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Chapter 6
**PHARMACOKINETIC-PHARMACODYNAMIC
MODELLING OF THE ELECTROENCEPHALOGRAM
EFFECTS OF MORPHINE:
INFLUENCE OF BIOPHASE DISTRIBUTION AND
P-GLYCOPROTEIN INTERACTION**

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SUMMARY

Background and purpose

The aim was to investigate the influence of biophase distribution including P-glycoprotein (Pgp) functionality on the pharmacokinetic-pharmacodynamic correlation of morphine.

Experimental approach

Male rats received a 10-min infusion of morphine as 4 mg/kg, combined with a continuous infusion of the Pgp inhibitor GF120918 or vehicle, 10 or 40 mg/kg. EEG signals were recorded continuously and blood samples were collected.

Key results

Profound hysteresis was observed between morphine blood concentrations and EEG effect. Only the offset of the EEG effect was influenced by GF120918. Biophase distribution was best described with an extended-catenary biophase distribution model, with a sequential transfer and effect compartment. The rate constant for transport through the transfer compartment (k_{1e}) was 0.038 min^{-1} , being unaffected by GF120918. In contrast, the rate constant for the loss from the effect compartment (k_{e0}) decreased 60% by GF120918, from 0.043 to 0.015 min^{-1} . The EEG effect was directly related to the concentrations in the effect compartment using the sigmoidal E_{max} model. The values of the pharmacodynamic parameters E_0 , E_{max} , EC_{50} and Hill factor were $45.0 \text{ } \mu\text{V}$, $44.5 \text{ } \mu\text{V}$, 451 ng/ml and 2.3 , respectively.

Conclusions and implications

The effects of GF120918 on the distribution kinetics of morphine in the effect compartment were consistent with recent observations on the distribution in brain extracellular fluid (ECF) as estimated by intracerebral microdialysis. However, the time-course of morphine concentrations at the site of action in the brain, as deduced from the biophase model, is distinctly different from the brain ECF concentrations.

INTRODUCTION

Mechanism-based pharmacokinetic-pharmacodynamic (PK-PD) models for the central action of opioids contain expressions for a) blood pharmacokinetics, b) biophase distribution, which is mainly determined by blood-brain barrier (BBB) transport, c) receptor interaction kinetics and d) signal transduction (Danhof *et al.* 2005). Especially for morphine, biophase distribution is an important determinant of the onset and duration of the effect because of its hydrophilic nature and the interaction with the efflux transporter P-glycoprotein (Pgp). Research on the influence of biophase distribution on morphine PK-PD relationships has so far primarily focussed on rather empirical biophase distribution model. Bouw and co-workers have proposed a single biophase compartment model to account for the delay of the anti-nociceptive effect of morphine relative to corresponding plasma concentrations (Bouw *et al.* 2000). It was found that BBB transport accounts for 84% of the observed hysteresis. For morphine, a limited number of studies have focused on the role of active transport mechanisms at the BBB. Specifically, it has been shown 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, presumably resulting from inhibition of Pgp as an active efflux mechanism (Letrent *et al.* 1998; 1999). Moreover, the role of transporters other than Pgp efflux at the BBB on brain distribution of morphine has been indicated by interaction studies with probenecid (Tunblad *et al.*, 2004).

In recent investigations, the brain distribution of morphine has been characterised in greater detail with intracerebral microdialysis (Groenendaal *et al.*, 2007 - chapter 5). Brain distribution was non-linear and was successfully described by a complex brain distribution model with specific expression for 1) passive diffusion, 2) active saturable influx and 3) active efflux which could in part be inhibited by GF120918. Against this background, it is of considerable interest to characterise in a mechanistic manner the biophase distribution kinetics of morphine in a PK-PD investigation.

Detailed characterisation of the biophase distribution kinetics 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). Meanwhile, 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. 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). 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 it has been demonstrated that the increase in the delta frequency band of the EEG reflect μ -opioid receptor activation (Cox *et al.* 1997; 1998; 1999). Moreover, in clinical studies, this biomarker has been validated as a surrogate marker for depth of anaesthesia (Egan *et al.* 1996; Lemmens *et al.* 1995; Scott *et al.* 1991; Scott *et al.* 1985).

