RESEARCH PAPER

Influence of biophase distribution and P-glycoprotein interaction on pharmacokinetic-pharmacodynamic modelling of the effects of morphine on the EEG

D Groenendaal[1,4], J Freijer[2], D de Mik[1], MR Bouw[3], M Danhof[1,2] and ECM de Lange[1]

[1]*Division of Pharmacology, Leiden Amsterdam Center for Drug Research, Leiden University, Leiden, The Netherlands;* [2]*LAP&P Consultants BV, Archimedesweg 31, Leiden, The Netherlands and* [3]*GlaxoSmithKline, Clinical Pharmacokinetics, Harlow, UK*

Background and purpose: The aim was to investigate the influence of biophase distribution including P-glycoprotein (Pgp) function on the pharmacokinetic-pharmacodynamic correlations of morphine's actions in rat brain.

Experimental approach: Male rats received a 10-min infusion of morphine as $4 \, mg \, kg^{-1}$, combined with a continuous infusion of the Pgp inhibitor GF120918 or vehicle, 10 or $40 \, mg \, kg^{-1}$. EEG signals were recorded continuously and blood samples were collected.

Key results: Profound hysteresis was observed between morphine blood concentrations and effects on the EEG. Only the termination 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_{eo}) decreased 60% after GF120918. The EEG effect was directly related to 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 $45.0 \, \mu V$, $44.5 \, \mu V$, $451 \, ng \, ml^{-1}$ and 2.3, respectively.

Conclusions and implications: The effects of GF120918 on the distribution kinetics of morphine in the effect compartment were consistent with 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.

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Introduction

Mechanism-based pharmacokinetic–pharmacodynamic (PK–PD) models for the central action of opioids contain expressions for (a) blood PK, (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 focused on rather empirical biophase distribution models. Bouw *et al.* (2000) have proposed a single biophase compartment model to account for the delay of the anti-nociceptive effect of morphine relative to corresponding plasma concentrations. Transport across the BBB accounted 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 pretreatment with the specific Pgp inhibitor GF120918, the anti-nociceptive effect of morphine

Correspondence: Dr ECM de Lange, Division of Pharmacology, Leiden/ Amsterdam Center for Drug Research, Leiden University, PO Box 9502, RA Leiden 2300, The Netherlands.
E-mail: l.lange@lacdr.leidenuniv.nl
[4]Current address: Astellas Pharma Europe BV, Leiderdorp, The Netherlands.
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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., 2003).

Recently, the brain distribution of morphine has been characterized in greater detail with intracerebral microdialysis (MD) (Groenendaal et al., 2007, companion paper). Brain distribution was nonlinear and was successfully described by a complex brain distribution model with specific expressions 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 characterize, in a mechanistic manner, the biophase distribution kinetics of morphine in a PK–PD investigation.

Detailed characterization of the biophase distribution kinetics requires the availability of high-density PD 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). Quantitative EEG parameters have been widely used as a PD end point in preclinical and clinical investigations on the PK–PD correlations of a variety of central nervous system (CNS) active drugs. The synthetic opioid alfentanil, which is frequently used in anaesthesia produces a progressive slowing of the EEG with a predominant increase in the delta frequency band (0.5–4.5 Hz) of the EEG power spectrum in both animals (Young and Khazan, 1984; Wauquier et al., 1988; Mandema and Wada, 1995; Cox et al., 1997) and humans (Wauquier et al., 1984; Young and Khazan, 1984; Scott et al., 1985). 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, an increase in the delta frequency band of the EEG reflected 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 (Scott et al., 1985, 1991; Lemmens et al., 1995; Egan et al., 1996).

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 MD.

Methods

The PKs in blood and PDs 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, companion paper).

