Mechanism-based pharmacokinetic-pharmocodynamic modelling of opioids : role of biophase distribution and target interaction kinetics
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Section 4

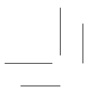
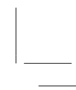
CONCLUSIONS AND GENERAL DISCUSSION

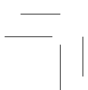
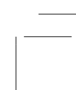
Sec4

Chapter 9

MECHANISM-BASED PHARMACOKINETIC-PHARMACODYNAMIC MODELLING OF OF OPIOIDS: SUMMARY, CONCLUSIONS AND PERSPECTIVES

Ch9

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1. SCOPE OF THESIS AND GENERAL BACKGROUND

The objective of the research described in this thesis was to develop a mechanism-based pharmacokinetic-pharmacodynamic (PK-PD) model for the electro-encephalogram (EEG) effects of opioids. The central effects of opioids are determined by four main processes: (1) blood/plasma pharmacokinetics, (2) biophase distribution, which is largely determined by blood-brain barrier (BBB) transport, (3) kinetics of target binding and activation and (4) transduction (figure 1).

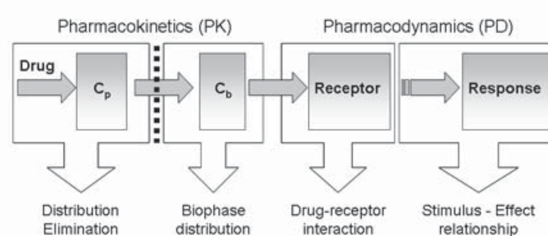

Figure 1: Determinants of in vivo drug effects

Under the assumption that within a single species the transduction mechanisms are similar for all opioids, the focus of this thesis was on the role of biophase distribution kinetics and the kinetics of target binding and activation on the pharmacokinetic-pharmacodynamic (PK-PD) relationships of opioids in the rat.
The opioids selected were alfentanil, fentanyl, sufentanil, morphine, butorphanol and nalbuphine because they differ widely in their brain distribution kinetics and efficacy.

Biophase distribution is considered to be a major determinant of the effects of opioids because they have to cross the blood-brain barrier (BBB) to enter the brain and to exert their central effect. Transport across the BBB can be a critical factor in the PK-PD relationships of opioids, since it determines the rate and extent of biophase distribution. For BBB transport, apart from restricted paracellular diffusion by the presence of tight junctions between the endothelial cells of the BBB, active transport mechanisms may play an important role, especially the efflux transport mediated by P-glycoprotein (Pgp).
In order to develop a mechanism-based PK-PD model, the influence of receptor binding and activation should also be considered. A distinction between affinity and intrinsic efficacy at the μ-opioid receptor can be made using concepts of receptor theory (i.e. the operational model of agonism).

In this chapter, the results of the investigations described in this thesis are reviewed and discussed. Furthermore, considerations and perspectives are presented.

2. MECHANISMS OF BIOPHASE DISTRIBUTION *IN VITRO*

The membrane transport characteristics of opioids were investigated *in vitro* with the focus on the relative contribution of passive permeability and P-glycoprotein (Pgp)-mediated efflux. Such information was also present in literature for some of the opioids (Schinkel *et al.* 1995; 1996; Wandel *et al.* 2002). However, in the context of the development of mechanistic PK-PD models for opioids, it is important to have comparative data for Pgp interaction on the selected opioids, obtained in a single test system an using an identical experimental design. To this end, for all selected opioids, studies were conducted in an *in vitro* cell system using monolayers of either the MDCK: MDR1 cells, which were transfected with the MDR1 gene encoding for human Pgp, or LLC-PK1:MDR1a cells, which were transfected with the MDR1a gene encoding for rodent Pgp. Moreover, unlike in previous studies, here the Pgp mediated transport relative to the passive membrane transport component was explicitely addressed. In these investigations, the passive permeability, as reflected by the Papp, of the different opioids was determined in the presence of a relatively high concentration of the potent and specific Pgp-blocker GF120918 (Hyafil *et al.* 1993).

2.1 Interaction with P-glycoprotein

The interaction of the opioids with Pgp was determined indirectly by investigating the inhibition of Pgp-mediated efflux of ^{3}H-digoxin and directly by a substrate assessment study. In the latter study, the transport of opioids was determined in the presence and absence GF120918.

The 3H-digoxin inhibition studies showed that alfentanil, fentanyl, sufentanil, morphine and loperamide were able to reduce the efflux transport of 3H-digoxin. The fact that loperamide is a strong Pgp substrate and therefore does not cross the BBB is a quantifiable extent (Schinkel *et al.* 1996; Wandel *et al.* 2002) explains why this anti-diarrhoeal agent with an opioid-like structure and receptor pharmacology does not exert any opioid-like effect *in vivo* (DeHaven-Hudkins *et al.* 1999; Niemegeers *et al.* 1979).