The aim of the present study was to investigate the influence of biophase distribution and Pgp interaction at the BBB on the PK-PD relationships of morphine and to compare the time course of the predicted effect-site concentrations with the time course of the brain extracellular fluid (ECF) concentrations as determined by intracerebral microdialysis.

METHODS

The pharmacokinetics in blood and pharmacodynamics were investigated in two sets of experiments, the classic EEG experiments and the EEG/MD experiments. The details of the EEG/MD experiments have been described previously (Groenendaal *et al.* 2007 - chapter 5).

Surgical procedures

Details on the anaesthesia and surgical procedures have been described previously (Groenendaal *et al.* 2007 - chapter 5). For the EEG experiments, seven cortical electrodes were stereotactically implanted into the skull of rats ten days before the start of the experiments as described before (Cox *et al.* 1996). Briefly, the electrodes were placed at the locations 11 mm anterior and 2,5 mm lateral (F_1 and F_r), 3 mm anterior and 3,5 mm lateral (C_1 and C_r) and 3 mm posterior and 2,5 mm lateral (O_1 and O_r) to lambda. A reference electrode was placed on lambda. Stainless-steel screws were used as electrodes and connected to a miniature connector. For the EEG/MD experiments, the rats were chronically instrumented with four EEG electrodes at the F_r , C_r , O_1 and reference position and with a CMA/12 guide cannula with a dummy probe placed in the striatum of the right brain hemisphere (anterior-posterior: +0.5 mm, lateral: +2.7 mm with bregma as reference and ventral: -3.5 mm ventral to the skull).

Experimental procedures

All experimental procedures were identical for both the EEG groups and the EEG/MD groups, as described previously (Groenendaal *et al.* 2007 - chapter 5). The EEG signal was continuously monitored via bipolar EEG leads on the left hemisphere (Cl-Ol) using a Nihon-Kohden AB-621G Bioelectric Amplifier (Hoekloos BV, Amsterdam, The Netherlands) and concurrently digitized at a rate of 256 Hz using a CED 1401plus interface (CED, Cambridge, United Kingdom). The digitized signal was transferred into a Pentium III computer and stored on hard disk for off-line analysis. For each 5-sec epoch, quantitative EEG parameters were obtained off-line by Fast Fourier Transformation

with a user-defined script within the software package Spike2 for Windows, version 3.18 (CED, Cambridge, United Kingdom). Changes in the amplitudes in the δ -frequency band of the EEG (0.5-4.5 Hz) averaged over 1-minute time intervals were used as a pharmacodynamic endpoint. Further reduction of the EEG data was performed by averaging the signals over predetermined time intervals using a user-defined script within the software package Matlab®, version 6.1 (The Mathworks Inc., Gouda, The Netherlands). The size of the intervals was dependent on the different periods of the experiment. The intervals were 3 min for baseline, 3 min between start of infusion (time = 0) and 75 min, 5 min between 75 and 200 min after start of infusion and 10 min between 200 and 360 min after start of the infusion. These intervals were chosen on the basis on visual inspection of the 1-min datafile.

Blood samples were analysed for morphine, GF120918 and midazolam as described previously (Groenendaal *et al.* 2005 - chapter 3).

Data analysis

The details on the general modelling procedures have been described previously (Groenendaal *et al.* 2007 - chapter 5). The EEG effects of morphine were analysed using non-linear mixed effect modelling as implemented in the NONMEM software version V, level 1.1 (Beal & Sheiner 1999). Population analysis was undertaken using the first-order conditional estimation method (FOCE interaction). Individual pharmacokinetic parameter estimates were used as input for the pharmacodynamic models. Individual blood concentrations were calculated at the times of the EEG measurements.

