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Mechanism-based pharmacokinetic-pharmacodynamic modeling of the efficacy and safety of (semi-)synthetic opioids

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**MECHANISM-BASED
PHARMACOKINETIC-PHARMACODYNAMIC
MODELING OF THE EFFICACY AND SAFETY OF
(SEMI-)SYNTHETIC OPIOIDS**

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MODELING OF THE EFFICACY AND SAFETY OF
(SEMI-)SYNTHETIC OPIOIDS**

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*"The mediocre teacher tells.
The good teacher explains.
The superior teacher demonstrates.
The great teacher inspires."*

William Arthur Ward

*Ter nagedachtenis aan mijn vader
Voor mijn moeder
Aan Nurçan*

The preclinical investigations described in this thesis were performed at the Division of Pharmacology of the Leiden/Amsterdam Center for Drug Research, Leiden University, Leiden, The Netherlands. The clinical Phase I studies were conducted in the Anesthesia and Pain Research Unit at the Department of Anesthesiology, Leiden University Medical Center, Leiden, The Netherlands.

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SECTION

I

GENERAL INTRODUCTION

Chapter

1

SCOPE AND OUTLINE OF THE THESIS

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BACKGROUND

Worldwide millions of people suffer from (severe) pain and are dependent on the intake of analgesic drugs. Different pain syndromes require a different approach to treatment. In contemporary medicine, strong opioid analgesics remain the cornerstone for the treatment of nociceptive pain of an acute (postoperative) or malignant origin. For many decades morphine was considered the first choice opioid for the treatment of acute and chronic (malignant) pain. Despite their analgesic effectiveness, morphine and other full μ -opioid agonists like fentanyl and methadone, have many side effects (Bowdle, 1998; Gan, 2006). Most of these side effects are mild of nature. However, acute respiratory depression remains a serious complication with a potentially fatal outcome (Baxter, 1994; Bailey *et al.*, 2000). At present, the design of opioids with improved efficacy-safety balances remains a major challenge in the development of novel opioid analgesics. In theory, partial agonists have a greater selectivity of action, and can be more easily titrated to the desired effect, without an increased risk of serious side effects (Van der Graaf *et al.*, 1999).

Buprenorphine is a semi-synthetic opioid analogue derived from the precursor thebaine (figure 1). In a variety of *in vitro* receptor binding test systems buprenorphine was characterized as a partial agonist at the μ -opioid receptor (Huang *et al.*, 2001). As such an improved selectivity of action profile might be expected for buprenorphine. However, the clinical utility of the improved balance between efficacy and safety is of minimal value when partial agonism for the analgesic effect coincides with partial agonism for the respiratory depressant effect. In other words, enhanced tissue selectivity for buprenorphine must be demonstrated in order for it to be an effective opioid analgesic for the treatment of severe pain.

The application of pharmacokinetic-pharmacodynamic (PK-PD) modeling in drug development is well established. The primary objective of PK-PD modeling is prediction of the time course of the drug effect *in vivo* (intensity and duration) in health and disease (Breimer and Danhof, 1997). As such PK-PD constitutes the scientific basis for optimization of the dosing and delivery profile of drugs in phase-2 clinical trials. Furthermore PK-PD modeling is also widely applied as the basis for the design (by clinical trial simulation) and the evaluation of phase-3 clinical trials (Peck *et al.*, 1994; Breimer and Danhof, 1997). In the meantime PK-PD modeling is increasingly applied in drug discovery and pre-clinical development. Here the objective is the prediction of the PK-PD properties of novel drugs in man on the basis of information from (high throughput) *in vitro* bioassays and *in vivo* animal studies (Danhof *et al.*, 2005, 2007a,b). Specific applications in this context include a) the selection of drug candidates with a predicted optimal PK-PD profile in man, b) acquisition of pertinent information for lead optimization and c) the use of preclinical PK-PD information in optimizing early clinical trials.

Thus the use of PK-PD modeling in drug discovery and early development relies on the prediction, in a strictly quantitative manner, of PK-PD properties in man. Not sur-

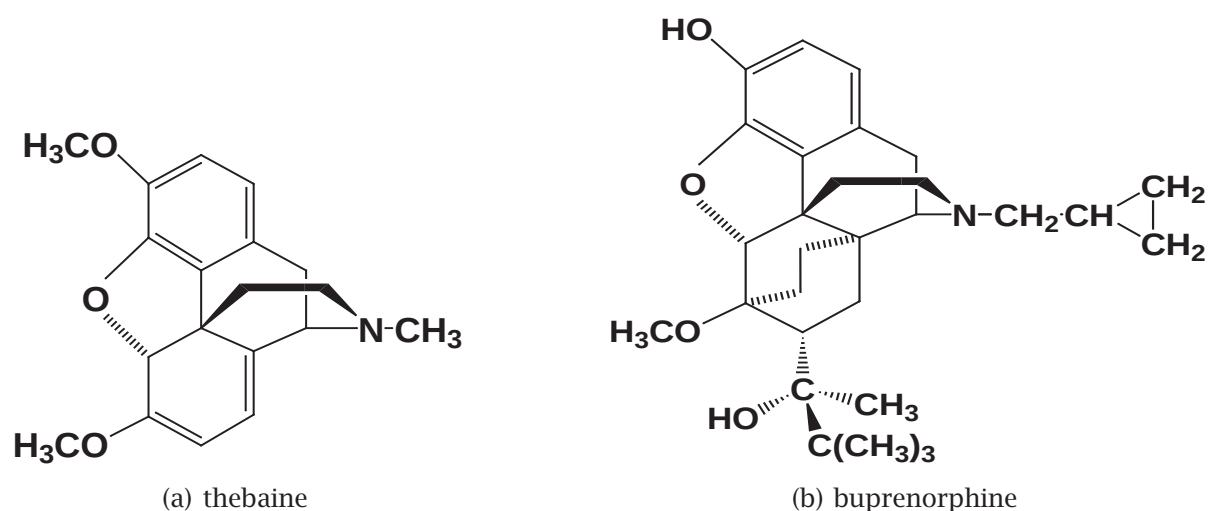


Figure 1. Chemical structures of the precursor thebaine and buprenorphine. The chemical name for buprenorphine is N-cyclopropylmethyl-7a-(1-S-hydroxy,1,2,2-trimethyl-propyl)-6,14-endoethano-6,7,8,14-tetrahydronororipavine. Buprenorphine has an empirical formula of C₂₉H₄₁NO₄, with a molecular weight of 467.6 g/mol. Buprenorphine hydrochloride occurs as a white crystalline powder, and is soluble in water (17 mg/ml) and alcohol (44 mg/ml) at room temperature (Reynold, 1982). This compound has two pK_a values of 8.2 and 9.9, corresponding to the amine and phenol chemical group, respectively (Garrett and Chandran, 1985).

prisingly there is a clear trend towards mechanism-based PK-PD models, which have much improved properties for extrapolation and prediction. Mechanism-based PK-PD models differ from empirical, descriptive models in that they contain specific expressions for processes on the causal path between drug administration and effect (Danhof *et al.*, 2007a,b). In recent years, several pharmacokinetic-pharmacodynamic (PK-PD) models have been proposed to characterize the time course of the analgesic and anesthetic effects of opioids *in vivo* using a variety of different pharmacodynamic endpoints including quantitative EEG parameters as surrogates for depth of anesthesia (Stanski, 1992; Cox *et al.*, 1998). In the various investigations, there has been a clear trend towards the development of mechanism-based PK-PD models. The incorporation of principles from receptor theory has been a key element in this development (Van der Graaf and Danhof, 1997).

At present mechanism-based PK-PD modeling of the effects of opioids on the respiratory system has not been accomplished. Another issue that has received little attention is the modeling of the slow receptor association/dissociation kinetics as a factor causing hysteresis between plasma concentration and effect. It is well established that buprenorphine's pharmacology is characterized by slow receptor association-dissociation kinetics at the μ -opioid receptor. This can be a complicating factor in optimizing the dosing regimen of buprenorphine and in the context of the reversal of buprenorphine-induced respiratory depression with naloxone (Gal, 1989).

The aim of the research described in this thesis was the development of a mechanism-based PK-PD model for the prediction of the efficacy and safety of buprenorphine, with

emphasis on the modeling of the slow receptor association-dissociation kinetics as a determinant of the time course of the pharmacological effects. Fentanyl was selected as a comparator opioid with full agonist activity at the μ -opioid receptor and fast receptor association/dissociation kinetics. The research described in this paper focuses on: 1) the characterization of the PK-PD relationships of buprenorphine and fentanyl for antinociception (efficacy) and respiratory depression (safety), 2) characterization of the PK-PD relationships of buprenorphine's major metabolite norbuprenorphine for respiratory depression and 3) characterization of the pharmacodynamic interaction between buprenorphine and the opioid antagonist naloxone. A unique property of our research on the selectivity of action of (semi-)synthetic opioids is that both preclinical data from rats and clinical data from healthy volunteers were used to determine the *in vivo* selectivity of action through PK-PD modeling. In this context, preclinical data were used to identify and validate the complex mechanistic PK-PD model using several buprenorphine dosages and infusion rates. In this respect it is important that the animal studies allow the testing of much wider dosing ranges, thereby exploring the pharmacological characteristics (i.e. full or partial agonist) of buprenorphine to the optimum. Subsequently, the gathered pharmacological and PK-PD information obtained from the preclinical studies was applied to clinical studies in healthy volunteers.

SCOPE AND OUTLINE OF THE THESIS

In the first section (**Chapter 2**) an overview is presented of mechanism-based PK-PD models with special emphasis to the integration of target association/dissociation in PK-PD analysis. In **chapter 3** a brief introduction is given on available techniques for the animal to human extrapolation of the pharmacokinetic and pharmacodynamic characteristics of drugs.