In the substrate assessment studies only morphine and loperamide were identified as Pgp substrates. For alfentanil, fentanyl and sufentanil no Pgp mediated transport could be detected, which is in accordance with literature (Wandel *et al.* 2002). For nalbuphine, literature values were included (Mahar Doan *et al.* 2002) to calculate a Pgp substrate efflux ratio, since in the present study no quantifiable transport could be obtained at the concentration tested. A transport ratio of 2 was found, which indicates that nalbuphine is a substrate for Pgp, while no inhibition of Pgp mediated transport of 3H-digoxin by nalbuphine was found. This apparent contradiction might be explained by the existence of several Pgp binding sites (Martin *et al.* 2000).

2.2 Apparent permeability and QSAR

From the Pgp substrate assessment data after pre-incubation with GF120918, the apparent passive permeability (P_{app}) could be calculated. It was shown that the P_{app} of alfentanil, fentanyl, sufentanil and butorphanol was high (>500 nm/sec) whereas for loperamide and nalbuphine the Papp was below 200 nm/sec. For morphine the lowest value of Papp (16 nm/sec) was observed. A schematic overview of the results of the *in vitro* transport studies is shown in table 1. It shows that the relative contributions of

Table 1: Results of the biophase distribution studies *in vitro*

Compound	Inhibitory activity	Substrate Assessment	Apparent permeability
Alfentanil	+	-	Very high
Fentanyl	++	-	Very high
Sufentanil	++	-	Very high
Morphine	+/-	+	Low
Butorphanol	-	-	Very high
Nalbuphine	-	+	Moderate
M3G	-	n.d.	n.d.
M6G	-	n.d.	n.d.
Loperamide	++	++	moderate

Inhibition/Substrate: ++ strong, + moderate, +/- weak, - none

Permeability: very high > 400 nm/sec, high 250-400 nm/sec, moderate 50-250 nm/sec, low <50 nm/sec

Pgp transport and passive permeability to the overall membrane transport were widely different between the opioids. Specifically, as a result of the high passive permeability, the relative contribution of Pgp-mediated transport to the overall transport of alfentanil, fentanyl and sufentanil are minimal. In contrast, for morphine, with a very low passive permeability, Pgp has a significant influence on the membrane transport of morphine, because the passive permeability is very low. This implicated the need for further *in vivo* BBB transport measurements in order to characterise the biophase distribution kinetics in the PK-PD relationships of morphine.

An interesting question is to what extent the passive permeability of opioids could be predicted on the basis of their physicochemical properties. To this end, regression analysis was performed to investigate the relationships between the physico-chemical properties and the P_{app} values. No statistically significant correlations were found in this analysis. This is in contrast with the results of an earlier study on the transport characteristics of adenosine A_1 receptor agonists. Here it has been found that the dynamic polar surface area (non-linear) and logBB as calculated by the Abraham equation (linear) were significantly related to the *in vitro* BBB clearance values of a set of 11 structurally highly related adenosine A1 receptor agonists (Schaddelee *et al.* 2003). An important

factor with the opioids tested here is that three clusters exist: four compounds with a very high passive permeability (alfentanil, fentanyl, sufentanil and butorphanol), two compounds with an intermediate permeability (nalbuphine and loperamide) and one with a low permeability (morphine). It is therefore expected that the *in silico-in vitro* correlations may improve upon inclusion of more opioids representing a more equal distribution of passive permeabilities.

3. MODELLING OF THE BIOPHASE DISTRIBUTION KINETICS OF MORPHINE *IN VIVO*

For morphine, the role of biophase distribution kinetics in the PK-PD correlation was investigated using a novel combined EEG/microdialysis technique that allows simultaneous characterisation of both the brain extracellular fluid (ECF) distribution and the EEG effect. In these investigations a wide dose range was investigated (4 to 40 mg/kg). The influence of Pgp was investigated by co-infusion of the Pgp blocker GF120918. In these investigations, the pharmacokinetics of morphine was investigated by non-linear mixed effect modelling (NONMEM).
The pharmacokinetics of morphine in blood was best described by a three-compartment model, whereas in previous studies plasma pharmacokinetics was described with a two-compartment model (Bouw *et al.*, 2000; Tunblad *et al.*, 2004). A possible explanation of this difference is that in the current investigations samples were collected for a much longer period of time (up to 350 minutes after the end of the infusion), whereas in previous studies samples were only collected up to 180 minutes. The blood pharmacokinetics of morphine was further shown to be independent of the dose administered. Moreover, co-administration of GF120918 did not influence the blood pharmacokinetic parameters.

3.1 Non-linear brain distribution model

To study BBB transport characteristics, intracerebral microdialysis is a valuable tool, since its allows the determination of detailed profiles of the free drug concentrations in the ECF as a function of time, which can be related to the (free) concentration-time profile in blood. However, the dialysate concentrations do not directly equal the real extracellular fluid (ECF) concentrations, because of the existence of a constant flow of the perfusion fluid. This results in a recovery value below 100%. The recovery can be determined *in vitro*, but to account for periprobe tissue processes, the recovery of the microdialysis should also be determined *in vivo* (Bungay *et al.*, 1990). For example, co-infusion of GF120918 can alter the elimination of morphine from the ECF and in addition, the *in vivo* recovery can also be changed by GF120918. It is therefore crucial the determine the *in vivo* recovery in the presence and absence of this Pgp efflux blocker.
For morphine, the *in vivo* recovery is typically determined using the retrodialysis-by-drug method (Bouw *et al.* 2000; Hyafil *et al.* 1993; Tunblad *et al.* 2004). In the current investigation, the *in vivo* recovery was determined with the retro-DNNF method (Bouw