The selection of the biophase distribution model

A profound delay in the EEG effect (hysteresis) of morphine was observed. The hysteresis was characterized on the basis of two biophase distribution models: A) the one-compartment biophase distribution model, also known as the effect-compartment model and B) the extended-catenary biophase distribution model (figure 1).

A) One-compartment biophase distribution model

Hysteresis is often characterised on the basis of the one-compartment biophase distribution model. With this 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} 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:

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

Where C_b represents the blood concentration and C_e represents the effect-site

concentration. Under the assumption that in equilibrium the effect-site concentration equals the blood concentration, this equation can be simplified to:

$$\frac{dC_e}{dt} = k_{eo} \cdot (C_b - C_e) \quad (2)$$

This model describes a symmetrical biophase. In contrast, when k_{1e} is not equal to k_{eo} , the biophase is considered to be a-symmetrical. Both models were investigated.

B) Extended-catenary biophase distribution model

The extended-catenary biophase distribution model consists of two sequential compartments, a transfer (e_t) and an effect (e) compartment which was based on the “tank-in-series” models described by Upton and co-workers and provides a simple method for accounting for dispersion of drug in transit through the brain (Upton *et al.* 2000). The rate of change of the concentrations in the effect compartments can then be described as follows:

$$\frac{dC_{e_t}}{dt} = k_{1e} \cdot C_b - k_{1e} \cdot C_{e_t} \quad (3)$$

$$\frac{dC_e}{dt} = k_{1e} \cdot C_{e_t} - k_{eo} \cdot C_e \quad (4)$$

where C_{e_t} and C_e describe the concentrations in the transfer and effect-compartment, respectively. The concentrations in the effect-compartment were then linked to the pharmacological effect. Both the symmetrical ($k_{1e}=k_{eo}$) and the asymmetrical ($k_{1e} \neq k_{eo}$) biophase models were investigated.

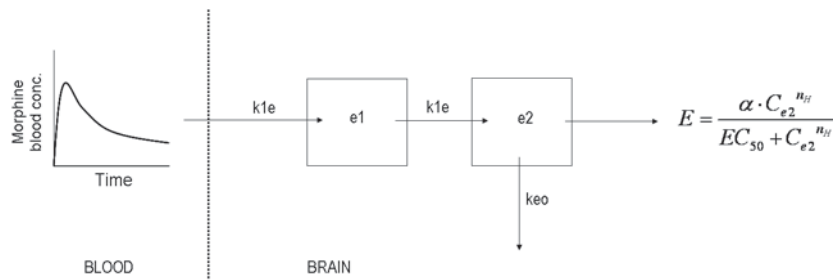


Figure 1: A schematic overview of the one-compartment distribution model (panel A) and the extended-catenary biophase distribution model (panel B) to describe the biophase kinetics of morphine. The blood pharmacokinetics were described with a three-compartment model and used as input function for morphine in the brain. The one-compartment distribution model consists of one effect compartment (e), whereas the biophase distribution model consists of two sequential compartments, the transfer (e_t) and the effect (e) compartment. The concentrations in the effect compartment were related to the EEG effect on the basis of the sigmoidal Emax model. Abbreviations: k_{1e} represents the rate constant for transport through the transfer compartment; k_{eo} represents the rate constant for loss from the effect compartment and is influenced by GF120918; Emax represent the intrinsic activity and EC_{50} represents the potency.

PK-PD analysis of the EEG effect

For the development of the structural PK-PD model for the EEG effect of morphine, the PREDPP subroutine ADVAN6 was used, which is a general nonlinear model that uses a numerical solution of the differential equations.

After hysteresis minimization, the individual concentration-effect relationships were fitted to the sigmoidal $E_{\max}$ model:

$$E = E_0 + \frac{E_{\max} \cdot C_e^{n_H}}{EC_{50}^{n_H} + C_e^{n_H}} \quad (5)$$

Where E_0 is the no-drug response, $E_{\max}$ is the intrinsic activity, EC_{50} is the potency and n_H is the slope factor.

