Mechanism-based pharmacokinetic-pharmocodynamic modelling of opioids : role of biophase distribution and target interaction kinetics

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Section 1

GENERAL INTRODUCTION

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Chapter 1

PHARMACOKINETIC-PHARMACODYNAMIC MODELLING OF OPIOIDS:
ROLE OF BIOPHASE DISTRIBUTION AND TARGET INTERACTION KINETICS

ABSTRACT

Mechanism-based pharmacokinetic-pharmacodynamic (PK-PD) models contain specific expressions for processes on the causal path between drug administration and response. These models include expressions to describe a) blood pharmacokinetics in blood or plasma, b) biophase distribution, c) kinetics or target binding, d) transduction and e) homeostatic feedback mechanisms.

Previously, the PK-PD correlations of the high efficacy μ-opioid receptor agonists alfentanil, fentanyl and sufentanil have been investigated using quantitative EEG parameters as pharmacodynamic endpoint. In these investigations, the hysteresis observed for fentanyl and sufentanil was described with an effect-compartment model. Furthermore, by simulation it was shown that, in mechanistic terms, the *in vivo* concentration-effect relationships could be explained on the basis of the operational model of agonism, under the assumption of considerable receptor reserve. A limitation of this analysis was however, that all investigated opioids behaved as full agonists. Moreover, complexities at the level of blood-brain barrier (BBB) distribution had not been taken into account.

The main focus of the research in this thesis is on mechanism-based PK-PD modelling of the EEG effects of opioids with special emphasis on 1) the biophase distribution kinetics, which is mainly determined by BBB transport, and 2) interaction with the μ-opioid receptor in the brain as determinants of the time course of the pharmacological effect. To this end, a wide range of opioids with different binding characteristics and intrinsic activities should be investigated. The impact of biophase distribution should be investigated *in vivo* in great detail using intracerebral microdialysis.

CONTENTS

1. PHARMACOKINETIC-PHARMACODYNAMIC MODELLING

The objective of pharmacokinetic-pharmacodynamic (PK-PD) modelling is the characterisation and prediction of the time course of drug effects *in vivo* under physiological and pathological conditions (Breimer & Danhof 1997).

1.1 Classical/empirical approach

Classical PK-PD models consist of three components: (1) a pharmacokinetic model describing the time-course of drug concentration in blood or plasma, (2) a pharmacodynamic model describing the relation between the observed effect and the (predicted) drug concentration and (3) a link model to account for the often observed delay between blood/plasma concentration and effect.

1.1.1 Pharmacokinetic models

In PK-PD modelling, compartmental models are most commonly used to describe the time course of the drug concentration in blood/plasma. In these models drug disposition is characterised as the transfer of drug between interconnected hypothetical compartments, which serves to mimic the drug absorption, distribution and elimination processes. A limitation of this approach is that, although useful for descriptive purposes, it is not truly mechanistic. As a result, it is of limited value for extrapolation and prediction (i.e. interspecies scaling).

1.1.2 Pharmacodynamic models

Pharmacodynamic models describe the relationship between blood or plasma concentration and effect. The most general pharmacodynamic model is the sigmoid E_{max} model. This model is mathematically expressed by the Hill equation (Hill 1910) according to:

$$E = E_0 + \frac{\alpha \cdot C^{n_H}}{EC_{50} + C^{n_H}} \qquad (1)$$

in which E_0 is the no-drug response (baseline), α is the maximum response (intrinsic activity), EC_{50} is the concentration at which 50% of the maximum effect is reached (potency) and n_H is a factor expressing the slope of the sigmoid relationship. A limitation of the Hill equation is that it is not a mechanistic model. Specifically, the model does not provide insight in the factors which determine the shape and the location of the drug concentration-effect relationship. In this respect, it is important that drug concentration-effect relationships are determined by a combination of drug-specific properties (affinity and intrinsic efficacy) and biological system-specific characteristics such as receptor density and the efficiency of receptor-effector coupling (van der Graaf & Danhof 1997), as is illustrated in figure 1. An important factor is that the system-specific properties may differ between biological systems. Moreover, they can be influenced by several

processes like disease, age, chronic treatment and by other drugs. This may explain differences in drug-concentration-effect relationships between biological systems (i.e. species) and individuals (inter-individual variability). Moreover, the parameters of the sigmoid E_{max} model are "mixed" parameters; potency of a drug is determined by both affinity and efficacy and the intrinsic activity is a function of both compound (intrinsic efficacy) and system (receptor density and signal transduction) characteristics. This complicates the prediction of *in vivo* drug concentration-effect relationships on the basis of information from *in vitro* bio-assays.

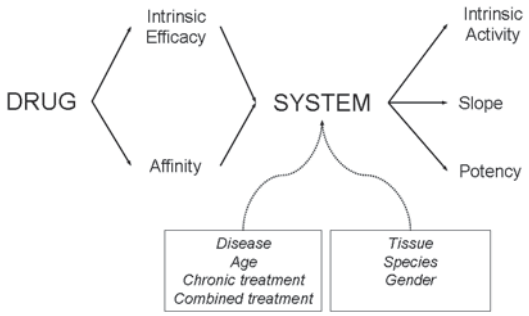

Figure 1: The pharmacodynamics of a drug (potency, intrinsic activity and Hill factor) are dependent on both drug (affinity and intrinsic efficacy) and system-related properties. These system related properties can be influenced by several processes like disease, age, chronic treatment and other drugs. Adapted from Van der Graaf and Danhof (1997).

