiratory epithelium, compounds diffuse into trigeminal nerves which may allow compounds to further enter the brain.

- **Olfactory epithelial pathway**
  Compounds that enter the brain by the olfactory epithelial pathway may diffuse into the perineural spaces that cross the cribriform plate, ending up in the cerebrospinal fluid of the subarachnoid space, and may further diffuse throughout the brain. The olfactory epithelial pathway also allows intracellular transport through the sensory neurons, into the olfactory bulb. On the cell surface of sensory neurons, transporter proteins (glycoproteins) are present allowing compounds to bind and to be absorbed via endocytosis. After intracellular transport, the compound is exocytosed in the olfactory bulb.

- ◼ **Intranasal administration of dopaminergic compounds**

  For quite a number of dopaminergic drugs the intranasal route has been investigated. A few examples are shortly described below.

- **Dopamine**
  Following intranasal administration of dopamine, higher plasma bioavailability was found compared to dermal, buccal, or rectal administration in beagle dogs (Ikeda, et al., 1992). For radiolabeled dopamine, direct transport of radioactivity from nose into brain was indicated in the olfactory tract and -bulb, while also radioactivity was found to be increased in the striatum, indicating distribution into deeper brain areas (Dahlin, et al., 2000). Interestingly, dopamine uptake transporters have been identified in rat- and bovine respiratory- and olfactory epithelia, indicating potential active uptake of dopamine (Chemuturi, et al., 2006).

- **L-DOPA**
  Being the precursor for dopamine, intranasal administration of L-DOPA might directly increase dopamine levels in the brain following intrabrain decarboxylation. De Souza Silva and colleagues found an increase in the extracellular dopamine concentrations in the neostriatum of the brain upon intranasal L-DOPA administration to anaesthetized rats, as determined by microdialysis (Souza Silva, et al., 1997). Recently, Kim and colleagues (Kim, et al., 2009) investigated intranasal dosing of L-DOPA together with carbidopa, compared to oral administration of these drugs. Plasma and brain data, analyzed by compartmental modeling, indicated that L-DOPA was rapidly transported into the brain, and nasal administration of the drugs resul-

ted in ~3-fold higher bioavailability than oral administration. Intranasal administration of L-DOPA and carbidopa was suggested as a good rescue therapy for Parkinson's disease patients who experience symptom fluctuation with oral L-DOPA administration.

- **Apomorphine**
  Several clinical results have been published on apomorphine following intranasal administration. Improved pharmacodynamics were found compared to subcutaneous administration in Parkinson patients (Kapoor, et al., 1990). In more longitudinal studies, however, nasal blockage, burning sensation and nasal vestibulitis were indicated (Kleedorfer, et al., 1991; Van Laar, et al., 1992). Several preclinical formulations have been tested to overcome these issues, and bioavailability could be increased up to ~70% (Ugwoke, et al., 1999). For the treatment of sexual dysfunctions, several clinical intranasal apomorphine formulations were found to result in a rapid time to maximal plasma concentration while CSF-plasma ratios increased significantly (from 5% to approximately 35%), compared to subcutaneous administration (Kendirci and Hellstrom, 2004). This was accompanied by an improvement of the effect (sexual activity) in the majority of the cases (Kendirci and Hellstrom, 2004).

  These studies indicate that intranasal administration may indeed be a useful route of administration, although also limitations should be considered.
  - Nasal administration is primarily suitable for potent drugs since only a limited volume can be administered into the nasal cavity (Dhuria, et al., 2009; Stevens, et al. 2009).
  - For continuous and frequent administration of drugs and vehicles, the risk of harmful long term effects on the nasal epithelium exists (like for apomorphine, as described previously).
  - For nasal administration, a high variability in the amount of drug absorbed may be encountered. Variability may result from variation of the method of nasal administration, e.g. position of the tip upon spraying, therefore exposing nasal tissues differentially, as well as by upper airway infections, irritation of the nasal mucosa, and by partial swallowing of the sprayed drug solution. As a note, such variability should always be put into perspective, and compared to that following oral (or other routes of) administration (Coda, et al., 2003; Studd et al., 1999; Kublik, et al., 1998).
  - Furthermore, following intranasal administration, compounds can be subjected to extensive metabolism in the nasal mucosa and tissue by several enzymes (e.g. cytochrome P450 isoenzymes, amino peptidases and carboxyl esterases (Minn, et al., 2005; Zhang et al., 2009; Benedetti, et al., 2009).