In the experiments described here, two experimental approaches were used, the EEG method and the EEG/MD method. Since the removal of three EEG electrodes and the subsequent implantation of a microdialysis probe could possibly result in a change in baseline EEG, a covariate was included in the analysis to validate the EEG/MD method. The following equation was used:

$$P_i = \theta_1 \cdot (1 - METHOD_i) + \theta_2 \cdot (METHOD_i) \quad (6)$$

where P_i is the individual value of model parameter and θ_1 and θ_2 are the parameter values obtained with METHOD=1 for EEG/MD and METHOD=0 for EEG.

The influence of co-infusion of GF120918 on the biophase distribution rate constants was tested with the following equation:

$$P_i = \theta_3 \cdot (1 + \theta_4 \cdot GF\ 120918_i) \quad (7)$$

Where P_i is the individual value of model parameter and θ_3 and θ_4 are the parameter estimate and Pgp inhibition value and GF120918 is a factor, set to 1 if GF120918 is co-infused and set to 0 if vehicle is co-infused.

Inter-animal variability on E_0 and $E_{\max}$ was described with a proportional variability model according to equation:

$$P_i = P_{typ} \cdot (1 + \eta_i) \quad (8)$$

with

$$\eta_i \sim N(0, \omega^2) \quad (9)$$

where P_i is the individual value of the model parameter P , P_{typ} is the typical value (population value) of parameter P in the population, and η_i is inter-animal random variable. The assumption was made that all other parameters were log normal distributed with mean zero and variance ω^2 .

The inter-animal variability on all other parameters was described with an exponential error model according to equation:

$$P_i = P_{typ} \cdot \exp(\eta_i) \quad (10)$$

with

$$\eta_i \sim N(0, \omega^2) \quad (11)$$

Inter-animal variability was investigated for each parameter and was fixed to zero when the MVOF did not improve. Correlations between the inter-animal variability of the various parameters were graphically explored. In addition, correlations between the PD parameters and dose and between the PD parameters and the co-infusion of GF120918 were also investigated graphically.

The residual error, which accounts for unexplained errors (such as measurement and experimental errors) in the EEG measurements, was best described with a proportional error model according to equation:

$$C_{obs,ij} = C_{pred,ij} \cdot (1 + \varepsilon_{ij}) \quad (12)$$

where $C_{obs,ij}$ is the j -th observation of the i -th individual, $C_{pred,ij}$ is the predicted concentration and ε_{ij} is a realisation from the normally distributed residual random variable with mean zero and variance σ^2 :

$$\varepsilon_i \sim N(0, \sigma^2) \quad (13)$$

RESULTS

Pharmacodynamics and hysteresis

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. The maximal effect was 60 μ V 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 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. In figure 2, the pharmacokinetics and the pharmacodynamics of a typical rat of each experimental group are shown. It was found that the derived blood concentration-EEG effect relationships showed profound hysteresis for all experimental groups (figure 3), which was counter clockwise.

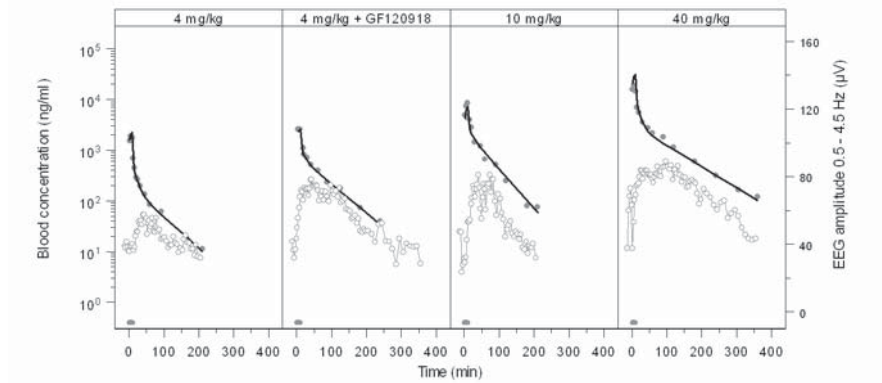


Figure 2: Pharmacokinetics and pharmacodynamics of a typical rat after administration of the opioids. Observed blood concentrations (grey dots), individual predicted blood concentrations (black line) and observed EEG effect (grey open dots) are depicted for each dose group. The grey bar indicates the infusion time.