Surgical procedures
Details of the anaesthetic and surgical procedures have been described previously (Groenendaal et al., 2007, companion

paper). For the EEG experiments, seven cortical electrodes were stereotaxically implanted into the skull of rats 10 days before the start of the experiments as described before (Cox et al., 1997). 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_1, C_1, 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, companion paper). The EEG signal was continuously monitored via bipolar EEG leads on the left hemisphere (C_1–O_1) 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, UK). The digitized signal was transferred into a Pentium III computer and stored on hard disk for offline analysis. For each 5 s epoch, quantitative EEG parameters were obtained offline by fast Fourier transformation with a user-defined script within the software package Spike2 for Windows, version 3.18 (CED, Cambridge, UK). Changes in the amplitudes in the δ-frequency band of the EEG (0.5–4.5 Hz) averaged over 1 min time intervals were used as a PD end point. 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 of visual inspection of the 1 min datafile.

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

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).
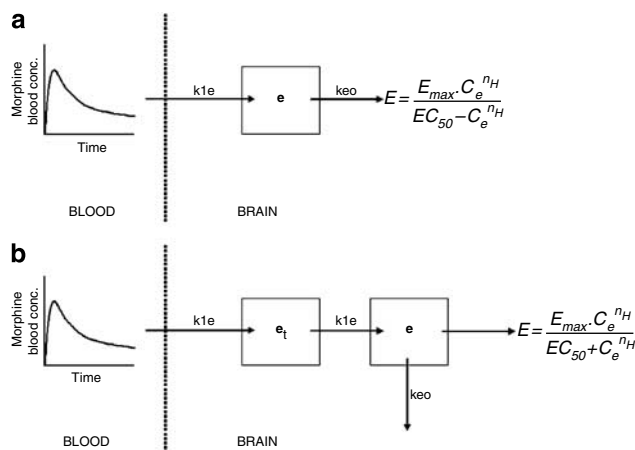
Figure 1 A schematic overview of the one-compartment distribution model (**a**) and the extended-catenary biophase distribution model (**b**) to describe the biophase kinetics of morphine. The blood PKs were described with a three-compartment model and used as input function for morphine in the brain. 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 E_{max} model. In the figure, 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 and E_{max} represent the intrinsic activity and EC_{50} represents the potency.

One-compartment biophase distribution model. Hysteresis is often characterized 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 the equation

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

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

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

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

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 *et al.* (2000) and provides a simple method for accounting for dispersion of drug in transit

through the brain. The rate of change of the concentrations in the effect compartments can then be described as follows:

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

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

where C_{et} 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.

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:

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

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 MD 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

$$P_i = \theta_1(1 - \text{METHOD}_i) + \theta_2(\text{METHOD}_i) \qquad (6)$$

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

$$P_i = \theta_3(1 + \theta_4 \cdot GF120918_i) \qquad (7)$$

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 is vehicle is co-infused.

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

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

with

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

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 lognormal 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 the equation

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

with

$$\eta_i \sim N(0, \omega^2) \tag{11}$$

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 the equation

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

where $C_{\mathrm{obs},ij}$ is the jth observation of the ith individual, $C_{\mathrm{pred},ij}$ is the predicted concentration and ε_{ij} is a realization from the normally distributed residual random variable with mean zero and variance σ^2

$$\varepsilon_i \sim N(0, \sigma^2) \tag{13}$$

Data analysis
The details of the general modelling procedures have been described previously (Groenendaal *et al.*, 2007, companion paper). The EEG effects of morphine were analysed using nonlinear mixed effect modelling as implemented in the NONMEM software version V, level 1.1 (Beal and Sheiner, 1999). Population analysis was undertaken using the first-order conditional estimation method (FOCE interaction).

Individual PK parameter estimates were used as input for the PD models. Individual blood concentrations were calculated at the times of the EEG measurements.

Results

PDs 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 μV and was observed around 20 min 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 min following the infusion of 4 and 10 mg kg^{-1} morphine whereas after a dose of 4 mg kg^{-1} combined with GF120918, or 40 mg kg^{-1} morphine alone, the duration of the effect was around 360 min. In Figure 2, the PKs and the PDs 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 counterclockwise.