& Hammarlund-Udenaes, 1998; Olson & Justice, Jr., 1993) in order to obtain concentration- as well as time-dependent recovery values of morphine. The retro-DNNF method consists of a retrodialysis period before intravenous administration of morphine to determine whether the loss of morphine from the probe was concentration dependent or influenced by GF120918. These perfusion concentrations were maintained until the end of the experiment, according to the DNNF principle (Olson & Justice, Jr., 1993). *In vivo* recovery was calculated from the retrodialysis period, in order to determine time-dependent values for *in vivo* recovery after iv administration of morphine. However, in contradiction to previous results for morphine in mice (Xie *et al.* 1999), in this case no conclusions could be drawn from the microdialysis data obtained for the DNNF rats after iv administration of morphine. Therefore these data were omitted from further analysis.

Upon administration of morphine, a rapid increase in morphine concentrations in the brain ECF was observed. A non-linearity was observed between the two dose groups as reflected in a reduction of the dose normalized AUC with increasing dose (chapter 5). Moreover, for the 4 mg/kg dose group a characteristic relatively stable plateau of the morphine concentration was reached in the brain ECF which was not observed for the 40 mg/kg dose group for which a clear decline was observed in time. To describe this non-linearity, a transport model was proposed which consisted of a passive diffusion, an active saturable influx and an active efflux component (figure 2). The active efflux was influenced by GF120918. This model is based on earlier indications for active uptake of morphine into the brain (Xie *et al.*, 1999), simulations of the influence of different transport processes on the ECF concentration-time profiles (Hammarlund-Udenaes *et al.* 1997) and the models proposed by Upton and Geldof for the brain distribution of fluvoxamine (Upton *et al.* 2000; Xie *et al.* 1999; Geldof *et al.*, 2007). The value of the passive diffusion rate constant was 0.0014 min^{-1} and the values of the active efflux rate constant were 0.0113 min^{-1} and 0.0195 min^{-1} in the presence and absence of GF120918, respectively. The active influx has a low capacity as indicated by the maximum transport rate of 0.66 $ng.min^{-1}.ml^{-1}$ and was readily saturated at low concentrations of morphine (C_{50} = 9.9 ng/ml). The active influx was not influenced by GF120918. Interestingly, the active efflux component could not be blocked completely with GF120918 indicating that BBB efflux of morphine is also mediated by transporters other than Pgp. Tunblad and co-workers showed that morphine is also substrate for the probenecid-sensitive transporters at the BBB. Specifically, co-administration of probenecid was found to result in a decrease in efflux clearance of morphine from the brain (Tunblad *et al.* 2004). Taken together, it can be concluded that the distribution of morphine is dependent by multiple transport mechanisms.

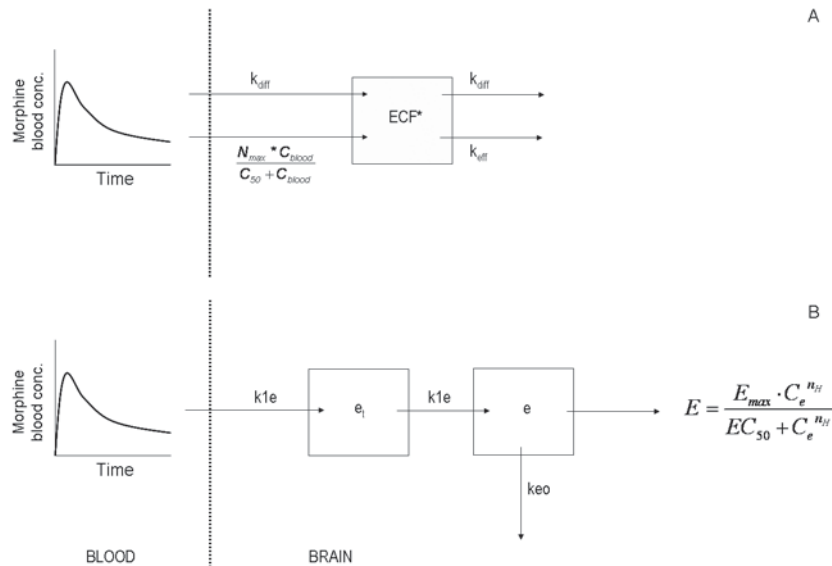

Figure 2: Schematic diagram of the non-linear brain distribution model as determined by microdialysis (panel A) and the extended-catenary biophase distribution model (panel B)

3.2 Biophase distribution model

In the current investigations, EEG monitoring was used as a pharmacodynamic endpoint. Quantitative analysis of drug effects on the electroencephalogram (EEG) yields an attractive biomarker, which is continuous, sensitive and reproducible (Dingemanse *et al.* 1988). It has 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). Meanwhile the increase in the delta frequency band of the EEG has been widely used as a biomarker in numerous studies on the PK-PD correlations of synthetic opioids. In preclinical studies evidence has been obtained that the increase in the delta frequency band of the EEG reflect μ-opioid receptor activation (Cox *et al.* 1997; 1998; 1999). However, it remains to be elucidated whether changes in the delta frequency band are solely caused by μ-opioid receptor activation or whether more complex interactions at multiple receptor subtypes are involved.