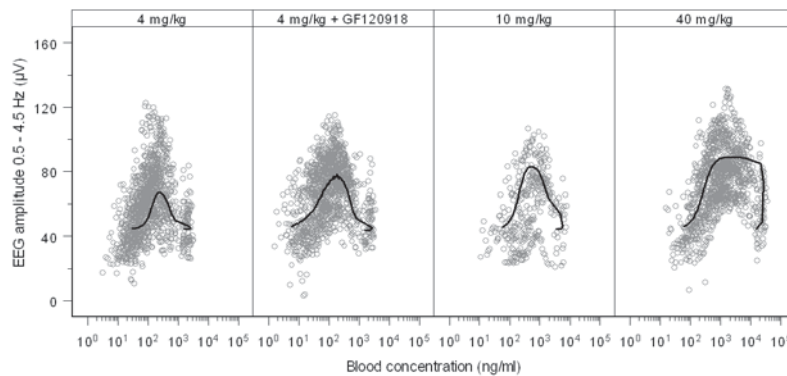


Figure 3: PK-PD relationship after administration of morphine. Observed (grey dots) and population predictions (black line) are depicted for each dose group. A clear counter clockwise hysteresis loop was observed for all morphine doses.

The selection of the biophase distribution model

In order to describe the observed hysteresis, two biophase distribution models were proposed: 1) the one compartment distribution model and 2) the extended-catenary biophase distribution model. First, the biophase distribution kinetics was fitted according to the one-compartment biophase distribution model. Both the symmetrical and a-symmetrical effect compartment model was tested. With the symmetrical ($k_{1e}=k_{e0}$) biophase distribution no results were obtained (minimisation terminated) whereas with the asymmetrical ($k_{1e}\neq k_{e0}$) effect compartment model no precise estimates could be obtained and bias was observed between the observed and predicted values. Therefore, the extended-catenary biophase distribution model was proposed, consisting of two sequential compartments; a transfer and an effect compartment. Both

the symmetrical and asymmetrical model was used. The asymmetrical model resulted in the lowest objective function, 24671 ($k_{1e} \neq k_{eo}$) versus 24936 ($k_{1e} = k_{eo}$) and precise estimates of the parameters of the biophase distribution kinetics were obtained (table 1).

Table 1: Population pharmacokinetic parameter estimates of the biophase distribution of morphine obtained with the extended-catenary biophase distribution model

Parameter	Estimate	CV%	LLCI – ULCI
<i>Structural model</i>			
k_{1e} (min^{-1})	0.038	8.4	0.0315 – 0.0441
k_{eo} (min^{-1})			
-GF120918	0.0426	10	0.0342 – 0.0510
+GF120918 [#]	0.0152		
Pgp inhibition factor	-0.644	-7.3	-0.736 – -0.552
<i>Interindividual variability</i>			
$\omega^2 k_{eo}$	0.237	20.2	0.143 – 0.331

[#] $k_{eo}(+GF120918) = k_{eo}(-GF120918) * (1 + \text{Pgp inhibition factor})$

Abbreviations: k_{1e} = rate constant for transport to the effect-site; k_{eo} = rate constant for the loss from the effect-site; CV% = coefficient of variation; LLCI = lower limit of confidence interval; ULCI = upper limit of confidence interval

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. The population predictions of blood and biophase concentration-time profiles are shown in figure 4.