The selection of the biophase distribution model
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 asymmetrical effect compartment models was tested. With the symmetrical ($k_{1e} = k_{eo}$) biophase distribution no results were obtained (minimization terminated), whereas with the asymmetrical ($k_{1e} \neq k_{eo}$) 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
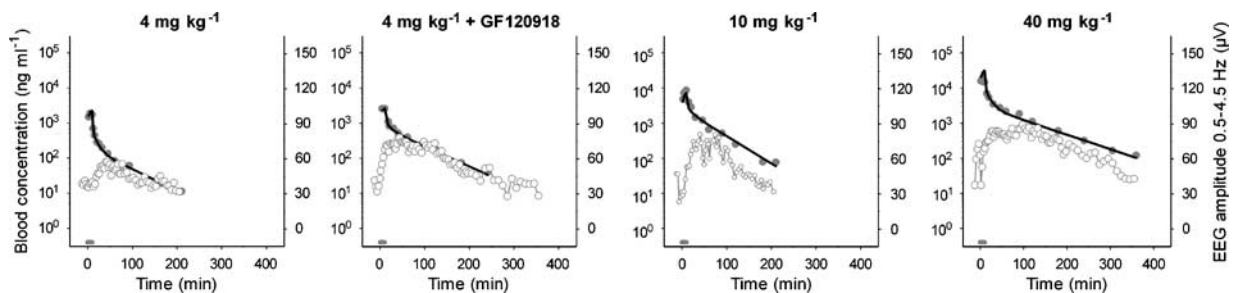
Figure 2 PKs and PDs of a typical rat after administration of the opioids. Observed blood concentrations (grey dots), individual predicted blood concentrations (black line) and observed EEG effect (grey open dots) are depicted for each dose group. The grey bar indicates the infusion time.

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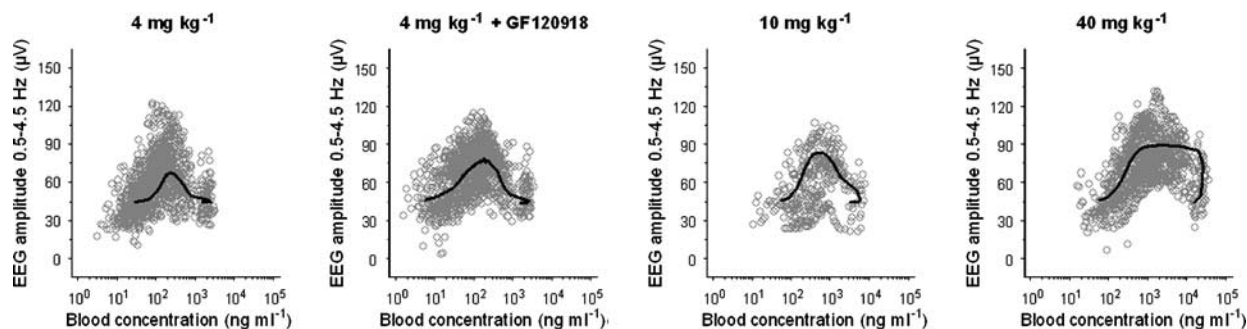
Figure 3 PK–PD relationship after administration of morphine. Observed (grey dots) and population predictions (black line) are depicted for each dose group. A clear counterclockwise hysteresis loop was observed for all morphine doses.

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
Structural model			
k_{1e} (min^{-1})	0.0378	8.4	0.0315–0.0441
k_{eo} (*min^{-1}*)			
−GF120918	0.0426	10.0	0.0342–0.0510
+GF120918[a]	0.0152		
Pgp inhibition factor	−0.644	−7.3	−0.736––0.552
Interindividual variability			
$\omega^2\ k_{eo}$	0.237	20.2	0.143–0.331

Abbreviations: CV%, coefficient of variation; k_{1e}, rate constant for transport to the effect-site; k_{eo}, rate constant for the loss from the effect-site; LLCI, lower limit of confidence interval; ULCI, upper limit of confidence interval.
[a]$k_{eo}(+\text{GF120918}) = k_{eo}(-\text{GF120918}) \cdot (1 + \text{Pgp inhibition factor})$.