1.1.3 Link models

For many drugs the relationship between blood/plasma concentration and pharmacological effect is not direct. Often a delay in pharmacological effect (hysteresis) is observed, which can be caused by time dependencies at the level of a) pharmacokinetics and b) pharmacodynamics. To account for delays between drug concentration and response, Segre introduced the concept of a "hypothetical effect compartment" (Segre 1968). Sheiner and co-workers were the first to formalize this concept into a model to describe hysteresis caused by distribution to the biophase (Holford & Sheiner 1982; Sheiner *et al.* 1979). With the effect compartment model the assumption is made that the rate of onset and offset of the drug effect is governed by the rate of drug distribution to the hypothetical "effect-site" (Sheiner *et al.* 1979). This effect-compartment is then linked to the blood concentrations with the rate constant k_{1e} for transport to the effect-site and the rate constant for drug loss k_{eo}. The effect-site distribution is considered to be symmetrical under the assumption that in equilibrium the effect-site concentration equals the blood concentration, where k_{1e} is equal to k_{eo}.

1.2 New approach – distinction between determinants of *in vivo* effects

At present there is a clear trend towards the development and application of mechanism-

based PK-PD models. Mechanism-based PK-PD models differ from the classical empirical descriptive models in that they contain specific expressions to characterise the processes on the causal path between drug administration and effect. These models should contain expressions for a) blood/plasma pharmacokinetics, b) biophase distribution c) kinetics of target binding, d) transduction and e) homeostatic feedback mechanisms (Danhof *et al.* 2005). A schematic diagram of drug action *in vivo* with the major determinants is shown in figure 2.

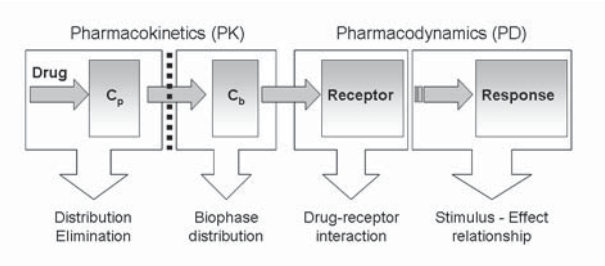

Figure 2: Schematic diagram of drug action in

1.2.1 Blood/plasma pharmacokinetics

To improve the prediction of pharmacokinetics, especially with regard to the extrapolation between species and the understanding of inter-individual variability, the concept of physiologically-based pharmacokinetic (PBPK) modelling has been proposed (Rowland *et al.* 2004). PBPK models are based on physiological principles and typically contain specific expressions for physiological variables such as blood flow to specific organs, binding to plasma proteins and/or tissue components and liver enzyme activity.

1.2.2 Biophase distribution

The concentration at the site of action (the biophase) is an important determinant of the drug effect *in vivo*. For drugs acting at extracellular targets, physicochemical properties (e.g. molecular size) and binding to plasma proteins and other blood constituents can restrict distribution to the biophase. Moreover, for drugs acting at intracellular targets and at targets in tissues that are protected by specific barriers (e.g. the brain), the distribution into the biophase can be influenced by the functionality of transporters. At present, these mechanisms are usually not taken into consideration when modelling biophase distribution kinetics. Yet, this is important since complexities at the level of biophase distribution may affect the derived shape of the concentration-effect relationship (Mandema *et al.* 1991; Visser *et al.* 2002b). Moreover, it may complicate the *in vitro* to *in vivo* extrapolation of parameters characterising the binding affinity of a drug to a specific target (Zuideveld *et al.* 2004). Another important consideration in relation to biophase distribution is whether it is indeed the free drug concentration

that drives the intensity of the pharmacological response. For a number of drugs (i.e. benzodiazepines, synthetic opioids) this seems to be the case (Cox *et al.* 1998; Mandema *et al.* 1991). However, there is still limited experimental evidence that the "free drug hypothesis" is valid under all circumstances. Particularly for drugs with a high affinity to their biological target and for drugs, which are transported by active transport mechanisms to the site of action, the biophase distribution may be non-restrictive.

In general, biophase distribution is dependent on both perfusion and distribution processes. Distribution processes include 1) passive diffusion and 2) transporter-mediated transport. Passive membrane diffusion is dependent on filtration (paracellular transport) and diffusion (transcellular transport), which can both be influenced by binding of the compound to proteins or other blood constituents. The main focus of this thesis is on the mechanisms of biophase distribution and this will be discussed in a separate section.

1.2.3 Kinetics of target binding

At the effect-site in the brain, the relationship between the concentration and the pharmacological effect is dependent on target interaction kinetics and signal transduction. Typically, when analysing concentration-effect relationships of a range of compounds in a given biological system, a single unique transducer function determines the effect and observed differences in concentration-effect relations are related to differences in receptor interaction kinetics in terms of target affinity and intrinsic efficacy.