In **section II**, a mechanism-based PK-PD model is proposed for characterization of the time course of the antinociceptive effect of (semi-)synthetic opioids. Initially, this model was applied to the concentration-antinociceptive effect relationships of buprenorphine and fentanyl in rats (**chapter 4**). To this end, the antinociceptive effect was quantified on the basis of the tail flick latency test, with a maximum latency cut-off at 10 sec to avoid tissue damage. The mechanism-based model combined a pharmacodynamic model to characterize the antinociceptive effect of (semi-)synthetic opioids with a statistical distribution model to enable prediction of censored antinociceptive outcomes. Moreover a combined biophase distribution-receptor association-dissociation model was applied to account for hysteresis between plasma concentration and effect. The proposed mechanism-based PK-PD model was subsequently applied to pertinent data on the antinociceptive effect in man to determine the *in vivo* concentration-antinociceptive effect relationship and to estimate the various rate constants which determine the time course of this effect in **chapter 5**. In this manner, the combined biophase distribution-receptor association/dissociation model

could be identified, yielding precise estimates of the rate constants of biophase equilibration and receptor association/dissociation. The results of this analysis showed that buprenorphine displays full agonistic activity in the studied 'therapeutic' concentration range.

Section III focuses on the PK-PD modeling of the respiratory depressant effect of buprenorphine and fentanyl. For this purpose, a novel animal model for respiratory depression was developed in **chapter 6**. PK-PD analysis revealed that both biophase distribution and receptor association/dissociation kinetics contribute to the observed hysteresis between plasma concentration and respiratory depression. In addition, buprenorphine was shown to act as a partial agonist for respiratory depression (ceiling effect), while fentanyl displays full agonistic respiratory depressant activity. In **chapter 7**, a nearly identical biomarker for respiratory depression was used to characterize the respiratory depressant effect of buprenorphine and fentanyl in healthy volunteers. Also in these investigations buprenorphine displayed ceiling respiratory depressant effect, while fentanyl induced apnea (100 % respiratory depression). The similarities in the estimated values of the rate constants of receptor association/dissociation between the animal and human investigations indicate that these rate constants are indeed drug-specific parameters. This indicates that the proposed mechanism-based PK-PD model could be used for animal to human extrapolation. The investigations in **chapter 8** focus on characterization of the PK-PD correlation of norbuprenorphine, the main metabolite of buprenorphine, both with regard to partial agonism and the rate constants for receptor association-dissociation. In animals, norbuprenorphine behaved as a full agonist displaying full respiratory depressant activity. Furthermore, through PK-PD modeling it was shown that following the administration of buprenorphine, only a small fraction of buprenorphine is converted into norbuprenorphine. Consequently, due to norbuprenorphine's low potency for respiratory depression and on the assumption of a competitive interaction, norbuprenorphine plasma concentrations appear to be low to contribute to the overall respiratory depressant effect of buprenorphine in humans.

Section IV discusses the application of the combined biophase distribution-receptor association/dissociation model in the integrated PK-PD analysis of buprenorphine's efficacy and safety outcomes. In **chapter 9** a PK-PD model is proposed for the characterization of the efficacy/safety balance of the opioids buprenorphine and fentanyl. To this end, therapeutic utility functions were constructed to assess the efficacy/safety balances of buprenorphine and fentanyl in rats. For buprenorphine, it was shown that the therapeutic utility function is positive in the therapeutic concentration range, suggesting a positive efficacy-safety balance, while for fentanyl the utility function was negative in the relevant concentration range. In **chapter 10** the combined biophase distribution-receptor association/dissociation model was applied to the animal to human extrapolation of buprenorphine's pharmacological effects at the μ -opioid receptor. To this end, the animal and human antinociceptive and respiratory depressant effect data were simultaneously analyzed. The outcome of the analysis showed that the rate constants of

receptor association and dissociation are indeed identical between species, but that the values differed between the antinociceptive and the respiratory depressant effect. The rate constant of biophase distribution was scaled from animal to human using allometry. Interestingly, the estimated exponent of the allometric function was -0.28, which is nearly identical to the value -0.25, which is generally believed to be appropriate for interspecies scaling of rate constants. In **chapter 11** a mechanism-based pharmacodynamic interaction model was proposed to characterize the complex interaction between buprenorphine and naloxone at the μ -opioid receptor. PK-PD analysis confirmed the difficulty of the reversal of buprenorphine-induced respiratory depression by naloxone using clinically well-established naloxone doses. Using the mechanism-based PK-PD modeling approach, new naloxone dosing regimens have been designed and tested prospectively in healthy volunteers. The results showed that reversal of respiratory depression can be accomplished when naloxone is administered as a continuous infusion rather than as intravenous bolus doses.

Finally, in **Section V, chapter 12** all the results of the various investigations are discussed and future perspectives are presented.

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Chapter 2

MECHANISM-BASED PHARMACOKINETIC-PHARMACODYNAMIC MODELING: TOWARDS THE INCORPORATION OF TARGET ASSOCIATION AND DISSOCIATION KINETICS

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INTRODUCTION

Pharmacology embraces the disciplines of pharmacokinetics (PK) and pharmacodynamics (PD). Pharmacokinetics describes the relationship between the dose of a drug and the time course of its concentration in the various body fluids and tissues, usually plasma, encompassing the biological processes of absorption, distribution, metabolism and excretion (ADME) (Bickel, 1996). Pharmacodynamics studies the mechanisms by which xenobiotics exert their biochemical and physiological effect in the living organism (Holford and Sheiner, 1982). For a long time, pharmacokinetics and pharmacodynamics were investigated separately and as consequence dose regimes were usually derived from the analysis of dose-effect relationships. However, over the years it has become clear that pharmacological effect correlates better with concentration than with dose and that more important relationships can be established between the concentration of a drug and the magnitude of the pharmacodynamic effect. Since then a more differentiated consideration of this relationship led to the systematic connection between pharmacokinetics and pharmacodynamics.

In the late 1970s the first principles of PK-PD modeling were introduced, which enabled the characterization of exposure *versus* pharmacological effect relationships in a strict quantitative manner (Sheiner *et al.*, 1979). Ever since, PK-PD modeling has evolved largely to become a highly appreciated and powerful tool which facilitates better understanding of drug efficacy and safety (Breimer and Danhof, 1997). PK-PD modeling is increasingly applied in drug discovery and (pre-)clinical development (Miller *et al.*, 2005; Danhof *et al.*, 2007a,b). Traditionally, drug development programs encompass sequential phases starting from lead finding/optimization to first-in-human studies and ultimately pivotal phase III clinical trials ending when all gathered information is submitted to regulatory authorities. Over the last years, PK-PD modeling has achieved a high level of acceptance in all stages of drug development to streamline the information gain and to support decision-making with the aim to increase the speed and success of drug development. To this end PK-PD modeling is very useful in (1) biomarker selection (Danhof *et al.*, 2005; Rolan *et al.*, 2007), (2) extracting information from data supporting 'go/no go' decisions and proof of concept studies (Gieschke and Steimer, 2000) and (3) optimizing clinical trial design (Sheiner, 1997).

Not only from the perspective of pharmaceutical industry the importance of PK-PD modeling in drug development is recognized, but also regulatory authorities are becoming more actively engaged in conducting PK-PD analysis to support and aid regulatory decisions (Machado *et al.*, 1999). Nowadays, population PK and PK-PD analysis are a regular part of regulatory submissions. It is expected that the trend in drug development will shift towards an *in silico* oriented development process, driven by both economic necessity and regulatory scrutiny. To this end, the application of integrated PK-PD models in drug development may facilitate selection of effective and safe dosage regimens tailored

to each patient's need and decision-making at key transition steps in drug development.

PHARMACOKINETIC MODEL

Pharmacokinetic-pharmacodynamic modeling comprises the use of mathematical and/or statistical models to describe and predict exposure-response relationships. Basically, PK-PD models consists of three components 1) a pharmacokinetic model to describe the time course of drug concentrations, 2) a pharmacodynamic model to characterize the relationships between drug concentration and pharmacological effect and 3) a link model to account for time-dependencies in pharmacodynamics or the use of time variant models to model tolerance or sensitization.

Pharmacokinetics in plasma

On the basis of their structure pharmacokinetic models can be subdivided into: i) non-compartmental PK models, ii) compartmental PK models and iii) physiologically-based PK models. Non-compartmental pharmacokinetic models require minimal *a priori* assumptions on the structure of the pharmacokinetic model and are used to describe the drug disposition characteristics in terms of C_{max} , T_{max} and AUC and the pharmacokinetic parameters volume of distribution at steady-state (V_{SS}) and clearance (Cl). However, non-compartmental PK models are of limited use for the characterization of the time course of drug concentration and are therefore of minimal value in PK-PD modeling.

To this end, the compartmental or physiologically-based pharmacokinetic models have more merit. Typically, pharmacokinetic modeling utilizes the time course of the drug concentration in plasma as a measure of internal exposure. This is important because drug concentration *versus* time profiles can differ widely between drugs, and for the same drug, between species and individuals. Compartmental models are the most commonly used pharmacokinetic models to describe the time course of the drug concentration in plasma. In a compartmental model, drug disposition is characterized as the transfer of drug between interconnected hypothetical compartments, which serves to mimic the drug absorption, distribution, and elimination processes. A limitation of this approach is that, although useful for descriptive purposes, it is not truly mechanistic. As a result, it is of limited value for extrapolation and prediction. This is particularly the case in relation to the interspecies extrapolation.

Physiologically based (PB)-PK modeling has been proposed to improve interspecies extrapolation of the pharmacokinetics (Blakey *et al.*, 1997; Rowland *et al.*, 2004; Rolan *et al.*, 2007). However, discussion of the various PB-PK modeling concepts is beyond the scope of this paper (Charnick *et al.*, 1995; Poulin and Theil, 2002; Nestorov, 2003), which focuses on the prediction of drug effects rather than concentrations.