After the start of the morphine infusion, a gradual increase in the EEG effect, expressed as the absolute amplitude in the 0.5-4.5 Hz frequency range, was observed. After the start of the morphine infusion, a gradual increase in the EEG effect was observed. The maximal effect was 60 μV and was observed around 20 minutes after the end of the morphine infusion. The duration of the effect (from the start of the infusion until the

return to baseline values) was dependent on dose and co-infusion of GF120918 and around 180 minutes following the infusion of 4 and 10 mg/kg morphine whereas after a dose of 4 mg/kg combined with GF120918 or 40 mg/kg morphine the duration of the effect was around 360 minutes. It was found that the derived blood concentration-EEG effect relationships showed profound hysteresis for all experimental groups. To describe this hysteresis, a biophase distribution model was developed. Initially, the biophase equilibration kinetics was fitted according to the one-compartment biophase distribution model. The utility of both a symmetrical (with identical values of the rate constants k_{1e} and k_{eo}) and an asymmetrical (with different values for k_{1e} and k_{eo}) effect compartment distribution model to describe the data was tested. However, neither the symmetrical nor the asymmetrical effect compartment model was able to estimate the biophase concentrations accurately. Therefore, the extended-catenary biophase distribution model was proposed, consisting of two sequential effect compartments; a transfer and an effect compartment (figure 2). The structure of this model is similar to the two-compartment "tank in series" model described by Upton and co-workers and provides a simple method to account for dispersion of drug in transit through the brain (Upton *et al.* 2000). The value of the rate constant for transport through the transfer compartment (k_{1e}) was 0.038 min^{-1} and was unaffected by the co-administration of GF120918. The values for transport rate constants for the loss from the effect compartment (k_{eo}) in the presence and absence of GF120918 were 0.0015 min^{-1} and 0.043 min^{-1}, respectively. Interestingly, the observation of the involvement of Pgp-mediated efflux in the brain distribution of morphine as observed on the basis of the EEG effect is consistent with observations on the basis of the brain ECF concentrations as obtained with intracerebral microdialysis.

3.3 Evaluation of the brain and biophase distribution models

In the investigations on the brain distribution kinetics and the PK-PD correlations, two structurally different models are proposed for characterization of the biophase distribution. The brain distribution model consisted of one brain ECF compartment with distinction between passive diffusion, active saturable influx and active efflux whereas the biophase distribution model consisted of two sequential biophase compartments with two rate constants for transport through the transfer compartment and efflux from the effect compartment . A schematic diagram of both models is shown in figure 2. When comparing the concentration-time profiles in brain ECF and biophase, it could be noted that they were distinctly different (figure 3). The concentration in brain ECF peaked early, whereas the maximum biophase concentration showed a profound delay. In addition, at the low dose of morphine a "plateau" was observed in brain ECF whereas in the biophase concentrations a clear decline over time was observed. These observations indicate that the brain ECF cannot be used to explain the hysteresis. This is in contrast with the observation by Bouw and co-workers where 85% of the observed hysteresis for the anti-nociceptive effect could be explained by distribution into the

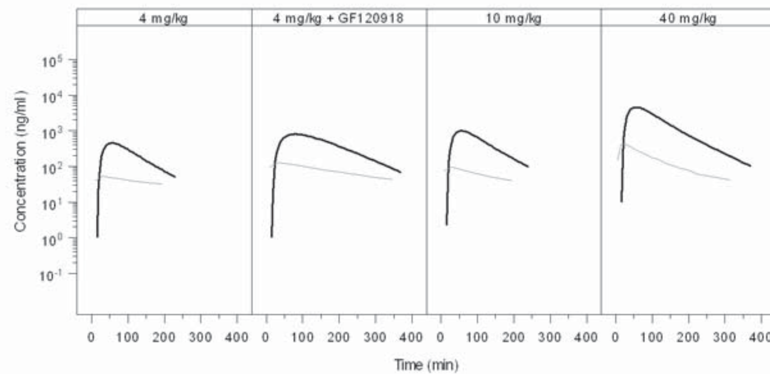

Figure 3: Comparison of the population predicted biophase concentration-time profiles (black lines) and the population predicted brain ECF fluid concentration-time profiles (grey lines) as obtained previously. It is shown that the time-course of the biophase concentrations differs substantially from the time-course of the brain ECF concentrations indicating that biophase distribution is slower than transport to the brain ECF.

brain ECF (2000). In addition, Bouw and co-workers did not identify the active uptake of morphine in the brain ECF. This indicates that the site of action for the anti-nociceptive effects is different from that for the EEG effect.