• Finally, also several transporter proteins have been identified, which are responsible for active efflux of compounds, for example P-glycoprotein, and organic anion/multidrug resistance associated proteins (Jones, 2001; Graff and Pollack, 2003).

■   **Considerations in preclinical studies on intranasal administration**

There are several issues that need to be considered in obtaining preclinical data following intranasal administration studies.

• **Impact of anesthesia and/or restrain stress**
Many preclinical PK–PD studies on intranasal administration so far have been performed on anaesthetized animals, whether or not in combination with complete isolation of the nasal cavity and cannulated trachea to aid breathing. Alternatively, restrained animals are used. Such experimental conditions have major influence on physiological parameters, like blood flow, mucocilliary clearance, nasal cycle, and airflow dynamics (Van den Berg, et al., 2002; Ross, et al., 2004; Thorne, et al., 2008). These are all factors that may influence the rate and/or extent of absorption and widens the gap between animal and human conditions. Altogether, this indicates the need for more refined, minimum-stress animal models for intranasal administration (Dhuria, et al., 2009; Stevens, et al., 2009).

• **Sampling techniques**
To have information on brain distribution of nasally administered drugs, the use of brain tissue allows only one time-point per animal, and thus large numbers of animals are required. Puncturing the cisterna magna induces a high risk of blood contamination, while also puts a limit to the number of samples that can be obtained (Van den Berg, et al., 2002). The use of a CSF cannula has provided a major improvement as it allows serial CSF-sampling and decreases the risk of blood contaminations (Van Bree, et al., 1989). However, still, by serial CSF sampling, the potential effects of CSF-removal on brain fluid pressure and brain physiology remain.

• **Parameters used for comparison of data on brain distribution**
For quantification of brain distribution following intranasal versus other routes of administration, often the ratio of AUC in CSF over AUC in plasma is used and compared to the ratio following other routes of administration (Van den Berg, et al., 2005). Such AUC ratios identify differences in total brain exposure (extent) after intranasal administration, rather than provide the quantitative distinction between brain distribution via the systemic circulation and direct nose–to–brain transport, in terms of absorption rate

and bioavailability. For that reason, such descriptive pharmacokinetic models are of limited value for translational purposes, especially for animal to human extrapolation.

TRANSLATIONAL MECHANISM-BASED PK–PD MODELING

Mechanism-based PK–PD models are based on principles from systems pharmacology and contain specific expressions to characterize processes on the causal path between drug exposure and drug response. An important feature of mechanism-based PK–PD models is the strict distinction between drug-specific and biological system-specific parameters to describe *in vivo* drug effects. In this regard PK–PD modeling has developed from an empirical and descriptive approach into a mechanistic science recognizing the (patho)-physiological mechanisms which are underlying PK–PD relationships.

With time, it has become clear that mechanism-based PK–PD models have indeed much improved properties for extrapolation and prediction (Danhof, et al., 2008). To that end, a mechanism-based approach should include specific expressions to describe target site distribution, target binding and activation, and the transduction, including the homeostatic control mechanisms (Danhof, et al., 2007; Ploeger, et al., 2009; Gabrielsson and Green, 2009). The use of more detailed biological system information will improve the accuracy in PK–PD relationships in humans (Danhof, et al., 2008). As in most cases direct measurement of the anticipated effect is difficult or impossible, the use of biomarkers of the effects may provide quantitative information on the causal path between drug PK and effect.

■   **Biomarker classification and application**

Biomarkers can be classified according to the biomarker classification scheme (Danhof, et al., 2005). In short:

• **Type 0 biomarkers** refer to the genotype- or phenotype as determinant of the drug response, that influences target site exposure or response due to variation in the expression of e.g. enzymes or receptors. They are commonly used as covariates in PK–PD models.

• **Type 1 biomarkers** refer to drug concentrations in general and at the target site in particular. As previously pointed out, quantitative biomarkers that represent the target site distribution of drugs and metabolites for compounds that act in the CNS are difficult to obtain in man, but readily available *in vivo* in animal (De Lange, et al., 2005).