Biophase distribution kinetics

PK-PD modeling studies typically utilize drug concentrations in plasma as a measure of internal exposure to the drug. Most drugs however have a target in peripheral tissues. For drugs producing their biological effect in a peripheral tissue, distribution to the site of action may represent a rate-limiting step for producing the biological effect. Typically this is reflected in a delay in the time course of the pharmacological effect relative to the plasma concentration, which is commonly referred to as 'hysteresis'. In order to account for hysteresis in PK-PD investigations the so-called effect compartment model has been introduced (Sheiner *et al.*, 1979). In this model the distribution of drug to a hypothetical effect site is described by the following differential equation:

$$\frac{dC_e}{dt} = k_{1e} \cdot C_p - k_{e0} \cdot C_e \quad (1)$$

where k_{1e} and k_{e0} are the first-order rate constants distribution into and out of the hypothetical effect compartment and C_p and C_e are the drug concentrations in plasma and the hypothetical effect compartment respectively. In this model the amount of drug entering the effect compartment is considered to be negligible and therefore not reflected in the pharmacokinetics of the drug. Furthermore, for reasons of identifiability, the values of k_{1e} and k_{e0} are usually set equal to each other. In this manner it is inherently assumed that in steady state the drug concentration in the biophase is identical to the (free) plasma concentration. In their seminal paper, Sheiner *et al.* (1979) demonstrated that for drugs that distribute to the site of action by passive diffusion, this can be a reasonable assumption. A number of semi-parametric and nonparametric approaches of modeling effect compartment distribution kinetics have been proposed. These approaches differ mainly with regard to the prior information required and the technique used for minimalization of the hysteresis between change in plasma concentration and change in effect (Fuseau and Sheiner, 1984; Unadkat *et al.*, 1986; Veng-Pedersen *et al.*, 1991; Veng-Pedersen and Modi, 1992). At the same time, the model has also been successfully applied in preclinical studies to derive meaningful *in vivo* drug concentration-effect relationships (Cox *et al.*, 1998).

Physiological biophase distribution model

Although for many drugs the assumption that in steady state the drug concentration in the effect compartment is identical to the (free) plasma concentration is plausible, this may not always be the case. Particularly for relatively large hydrophilic molecules and for compounds that are substrates for specific transporters the distribution to target site be restricted. This concerns especially drugs with an intracellular target (i.e. cytostatic drugs) and drugs, which act in tissues that are protected by specific barriers (i.e. the central nervous system) (Danhof *et al.*, 2005, 2007a,b). Recently several specific transporters

have been discovered which may restrict the access of a drug to the site of action (Jonker and Schinkel, 2004). For the drugs acting in central nervous system measures of target exposure can be indispensable in PK-PD modeling. However, very few PK-PD investigations have considered the functional role of these transporters in the biophase distribution. An interesting example is the investigation by Letrent *et al.* (1999) of the PK-PD correlation of the antinociceptive effect of morphine, in which the functional role of active extrusion at the blood-brain barrier was determined on the basis of the interaction with the selective P-glycoprotein inhibitor GF120918. A novel technique to obtain information on target site exposure in the central nervous system is by intra-cerebral microdialysis (de Lange *et al.*, 1997; Xie *et al.*, 1999). An example of the application of intra-cerebral microdialysis is in the investigations on the PK-PD correlation of morphine, where P-glycoprotein and possibly other transporters restrict the distribution into the central nervous system (Bouw *et al.*, 2001). Ultimately, this may lead to the development of novel mechanism-based biophase distribution modeling concepts (Groenendaal *et al.*, 2007a,b).

PHARMACODYNAMIC MODEL

Steady-state concentration-effect relationships

The simplest model which describes the relationship between concentration and effect is the linear model:

$$E = E_0 + S \cdot [A] \quad (2)$$

where E is the biological effect intensity, E_0 is the baseline effect in the absence of drug A and S is a slope parameter. However, in many cases the relationship between concentration and effect which can describe a range of effect is non-linear. To this end, the application of the linear model may well underestimate the intensity of the pharmacological effect. Therefore, in PK-PD modeling, steady-state drug concentration-effect relationships are commonly described using a hyperbolic function (i.e. the Hill equation):

$$E = \frac{\alpha \cdot [A]^n}{EC_{50}^n + [A]^n} \quad (3)$$

where E is the observed drug effect intensity, α is the observed maximum effect, $[A]$ is the drug concentration, EC_{50} is the drug concentration at half-maximal effect and n is the Hill factor, which is the parameter denoting the steepness of the concentration-effect curve. The usefulness of the Hill equation to describe *in vivo* drug concentration-effect relationships is well established. However, a limitation is that it is not mechanistic. Specifically, the Hill equation does not provide insight into the factors that determine the shape and the location of the concentration-effect relationship. In theory, the relationship between the concentration of a drug and the intensity of the biological response depends

on several factors related to the drug and the biological system (figure 1). Descriptive models are only applicable to restricted and well-defined conditions. In fact, the rather poor behavior of the descriptive models in terms of prediction and extrapolation make these models not useful for studying different study designs, scenarios or patient groups beyond the study design on which the original PK-PD model is established. Specifically, according to receptor theory, the potency (i.e. EC_{50}) and intrinsic activity (i.e. α) are dependent on properties of the drug (i.e. the receptor affinity and the intrinsic efficacy) and the biological system (i.e. the receptor density and the transducer function, relating receptor activation to pharmacological response). Therefore, the prediction of *in vivo* drug concentration-effect relationships requires the distinction between drug-specific and biological system-specific parameters (Van der Graaf and Danhof, 1997). Recently, a PK-PD modeling strategy has been developed based on concepts from receptor theory and that constitutes a scientific basis for the prediction of *in vivo* drug concentration-effect relationships (Danhof *et al.*, 2007a,b).

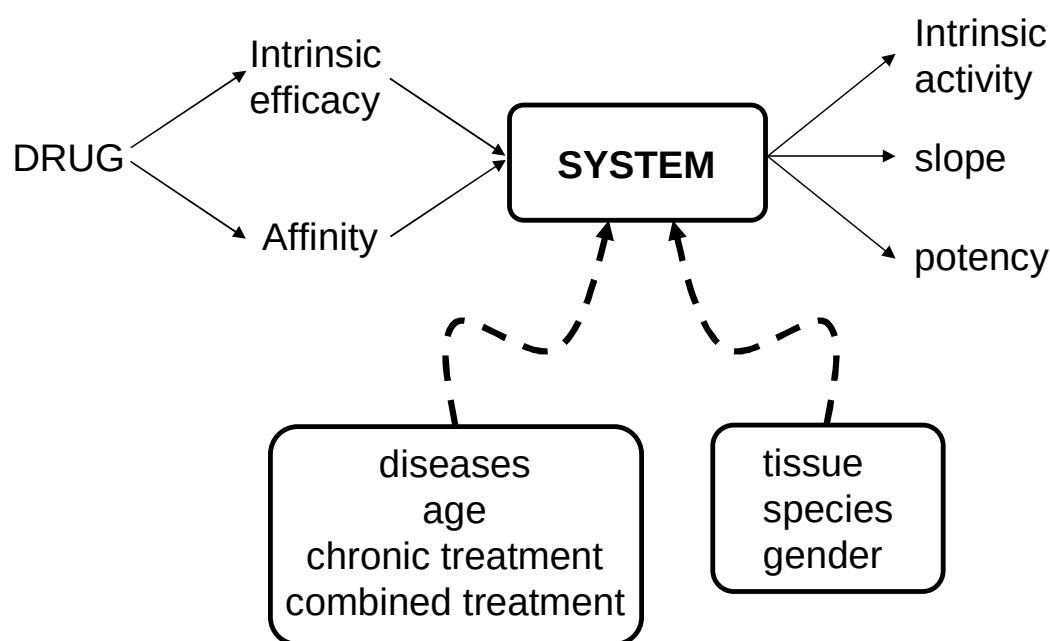


Figure 1. The shape and location of *in vivo* drug concentration-effect relationships is determined by drug-specific (affinity, intrinsic efficacy) and biological system-specific (transducer function relating receptor activation to effect) properties. Reproduced from (Van der Graaf and Danhof, 1997).

Receptor theory for the prediction of concentration-effect relationships

Classical receptor theory combines two independent parts to describe drug actions: (a) an agonist-dependent component, which describes the interaction between the drug and the biological system in terms of target affinity and activation, and (b) a biological system-dependent component, which is determined by receptor concentration and the nature of

the stimulus-response relationship. Typically the interaction of the drug with the biological system is described by a hyperbolic function:

$$\frac{[AR]}{[R_{tot}]} = \frac{[A]}{K_A + [A]} \quad (4)$$

where $[AR]$ is the concentration of agonist-receptor complex, $[R_{tot}]$ is the total receptor concentration, $[A]$ the agonist concentration and K_A is the dissociation equilibration constant of the agonist-receptor complex.

In contrast, the stimulus-response relationship can in principle take any shape. Dependent on the behavior of the drug in the biological system a linear, hyperbolic or an even more complex transduction function is selected. Black and Leff (1983) considered a hyperbolic stimulus-response relationship for systems with high receptor reserve, which can be represented as follows:

$$E = \frac{E_m \cdot [AR]}{K_E + [AR]} \quad (5)$$

where E is the response, E_m is maximum system response and K_E is the concentration of $[AR]$ that elicits half the maximal tissue response. Combining equation 4 with equation 5 yields:

$$E = \frac{E_m \cdot [R_{tot}] \cdot [A]}{K_A \cdot K_E + ([R_{tot}] + K_E) \cdot [A]} \quad (6)$$

Black and Leff postulated that the efficacy of a drug is determined by drug-dependent properties and system-specific characteristics represented by K_E and $[R_{tot}]$, respectively. Subsequently, a transduction constant $\tau = [R_{tot}]/K_E$ was introduced as a measure of efficiency of the transduction pathway. Since, τ is inherent to drug- and system-related characteristics, it should be noted that τ is not a direct measure of intrinsic efficacy, which is commonly defined as a drug-dependent property unique for each agonist-receptor complex. Henceforth, by redefining $[R_{tot}]/K_E$ as τ equation 6 can be rewritten as:

$$E = \frac{E_m \cdot \tau \cdot [A]}{K_A + [A] \cdot (\tau + 1)} \quad (7)$$

To demonstrate the complex (inter)relationship between the empirical parameters intrinsic activity (α) and potency (EC_{50}) and the parameters obtained from the operational model of agonism, equation 7 can be rewritten as:

$$E = \frac{[A] \cdot \left(E_m \cdot \frac{\tau}{\tau + 1} \right)}{\frac{K_A}{1 + \tau} \cdot [A]} \quad (8)$$