A discrepancy between the predicted biophase concentration and the measured CNS concentration-time course has also been observed for the EEG effects of amobarbital and baclofen, where the biophase concentrations did not reflect the measured cerebrospinal fluid concentrations (Mandema *et al.* 1991; 1992). In addition, Chenel and co-workers showed that the extensive time delay between EEG effect and plasma concentrations of norfloxacin, best described with an effect-compartment model, could not be explained by slow distribution to the biophase (Chenel *et al.* 2004). For norfloxacin the brain ECF concentrations peaked very early, whereas the EEG effect was delayed, which was also seen for morphine. For norfloxacin the brain ECF profiles were parallel to the plasma profiles whereas for morphine a non-linearity was observed at the low dose (4 mg/kg). Chenel and co-workers showed that the keo did not decrease when the ECF data were included in the PK-PD analysis, whereas for morphine the brain ECF and EEG effects could not be analysed simultaneously.

A number of possibilities can explain these observations. First, from a pharmacokinetic perspective it may be that the biophase distribution of morphine is not mainly determined by BBB transport but also to a significant extent by distribution and elimination processes in relation to the regional distribution of the target (de Lange & Danhof 2002). The location of the microdialysis probe in the specific area of the brain, and the concentrations measured in that region, may differ from the biophase as determined by receptor density (Mansour *et al.* 1988). In addition, while the free concentrations in the brain ECF are quantified by intracerebral microdialysis, also intracellular concentrations of morphine may contribute to the EEG effects.

Alternatively, from a pharmacodynamic perspective, it could be that morphine does not exclusively exerts its EEG effects by binding to the μ-opioid receptor. It is known that morphine has affinity for both the μ and κ receptor (Chen *et al.* 1993; Kilpatrick & Smith 2005; Ulens *et al.* 2001) and therefore it is possible that although the EEG is considered to be a biomarker for μ-opioid receptor activation, the other receptor subtypes can influence the EEG effect of morphine.

In addition, the observed discrepancy could also be explained by the influence of receptor association-dissociation kinetics. Recently it has been shown that the onset and offset of the anti-nociceptive and respiratory depressant effects of buprenorphine are determined by both biophase distribution and receptor association-dissociation kinetics, although the major determinant was the biophase distribution kinetics (Yassen *et al.* 2005; 2006). Buprenorphine is an opioid which is structurally related to morphine and kinetics of binding to and dissociation from the μ-opioid receptor is slow (Boas & Villiger 1985; Cowan *et al.* 1977). In contrast, for fentanyl it was shown that the hysteresis could be completely explained by biophase distribution kinetics. Since buprenorphine is structurally related to morphine, it may be possible that receptor association-dissociation kinetics also influence the onset and offset of the EEG effect.

Finally, transduction processes can also influence the time-course of the EEG effect. In the current investigations, the biophase concentration-effect relationships were described with the sigmoidal E_{max} model. However, in theory, the transduction function may take any shape. Depending on the behaviour of the drug in the biological system, a hyperbolic or linear transduction is chosen (Black & Leff 1983). The receptor theory by Clark states that for partial agonists, the pharmacological response is directly related to the number of receptors occupied (Clark 1937). So, when information is available in the *in vivo* receptor binding, a linear transduction function is able to describe the receptor occupancy-effect relationship.

4. PK-PD MODELLING OF THE EEG EFFECTS OF OPIOIDS

In addition to morphine, the EEG effects of the opioids alfentanil, fentanyl, sufentanil, nalbuphine and butorphanol have been investigated. The data for alfentanil, fentanyl and sufentanil have been collected and published previously (Cox *et al.* 1998). The blood pharmacokinetics of all opioids could be successfully described using population PK analysis. For fentanyl, sufentanil and butorphanol, a two-compartment model best described the data whereas for alfentanil, morphine and nalbuphine a three-compartment model was most suitable.

4.1 Role of complex biophase distribution kinetics

From the previous studies it can be concluded that the modelling of complex biophase distribution kinetics of opioids is important, given the potential interaction with active transporters and the wide range in lipophilicity. It was shown the biophase distribution

kinetics of morphine could be best described with the extended-catenary biophase distribution model which consists of two sequential compartments (chapter 6). To describe the time-course of the EEG effect of fentanyl, sufentanil, butorphanol and nalbuphine two structurally different biophase distribution models were investigated: 1) the one-compartment biophase distribution model (the effect-compartment model) and 2) the extended-catenary biophase distribution model. For alfentanil, a direct relationship between the blood concentrations and the EEG effect was observed. For fentanyl, sufentanil and butorphanol, the one-compartment distribution model, with identical values of the distribution rate constants k_{1e} and k_{eo} yielded the most accurate description of the pharmacodynamics. This was expected since the delay in pharmacodynamic effect was relatively short, as is confirmed by the k_{1e} values of 0.47 min^{-1}, 0.17 min^{-1} and 0.21 min^{-1} for fentanyl, sufentanil and butorphanol, respectively. The biophase distribution kinetics of nalbuphine could be equally well described with both distribution models. However, since the accuracy of the parameter estimates did not change when the model was simplified from the extended-catenary to the one-compartment distribution model, the latter model was accepted as the most appropriate model to describe the biophase distribution kinetics of nalbuphine. In addition, asymmetrical distribution could not be identified for nalbuphine and the estimated k_{1e} (and k_{eo}) value was 0.20 min^{-1}.
Interestingly, a statistically significant correlation was observed between the values of the *in vivo* k_{1e} and the *in vitro* P_{app} as determined in the *in vitro* assays (chapter 4). However, it should be taken into consideration that for biophase distribution processes, apart from BBB transport, also distribution within the brain can influence the hysteresis (Liu *et al*. 2005). An important question is whether the estimates of the biophase concentrations that have been obtained with this PK-PD modelling approach indeed reflect the true biophase concentrations. In this respect, it is important that the role of active transporters at the BBB other than Pgp has not been taken into considerations. The findings on the effect of the Pgp blocker GF120918 on the biophase distribution and the EEG effect of morphine indicate that such an effect might be significant. Whether this also applies to the other opioids will be discussed in a subsequent paragraph.