In recent years, there has been considerable interest in the incorporation of receptor theory in PK-PD modelling for the prediction of *in vivo* concentration-effect relationships (van der Graaf & Danhof 1997). This is important since this enables a separation between drug-specific and biological-specific properties as determinants of the concentration-effect relationships. Modern receptor theory is based on the concept of the occupancy theory as first proposed by Clark (1937). This theory was further refined to describe the effects of partial agonists (Ariens 1954; Stephenson 1956) and receptor reserve (Furchgott 1966). Black and Leff have proposed the operational model of agonism to describe the relationship between drug concentration, receptor interaction and response (Black & Leff 1983). This model consists of two hyperbolic functions to describe the concentration-receptor occupancy and the receptor occupancy-response relationship, respectively, according to:

$$E = E_0 + \frac{E_m \cdot \tau^n \cdot C^n}{(K_A + C)^n + \tau^n \cdot C^n} \qquad (2)$$

where E_m is the maximum effect achievable in the system, K_A is the agonist dissociation equilibrium constant, n is the slope index for the occupancy-effect relationship and τ is the efficacy parameter. This efficacy parameter expressed according to equation:

$$\tau = \frac{R_0}{K_E} \quad (3)$$

where R_0 is the total number of available receptors and K_E is the concentration of the drug-receptor complex required to produce half-maximal effect. The drug-specific properties, the intrinsic activity (α) and the potency (EC_{50}) can than be derived with the following equations:

$$\alpha = \frac{E_{max} \cdot \tau^n}{\tau^n + 1} \quad (4)$$

$$EC_{50} = \frac{K_A}{\left(2 + \tau^n\right)^{1/n} - 1} \quad (5)$$

Recently, the principles of receptor theory have been successfully applied in the PK-PD analysis of neuroactive steroids (Visser *et al.* 2002a), benzodiazepines (Tuk *et al.* 1999; 2003; Visser *et al.* 2001), adenosine A_1 receptor agonists (van der Graaf *et al.* 1997) and 5-HT_{1A} receptor agonists (Zuideveld *et al.* 2004). PK-PD analysis on the basis of the operational model of agonism of the concentration-effect relationships of adenosine A_1 receptor agonists (van der Graaf *et al.* 1997) and 5-HT_{1A} receptor agonists (Zuideveld *et al.* 2004) have shown that a distinction can be made between drug-related and system-related parameters. For the adenosine A_1 receptor agonists a good correlation was found between the *in vivo* pK_A and the in *vitro* pK_i and between the *in vivo* efficacy parameter τ and the *in vitro* GTP shift. In contrast, for the 5-HT_{1A} receptor agonists a poor correlation was found between the *in vivo* pK_A and the *in vitro* pK_i, whereas a good correlation was found between *in vivo* efficacy parameter τ and the *in vitro* GTP shift. This poor correlation between *in vivo* pK_A and *in vitro* pK_i could in part be explained by differences in blood-brain distribution of the 5-HT_{1A} receptor agonists.

1.2.4 Signal transduction

Within the context of PK-PD modelling, transduction is defined as the cascade of processes that govern the time course of the pharmacological response *in vivo* following drug-induced target activation.

In addition to biophase distribution, time-dependent processes like the synthesis or degradation rate of an endogenous compound can explain hysteresis. To link this time delay between blood/plasma concentration and effect, a family of four physiological indirect response models have been proposed (Dayneka *et al.* 1993), which are based on the following differential equation:

$$\frac{dR}{dt} = k_{in} - k_{out} \cdot R \quad (6)$$

Where *R* is a physiological entity, which is constantly being produced and eliminated

in time, k_{in} is the zero-order rate constant for production of the physiological entity and k_{out} is the first-order rate constant for its loss. These models have been applied to describe the time-courses of a wide array of different drugs (Jusko & Ko 1994), although these models are often not properly validated.
A recent development has been the incorporation of dynamic system analysis to describe complex *in vivo* transduction processes (Zuideveld *et al.* 2001; 2004).

1.2.5 Homeostatic feedback mechanisms

The time course of the pharmacological response is often influenced by *in vivo* homeostatic feedback mechanisms, which may be operative. Such mechanisms may explain observations such as complex pharmacological effect *vs* time profiles. Recently, a model has been developed to describe the complex effect vs time profiles of the hypothermic response following the administration of 5-HT_{1A} receptor agonists to rats (Zuideveld *et al.* 2001; 2004). This model describes the hypothermic effect based on the concept of a set-point and general physiological response model.

2. BIOPHASE DISTRIBUTION OF CNS DRUGS

CNS active drugs have to pass the blood-brain barrier (BBB) to reach their target in the brain to be able to exert their pharmacological effect. This often results in biophase kinetics which are substantially different from plasma pharmacokinetics since BBB transport and brain distribution is often neither instantaneous nor complete (Welty *et al*. 1993).

2.1 Blood-brain barrier

2.1.1 Blood-brain barrier morphology

The BBB is situated at the interface between blood and brain and its main functions are (1) to maintain homeostasis in the brain (Abbott & Romero 1996), (2) selective transport of essential compounds like amino acids and glucose and (3) metabolism and modification of substances before entering the brain, for example proteins and peptides (de Boer & Breimer 1992). The BBB is primarily formed by brain capillary endothelial cells (BCEC) (de Boer & Breimer 1992; Rubin & Staddon 1999), although cells like astrocytes, pericytes and neuronal cells also play an important role in the function of the BBB (Pardridge 1991). The BCECs are distinctly different from peripheral endothelial cells in functional and morphological aspects (Bradbury 1993; de Boer *et al.* 2003). The most specific feature is the presence of tight junctions which prevents paracellular transport of hydrophilic compounds (Brightman & Reese 1969). Moreover, the BCECs express numerous influx and efflux transporters (de Boer *et al.* 2003; Golden & Pollack 1998; Lee *et al.* 2001).