A specific feature of the operational model of agonism is that maximum effect observed with a particular agonist is not necessarily equal to the maximum system response, but

approaches maximal achievable effect in a biological system as $\tau \rightarrow \infty$, i.e.:

$$\alpha = \frac{E_m \cdot \tau}{\tau + 1} \quad (9)$$

Full agonists (large value of τ) yield α values close to E_m , whereas partial agonists (small values of τ) yield α values lower than E_m . Similarly, the potency parameter EC_{50} does not equal K_A but rather $K_A/(1+\tau)$ (equation 10). In other words, a full efficacy agonist displays half-maximal effect at agonist concentrations below the required concentration for 50 % receptor occupation (i.e. $EC_{50} < K_A$):

$$EC_{50} = \frac{K_A}{\tau + 1} \quad (10)$$

For concentration *versus* effect relationships which are different from the general rectangular hyperbola, the operational model of agonism can be extended by including a slope parameter n to allow for variable steepness in concentration *versus* effect relationships. The extended form of the operational model of agonism is:

$$E = \frac{E_m \cdot \tau^n \cdot [A]^n}{(K_A + [A])^n + \tau^n \cdot [A]^n} \quad (11)$$

The relationship between this operational model of agonism and the geometric parameters (α and EC_{50}) characterizing the empirical $E_{\max}$ model is as follows:

$$\alpha = \frac{E_m \cdot \tau^n}{\tau^n + 1} \quad (12)$$

$$EC_{50} = \frac{K_A}{(2 + \tau^n)^{1/n} - 1} \quad (13)$$

It should be noted that for $n=1$ (i.e. symmetric curve) the original operational model of agonism is obtained (equation 7). Consequently, equations 12 and 13 can be simplified to equations 9 and 10, respectively. The introduction of the transducer constant τ and slope parameter n allows for characterization of receptor reserve, a phenomenon wherein sub-maximal receptor occupancy causes maximal response. However, the operational model of agonism may predict receptor reserve for partial agonists. In theory, partial agonists produce maximal effect at 100 % receptor occupancy. From equation 4, at half-maximal response it follows:

$$[AR] = \frac{[R_{tot}]}{2} = \frac{[AR]}{[R_{tot}]} = 0.5 = \frac{[A]}{K_A + [A]} \quad (14)$$

In this case it can be simply derived that $[A] = K_A$. According to equation 10 $[A] = K_A$ means that τ approaches to 0. However, equation 7 defines $\tau = [R_{tot}]/K_E$, which never can be zero, since a zero values for $[R_{tot}]$ and K_E are not rational. Therefore, it seems that

if a drug displays partial agonist activity, the use of the operational model of agonism is inappropriate.

Notwithstanding, the operational model of agonism can be used to obtain estimates of drug-dependent characteristics, like affinity and efficacy, for partial agonist by comparison with full agonists. With the comparative method the value of the maximum system response is set to a value which is identical to the intrinsic activity of the full agonist producing the highest response (Barlow *et al.*, 1967; Leff *et al.*, 1990; Van der Graaf *et al.*, 1999; Danhof *et al.*, 2007a). Accordingly, K_A and τ for a partial agonist can be estimated by directly fitting the concentration *versus* effect data to the operational model of agonism. The operational model of agonism has been successfully applied in a series of *in vivo* investigations of the PK-PD correlations of A_1 adenosine receptor agonists (Van der Graaf *et al.*, 1999), μ -opioid receptor agonists (Cox *et al.*, 1998; Garrido *et al.*, 2000), and 5-HT_{1A} receptor agonists (Zuideveld *et al.*, 2004). The most recent application is in the analysis of drug effects on QTc interval prolongation (Jonker *et al.*, 2005a).

A limitation of the operational model of agonism is that it contains a strict assumption on the shape of the transducer function. In situations where the stimulus-response relationship is not linear or hyperbolic, the operational model of agonism is sensitive to pharmacodynamic model misspecifications, yielding biased model parameters estimates. Specifically, the operational model of agonism does not provide any flexibility with respect to the identification of the most appropriate transduction function other than the linear or hyperbolic function. For this reason a semi-parametric approach to the incorporation of receptor theory in PK-PD modeling have been proposed. In this approach the interaction between the drug and its receptor is still described by a hyperbolic function (equation 4), but no specific assumptions are made on the shape of the transducer function. Receptor activation is incorporated in the model on the basis of Stephenson's concept of a stimulus to the biological system, which results from the drug-receptor interaction (Stephenson, 1956). This stimulus is defined as:

$$S = \frac{e \cdot [AR]}{[R_{tot}]} = \frac{e \cdot [A]}{[A] + K_A} \quad (15)$$

where S is the stimulus to the biological system and e is a dimensionless proportionality factor denoting the power of a drug to produce a response in a tissue. Accordingly, the stimulus is directly related to the fraction of receptors occupied. The stimulus-response relationship can take any shape and can be described by an unspecified, but monotonously increasing and continuous function f :

$$\frac{E_A}{E_{max}} = f(S) = f\left(\frac{e \cdot [A]}{[A] + K_A}\right) \quad (16)$$

where e is a proportionality factor referring to the efficacy of a drug to produce a response in a tissue. The introduction of function f dissipates receptor stimulus and tissue response as directly related quantities, providing unique drug- and system-related

pharmacodynamic parameter estimates. Therefore, Stephenson's concept can be used to explain receptor reserve and partial agonism. For instance, agonists displaying high efficacy can produce maximal effect while occupying a relatively small fraction of receptors, while agonists of lower efficacy cannot activate the receptors to the same degree and may not be able to produce the same maximal response even when they occupy the entire receptor population, thereby behaving as partial agonists. A restriction to the model is that it predicts the response of a drug after generation of a stimulus in a certain tissue. Therefore, efficacy is on one hand drug-specific but on the other hand it can be different in various tissues, since receptor density may differ across tissues. Furchgott (Furchgott, 1966) refined the model by introduction of intrinsic efficacy ε . This term was defined as a quantal unit for the stimulant activity of a drug to initiate a stimulus from one receptor:

$$\varepsilon = e/[R_{tot}] \quad (17)$$

where $[R_{tot}]$ is the total receptor concentration in a tissue, ε is term to describe the stimulant activity of the drug itself, independent of tissue. The importance of the parameter ε is that it is unique for each drug, independent of tissue. The incorporation of ε into equation 16 results in:

$$\frac{E_A}{E_{\max}} = f(S) = f\left(\frac{\varepsilon \cdot [R_{tot}] \cdot [A]}{[A] + K_A}\right) \quad (18)$$

Integration of equation 18 in mechanism-based PK-PD models allow characterization and prediction of concentration-effect relationships in any tissue on the basis of two drug- and two system-related parameters. The drug-specific part is displayed by the equilibrium dissociation constant K_A and intrinsic efficacy ε and are unique for each drug-receptor interaction. The tissue factors are $[R_{tot}]$ and function f , which determines the shape and location of the stimulus *versus* response relationship.

The semi-parametric approach uses a parametric (i.e., hyperbolic) function to describe the receptor activation process in combination with a nonparametric function to describe transduction. A semi-parametric approach to the incorporation of receptor theory in PK-PD modeling has been successfully applied in investigations on GABA_A receptor agonists (Tuk *et al.*, 1999). In the meantime, a full parametric PK-PD model for GABA_A receptor agonists was proposed, featuring a hyperbolic model to describe the receptor activation process in combination with a parameterized biphasic transducer function (i.e., stimulus-response relationship) (Visser *et al.*, 2002). In this model, the transduction function f (equation 16) is described by a parabolic function according to:

$$E = E_{top} - a \cdot (S^d - b)^2 \quad (19)$$

where S represents the stimulus to the biological system resulting from GABA_A receptor activation, E_{top} represents the top of the parabola, and a is a constant reflecting the slope

of the parabola. Furthermore, $b^{1/d}$ is the stimulus for which the top of the parabola (i.e. the maximal effect, E_{top}) is reached, and the exponent d determines the asymmetry of the parabola. When no drug is present, the effect is equal to its baseline value (E_0). Equation 19 then reduces to:

$$E_0 = E_{top} - a \cdot b^2 \quad (20)$$

Substituting Equation 20 in equation 19 and rearranging yields:

$$E = E_0 - a \cdot ((S^d)^2 - 2 \cdot b \cdot S^d) \quad (21)$$

Thus, the shape and location of the parabolic function is determined by three parameters: a , b and d . Specifically, the value of parameter a determines the height of the maximal achievable response (E_{top}) as well as the steepness of the increasing and decreasing wing. Parameter b determines the location of E_{top} and d determines the asymmetry of the parabola (figure 2).

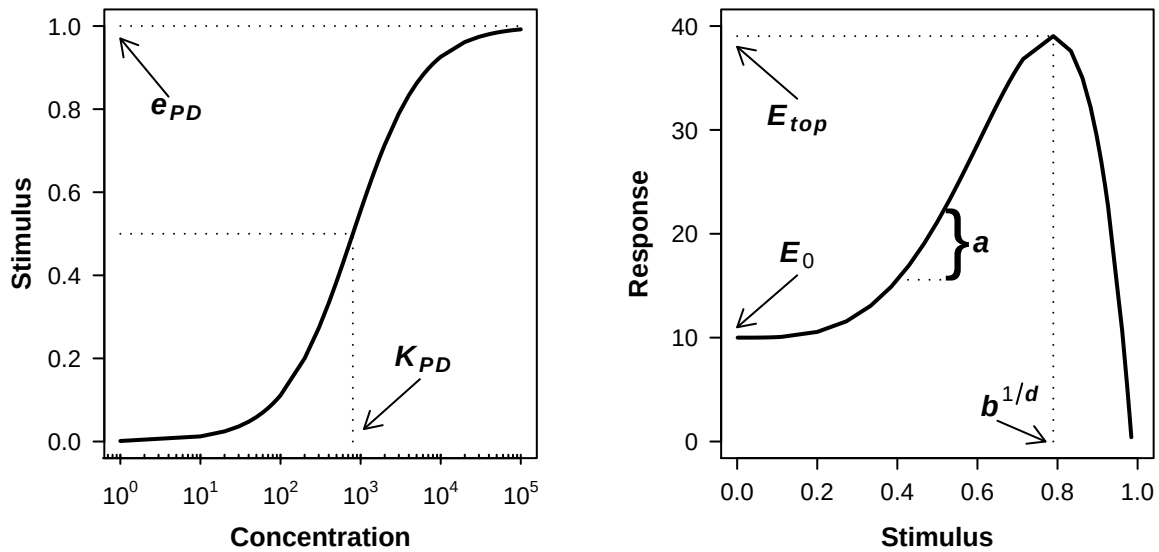


Figure 2. Full-parametric receptor model for characterization of the concentration-EEG effect relationships of GABA_A receptor agonists. The full parametric model features a hyperbolic function to describe the drug-receptor interaction in combination with a parabolic transducer function. Reproduced from Visser *et al.* (2002).