(Table 1). The value of the rate constant for transport through the transfer compartment (k_{1e}) was unaffected by the co-administration of GF120918. The population predictions of blood and biophase concentration–time profiles are shown in Figure 4. 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 indicating that in the presence of GF120918, the k_{eo} was decreased by 64%. The inter-animal variability (ω^2) on k_{eo} was estimated with an exponential error model. The inter-animal variability could not be estimated for the other parameters and were therefore fixed to zero.

PK–PD analysis of the EEG effect
The individual predicted biophase concentrations were related to the EEG effect on the basis of the sigmoidal E_{max} PD model. As the EEG and EEG-MD experiments were performed in parallel, covariate analysis was included to investigate the influence of the MD probe on the PD 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. Co-infusion of GF120918 did not influence the PD parameters. Inter-animal variability (ω^2) was estimated with a proportional error model for E_0 and E_{max}. 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.

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 MD.

So far the PK–PD investigations of morphine have focussed on the anti-nociceptive effects (Letrent *et al.*, 1998; Bouw *et al.*, 2000). 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_{eo}. In these models, a wide difference in k_{eo} values (hysteresis) was observed; the k_{eo} values were 0.228 and 0.022 min^{-1}, for the doses of 1 mg kg^{-1} and 10 or 40 mg kg^{-1}, respectively. This difference may be explained by the different dose used (1 mg kg^{-1} versus 10 or 40 mg kg^{-1}), 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 vocalization 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)
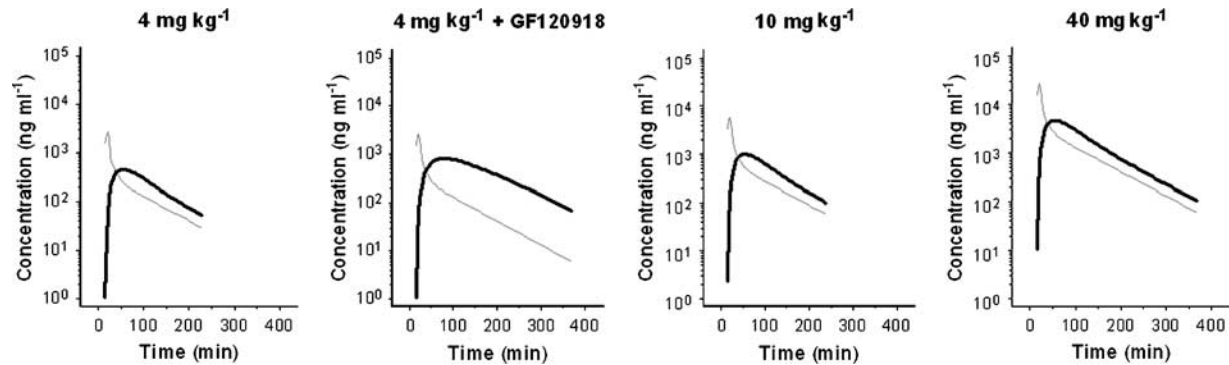
Figure 4 Population predicted blood and biophase concentration–time profiles of morphine obtained with the extended catenary biophase distribution model. The grey lines represent the blood concentration–time profiles and the black lines represent the biophase concentration–time profiles.
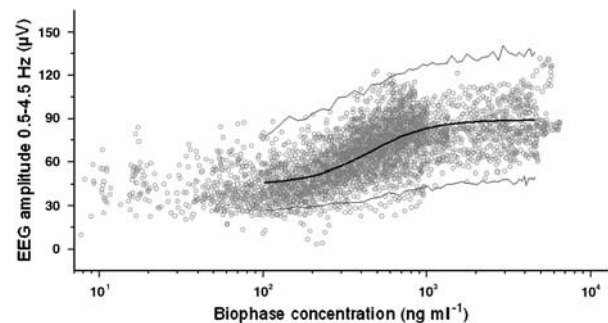
Table 2 Population pharmacodynamic parameter estimates of morphine EEG effect obtained with the sigmoidal E_{max} model after hysteresis minimisation with the extended catenary biophase distribution model