4.2 Identification of the kinetics of target binding and activation

As the first step in the pharmacodynamic analysis, the unbound biophase concentration effect relationships were analysed with the Hill equation. This analysis showed that alfentanil is the opioid with the highest intrinsic activity (123 ± 13 μV). This analysis was performed in order to enable a ranking in intrinsic activity for the set of opioids. The relative intrinsic activity of the various opioids (α) ranged from 0.81 to 0.10 for sufentanil and butorphanol, respectively which corresponds to an E_{max} value of 100 μV for sufentanil and 12 μV for butorphanol. In addition, the $EC_{50,u}$ ranged from 0.21 nM for sufentanil to 1223 nM for morphine. Thus, a wide range of both E_{max} and EC50,u values was observed (figure 4).

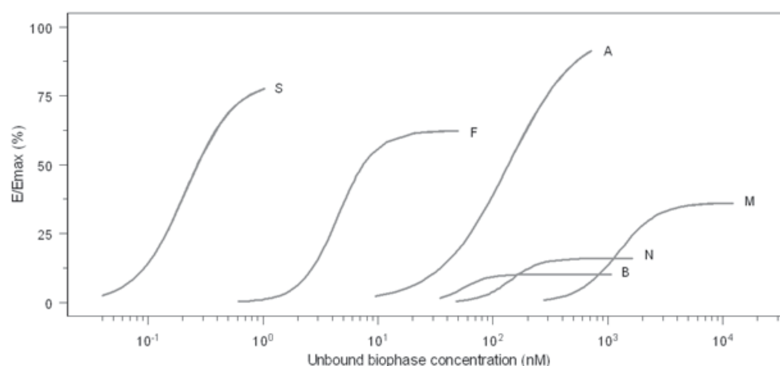

Figure 4: Unbound biophase concentration-EEG effect relationships of opioids as obtained by simultaneous analysis with the Hill equation. From left to right the opioids are: sufentanil, fentanyl, alfentanil, butorphanol, nalbuphine and morphine. The EEG effect is presented as the net EEG amplitude as a percentage of the maximum achievable effect in the system (123 μV).

A limitation of the Hill equation is that, although very useful for descriptive purposes, it is only of limited value to understand factors which determine the shape and location of the concentration-effect relationship. Specifically, the pharmacodynamic parameters of the Hill equation are mixed parameters which depend on both the properties of the drug and the biological system (van der Graaf *et al.* 1997; van der Graaf & Danhof 1997). As a result, observed values of the E_{max} may depend on the drug or the biological system. Moreover, values of the EC_{50} depend on both the target affinity and the intrinsic efficacy. Recently, the concepts of receptor theory have been used to make a strict distinction between drug-specific and biological-system specific factors as determinants of *in vivo* concentration-effect relationships. Especially, the operational model of agonism (Black & Leff 1983;) has been successfully applied for explaining and predicting the effects of differential expression of agonism *in vivo* (Black & Leff 1983; van der Graaf *et al.* 1997; Zuideveld *et al.* 2004).

The comparative method (Black & Leff 1983; Leff *et al.* 1990; van der Graaf *et al.* 1997) was applied for analysis of the concentration-effect relationships by the operational model of agonism with the values of E_{max} (123 μV) and n (1.44) constrained to the values of alfentanil which displays the highest intrinsic activity *in vivo*. In addition, the pK_A of sufentanil, as the opioid with the highest receptor affinity, was fixed to the *in vitro* pK_i (9.15). The constraint of the *in vivo* pK_A to the *in vitro* pK_i has also been applied in previous analyses of concentration-effect relationships (Black & Leff 1983; Jonker *et al.* 2005; van der Graaf *et al.* 1997; Zuideveld *et al.* 2004). When analyzing the concentration-effect relationships of the opioids, the efficacy parameter τ ranged from 0.452 for nalbuphine to 2.618 for alfentanil. In addition, the K_A ranged from $4.3*10^{-10}$ to $2.3*10^{-6}$ M for sufentanil and morphine, indicating that large differences existed in the set of opioids.