Time-dependent pharmacodynamics

Turnover models

Binding of a drug molecule to a biological target initiates a cascade of biochemical and/or electrophysiological events resulting in the observable biological response. For most re-

ceptors (i.e. G-protein coupled receptors) second messengers such as phospholipases (i.e. 1,4,5 inositol triphosphate, diacylglycerol) and nucleotide cyclases (i.e. cAMP) serve as second messengers. For other receptors (i.e. glucocorticoid receptors) transduction is mediated through the interaction with DNA thus regulating the expression of second messengers, proteins or enzymes. Within the context of mechanism-based PK-PD modeling, transduction is defined as the cascade of processes that govern the time course of the pharmacological response *in vivo* following drug-induced target activation. This is therefore a much broader definition than the more traditional definition that is used in biochemical pharmacology (Danhof *et al.*, 2007b).

Large differences exist in the rates at which the various transduction processes occur *in vivo*. In many instances transduction is fast (i.e. operating with rate constants in the range of milliseconds to seconds) relative to the rate constants governing the disposition processes (typically minutes to hours). In that situation the transduction process determines the shape and the location of the *in vivo* concentration-effect relationship, but it does not influence the time course of the drug effect relative to the plasma concentration. In contrast, transduction *in vivo* can also be slow, operating with rate constants in the order of hours to days, in which case transduction can become an important determinant of the time course of drug action (Danhof *et al.*, 2007b). As an approach to account for delays between the drug concentration and effect, Dayneka *et al.* (1993) have proposed a family of four indirect response models on the basis of the following equation:

$$\frac{dR}{dt} = k_{in} - k_{out} \cdot R \quad (22)$$

where R is a physiological entity, which is constantly being produced and eliminated in time, k_{in} is the zeroth-order rate constant for production of the physiological entity and k_{out} is the first-order rate constant for its loss. In this model the drug effect is described as stimulation or inhibition on the factors controlling either the input or the dissipation of drug response in a direct concentration-dependent manner. Nagashima *et al.* (1969) were the first to apply an indirect response model for the characterization of the time course of the anti-coagulant effect of warfarin in man. In this model, the inhibition of synthesis of prothrombin complex was used as a biomarker of the anti-coagulant effect. The basic properties of these models and their applications are discussed in detail in two reviews (Sharma and Jusko, 1996; Krzyzanski and Jusko, 1998).

The direct and indirect response or turnover models are complementary and can be applied to a wide range of drugs describing a variety of responses under different exposure circumstances. Selection of an appropriate PK-PD model is based on the underlying mechanisms dictating the rate-limiting steps for observed delay between concentration and effect. For instance, biophase equilibration is the rate-limiting step for time-dependent pharmacodynamics, the peak effect will be reached at the same time irrespective of dose. In the case, drug effect is not directly mediated by drug-receptor interaction but rather by affecting the fate of endogenous substances an indirect response model would be more

relevant. This type of models predicts that the peak effect will be reached later at higher doses (Dayneka *et al.*, 1993). The indirect response model has been successfully applied in preclinical investigations to derive concentration-effect relationships of drugs with an indirect mechanism of action (van Schaick *et al.*, 1997a,b; Cleton *et al.*, 1999).

Cascading models

Complex transduction mechanisms can be modeled on the basis of cascading turnover models describing intermediary processes between the pharmacokinetics and the ultimate biological response. In terms of mathematical modeling, the so-called transit compartment model has been proposed. This model relies on a series of differential equations to describe the cascade of events between receptor activation and final response (Sun and Jusko, 1998). The transit compartment model is attractive because of its flexibility, but for it to become fully mechanistic, pertinent information on the processes of the causal path is required. This underscores the need for biomarkers to characterize *in vivo* transduction mechanisms. The most well-known example of a mechanism-based transduction model is the so-called fifth generation model for corticosteroid pharmacodynamics (Ramakrishnan *et al.*, 2002). This model describes the pharmacodynamics of the receptor/gene-mediated effects of methylprednisolone on the basis of a series of differential equations for the receptor regulation and the turnover of serum tyrosine aminotransferase (TAT) activity as the ultimate pharmacodynamic endpoint. The differential equations for the various components of the model controlling the receptor regulation are the following:

$$\frac{dR_{in}}{dt} = k_{syn_Rm} \cdot \left(1 - \frac{DR(N)}{IC_{50_Rm} + DR(N)}\right) - k_{dgr_Rm} \cdot R_{in} \quad (23)$$

$$\frac{dR}{dt} = k_{syn_R} \cdot R_{in} + R_f \cdot k_{re} \cdot DR(N) - k_{on} \cdot D \cdot R - k_{dgr_R} \cdot R \quad (24)$$

$$\frac{dDR}{dt} = k_{on} \cdot D \cdot R - k_T \cdot DR \quad (25)$$

$$\frac{dDR(N)}{dt} = k_T \cdot DR - k_{re} \cdot DR(N) \quad (26)$$

where R_{in} represents mRNA for the receptor, R is the free cytosolic receptor concentration, DR is the cytosolic drug-receptor complex concentration, $DR(N)$ is the nuclear activated drug-receptor complex concentration and k_{on} and k_{dgr_Rm} are the first-order rate constants of synthesis and degradation of the response respectively. Another well-known

example of applications of this type of modeling is the modeling of hematologic toxicity in cancer (Sandstrom *et al.*, 2005).

Physiological counter-regulation

A shift towards the development of PK-PD models describing and predicting complex time profiles of pharmacological effects arises from the observation that biological systems evolve over time, showing transformations in pharmacological and/or physiological behavior and an increase in complexity under normal and disease conditions (Zuideveld *et al.*, 2001). For example, desensitization and tolerance upon chronic administration of drugs, involvement of counter regulatory or homeostatic feedback mechanisms and other factors may have major implications on the behavior of biological systems upon acute or chronic exposure. In these situations, PK-PD models based on physiological principles are useful since they mimic the underlying mechanisms of drug action *in vivo* more closely than rather empirical descriptive models. Over the years several mathematical models for the characterization of homeostatic feedback have been proposed. A useful model to describe complex effect versus time profiles is the so-called 'push-and-pull' model:

$$\frac{dR}{dt} = k_{in} \cdot (1 - f(C)) - k_{out} \cdot R \cdot M \quad (27)$$

$$\frac{dM}{dt} = k_m \cdot R - k_m M \quad (28)$$

where C the drug concentration, M is a response modifier value and k_m is the rate constant for formation and dissipation of the response modifier. Due to its plasticity the 'push-and-pull' model could be successfully applied to describe tolerance to the diuretic response upon repeated administration of furosemide (Wakelkamp *et al.*, 1996). Another example of a PK-PD model describing tolerance is the 'precursor pool' model.

$$\frac{dPool}{dt} = k_{in} - k_{loss} \cdot Pool \cdot (1 - f(C)) \quad (29)$$

$$\frac{dR}{dt} = k_{loss} \cdot Pool \cdot (1 - f(C)) - k_{out} \cdot R \quad (30)$$

where $Pool$ is the precursor pool value and k_{loss} is the first-order rate constant for release of precursor into the central compartment. The precursor pool model can conceptually be considered a description of a tachyphylactic system and has been successfully applied to describe the effects of neuroleptic drugs on the prolactin balance (Movin-Osswald and Hammarlund-Udenaes, 1995). Attempts to model physiological counter regulatory mechanisms have resulted in a series of advanced models describing complex behavior. These models are in part based on the work by Ekblad and Licko (Ekblad and Licko, 1984). An example is the model proposed by Bauer *et al.* (1997) to characterize tolerance to the

hemodynamic effects of nitroglycerin in experimental heart failure. In the meantime this type of physiological counter-regulatory effect models has been successfully applied to describe tolerance and rebound to the effects of drugs such as alfentanil and omeprazole (Veng-Pedersen and Modi, 1993; Mandema and Wada, 1995; Abelo *et al.*, 2000). Moreover, a dynamical systems model has been proposed, which can account for the complex hemodynamic effects of arterial vasodilators (i.e. nifedipine) for which rate of administration is a major determinant of the effects (Kleinbloesem *et al.*, 1987; Francheteau *et al.*, 1993).

The most recent development in the incorporation of dynamical systems analysis in PK-PD modeling has been the conceptualization of the so-called 'set-point' model (Figure 3). This model was designed to describe complex effect *versus* time profiles of the hypothermic response following the administration of 5-HT_{1A} receptor agonists to rats (Zuideveld *et al.*, 2001). In order to characterize 5-HT_{1A}-agonist induced hypothermia in a mechanistic manner a mathematical model which describes the hypothermic effect based on the concept of a set-point and a general physiological response model has been proposed (Cabanac, 1975; Dayneka *et al.*, 1993; Zeisberger, 1998; Zuideveld *et al.*, 2001). Briefly, as an agonist binds to the 5-HT_{1A} receptor a stimulus, S is generated, which in turn drives physiological processes that lower the temperature. This stimulus, which is determined by the drug receptor interaction and hence the drugs' affinity and efficacy, can be described by a sigmoid function where $f(C)$ for example equals equation 3. As S is assumed to be inhibitory, it is defined as $S=1-f(C)$. As the drug concentration changes with time, S changes, governing the first time-scale of the model. The second time-scale on which the model operates is governed by physiological principles.