Parameter	Estimate	CV%	LLCI–ULCI
Structural model			
E_0 (μV)	44.6	2.3	42.6–46.6
E_{max} (μV)	44.5	8.0	37.5–51.5
EC_{50} (ng ml^{-1})	451	17.3	298–604
n_H	2.32	10.4	1.85–2.79
Interindividual variability			
$\omega^2 \, k_{E0}$	0.034	17.8	0.022–0.045
$\omega^2 \, k_{Emax}$	0.121	24.1	0.064–0.178
Residual variability			
Proportional error	0.027	7.6	0.023–0.031

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

Figure 5 PK–PD relationships of the opioids after hysteresis minimization 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 E_{max} model to relate the biophase concentrations to the EEG effect.

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 and Smith, 1995; Loser et al., 1996; de Jong et al., 2005), 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 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_{eo}). These rate constants are distinctly different since only the k_{eo} could be influenced by co-infusion of the specific and potent Pgp inhibitor GF120918.

The biophase distribution observed for morphine is more complex than those of 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_{eo} values of 0.32 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.

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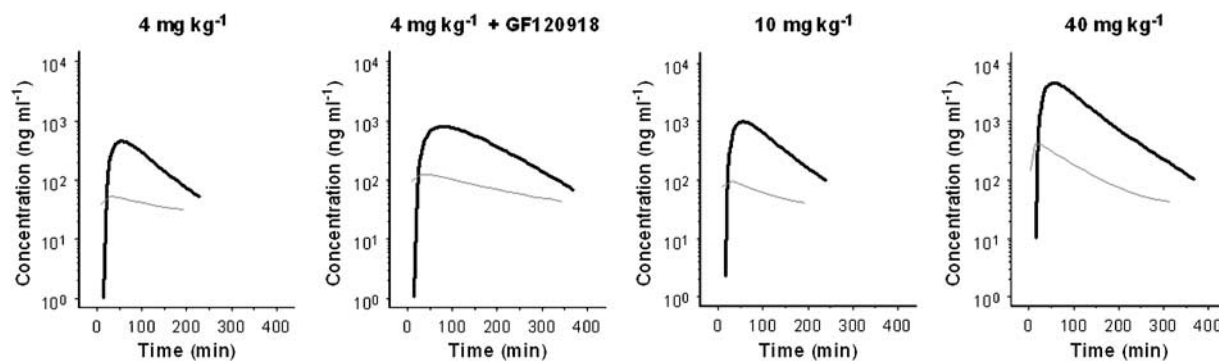
Figure 6 Comparison of the population predicted biophase concentration–time profiles (black lines) and the population predicted brain ECF fluid concentration–time profiles (grey lines) as obtained previously (see Groenendaal et al., 2007, companion paper). The time-course of the biophase concentrations differs substantially from the time course of the brain ECF concentrations indicating that biophase equilibration is slower than transport into the brain ECF.

The PD 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 PKs (Groenendaal et al., 2007, companion paper). To describe the brain ECF distribution kinetics, a nonlinear 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 nonlinearity 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, companion paper). When comparing the concentration–time profiles in brain ECF and biophase, it was noted that they were distinctly different (Figure 6). 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 et al. (2000) where 85% of the observed hysteresis for the anti-nocicep-

tive effect could be explained by distribution into the brain ECF. In addition, Bouw et al. (2000) 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 et al. (2004) 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. 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 nonlinearity was observed at the low dose ($4\,mg\,kg^{-1}$). Chenel et al. (2004) 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 nonlinear 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 MD.

Conflict of interest

The authors state no conflict of interest.

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