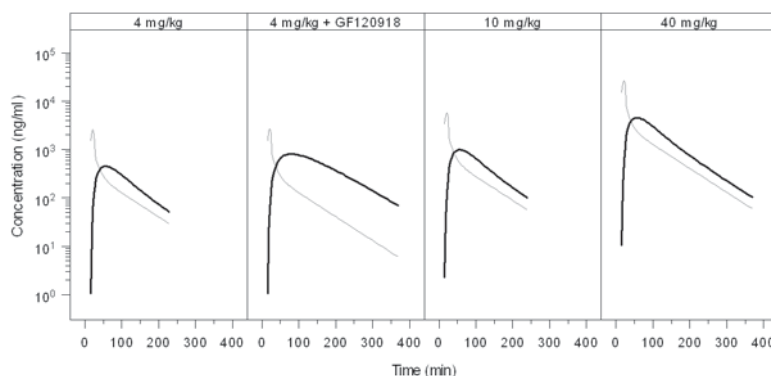


Figure 4: Population predicted blood and biophase concentration-time profiles of morphine obtained with the extended-catenary biophase distribution model. The grey lines represent the blood concentration-time profiles and the black lines represent the biophase concentration-time profiles.

The best fit was obtained when the influence of GF120918 was described with a Pgp inhibition factor that influences the k_{eo} . This Pgp inhibition factor was estimated at a value of -0.64 indicating that in the presence of GF120918, the k_{eo} is decreased with 64% from 0.043 to 0.015 min^{-1} . The inter-animal variability (ω^2) on k_{eo} was estimated with an exponential error model and was equal to 0.237 . The inter-animal variability could not be estimated for the other parameters and were therefore fixed to zero.

Pharmacokinetic-pharmacodynamic analysis of the EEG effect

The individual predicted biophase concentrations were related to the EEG effect on the basis of the sigmoidal E_{max} pharmacodynamic model. Since the EEG and EEG/MD experiments were performed in parallel, covariate analysis was included to investigate the influence of the microdialysis probe on the pharmacodynamic parameters. Since no differences were observed in E_0 and E_{max} values between the EEG and the EEG/MD group, a single parameter value was estimated. Morphine PK-PD relationships were accurately described as shown in figure 5 and table 2.

Table 2: Population pharmacodynamic parameter estimates of morphine EEG effect obtained with the sigmoidal E_{max} model after hysteresis minimisation with the extended-catenary biophase distribution model.

Parameter	Estimate	CV%	LLCI – ULCI
<i>Structural model</i>			
E_0 (μV)	44.6	2.3	42.6 – 46.6
E_{max} (μV)	44.5	8	37.5 – 51.5
EC_{50} (ng ml^{-1})	451	17.3	298 – 604
N	2.32	10.4	1.85 – 2.79
<i>Interindividual variability</i>			
$\omega^2 k_{E0}$	0.034	17.8	0.022 – 0.045
$\omega^2 k_{Emax}$	0.121	24.1	0.064 – 0.178
<i>Residual variability</i>			
Proportional error	0.027	7.6	0.023 – 0.031

Abbreviations: E_0 is the no-drug response; E_{max} is the intrinsic activity; EC_{50} is the potency; n_H is the slope factor; CV% = coefficient of variation; LLCI = lower limit of confidence interval; ULCI = upper limit of confidence interval

Co-infusion of GF120918 did not influence the pharmacodynamic parameters. The values of the pharmacodynamic parameters E_0 , E_{max} , EC_{50} and n_H were $45.0 \mu\text{V}$, $44.5 \mu\text{V}$, 451 ng/ml and 2.3 , respectively. Inter-animal variability (ω^2) was estimated with a proportional error model for E_0 and E_{max} and was equal to 0.034 and 0.121 , respectively. Inter-animal variability could not be estimated for the other parameters and were therefore fixed to zero. Graphical analysis showed that no significant correlations

were observed between the estimates of the PD parameters and dose and between PD parameter estimates and co-infusion of GF120918.