The model that describes the hypothermic response utilizes the concepts of the indirect physiological response model as described by Dayneka *et al.* (1993) (equation 22). In this model the change in temperature (T) is described as an indirect response to either the inhibition of the production of body heat or the stimulation of its loss, where k_{in} represents the zero-order fractional turnover rate constant associated with the warming of the body and k_{out} is a first-order rate constant associated with the cooling of the body. The indirect physiological response model is combined with the thermostat-like regulation of body temperature. This regulation is implemented as a continuous process in which the body temperature is compared with a reference or set point temperature (T_{SP}). It is accepted that 5-HT_{1A} agonists elicit hypothermia by decreasing the value of the set-point temperature T_{SP} , and hence T_{SP} depends on the drug concentration C : $T_{SP}=T_{SP}(C)$. It is assumed that T_{SP} is controlled by the drug concentration through equation 31:

$$T_{SP} = T_0[1 - f(C)] \quad (31)$$

where T_0 is the set-point value in the absence of any drug: $T_0=T_{SP}(0)$. Combining the

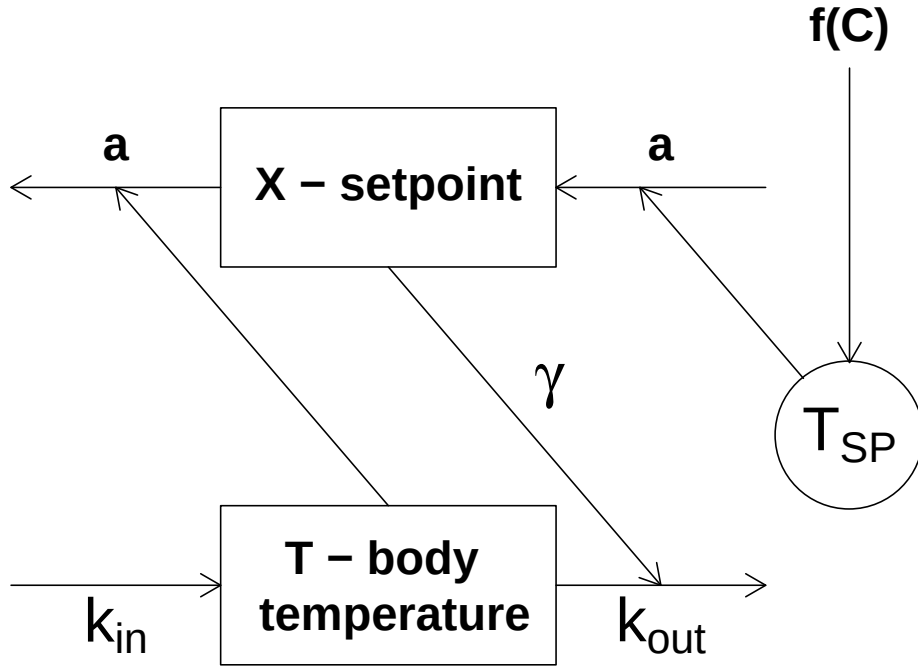


Figure 3. Full model to describe 5-HT_{1A}-receptor-mediated hypothermia. The model is based on the concept of an indirect response model and takes into account rate constants associated with the warming (k_{in}) and the cooling (k_{out}) of the body. The indirect physiological response model is combined with a thermostat-like regulation of body temperature in which the body temperature (T) is compared with a fixed reference or set point temperature (T_{SP}) at rate a , generating a set point signal X . The extent to which the set point value decreases is a function of the drug concentration $f(C)$, which decreases X by the amplification factor γ . Reproduced from Zuideveld *et al.* (2001).

indirect physiological response model with the thermostat-like regulation then yields:

$$\frac{dT}{dt} = k_{in} - k_{out} \cdot T \cdot X^{-\gamma} \quad (32)$$

$$\frac{dX}{dt} = a(T_0 \cdot [1 - f(C)] - T) \quad (33)$$

in which X denotes the thermostat signal. The change in X is driven by the difference between the body temperature T and the set-point temperature T_{SP} on a time-scale that is governed by a . Hence when the set-point value is lowered, the body temperature is perceived as too high and X is lowered. To relate this decreasing signal to the drop in body temperature, an effector function $X^{-\gamma}$ was designed, in which γ determines the amplification. Raising this function to the loss term $k_{out} \cdot T$ therefore facilitates the loss of heat. In equations 32 and 33, body temperature and set-point temperature are inter-dependent and a feedback loop is created that can give rise to oscillatory behavior, as has been shown. The model is able to reproduce the observed complex effect *versus* time profiles, which are typically observed upon the administration of 5-HT_{1A}-receptor partial agonists.

INCORPORATION OF TARGET ASSOCIATION/DISSOCIATION KINETICS IN PK-PD MODELING

Rationale

The majority of the pharmacodynamic models derived from receptor theory are based on the assumption that reaction mechanisms (i.e. drug-receptor binding) occur very rapidly and henceforth are described under equilibrium conditions. However, there are drugs which don't bind instantaneously to the target receptor. It is anticipated that dynamic receptor models describing time-dependencies in pharmacodynamics caused by slow receptor association/dissociation at the receptor can be of great value in the PK-PD analysis of drugs where slow target association-dissociation has been demonstrated (i.e. calcium antagonists, opioids, calcitonin gene-related peptide (CGRP) receptor ligands). Modeling of slow receptor association-dissociation kinetics involves the estimation of the first- and second-order rate constant for receptor dissociation and association. In this respect it is important that for certain receptor-drug combinations the rate constants for association-dissociation have been shown to be slow relative to their pharmacokinetics and/or rate constants for biophase equilibration (Shimada *et al.*, 1996; Yassen *et al.*, 2005). Therefore, it is believed that receptor binding kinetics constitutes a correct representation of the rate-limiting step in the *in vivo* pharmacodynamics for those drug-receptor combinations.

Theory of target association-dissociation kinetics

In the occupation theory of Clark (Clark, 1937) generation of an biological effect by agonists is directly related to the formation of drug-receptor complex according to:



The binding of a drug molecule A to its receptor R and subsequent dissociation of the drug-receptor complex is described by the second-order rate constant k_{on} and first-order rate constant k_{off} , respectively. The change in the concentration of the drug-receptor complex over time can be described by the following differential equation:

$$\frac{d[AR]}{dt} = k_{\text{on}} \cdot [A] \cdot [R] - k_{\text{off}} \cdot [AR] \quad (35)$$

Consequently, in equilibrium, the rate of binding of an agonist to the receptor equals the rate of dissociation of the existing agonist-receptor complex:

$$\frac{d[AR]}{dt} = 0 \implies k_{\text{on}} \cdot [A] \cdot R = k_{\text{off}} \cdot [AR] \quad (36)$$

In equilibrium the dissociation constant K_D can be described as follows:

$$K_D = \frac{k_{\text{off}}}{k_{\text{on}}} = \frac{[A] \cdot [R]}{[AR]} \quad (37)$$

The equilibration dissociation constant K_D represents the concentration of agonist that results in 50 % receptor occupancy and reflects the affinity of an agonist for its receptor. The K_D is dependent on the relative rates of receptor association and dissociation kinetics of a drug and is therefore unique for each drug in a biological system. As such the K_D is often used as a quantitative parameter to classify drugs on the basis of potency. Furthermore, K_D is of important value for the prediction of the *in vivo* effect of drugs using concepts from receptor theory.

Under the assumption that the free drug concentration is large compared to the unbound receptor concentration ($[A] \gg [R]$) and since that the total amount of receptor present ($[R_{\text{tot}}]$) equals the sum of the agonist-receptor complex ($[AR]$) and the free receptor concentration ($[R]$):

$$[R] = [R_{\text{tot}}] - [AR] \quad \text{and} \quad \frac{[R]}{[R_{\text{tot}}]} = \frac{[R_{\text{tot}}]}{[R_{\text{tot}}]} - \frac{[AR]}{[R_{\text{tot}}]} \quad (38)$$

equation 35 can be rearranged:

$$\frac{d[AR]}{dt} = k_{\text{on}} \cdot [A] \cdot ([R_{\text{tot}}] - [AR]) - k_{\text{off}} \cdot [AR] \quad (39)$$

and subsequently into:

$$\frac{d\rho_{\text{app}}}{dt} = k_{\text{on}} \cdot [A] \cdot (1 - \rho_{\text{app}}) - k_{\text{off}} \cdot \rho_{\text{app}} \quad (40)$$

where ρ_{app} is the apparent fractional receptor occupancy ($[AR]/[R_{\text{tot}}]$). In theory the proposed model allows characterization of the *in vivo* receptor association/dissociation kinetics as a determinant of the time course of drug effect on the basis of PK-PD analysis of the time course of the drug effect in conjunction with plasma concentrations. As such, the PK-PD analysis of slow receptor association-dissociation kinetics provide an unique opportunity to calculate the *in vivo* K_D on the basis of the estimates of the rate constants of k_{on} and k_{off} .

Identification of receptor occupancy-response relationships

In the previous paragraphs it has been shown that transduction functions can in principle take any shape. Dependent on the behavior of a drug in a particular biological system (i.e. partial or full agonism) the transduction function can be linear, hyperbolic or even more complex. Previously it has been shown that transducer functions can be identified on the basis of a simultaneous analysis of concentration-effect relationships of multiple drugs interacting at the same receptor (Tuk *et al.*, 1997; Van der Graaf *et al.*,

1999; Visser *et al.*, 2003; Zuideveld *et al.*, 2004). According to Stephenson (Stephenson, 1956) stimulus is a description of the relative strength of the response-inducing signal by agonist-bound receptors (receptor occupancy). However, a complicating factor in identifying the stimulus-response relation (transducer function) is that typically no information on the degree of target receptor occupancy is available. PK-PD analysis of the time course of the pharmacological effects of drugs displaying slow receptor association-dissociation kinetics provides unique opportunities to derive information on the target occupancy in conjunction with the intensity of the drug effect. This enables identification of the transduction function of a single drug. In the next paragraphs the mathematical equations describing the relationships between target occupancy and effect are discussed.