When taking all compounds together, the correlations between the *in vivo* pK_A and the *in vitro* pK_i determined in the presence of 1 mM GTP or 100 mM NaCl were not statistically significant (P>0.05). However, there were clear indications for two (sub-)populations of opioids. The estimated *in vivo* pK_A for alfentanil, fentanyl and sufentanil were similar to the values obtained *in vitro*, whereas for morphine, butorphanol and nalbuphine, the pKa was higher. For these opioids, this may be explained by biophase distribution processes, including BBB transport, as has been specifically addressed for morphine. Another explanation may be that these opioids interact not only with a specific μ-opioid receptor subtype, but also with other μ, κ and δ-opioid receptor subtypes.

a) Biophase distribution processes

As discussed in section 3, for morphine it has been shown that biophase distribution is influenced by the functionality of Pgp at the BBB. Specifically, co-infusion of GF120918 reduced the rate constant for efflux of morphine from the effect compartment with 60%. As a result, in the absence of Pgp blockers the free drug concentrations in the brain ECF are substantially lower than the corresponding free blood concentrations. This could well explain the observed discrepancy between the *in vitro* and *in vivo* K_A values. However, it is important to note that morphine is not only transported by Pgp, but also by other transporters like the probenecid-sensitive transporter which is also present at the BBB (Tunblad *et al.* 2004). The influence of active transport mechanisms as a confounder of the analysis of the *in vitro-in vivo* corrlations of pK_A values has also been identified for 5-HT_{1A} receptor agonists in particular with regard to flesinoxan (Zuideveld *et al.* 2004). In addition, regional differences in brain kinetics can also explains the differences between the local pharmacokinetic in the brain ECF and the overall kinetics responsible for the EEG effect.

b) Interaction with μ opioid receptor subtypes and κ and δ opioid receptors

Another possible explanation of the existence of two (sub-)populations is the interaction with different μ-opioid receptor subtypes or the interaction with κ and δ opioid receptors. Upon reducing the number of available μ-opioid receptors by irreversible binding by β-funaltrexamine (β-FNA), it has been found that the Hill factor of alfentanil is increased to 2.75 (Garrido *et al.* 2000). It has been speculated that antagonist-induced curve-steepening could be indicative for receptor heterogeneity (van der Graaf *et al.* 1996) and that the EEG effect of alfentanil is mediated via multiple receptor types which differ in their sensitivity to β-FNA (Garrido *et al.*,2000). Recently, several spliced μ-opioid receptor isoforms have been identified, which might be involved with different aspects of the pharmacology of opioids (Pasternak 2005; Zernig *et al.* 1994). Specifically, for the opioids morphine, butorphanol and nalbuphine it is known that they have affinity for both the μ- and κ-opioid receptor, whereas fentanyl is a specific μ-opioid receptor agonist (Chen *et al.* 1993). In literature, little is known about the receptor affinity of alfentanil and sufentanil, but they have been specifically designed to bind exclusively

to the μ-opioid receptor (Chen *et al.* 1993; Yeadon & Kitchen 1988). Furthermore, it is known that heterodimerisation of opioid receptors can potentiate the effects of opioids (Gomes *et al.* 2000).

5. CONCLUSIONS AND PERSPECTIVES

The objective of the research described in this thesis was to develop a PK-PD model for the EEG effects of opioids, to get insight into the mechanisms that determine the pharmacological effects of opioids. The results described in this thesis indicate that the major determinants of the EEG effects of opioids are biophase distribution processes and kinetics of target binding. This illustrates that the biophase distribution kinetics should be explicitly addressed in great detail as an integral part of the investigations into the pharmacodynamics of opioids.

Biophase distribution kinetics

In the current investigations, the biophase distribution kinetics has been studied with emphasis on BBB transport and the influence of Pgp. However, multiple (efflux) transporters are present at the BBB which could influence biophase distribution. These transporters include the multidrug resistance proteins (MRP) (Zhang *et al.* 2000) and other probenicid-sensitive transporters, which have shown to be involved in the transport of morphine and morphine-3-glucuronide (Tunblad *et al.* 2004; Xie *et al.* 2000). In addition, modelling of the BBB transport of morphine has revealed the influence of a yet unknown influx transporter (chapter 5). Active influx was also found for oxycodone (Bostrom *et al.* 2006). In addition, other yet unknown transporters could also play a role in the BBB transport of opioids. In order to be able to exclude the influence of such transporters a cocktail of different selective blockers could be administered in each experiment or single or multiple transporter knock-out animals (e.g. mdr1a/1b(-/-), mrp1(-/-) mice (Johnson *et al.* 2001)). As an alternative, detailed information on the brain concentrations should be obtained, for example including intracerebral microdialysis combined with EEG monitoring (chapter 5 and 6), though the location of the biophase does not need to be represented by that of the ECF surrounding the microdialysis probe as shown for morphine in relation to the EEG effect.

Other *in vivo* techniques could be considered as well. For example, Positron Emission Tomography (PET) would be very useful to study the distribution of a (radio labelled) opioid across the brain thereby enabling detailed information on total brain concentration distribution of the radiolabel and its specific binding within the brain as a function of time and location. Recently, Liefaard and co-workers have established a population pharmacokinetic model to describe and predict these processes for 11C-flumazenil (2005). Important disadvantages of PET are that it requires radioisotopes which are often instable resulting in a very short half-life and that it cannot distinguish between parent compound and metabolites.