2.1.2 Transport characteristics

Transport across the BBB can be divided into passive and active transport processes (de Lange & Danhof 2002). Passive transport of compounds across the BBB is dependent on physicochemical properties, such as lipophilicity, degree of ionisation and number of hydrogen bonds (van Bree *et al.* 1988). Passive transport (diffusion) across the BBB can either be permeability-limited or cerebral blood flow limited. Permeability-limited BBB transport is applicable for the more hydrophilic drugs that depend on the paracellular route for exchange between blood and brain. This route is restricted by the above-mentioned presence of tight junctions. Lipophilic, small and non-charged drugs more easily diffuse via the transcellular route and in such case blood-flow will mainly determine the transport rate. Active transport can be divided into carrier mediated transport, receptor mediated transport and endocytosis (de Boer *et al.* 2003). A schematic diagram of the transport processes across the BBB is shown in figure 3.

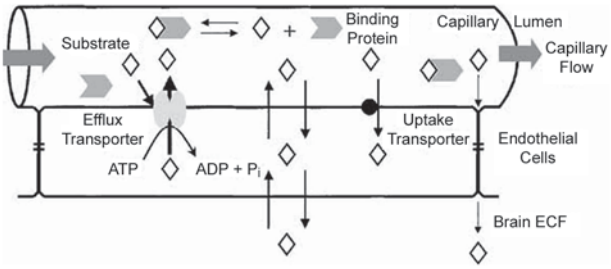

Figure 3: Schematic diagram of substrate flux through the BBB, indicating factors and processes that determine net brain uptake. Uptake and efflux transporters are illustrated on the luminal membrane of the endothelial cells for illustrative purposes only (Golden and Pollack, 2003)

2.1.3 P-Glycoprotein

An important efflux transporter expressed at the luminal face of the BBB is P-glycoprotein (Pgp) (Cordon-Cardo *et al.* 1989). Pgp is a member of the adenosine triphosphate-binding cassette (ABC) super family and is encoded by the multidrug resistance gene (MDR1) (Thiebaut *et al.* 1987). Pgp is a 170 kDa glycosylated membrane protein that consists of an integral membrane protein with twelve putative transmembrane α-helical domains and an energy-coupling domain localized at the cytoplasmatic side of the membrane (Fath & Kolter 1993; Pigeon & Silver 1994). The proposed function of Pgp is mainly to protect the brain from exogenous toxins, to excrete metabolites and furthermore to transport hormones from the brain to the periphery (Borst & Schinkel 1996; Karssen *et al.* 2001). The use of *in vitro* cell systems comprising of MDCK or LLC-PK1 cells transfected with the human MDR1 gene and MDR1a(-/-) (Pgp knock-out) mice (Schinkel *et al.* 1994) has clarified the impact of this efflux transporter on brain concentrations of many drugs including dexamethasone, domperidone, indinavir, digoxin, vinblastine, sparfloxacin, amitryptyline and cyclosporin (de Lange *et al.* 2000; de Lange & Danhof

2002; Kim *et al.* 1998; Meijer *et al.* 1998; Schinkel *et al.* 1995; Schinkel *et al.* 1996; Uhr *et al.* 2000; van der Sandt *et al.* 2001b). Alternatively, co-administration of Pgp inhibitors such as GF120918 (Hyafil *et al.* 1993) and SDZ-PSC 833 (Desrayaud *et al.* 1998; Mayer *et al.* 1997) can change drug distribution into the brain. For example, for the 5-HT_{1A} receptor agonist flesinoxan it was shown that a 5 to 6 fold increase in C_{max} and AUC was observed in brain pharmacokinetics when co-infused with the Pgp inhibitor SDZ-PSC 833 (van der Sandt *et al.* 2001a).

2.2 Distribution processes within the brain

The brain cannot be considered a homogeneous tissue, because it is composed of many anatomical structures with different characteristics (Collins & Dedrick 1983; Gross *et al.* 1986). In general, the main compartments are the brain extracellular fluid (ECF), the intracellular space (ICS) and the brain cerebrospinal fluid (CSF). After passage of the BBB, a drug enters the brain ECF and may thereafter distribute into brain ICS and the CSF (de Lange & Danhof 2002; Walker *et al.* 2000; Wong *et al.* 1993). Brain intracellular distribution is, in general, quantitatively more profound for the more lipophilic drugs and as a consequence the brain ECF concentrations will be relatively lower.

The interplay between the kinetics of BBB transport and intracellular distribution determines the time to equilibrium between plasma and biophase kinetics (Liu *et al.* 2005). With regard to the brain ECF concentrations, active transport out of the brain decreases whereas brain tissue binding increases the time to equilibrium. It should be noted that other than BBB transport, active transporters may also play a role in the intracellular distribution in the brain as indicated by localisation and functional expression of Pgp and MRP in the brain parenchyma (Lee *et al.* 2001).

2.3 Intracerebral microdialysis

Intracerebral microdialysis is very valuable technique for characeterisation of brain distribution kinetics, since it allows the determination of the free drug in the ECF as a function of time. It involves the implantation of a microdialysis probe into tissue, for example a specific region of the brain. The probe, consisting of a hollow tube and a semi-permeable membrane, is constantly perfused with a physiological solution. During perfusion, compounds that are small enough to traverse the membrane will diffuse from higher to lower concentration into the dialysate (Benveniste & Huttemeier 1990; de Lange *et al.* 1999a).