- **Type 2 biomarkers** refer to the degree of target occupancy. In theory, effects may occur at different degrees of target occupancy and may be species dependent. The relationship between target occupancy and effect is therefore important for the understanding of inter- and intra-individual variability. Information on target occupancy is available by bioassays *in vitro* and can also be non-invasively measured in humans by positron emission tomography (Kapur, et al., 2000).

- **Type 3 biomarkers** refer to quantification of the target site activation. By means of *in vitro* bioassays information can be obtained on receptor activation in animal and man. Techniques like electroencephalograms (Kropf and Kuschinsky, 1993; Vorobyov, et al., 2003) and functional-magnetic resonance imaging can obtain specific receptor activation in a clinical *in vivo* setting.

- **Type 4 biomarkers** refer to physiological measures in the integral biological system, which are often controlled by homeostatic feedback mechanisms. Since dopamine is an important neurotransmitter in hypothalamic control, pituitary hormones are have high potential as (translational) type 4 biomarkers for dopaminergic activity in the brain (Freeman et al., 2000).

- **Type 5 biomarkers** characterize disease processes and are particularly use ful in clinical settings. An important question is whether type 5 biomarkers can be identified in animal models of disease.

- **Type 6 biomarkers** refer to clinical endpoints and lie outside the scope of this review.

Obtaining information concerning multiple types of biomarkers allows better quantification of the causal chain of events, and increases the accuracy of the model. It is however, not always necessary to obtain information on each step/type of biomarker, as the parsimony of the biological system can be applied in mechanism-based PK–PD modeling. Still, to obtain enough information from the clinical setting is extremely costly, and for clear reasons limited. This makes that we have to make use of *in vivo* animal studies and apply translational pharmacology approaches. To that end, quantitative preclinical PK data should be obtained in refined animal models and, for biomarkers of the effect, emphasis should be on the ones that can be measured in both animal and human.

■ **Drug concentrations at the target site**

As the aim is for the drug to reach its target site to be able to interact with its target, it is important to have information on target site concentrations. CSF concentrations do not provide direct information on the target site concentration of dopaminergic drugs, as dopamine receptors are facing the ECF of the brain parenchyma. Indeed many factors contribute to the distribution between CSF and the target site concentrations, indicating that CSF concentrations do not necessarily reflect brain target site concentrations (De Lange and Danhof, 2002). In many cases, brain targets are membrane receptors facing the brain ECF, or enzymes within the brain ECF. This makes information on brain ECF concentrations highly valuable (De Lange and Danhof, 2002; Watson, et al., 2009). For intracellular targets, things get more complicated. There are no means to directly obtain brain intracellular concentration-time profiles. Moreover, the intracellular space is highly heterogeneous. Total brain concentrations may provide some information, but without temporal resolution. Information on brain cell exposure by brain ECF might still be of value.

Altogether, measurement of brain ECF pharmacokinetics is anticipated to provide a better basis to describe PK–PD relations (Jeffrey and Summerfield, 2010). To this end, the intracerebral microdialysis technique is of special interest, as it offers the advantage of multiple samples collected over time from the brain ECF, without removal of fluid, therefore maintaining normal fluid pressure and physiological conditions (De Lange, et al., 1994; De Lange, et al., 1999; Chaurasia, et al., 2007). It should however be mentioned that proper quantification of microdialysate concentrations relative to brain ECF concentrations is important. The microdialysis technique has been applied to date in a few studies following intranasal administration (Souza Silva, et al., 1997; Bagger and Bechgaard, 2004; Shi, et al., 2005; Yang, et al., 2005; Souza Silva, et al., 2008).

■ **Translational biomarkers on the activity of the dopaminergic system**

Dopamine is an important neurotransmitter in hypothalamic control of the pituitary. Therefore, pituitary hormones have a high potential as biomarker for dopaminergic activity in the brain. Of special interest are prolactin and oxytocin. These hormones are released in plasma, and blood sampling may provide information on the functioning of the dopaminergic system.
As blood sampling can be performed in man and in animals, prolactin and oxytocin are of interest as translational biomarkers in investigations on (intranasal) administration of dopaminergic compounds.

- **Prolactin**

  Prolactin is mainly associated with reproductive and metabolic functions. It is synthesized and stored in lactotrophs located in the anterior lobe of the pituitary. The release of prolactin is predominantly under hypothalamic inhibitory control by dopaminergic neurons.