Simultaneous analysis of biophase distribution and kinetics of target binding

Biophase distribution models were proposed for all opioids and biophase concentration-effect relationships were derived. Subsequently, the estimated biophase concentration-effect relationships were analysed according to the operational model of agonism to get insight into the kinetics of target binding and activation. As an alternative to this sequential approach, simultaneous analysis of the biophase distribution and kinetics of target binding could be performed. Recently, Yassen and co-workers have presented a mechanism-based PK-PD model that is based on receptor theory and aims at the separate characterisation of biophase distribution and receptor association/dissociation kinetics as determinants of hysteresis between plasma concentrations and effect (Yassen *et al*. 2005; 2006). It was shown that for the anti-nociceptive and respiratory depressant effects of opioids, reliable parameter estimates could be obtained for both biophase distribution and receptor association/dissociation. However, for fentanyl, no reasonable values could be obtained for the receptor association/dissociation kinetics, indicating that receptor binding for fentanyl is instantaneous and therefore does not contribute to the hysteresis. A prerequisite for this approach is that receptor binding is not instantaneous. On that basis it is expected that this approach cannot be applied for fentanyl-like opioids. Since buprenorphine is structurally related to morphine, it may be possible that receptor association-dissociation kinetics also influence the onset and offset of the EEG effect of morphine, butorphanol and nalbuphine.

Specificity of EEG as biomarker of μ-opioid receptor activation

In this thesis, EEG is used as a biomarker for μ-opioid receptor activation. EEG is an attractive biomarker because it can be monitored continuously. However, it remains to be elucidated whether changes in the δ-frequency band are solely caused by μ-opioid receptor activation. In order to investigate this, EEG experiments should be performed with specific agonists for the different receptor subtypes. A list of specific agonists and antagonists as derived from available literature is shown in table 2. See (Alexander *et al.* 2000; Baker & Meert 2002; Chen *et al.* 1993) for additional references.

Table 2: Selective agonists and antagonists for the different opioid receptor subtypes

Receptor subtype	Agonists	Antagonists
μ	Endomorphine-1 and -2	Naloxone
	DAMGO	β-funaltrexamine (irreversible)
		naloxonazine (μ_1)
κ	U50,488H	Nor-binaltorphine
	U69,593	DIPPA (irreversible)
δ	DPDPE	Naltrindole
	DSBULET	BNTX (δ_1)
	[dAla2]deltorphine I or II	Naltriben (δ_2)
	SNC80	

Influence of other μ-opioid receptor subtypes

It is known from literature that opioids bind to different opioid-receptor subtypes. First, the receptor binding characteristics at these receptor subtypes should be investigated *in vitro* using radioligand binding studies as described in section 3 of this discussion and by Chen and co-workers (1993).

Next, the influence of the different receptor subtypes should be investigated by co-administration of selective blockers for each receptor subtype (table 3). Recently, a study has been described which investigates the roles of peripheral and central μ, δ and κ receptors and their subtypes in opioid-induced hypothermia in mice (Baker & Meert 2002). The effects of morphine and selective agonists for the opioid receptor subtypes, (fentanyl, SNC80, U50,488H and loperamide), on the body temperature were assessed directly. All selected opioids produced hypothermia, which was (partly) counteracted by a selection of receptor specific antagonist (naloxone, β-funaltrexamine, naloxonazine, naltrindole, BNTX, naltriben, nor-binaltorphine and DIPPA). For morphine and fentanyl, the hypothermia was shown to involve a composite action on μ, κ and possible δ receptors after initial activation. Such findings indicate that for the development of a mechanistic PK-PD model also the influence of the interaction between different receptor subtypes has to be considered.

Other pharmacodynamic end-points

In the current investigations, EEG was used as a pharmacodynamic endpoint. It has the advantages of being continuous and subjective. However, for opioids to produce changes in the EEG effect, relatively high concentrations are required, often resulting in the occurrence of respiratory depression. In addition, for example for buprenorphine, no clear effect on the EEG could be observed (Yassen, personal communication), while also for butorphanol and nalbuphine, the EEG effect was difficult to quantify. The high dose group of nalbuphine had to be excluded because of severe systemic side effects during and after the opioid infusion. Another disadvantage of EEG in rats is that it is a rather invasive technique involving the implantation of cortical electrodes. For research of opioids, antinociception is often used as a pharmacodynamic endpoint. Antinociception can be investigated using the tailflick latency test (Letrent *et al.* 1998; 1999; Yassen *et al.* 2005) or the electrical stimulation-vocalisation test (Bouw *et al.* 2000; 2001; Ekblom *et al.* 1993). Disadvantages of these tests are censoring of the data (values above cut-off value), possible interference between measurement and the limited number of observations that can be obtained in one animal.

All opioids produce respiratory depression to some extent. Respiratory depression is often measured using arterial carbon dioxide tension (P_ACO_2) as a surrogate biomarker of minute ventilation (V_i) (Megarbane *et al.* 2006; Ohtani *et al.* 1997). Recently, Yassen and co-workers have developed a more sensitive method in which minute ventilation is measured by whole body plethysmography at a fixed inspired CO_2 concentration of 6.5% (Yassen *et al.* 2006).

Conclusions

The effects of opioids are mainly determined by biophase distribution kinetics and kinetics of target binding. For the development of a mechanism-based PK-PD model which also has predictive value, these processes should be investigated in great detail. In addition, supportive data for each of the processes are essential for model validation and prediction.

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