An important aspect of microdialysis is the recovery of the microdialysis probe. The dialysate concentrations do not equal the real ECF concentrations, because of the existence of a constant flow of the perfusion fluid. At early stages of microdialysis research, the *in vitro* recovery was used to calculate the ECF concentrations. However, *in vivo*, several tissue processes influence the recovery (Bungay *et al.* 1990) and therefore *in vivo* recovery methods have been developed including retrodialysis, no-net-flux and the dynamic-no-net-flux (Bouw & Hammarlund-Udenaes 1998; de Lange *et al.* 1997; 1999a; 1999b; 2000; Olson & Justice, Jr. 1993). Many CNS active drugs have their target

at extracellular recognition sites and therefore the ECF concentrations are most closely related to the biophase concentrations.

3. BRAIN AND BIOPHASE DISTRIBUTION MODELS

For PK-PD modelling, often only blood/plasma and effect data are available. In this case, a hypothetical effect-compartment is commonly applied to describe the biophase distribution kinetics. Recently, important progress has been made with the development of the technique of intracerebral microdialysis. It has been demonstrated that the mechanisms of brain distribution kinetics can be investigated in detail *in vivo* using this technique. It is proposed that this may provide novel insights in the mechanisms of the biophase distribution kinetics of CNS active drugs.

3.1. Brain distribution models

Recently, pharmacokinetic models have been developed that allow integrated analysis of microdialysis data which means that recovery calculations are included into the models (Schaddelee *et al.* 2004; Tunblad *et al.* 2004). In addition, population pharmacokinetic modelling of the microdialysis data has provided insight into the BBB transport characteristics of several drugs. These drugs include adenosine A_1 receptor agonists (Schaddelee *et al.* 2004), gabapentin (Wang & Welty 1996), norfloxacin (Chenel *et al.* 2004), fluvoxamine (Geldof *et al.* 2007) and opioids, which will be discussed in a separate section.

3.1.1 Application to adenosine A_1 receptor agonists

Schaddelee and co-workers have proposed a population pharmacokinetic model for estimation of the brain distribution clearance of the synthetic adenosine A_1 receptor agonists, 2'dCPA and MCPA. The model consisted of three compartments for description of the time course of the concentration in blood in combination with three compartments for the brain ECF concentrations (figure 4).

The mass balance in the brain compartments is described with the following differential equations:

$$\frac{dA_4}{dt} = K_{14} \cdot A_1 - K_{41} \cdot A_4 - K_{45} \cdot A_4 + K_{54} \cdot A_5 - K_{46} \cdot A_4 + K_{64} \cdot A_6 \quad (7)$$

$$\frac{dA_5}{dt} = K_{45} \cdot A_4 - K_{54} \cdot A_5 \quad (8)$$

$$\frac{dA_6}{dt} = K_{46} \cdot A_4 - K_{64} \cdot A_6 \quad (9)$$

Where A_1 represents the amount in the central blood compartment, A_4 – A_6 represent the amounts in the respective brain compartment and K_{mn} represents the first-order

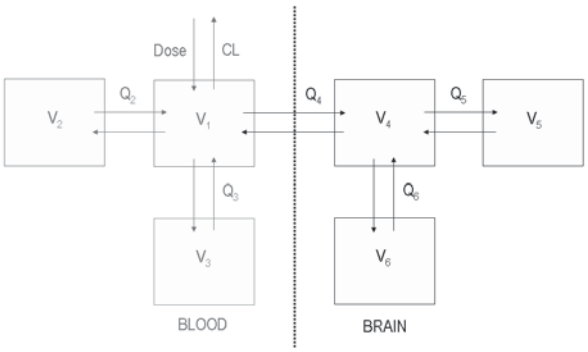

Figure 4: The population pharmacokinetic model for synthetic adenosine A_1 receptor agonists as proposed by Schaddelee and co-workers (2003). The model consists of three compartments to describe the blood pharmacokinetics (grey) and three compartments to describe the brain pharmacokinetics (black). Abbreviations: V= volume of distribution, Q = intercompartmental clearance and CL = body clearance.

transport rate constants from compartment *m* to compartment *n*. The rate constants were related to the inter-compartmental clearances (Q) and compartment volume (V) according to the following equations:

$$K_{mm} = \frac{Q_{m+1}}{V_m} \tag{10}$$

$$K_{mn} = \frac{Q_{m+1}}{V_n} \tag{11}$$

Low distribution clearances into the brain (Q_4) were found, 1.6 ± 0.3 µl/min and 1.9 ± 0.4 µl/min for 2'dCPA and MCPA, respectively, which were consistent with the results from *in vitro* tests. Furthermore, a slow elimination from the brain compartment was observed, indicating that the duration of the CNS effect might be much longer than expected on the basis of the terminal half-life in blood.

3.1.2 Application to the 5-HT$_{1A}$ receptor agonist fluvoxamine

The pharmacokinetics of the 5-HT_{1A} receptor agonist fluvoxamine, were described by simultaneous analysis of plasma, brain ECF and total brain concentrations on the basis of a more physiologically-based pharmacokinetic model. A three compartment model was used to describe the pharmacokinetics in plasma. The brain model to describe both brain ECF and total brain concentrations considers the brain to be composed of two areas (figure 5) and was based on the models proposed by Upton and co-workers (2000). The first area is the perfusion compartment, considered to be in direct contact with the blood flow and in which mass exchange is perfusion limited (shallow perfusion-limited compartment). The second area is the diffusion limited brain ECF compartment (deep brain compartment) in which the concentration is equal to the measured fluvoxamine ECF concentration. This compartment is poorly perfused and distribution is diffusion

limited. Fluvoxamine is not able to enter this compartment directly by perfusion, but only indirectly from the shallow perfusion-limited compartment by diffusion or active transport processes.