  Dopaminergic neurons project dopamine into the anterior lobe of the pituitary via several pathways (Figure 5). Activation of dopamine D2-receptors on the cell surface of lactotrophs inhibits the release of prolactin into plasma. Likewise, blockade of D2-receptors leads to release of prolactin. Besides the dopaminergic control on prolactin release, prolactin concentrations in plasma are also influenced by changes in synthesis rate, lactotroph storage capacity, homeostatic feedback mechanisms and rate of plasma elimination (Freeman, et al., 2000; Ben Jonathan and Hnasko, 2001; Fitzgerald and Dinan, 2008).

Figure 5 Neurons in the periventricular nucleus (PVN) and arcuate nucleus (AN) of the hypothalamus influence the release of prolactin (+) and oxytocin (o) by the pituitary. Prolactin release into peripheral veins by lactotrophs (L) is under inhibitory control of tuberoinfundibular (TIDA), periventricular hypothalamic (PHDA) and tuberohypothalamic (THDA) dopamine pathways. Oxytocin is released by terminals of magnocellular neurons (MC) into short portal vessels that transport oxytocin to the peripheral vein (adapted from Freeman, et al., 2000).
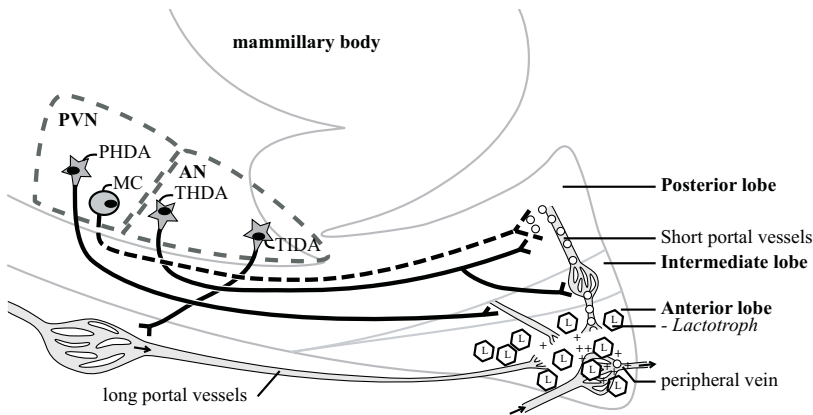
Interestingly, for prolactin, the synthesis, pathways of release and homeostatic feedback, and elimination half-life are similar in rats when compared to man. This makes prolactin concentrations in plasma an interesting candidate for evaluation as a translational biomarker for D2-receptor activity (Ben Jonathan, et al., 2008), in particular for dopamine receptor antagonists and possibly also partial agonists.

- **Oxytocin**
  The synthesis and release of oxytocin into plasma is mainly associated with birth, lactation, parenting, sexual behavior and stress management. Oxytocin is synthesized and stored in magnocellular neurons located in the periventricular- and supraoptic nucleus of the hypothalamus. Activation of dopamine D1- but primarily dopamine D2-receptors present on the cell surface of these neurons, induces oxytocin release from the terminals located in the pituitary. Also, dopamine release from the tuberohypothalamic axons in the posterior lobe of the pituitary activate dopamine receptors on the magnocellular neurons, leading to the release of oxytocin in plasma. This makes also oxytocin an attractive biomarker for dopamine D1/D2-receptor activation. A limitation however is that the physiology of oxytocin release is much less understood than that of prolactin. Although baseline oxytocin concentrations appear to be similar in rat and man (Uckert, et al., 2003; Kramer, et al., 2004), comparative information on oxytocin synthesis, pathways of release, and feedback mechanisms in animal and man is currently lacking.

■ Clinical PK–PD models for dopaminergic action and prolactin response

In general, the physiology of prolactin release and the discrepancies in the biological systems between animal and man (Ben Jonathan, et al., 2008) are better described for prolactin compared to oxytocin.