Linear transducer function

For partial agonists, the transduction function can be simplified by directly relating the change in fractional receptor occupancy to response in a linear fashion. In steady-state, equation 40 can be simplified to:

$$\frac{[AR]}{R_{\text{tot}}} = \rho_{\text{app}} = \frac{[A]}{\frac{k_{\text{off}}}{k_{\text{on}}} + [A]} = \frac{[A]}{K_D + [A]} \quad (41)$$

Clark (Clark, 1937) envisioned that the biological effect intensity of a drug (E) is directly related to the number of receptors occupied by the drug:

$$\frac{[AR]}{[R_{\text{tot}}]} = \frac{E}{E_{\text{max}}} = \frac{[A]}{K_D + [A]} \quad (42)$$

Since for partial agonist K_D is equal to EC_{50} , substitution of ρ_{app} in the inhibitory fractional sigmoid E_{max} model:

$$E = E_0 \cdot \left(1 - \frac{\alpha \cdot C_p}{EC_{50} + C_p} \right) \quad (43)$$

yields a linear transduction function:

$$E = E_0 \cdot (1 - \alpha \cdot \rho_{\text{app}}) \quad (44)$$

It should be noted that the steady-state solution of equation 40 and equation 44 is equal to inhibitory fractional sigmoid E_{max} model (equation 43). Accordingly, for the curve-linear transduction function:

$$E = \frac{E_0}{1 - \rho_{\text{app}}} \quad (45)$$

substitution of equation 41 in equation 45 yields:

$$E = \frac{E_0}{1 - \frac{[A]}{K_D + [A]}} = \frac{E_0}{1 - \frac{1}{1 + \left(\frac{EC_{50}}{[A]}\right)}} = \frac{E_0}{1 + \left(\frac{[A]}{EC_{50}}\right)} \quad (46)$$

For partial agonists, equation 46 is equal to the "power" pharmacodynamic model:

$$E = E_0 \cdot \left[1 + \left(\frac{[A]}{EC_{50}} \right) \right] \quad (47)$$

The steady-state and non-steady-state relationships are complementary and can be used to characterize the concentration *versus* effect relationship of all drug-receptor combinations, of which the effect is governed by biophase distribution, receptor association/dissociation kinetics or a combination of both processes. In that sense, K_D and EC_{50} are a direct measure of drug potency with a unique value for each drug-receptor complex. The most important aspect is that K_D and EC_{50} can be used to classify drugs acting at the same receptor in terms of potency. The term α is introduced to account for differences in maximal response observed between different drugs. The value of α can range from zero (i.e. antagonists) to one (i.e. full agonists).

Hyperbolic transducer function

The hyperbolic transduction function is defined as:

$$E = \frac{E_m \cdot \rho_{app}^n}{\rho_{app50}^n + \rho_{app}^n} \quad (48)$$

where ρ_{app} is the fractional receptor occupancy and ρ_{app50} is the value of fractional receptor occupancy eliciting half-maximal response. In this respect it is important that the rate constant of receptor association k_{on} and ρ_{app50} are not identifiable from a single concentration *versus* effect relationship. Hence, additional data is required to identify both parameters. For instance, receptor association-dissociation kinetics can be explored *in vivo* by using positron emission tomography (PET) (Liefwaard *et al.*, 2005). However, the costs and sophisticated operation of a PET scanner limit its use for daily routine in laboratory settings. Furthermore, in order to perform *in vivo* receptor binding studies using PET the availability of a tracer is required, which is the compound of interest labeled with a positron emitting isotope. As an alternative, *in vitro* or *ex vivo* receptor binding studies can be conducted (Geldof *et al.*, unpublished observations). Despite the fact that extrapolation of *in vitro* receptor binding data to the *in vivo* situation rely on a number of assumptions, several PK-PD studies have demonstrated good *in vitro-in vivo* correlations for the receptor binding affinity constant (K_D) (Van der Graaf *et al.*, 1999; Visser *et al.*, 2003).

Application of the receptor association/dissociation model in PK-PD analysis

The first application of the target association-dissociation kinetics in PK-PD modeling was in the analysis of the time course of the antihypertensive effect of calcium channel blockers. Specifically, an ion-channel binding model was proposed to explain time-dependencies in the pharmacodynamics of long-acting calcium antagonists (Shimada *et al.*, 1996). In this manner, estimates of the *in vivo* binding affinity of eight calcium antagonists could be obtained. Interestingly, a close correlation between the *in vitro* K_D and the *in vivo* estimates of target affinity was observed confirming the validity of the receptor binding model as a correct representation of the rate-limiting step in time-dependent pharmacodynamics. The binding model was also successfully used to describe the anti-secretory effect of the gastric acid pump inhibitor H 333/25 (Abelo *et al.*, 2001).

Target interaction kinetics also play an important role in the selection of therapeutic monoclonal antibodies (Copeland *et al.*, 2006). Specifically, the target dissociation kinetics is an important determinant of the pharmacological activity of the antibodies. The merits of the target binding model in the prediction of the kinetics of antigen-antibody interactions was recently demonstrated in the PK-PD analysis of the binding of a monoclonal antibody, Xolair, to immunoglobulin E (IgE) (Meno-Tetang and Lowe, 2005).

Most of these discussed applications of the receptor binding assume that the association/dissociation kinetics are mainly responsible for the observed time-dependent pharmacodynamics. However, considering the time course of drug action, usually a combination of different processes is involved in time delays of biological effect intensity relative to plasma concentration (Jusko *et al.*, 1995; Verotta and Sheiner, 1995; Danhof *et al.*, 2005, 2007b). When the rate-limiting process is a combination of slow biophase distribution and slow receptor association-dissociation kinetics, the kinetics of drug action is governed by rate constants k_{e0} , k_{on} and k_{off} . In this respect, the development of a PK-PD model allowing for separated characterization of the kinetics of target site distribution and receptor association-dissociation kinetics as determinants of the time course of drug action is of great importance.

Separation of biological processes, causing hysteresis (i.e. biophase distribution, receptor binding, transduction and active metabolite), frequently results in the inability to obtain unique parameter estimates expressing rate-limiting steps (Tuk *et al.*, 1997, 1998; Cleton *et al.*, 1999). A detailed data set including different doses and infusion schemes is required to elucidate and quantify more than one biological process underlying the time-dependencies in the *in vivo* pharmacodynamics. Recently, a mechanism-based PK-PD model has been proposed for the characterization of the kinetics of onset and offset of buprenorphine's antinociceptive and respiratory depressant effect on the basis of biophase distribution and receptor association/dissociation kinetics (Yassen *et al.*, 2005, 2006)

Pharmacodynamic drug-drug interaction

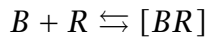
Mechanism-based PD models constitute a unique basis for the characterization and prediction of pharmacodynamic drug interactions (Jonker *et al.*, 2005b). The modeling of pharmacodynamic interactions is not limited to situations in which two drugs are given in combination or where a drug is converted into an (inter) active metabolite. Modeling of pharmacodynamic interactions is also relevant when (a) a drug interacts with an endogenous ligand, (b) a drug interacts at multiple targets, thereby activating multiple pathways and (c) when homeostatic feedback occurs. Moreover, modeling of the influence of baseline effects is also based on theoretical concepts of interaction modeling.

Pharmacodynamic drug-drug interactions are of various nature and comprise 1) competitive, 2) allosteric and 3) non-competitive interactions. The effect of a combination of drugs depends on the type of interaction. Typically, the nature of a pharmacodynamic interaction is characterized quantitatively in terms of addition, synergy or antagonism (Berenbaum, 1989). A number of papers has been published on the theoretical aspects of pharmacodynamic drug-drug interactions in terms of synergy and antagonism (Chou and Talalay, 1984; Koizumi *et al.*, 1993; Greco *et al.*, 1995; Carter, 1995; Gessner, 1995). Synergy occurs when the combined effect is larger than is expected under the assumption of additivity (no interaction) of the two effects separately. In contrast, antagonism occurs when the combined drug effect is smaller.

In order to characterize pharmacodynamic drug-drug interactions various PK-PD interaction models have been proposed. In general, these interaction models are derived from the Hill equation. The empirical character of the interaction models based on the classical $E_{\max}$ model does not allow for accurate prediction of the contribution of two separately administered drugs to the overall pharmacological effect after concomitant administration of two drugs. The rather poor predictive behavior of the empirical interaction model is harrowing in cases where both drugs display almost equal intrinsic activity at their target receptor. Recently it has been proposed to incorporate concepts from receptor theory into a novel class of mechanism-based pharmacodynamic interaction models (Jonker *et al.*, 2005b). Meanwhile, mechanism-based PK-PD modeling concepts have been applied in a number of investigations. For instance, the pharmacodynamic interaction between midazolam and its active metabolite α -hydroxy-midazolam was characterized using a mechanism-based PK-PD interaction model based on the concept of Furchgott/Stephenson (Tuk *et al.*, 1999), allowing the estimation of unique and independent values for *in vivo* potency and efficacy for both compounds. Furthermore, a single and unique transducer function was identified (Tuk *et al.*, 1999). Using a similar approach the allosteric interaction of ethanol with benzodiazepines was characterized in rats (Tuk *et al.*, 2002). In a different study, allosteric modulation of the anti-seizure activity of tiagabine by midazolam was investigated using a mechanism-based PK-PD interaction model on the basis of the operational model of agonism (Jonker *et al.*, 2003). Finally, a mechanism-based PK-PD model was proposed for the competitive interaction between buspirone and

its active metabolite (Zuideveld *et al.*, 2002).