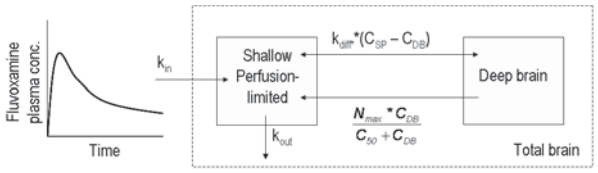

Figure 5: The physiologically-based pharmacokinetic model for fluvoxamine as proposed by Geldof and co-workers (2007). Plasma fluvoxamine concentrations were predicted with a three-compartment model and used as input function for fluvoxamine in the brain. The brain model consists of a shallow perfusion-limited and a deep brain compartment and mass exchange between the shallow perfusion-limited and the deep brain compartment is described by a passive diffusion term and an active saturable efflux process. Abbreviations: k_{in} is the rate constant into the shallow perfusion compartment, k_{out} is the rate constant out of shallow perfusion compartment; k_{diff} is the diffusion rate constant between the shallow perfusion-limited and the deep brain compartment; N_{max} is the maximal active efflux; C_{50} is the fluvoxamine concentration in the perfusion compartment at which 50% of the maximal active efflux is reached and k_{eff} is the active efflux rate constant which is influenced by GF120918.

In this model, the BBB is located between the perfusion and ECF compartment. The mass balance in the brain is determined by both the perfusion and the ECF compartment according to the following equations:

$$\frac{dA_{SP}}{dt} = Q_B \cdot C_{in} - Q_B \cdot C_{out} + N_{SP-DB} \tag{12}$$

$$\frac{dA_{DB}}{dt} = -N_{SP-DB} \tag{13}$$

where A_{SP} is the amount of morphine in the shallow perfusion-limited compartment, Q_B is the effective blood perfusion rate, C_{in} is the concentration entering the perfusion compartment en C_{out} is the concentration leaving the perfusion compartment. $N_{SP\text{-}DB}$ is the net mass exchange between the shallow perfusion-limited and the deep brain compartment and A_{DB} is the amount of fluvoxamine in the deep brain compartment and can include both passive and active transport processes. In case of fluvoxamine, mass exchange between the perfusion and ECF compartment consisted of passive diffusion and an active saturable efflux process, resulting in the following relation:

$$N_{SP-DB} = -k_{diff} \cdot (C_{SP} - C_{DB}) + \frac{N_{max} \cdot C_{DB}}{C_{50} + C_{DB}} \tag{14}$$

in which k_{diff} is the diffusion rate constant between the shallow perfusion-limited and deep brain compartment, C_{SP} is the concentration in the deep brain compartment, N_{max}

is the maximal active removal flux and C_{50} is the fluvoxamine concentration in the deep brain compartment at which 50% of saturation of the active removal flux is reached. Under the assumption that rapid equilibrium between fluvoxamine concentrations in the shallow perfusion-limited and the deep brain compartment is reached, the relationship between both compartments can be described as follows:

$$C_{SP} = C_{DB} + \frac{N_{max}}{k_{dif} f} \cdot \frac{C_{DB}}{C_{50} + C_{DB}} \quad (15)$$

The total amount of fluvoxamine in the brain can be described by:

$$\frac{dA_T}{dt} = \frac{dA_{SP}}{dt} + \frac{dA_{DB}}{dt} = Q_B \cdot C_{in} - Q_B \cdot C_{out} \quad (16)$$

in which A_T represents the total amount of fluvoxamine in the brain. The concentration entering the perfusion compartment (C_{in}) is assumed to be equal to the plasma concentration (C_{plasma}), whereas the concentration leaving the perfusion compartment is determined by the partition coefficient (P) between fluvoxamine in blood and the concentration in the shallow perfusion-limited compartment (C_{SP}).
When aggregating the perfusion rates, partition coefficient and brain distribution volume to the rate constants k_{in} and k_{out}, the differential equation for the total fluvoxamine in the brain can be described by:

$$\frac{dC_T}{dt} = k_{in} \cdot C_{plasma} - k_{out} \cdot C_{SP} \quad (17)$$

in which C_T is the total fluvoxamine concentration in the brain. The relationships between the total brain concentrations and both compartments could be derived on the basis of the partition coefficients for the shallow perfusion-limited and the deep brain compartment.
It was shown that the proposed model could accurately describe the plasma and brain pharmacokinetics of fluvoxamine. Active saturable efflux could be identified, although it remains unclear what transporter at the BBB is involved in the active efflux of fluvoxamine.