Several clinical PK–PD models have been published on the relationships between dopaminergic drug concentrations in plasma and prolactin concentrations in plasma, as a biomarker for dopaminergic activity, following intravenous administration. The corresponding feature of these models is a precursor pool model describing prolactin synthesis, storage in lactotrophs, release into- and elimination from plasma (Movin-Osswald and Hammarlund-Udenaes, 1995). In this first publication, a clinical PK–PD model described the drug effects of two consecutive intravenous doses of the dopamine D2/D3-antagonist remoxipride on the prolactin release (Movin-Osswald and Hammarlund-Udenaes, 1995). Since this model was less able to describe the data of subsequent clinical studies, another PK–PD model (Bagli, et al., 1999) integrated a physiological indirect-response PD/tolerance model, including putative concentrations of the agonist dopamine, following administration of the D1/D2/D3-dopamine receptor antagonist chlorprothixene. However, actual dopamine concentrations could not be obtained, hence limiting quantitation of biological system-specific parameters. More recently, circadian rhythmicity in prolactin release has also been included in the model (Friberg, et al., 2008; Ma, et al., 2010). From these studies it is clear that pro-

lactin release is highly regulated in the brain, and that some feedback model is required to accurately describe prolactin concentrations in plasma. However, such a feedback model remains to be optimized in a quantitative, mechanistic manner. Also, these models interconnect plasma drug concentrations to prolactinergic effects. As previously mentioned, a more mechanistic approach should be aimed at understanding the drug concentrations at the target site (brain ECF).

In animals, obtaining *in vivo* information on (part of) the seven biomarker types over time is more straightforward than in humans. Animal studies also provide the opportunity to investigate broader dosing regiments and different administration routes. Consequently, preclinical mechanism-based PK–PD models have high accuracy in quantitatively describing the drug specific- and the biological system specific characteristics that lie at the basis of drug–effect relationships and biological system responses. Challenging the model with other dopaminergic compounds provides an even stronger basis for the estimation of the biological system parameters, and should be pursued in order to validate the biological system in a proposed model structure. Figure 6 provides an example of such a mechanism-based PK–PD approach for intravenous and intranasal administration of dopamine receptor antagonists. The pharmacokinetics of the compound are described by a three compartmental approach; i) a central (plasma) compartment that describes the plasma drug concentrations over time, ii) a brain compartment, that represents the brain ECF concentrations over time, and iii) a peripheral compartment, describing the concentration-time profiles of compound in peripheral tissues. Modeling of obtained plasma- and brain ECF compound concentrations following intravenous administration provides an accurate description of the PK at the target site. When comparing intravenous and intranasal administration, pharmacokinetic modeling of brain ECF concentrations provides another unique feature. The predictive properties of mechanism-based PK–PD models that either include or exclude direct nose–to–brain transport can be compared. This would provide, for the first time, quantitative information on different intranasal absorption routes.

As the target site (brain ECF) PK is accurately described, this will also positively affect the accuracy of pharmacodynamic parameter estimates. In figure 6, the biological system of prolactin secretion is depicted as a two compartmental pool model (Movin-Osswald and Hammarlund-Udenaes, 1995). Modeling of target site PK of the compound (type 1 biomarker) and plasma concentrations of prolactin (type 4 biomarker) provides quantitative information on drug–effect relations, in which information on type 2 and 3 biomarkers can be included. Also, based on empirical approaches, direct

homeostatic feedback mechanisms that act on the prolactin secretion, can be separately and quantitatively investigated. The drug-specific- and system-specific parameters thus acquired can be used in a translational approach to predict the effect in humans.

Figure 6 An example of a structural PK-PD model (solid lines) and hypotheses that can be tested (dotted lines) in a mechanism-based PK-PD approach. Information on dose and route of absorption is provided following intravenous-(IV) or intranasal (by systemic- or direct nose-to-brain transport) administration of compound. Three compartments describe the pharmaco-kinetics, and the pharmacodynamics are described by synthesis (k_S), storage (lactotroph) and secretion (k_r) of prolactin into plasma (effect), and plasma elimination (k_{el}). Brain ECF concentrations (brain) induce a drug-effect via the k_r. Homeostatic biological systems effects can be investigated by feedback of prolactin plasma concentrations on k_S, storage, or k_r. k_{30} represents the elimination rate constant.
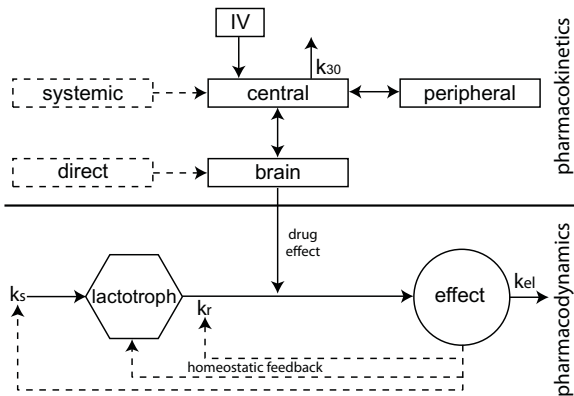
■ **Link between animal and human**