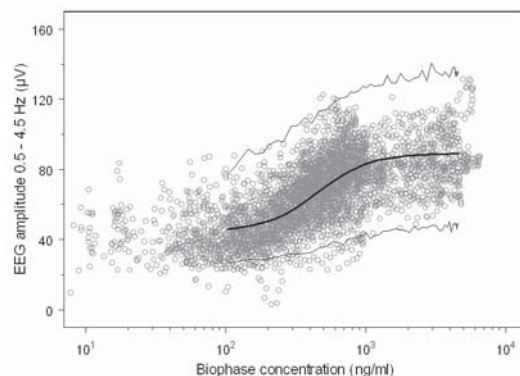


Figure 5: PK-PD relationships of the opioids after hysteresis minimisation with the extended-catenary biophase distribution model. Observed (grey dots), population predicted (solid line) and 2.5% and 97.5% quantiles (dotted lines) are depicted versus the predicted biophase concentration as shown in figure 4. The PK-PD relationship was obtained using the extended-catenary biophase distribution model to describe the distribution to the effect-site and the sigmoidal Emax model to relate the biophase concentrations to the EEG effect.

DISCUSSION

Biophase distribution can be defined as the distribution processes between the blood and the effect-site. The aim of the present study was to investigate the influence of biophase distribution and Pgp interaction at the BBB on the PK-PD relationships of morphine and to compare the time course of the predicted effect-site concentrations with the time course of the brain ECF concentrations as determined by intracerebral microdialysis.

So far the PK-PD investigations of morphine have focussed on the anti-nociceptive effects (Bouw *et al.* 2000; Letrent *et al.* 1998). In these studies, the hysteresis has been described with the standard symmetrical effect compartment model consisting of a single effect compartment, where k_{1e} is equal to k_{e0} . In these models, a wide difference in k_{e0} values (hysteresis) was observed; the k_{e0} values were 0.228 min^{-1} and 0.022 min^{-1} , for the doses of 1 mg/kg and 10/40 mg/kg, respectively. This difference may be explained by the different dose used (1 mg/kg versus 10/40 mg/kg), the difference in infusion speed (bolus versus 10 min infusion) and the differences in experimental set-up to measure the anti-nociceptive effect (hot-lamp tail-flick latency assay versus electrical stimulation vocalisation method). With anti-nociceptive effect measurements only a limited number of data points can be obtained, which may limit a detailed PK-PD analysis.

Morphine induces both analgesia and sedation. Changes in EEG are often used as a measure to reflect the depth of sedation or anaesthesia (Stanski 1992). As EEG effect

measurements have the advantage of being continuous, sensitive, objective and reproducible, EEG has been used in this study to investigate the influence of biophase distribution on the PK-PD relationship of morphine. Between rats, only very small differences were observed in baseline (E_0) values, indicating the robustness of the EEG model. The method, either EEG or EEG/MD, had no influence on the E_0 and $E_{\max}$.

A profound counter clockwise hysteresis was observed for the concentration-effect relationships of each group, which may result from the formation of metabolites that influence the effect of morphine. In rats, only the metabolite M3G is formed in significant amounts. Since the affinity of M3G for the μ -opioid receptor is much lower compared to morphine (Bartlett & Smith 1995; de Jong *et al.* 2005; Loser *et al.* 1996), it was concluded that the influence of M3G on the observed hysteresis could be neglected. Therefore, M3G was not quantitated in the present study. The hysteresis of the EEG effects of morphine was characterized on the basis of two biophase distribution models: A) the one-compartment biophase distribution model, also known as the effect-compartment model and B) the extended-catenary biophase distribution model. The biophase distribution kinetics could neither be described with the symmetrical nor the asymmetrical one-compartment biophase distribution model, indicating that the biophase distribution process of the EEG effect included multiple distribution processes. Therefore the extended-catenary biophase distribution model was developed. This model consists of two sequential biophase-compartments; the transfer and the effect compartment. The extended-catenary biophase distribution model is based on a "tank-in-series" model as proposed by Upton and co-workers (Upton *et al.* 2000). This model provides a simple method for accounting for dispersion of drug in the transit through the brain (Upton *et al.* 1999). The concentrations in the effect compartment were related to the EEG effects, defined by the rate constant for transport through the transfer compartment (k_{1e}) and for loss from the effect compartment (k_{e0}). These rate constants are distinctly different since only the k_{e0} could be influenced by co-infusion of the specific and potent Pgp inhibitor GF120918.