3.2 Biophase distribution models

In PK-PD investigations, the biophase distribution kinetics is often described by a 1-compartment biophase distribution model, also known as the effect-compartment model. With the effect compartment model, the assumption is made that the rate of onset and offset of the drug effect is governed by the rate of drug distribution to the hypothetical "effect-site" (Sheiner *et al.* 1979). This effect-compartment is then linked to the blood/plasma concentrations with the rate constant for transport to the biophase k_{1e} and the rate constant for drug loss k_{eo}. The rate of change of the drug concentration in the effect compartment can then be expressed by equation:

$$\frac{dC_e}{dt} = k_{1e} \cdot C_b - k_{eo} \cdot C_e \quad (18)$$

Where C_b represents the blood/plasma concentration and C_e represents the effect-site concentration. Under the assumption that in equilibrium the effect-site concentration equals the blood/plasma concentration, this equation can be simplified to:

$$\frac{dC_e}{dt} = k_{eo} \cdot (C_b - C_e) \quad (19)$$

However, more complex biophase distribution models have also been proposed. For example, for the neuroactive steroid alphaxolone the value of k_{eo} was concentration dependent (Visser *et al.* 2002b). In addition, Mandema and co-workers have reported two equilibration rate constants for the EEG effects of heptabarbital and have shown that the equilibration kinetics of amobarbital were best described with a bi-exponential equilibration function instead of a simple first-order mono-exponential equilibration model (Mandema & Danhof 1990; Mandema *et al.* 1991).

Recently, Chenel and co-workers have simultaneously investigated central nervous system distribution and the PK-PD relationship of the EEG effects of norfloxacin (2004). For this purpose, the combined EEG/microdialysis technique has been used. It was shown that the extensive time delay between EEG effect and plasma concentration of norfloxacin could be best described with an effect compartment model. However, this delay could not be accounted for by restricted BBB transport. Presumably, the drug concentration in brain ECF is not representative for the effect-site concentration of norfloxacin. In contrast, for morphine 80% and for morphine-6-glucuronide 50% of the delay in anti-nociceptive effect could be explained by restricted transport across the BBB (Bouw *et al.* 2000; 2001).

4. PHARMACOKINETIC-PHARMACODYNAMIC MODELLING OF OPIOIDS

4.1 Biophase distribution kinetics of opioids

For the analysis of the PK-PD correlations of opioids, modelling of complex biophase distribution kinetics is important, given the (potential) interaction with active transporters and the wide range in lipophilicity. In previous investigations, morphine and loperamide have been identified as Pgp substrates in two *in vitro* models, comprising of either BCECs or LLC-PK1:MDR1 cells, and in *in vivo* models, in rats and mice (Henthorn *et al.* 1999; Letrent *et al.* 1998; 1999a; 1999b; Mahar Doan *et al.* 2002; Schinkel *et al.* 1995; 1996). Alfentanil and sufentanil were not identified as Pgp substrates within the abovementioned investigations in *in vitro* models, whereas inconsistencies have been reported for fentanyl (Henthorn *et al.* 1999; Wandel *et al.* 2002). For fentanyl, *in situ*

brain perfusion studies have indicated a minimal contribution of Pgp mediated efflux as it was found that the brain uptake of fentanyl was only marginally increased (1.2 fold) in MDR1a (-/-) mice when compared to MDR1a (+/+) mice (Dagenais *et al.* 2004). Nalbuphine, a semi-synthetic opioid analgesic, was also found to be a Pgp substrate in an MDCKII-MDR1 cell-system (Mahar Doan *et al.* 2002).

The BBB transport characteristics of morphine and its metabolites have been studied in great detail using intracerebral microdialysis (Bouw *et al.* 2000; 2001; Xie *et al.* 2000). The brain pharmacokinetics of morphine, morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) were best described with a two compartment brain distribution model. The half-life of morphine in the brain was 44 ± 11 minutes for a dose of 10 mg/kg and was dependent on dose. For M3G and M6G the half-life in the brain was around 70 and 60 minutes, respectively. The brain-to-plasma concentration ratios were 0.28, 0.11 and 0.27 for morphine, M3G and M6G, respectively. All ratios are below 1 indicating that these compounds are actively removed at the BBB. Furthermore, PK-PD studies in rats have revealed that after oral pre-treatment with the specific Pgp inhibitor GF120918, the anti-nociceptive effect of morphine was prolonged due to its prolonged half-life in the brain (Letrent *et al.* 1998; 1999a). Tunblad and co-workers investigated the influence of probenecid on the BBB transport of morphine and found that in the presence of probenecid the steady state brain-to-plasma ratio was increased from 0.29 to 0.39 and that the morphine half-life in the brain increased from 58 to 115 min (2004). In addition, probenecid also decreased the systemic clearance of morphine and decreased the formation of M3G. M3G is also identified as a substrate for the probenecid-sensitive transporters (Xie *et al.* 2000).
In contrast, intracerebral microdialysis studies with the highly lipophilic opioid codeine have shown that a distributional equilibrium is reached rapidly with equal unbound concentrations in blood and brain and without dose dependency (Xie & Hammarlund-Udenaes 1998).

4.2 EEG as a biomarker for opioid receptor activation

Detailed characterisation of the role of biophase distribution kinetics in PK-PD investigations requires the availability of high density pharmacodynamic data. In this respect, quantitative analysis of drug effects on the electroencephalogram (EEG) yields attractive biomarkers, which are continuous, sensitive and reproducible (Dingemanse *et al.* 1988). Another advantage is that EEG effect measurements can be obtained in both laboratory animals and humans which enables interspecies extrapolation of the pharmacodynamics. On the basis of spectral analysis, the EEG can be subdivided into five distinct frequency bands: delta (0.5-4.5 Hz), theta (3.5-8 Hz), alpha (8-12 Hz), beta (12-30 Hz) and gamma (30->70 Hz) (Faulkner *et al.* 1999). Frequencies ranging from below 1 Hz up to around 12 Hz (delta – alpha bands) are readily observable in recordings from subjects during sleep, sedation or relaxed wakefulness. Higher frequencies from 12 Hz

to >70 Hz (beta – gamma bands) can also be seen, but at much lower amplitudes during intense mental activity and following sensory stimulation (Visser 2003).