Quantification of drug specific- and biological system specific parameters in mechanism-based PK–PD models provides the opportunity to scale the animal PK–PD model to man. Allometric scaling of drug PK and biological system specific parameters has been used in translational investigations, with reasonable degree of success, to predict drug effects in humans (Yassen, et al., 2007; Zuideveld, et al., 2007).

Pharmacodynamic properties are more difficult to scale compared to PK properties, since parameters that establish an effect are often not related to bodyweight (e.g. receptor occupancy, transduction, maximal effect, etc.).

However, this information can be obtained by *in vitro* bioassays, and, for many drugs and endogenous compounds, clinical information is readily available in literature (e.g. target binding characteristics of dopaminergic compounds (Kvernmo, et al., 2006)). This provides the opportunity to replace the drug- and biological system parameters estimated in rat by the human values, and thus provide an extrapolation step in man. Subsequent simulation studies can provide early insight on the clinical applicability of a drug, at an early stage in drug development. Also, as the preclinical derived translational mechanism-based PK–PD model describes the drug–effect relationship and the biological system, clinical studies suffice with fewer individuals and less samples per individual, for proof of concept in man. Ultimately, simultaneous modeling of relatively large animal- and small clinical datasets, allows further investigations on critical factors of animal-to-human extrapolation in a strict quantitative manner.

CONCLUSIONS

To develop treatments with improved safety and efficacy, one of the scientific challenges is to understand the biological mechanisms underlying the PK–PD relationships of (partial-) dopamine agonists and antagonists. PK–PD modeling is the golden standard to investigate such complex mechanisms. Often, these models include plasma drug concentration–effect relationships. However, for dopaminergic compounds, the target site is the brain ECF that surrounds the dopamine receptors. Consequently, a more mechanistic approach should be aimed at understanding the drug concentrations at the target site.

A second challenge is to achieve a more rapid onset of action and/or increased therapeutic window for dopaminergic compounds that have high clearance and/or low oral bioavailability characteristics. Intranasal administration of dopaminergic compounds avoids gastrointestinal- and hepatic first-pass elimination and is hypothesized to circumvent the BBB by direct nose–to–brain transport. This administration route is therefore anticipated to improve the time to onset- and/or the selectivity of action, specifically for drugs acting on the CNS.

To study (intranasal) administration of dopaminergic compounds, more advanced animal models are required that preferably; i) make use of freely moving animals under minimum stress conditions, ii) allow measurement of PK and PD parameters in serially obtained plasma- and brain ECF samples, and iii) allow intranasal and, for comparison, intravenous administration within the same individual. PK modeling of thus acquired rich datasets would allow

quantification of PK parameters in plasma and brain ECF following intra-nasal and intravenous administration. Such an approach has high potential to identify direct nose–to–brain transport in a quantitative manner.

Several clinical PK–PD models have been published on the relationships be-tween dopaminergic drug- and prolactin concentrations, as a biomarker for dopaminergic activity, following intravenous administration. From these studies it is clear that prolactin release is highly regulated in the brain, and that some homeostatic feedback model is required to accurately describe prolactin concentrations in plasma. As measurement at the target site is easier in animal studies, preclinical models allow for more mechanistic PK–PD approaches and can hence contribute to more accurate identification of drug-specific and biological system-specific parameters (including homeo-static feedback). This approach would allow for confirmation of potential improved onset- and/or selectivity of action following intranasal administra-tion of dopaminergic drugs.

An important feature of mechanism-based PK–PD models is the strict dis-tinction between drug-specific and biological system-specific parameters to describe *in vivo* drug responses. This is important since pertinent informa-tion on differences in biological system parameters enables the prediction of drug effects in biological systems other than the system in which the model has been developed. Consequently, translational modeling and simulation can be applied to quantitatively predict the time course of effect in man. Ultimately, preclinical derived mechanistic PK–PD models can provide the scientific basis for the translational pharmacology of dopaminergic drugs after intravenous and intranasal administration in humans.

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