The biophase distribution observed for morphine is more complex compared to other opioids. For alfentanil, the biophase distribution was too fast to be identified, while for fentanyl and sufentanil, the hysteresis could be described with simple k_{e0} values of 0.32 min^{-1} and 0.17 min^{-1} , for fentanyl and sufentanil, respectively (Cox *et al.*, 1998). These observations confirm that application of morphine as an anaesthetic is more difficult compared to fentanyl-like opioids.

The pharmacodynamic parameters of the EEG effects of morphine could be accurately described with the sigmoidal $E_{\max}$ model. All EEG experiments were performed in the presence of a steady state infusion of midazolam to prevent opioid induced seizure activity. Since a constant midazolam concentration was present in all treatment groups, the comparison of the biophase distribution and EEG effect is still valid. In addition, midazolam is a weak Pgp inhibitor, but is not transported by Pgp (Mahar Doan *et al.*

2002) and therefore the influence of midazolam could be neglected.

The next step was to correlate the biophase distribution kinetics with the previously investigated brain ECF distribution pharmacokinetics (Groenendaal *et al.*, 2007 – chapter 5). To describe the brain ECF distribution kinetics a non-linear transport model was proposed consisting of one brain compartment with distinction between passive diffusion, active linear efflux which is partly mediated by Pgp and active saturable influx by a yet unknown transport mechanism. In contrast, the extended-catenary biophase distribution model consists of two sequential compartments. There were no indications for non-linearity in the biophase distribution kinetics of morphine. The difference between the models indicates that transport into the brain ECF is distinctly different from transport to the effect-site. Transport into the brain ECF is dependent on both passive diffusion and active saturable influx, whereas for biophase distribution the transport to the effect-site is a linear process. The effects of GF120918 on the distribution kinetics of morphine in the effect compartments were consistent with recent observations on the distribution in brain ECF (Groenendaal *et al.*, 2007 – chapter 5). When comparing the concentration-time profiles in brain ECF and biophase, it could be noted that they were distinctly different (figure 6). The concentration in brain

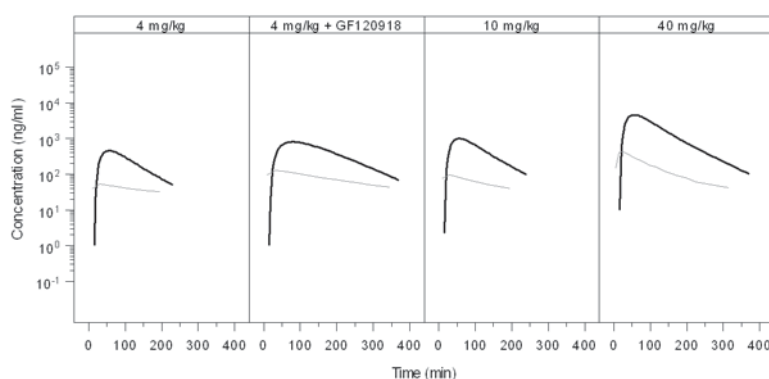


Figure 6: Comparison of the population predicted biophase concentration-time profiles (black lines) and the population predicted brain ECF fluid concentration-time profiles (grey lines) as obtained previously (see companion paper). It is shown that the time-course of the biophase concentrations differs substantially from the time-course of the brain ECF concentrations indicating that biophase equilibration is slower than transport into the brain ECF.

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 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 distinctly different from that for the EEG effect.

A discrepancy between the predicted effect-site concentration and the measured CNS time course has also been observed for the EEG effects of amobarbital, where the amobarbital effect-site concentrations did not reflect the measured cerebrospinal fluid concentrations (Mandema *et al.* 1991). 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 k_{eo} 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.

In conclusion, the biophase distribution kinetics of morphine was adequately described with the extended-catenary biophase distribution model. Comparison with the previously developed non-linear distribution model for morphine distribution into the brain showed that the time-course of morphine at the site of action in the brain is distinctly different from the brain ECF concentrations as estimated by intracerebral microdialysis.

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