Quantitative EEG parameters have been widely used as a pharmacodynamic endpoint in pre-clinical and clinical investigations on the PK-PD correlations of a variety of CNS active drugs. This includes barbiturates (Ebling *et al.* 1991), benzodiazepines (Mandema, *et al.* 1991a; 1991b; 1992b), neurosteroids (Visser *et al.* 2002a) and baclofen (Mandema *et al.* 1992a). It has also been shown that the synthetic opioid alfentanil, which is frequently used in anesthesia, produces a progressive slowing of the EEG with a pre-dominant increase in the delta frequency band (0.5-4.5 Hz) of the EEG power spectrum in both animals (Cox *et al.* 1997; Mandema & Wada 1995; Wauquier *et al.* 1988; Young & Khazan 1984) and humans (Scott *et al.* 1985; Wauquier *et al.* 1984; Young & Khazan 1984). After administration of alfentanil the EEG profile changes from high-frequency and low-amplitude to low-frequency and high-amplitude as is illustrated in figure 6.

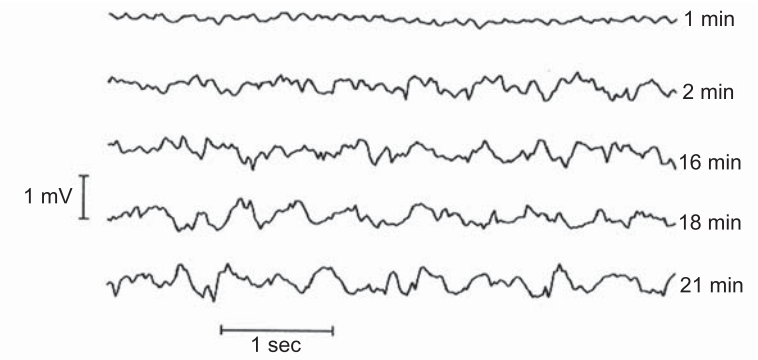

Figure 6: Typical EEG changes in rats upon alfentanil administrations. After administration of alfentanil, a progressive slowing of the EEG is observed with pre-dominant high-amplitude, low-frequency activity (0.5-4.5 Hz) (Cox *et al.* 1997).

4.3 Pharmacokinetic-pharmacodynamic modelling of the EEG effects of opioids

Previously, Cox and co-workers investigated the PK-PD correlations of the EEG effects of synthetic opioids alfentanil, fentanyl and sufentanil after intravenous administration in rats (1998). In these investigations, hysteresis was observed between the pharmacokinetics in blood and the pharmacodynamics for fentanyl and sufentanil whereas a direct correlation was observed for alfentanil. The hysteresis was described with the effect compartment model (equation 9), resulting in k_{eo} values of 0.32 min^{-1} ($t_{1/2,keo}$ = 2.2 min) and 0.17 min^{-1} ($t_{1/2,keo}$ = 4.2 min) for fentanyl and sufentanil, respectively. The pharmacodynamics was described using the sigmoid E_{max} model (equation 1). The interaction at the opioid μ receptor was determined *in vitro* on the basis of displacement of [^{3}H]-naloxone binding in washed rat brain membranes. The value of the 'sodium shift', being the ratio between affinities in the presence and the absence of sodium

chloride, was used as a measure of *in vitro* efficacy. Combination with the *in vitro* receptor binding characteristics showed that the *in vivo* concentration-effect relationships could be explained by the operational model of agonism according to Black and Leff (equation 2) under the assumption of a considerable receptor reserve (Cox *et al.* 1998).
The operational model of agonism has subsequently been used to explain the functional adaptation observed upon repeated administration of the selective μ-opioid receptor agonist, alfentanil. It was proposed that the ~2-fold decrease in potency observed following repeated administration of alfentanil can be explained by a ~40% decrease in the efficacy parameter of the operational model of agonism, τ, which includes both receptor density and coupling efficiency (Cox *et al.* 1998).
Garrido and co-workers further investigated the concept of receptor reserve for the full μ-opioid agonist, alfentanil, *in vivo* by pre-treatment with the irreversible μ antagonist, β-funaltrexamine (β-FNA) (2000). After pre-treatment with β-FNA the *in vivo* concentration-effect relationship of alfentanil was steeper and shifted to higher concentrations. Analysis with the operational model of agonism revealed that the observed changes could be explained by a 70-80% reduction in the alfentanil efficacy. This was consistent with the 40-60% reduction in the number of μ-opioid binding sites in the brain, as determined in an *in vitro* binding assay.
However, so far only simulations have been performed to investigate the role of μ-opioid receptor interaction kinetics. A complication in this analysis was however, that all investigated opioids behaved as full agonists.

5. CONCLUSIONS

In conclusion, to be able to develop a mechanism-based PK-PD model for opioids, a wide range of opioids should be investigated with different binding affinities and intrinsic activities. Moreover, potential complexities caused by interactions with specific transporters at the level of the brain distribution need to be taken into consideration.

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