So far the kinetics of target association and dissociation have not been included in analysis of pharmacodynamic interactions. This is of great interest, particularly in relation to the reversal of the effects of (semi-)synthetic opioids with naloxone. Particularly for buprenorphine complexities may arise as results from the slow receptor association/dissociation kinetics (Gal, 1989). The pharmacodynamic interaction of two drugs A and B competing at the same receptor is described as follows:



The binding of a drug molecule A or B to the receptor R and subsequent dissociation of the drug-receptor complex is described by the second-order rate constant k_{on} and first-order rate constant k_{off} , respectively. The change in drug-receptor complex over time can be described by the following differential equations:

$$\frac{d[AR]}{dt} = k_{on,A} \cdot [A] \cdot [R] - k_{off,A} \cdot [AR] \quad (50)$$

$$\frac{d[BR]}{dt} = k_{on,B} \cdot [B] \cdot [R] - k_{off,B} \cdot [BR]$$

with $[R_{tot}] = [R] + [AR] + [BR]$, substitution in equation 50 yields:

$$\frac{d[AR]}{dt} = k_{on,A} \cdot [A] \cdot ([R_{tot}] - [AR] - [BR]) - k_{off,A} \cdot [AR] \quad (51)$$

$$\frac{d[BR]}{dt} = k_{on,B} \cdot [B] \cdot ([R_{tot}] - [AR] - [BR]) - k_{off,B} \cdot [BR]$$

The developed mechanism-based pharmacodynamic interaction model constitutes a unique basis for the characterization of pharmacological response to a combination of drugs with distinct rate-limiting steps in the onset and offset of their respective pharmacological effects. In this respect, for certain drug-receptor combinations the rate constants for receptor association/dissociation have been shown to be relative shown relative to biophase equilibration kinetics, albeit the majority of drugs bind rapidly to their target receptor (Yassen *et al.*, 2005). Consider that the antagonist binds very rapidly to the

receptor (Ngai *et al.*, 1976). In steady-state, it follows:

$$\rho_{\text{app}_B} = \frac{k_{\text{on},B} \cdot [B]}{k_{\text{off},B} + k_{\text{on},B} \cdot [B]} \cdot (1 - \rho_{\text{app}_A}) \quad (52)$$

$$\rho_{\text{app}_B} = \frac{[B]}{EC_{50} + [B]} \cdot (1 - \rho_{\text{app}_A}) \quad (53)$$

$$\frac{d\rho_{\text{app}_A}}{dt} = k_{\text{on},A} \cdot [A] \cdot \left(1 - \rho_{\text{app}_A} \cdot \left[1 - \frac{[B]}{EC_{50} + [B]}\right]\right) - k_{\text{off},A} \cdot \rho_{\text{app}_A} \quad (54)$$

Experimental data presented in this thesis show that the competitive interaction model (equation 54) successfully predicted the reversal of buprenorphine-induced respiratory depression by naloxone.

CONCLUSION

This chapter presents an overview of the current status of PK-PD modeling concepts. It is shown that PK-PD modeling has progressed from an empirical descriptive discipline into a mechanistic science. Specific highlights in this development have been the recent incorporation of the principles of receptor theory and dynamical systems analysis in mechanism-based PK-PD modeling. This has resulted in a novel class of mechanism-based PK-PD models with much improved properties for extrapolation and prediction. In this thesis, the application of receptor theory in PK-PD modeling was further extended to cover receptor association and dissociation kinetics as main determinant of the kinetics of onset and offset of drug action *in vivo*.

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Chapter 3

INTERSPECIES EXTRAPOLATION OF PK-PD RELATIONSHIPS

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INTRODUCTION

Pharmacokinetic-pharmacodynamic modeling is an important tool for optimization of drug development and for optimization of drug treatment in clinical practise. An evolving topic in the field of mechanism-based pharmacokinetic-pharmacodynamic modeling is the ability to scale pharmacokinetic and pharmacodynamic parameters from animal species to human with the aim to obtain essential information on human pharmacology in an early stage of drug development. This is important for drug candidate selection and for the optimization of the design of early clinical 'proof of concept' studies.

The last decade has seen a growing interest in scaling pharmacokinetic characteristics from animal species to human. Especially, pharmacokinetic information obtained in animals can be of considerable relevance for the prediction and establishment of optimal doses for first-in-man human studies. Unfortunately, preclinical development is still largely focused on pharmacokinetics and drug metabolism, thereby ignoring the relationship with pharmacodynamics. In the meantime, it is well accepted that early information on the pharmacokinetic-pharmacodynamic relationship is invaluable in the design of phase II proof of concept studies and pivotal phase II/III trials. Not only is the incorporation of pharmacodynamics essential for characterization of the relationship between pharmacological effect intensity and drug exposure in terms of concentration, but also to understand potential sources of variability in the pharmacodynamics of a drug relative to its respective pharmacokinetics. Failure to appreciate the magnitude of inter- and intra-individual variability in the pharmacokinetics and pharmacodynamics can compromise clinical trial outcomes, making a drug appear less effective or more toxic (Levy, 1998).

Non-linear mixed effects models take into consideration both inter- and intra-individual variability and can therefore be used to identify characteristics which influence the magnitude of inter-individual variability in the PK-PD relationship. Factors which influence the biological response intensity include disease, age and concomitant use of other drugs, but also factors like receptor density (receptor down-regulation), receptor affinity (polymorphism in target receptor), post-receptor transduction processes, homeostatic responses, the kinetic characteristics of transporters involved in drug transfer between blood and the biophase and kinetics of receptor equilibration (Levy, 1994).

In recent years, there is growing interest in the application of mechanism-based PK-PD modeling in preclinical development. Evidently, mechanism-based PK-PD models have improved properties in terms of extrapolation and prediction, since rate-limiting biological processes involved the mechanism of action are integrated in PK-PD concepts. For instance, incorporation of receptor theory in PK-PD models allows for prediction of *in vivo* concentration-effect relationships on the basis of a strict distinction between drug- and system-dependent characteristics. In that sense, the functioning of the biological system is characterized in more detail. Henceforth, factors like changes in receptor density can be explored to understand intra- and inter-individual variability in drug response (Gar-

rido *et al.*, 2000). The most recent development in mechanism-based PK-PD modeling has been the incorporation of dynamical system analysis in order to characterize and predict time-dependencies in the behavior of the physiological system upon acute drug treatment. Changes in disease state or as a result of chronic drug treatment may have important implications on the functioning of the physiological system and constitute therefor potential sources for inter- and intra-individual variability. Although mechanism-based PK-PD models have been extremely valuable for the prediction of *in vivo* concentration *versus* pharmacological response relationships in animals, their role in the extrapolation from animal to human is currently minimal.

The interfacing of preclinical and clinical research is a major challenge in modern drug discovery. In the meantime, initiatives are being undertaken to address the prediction of 1) the relationship between dose and exposure, 2) the relationship between exposure and response and 3) the intra- and inter-individual variability in these relationships. These initiatives resulted in novel developments in pharmacokinetics and PK-PD modeling with an important impact on interspecies extrapolation, including i) the transition from compartmental to physiological PK models and ii) the transition from descriptive to mechanism-based PD models.

PHARMACOKINETIC MODELS

Compartmental models

In PK-PD modeling the time course of the drug effect *in vivo* is analyzed in conjunction with the time course of the drug concentration in plasma (i.e. the pharmacokinetics of the drug). Traditionally compartmental pharmacokinetic models have been used to characterize the time course of drug concentrations. In these type of pharmacokinetic models, drug disposition is described as the transfer of drug between interconnected compartments, serving to mimic the drug absorption and elimination processes. This type of analysis is considered routine and straightforward (Mager *et al.*, 2003). Typically, compartmental pharmacokinetic modeling relies on the estimation of parameters which are associated with hypothesized and thus unknown physiological processes. Therefore, the merits of compartmental pharmacokinetic models for animal to human extrapolation using allometric scaling is considered minimal. Consistency in the use of allometry to accurately predict the pharmacokinetics of a variety of drugs in man is lacking. This explains the varying degrees of success of allometric scaling in forecasting the PK in man on the basis of animal data. In some situations, however, the application of compartmental models in combination with allometry can produce satisfactory predictions. Particularly for compounds with a simple and well-defined elimination pathway (i.e. high extraction ratio drugs) pharmacokinetics have been successfully scaled from animal to human using allometry (Knibbe *et al.*, 2005; Yassen *et al.*, 2007).

Allometry

Basic allometric equations

The term allometry reflects the relationship between biological processes and body size. In general, three subtypes of allometry are recognized, including 1) ontogenetic allometry, 2) static allometry and 3) evolutionary or phylogenetic allometry. Ontogenetic allometry refers to the relative growth in individuals over time while static allometry describes changes in relationships between variously sized individuals within a population at a moment in time (Cock, 1966; Gould, 1966). Evolutionary allometry describes the size relationship across species (Stern and Emlen, 1999) and is therefore of specific interest for interspecies scaling of pharmacokinetics and pharmacodynamics. The existence of interspecies similarities in biochemical, physiological and anatomical structures provides the fundamental basis for relating various biological processes to body mass (Adolph, 1949). It has been shown that blood flow, organ size, metabolic rate, respiratory rate and many other physiological and anatomical characteristics are related to body mass. The general allometric approach is based on the power law of the form (Boxenbaum, 1982):

$$P = a \cdot BW^b \quad (1)$$

where P is a physiological or pharmacological variable correlated to bodyweight BW , a and b are the coefficient and exponent of the allometric equation, respectively. The exponent b is called the scaling factor because it describes the effect of a change in bodyweight. If $b=0$, bodyweight has no effect. If $b=1$, the variable increases linearly to bodyweight (e.g. heart mass, blood volume). If $0 < b < 1$, the variable does not increase as much as bodyweight (metabolic rate). If $b > 1$, the variable is increasing more than proportional to the increase in bodyweight (skeletal mass). The shape of the allometric relationship complicates quantitative interpretation. Therefore, the allometric equation can be log-transformed, yielding the linear form:

$$\log P = \log a + b \cdot \log BW \quad (2)$$

Incorporation of neoteny

The last decade, many studies have been published on the allometric scaling of pharmacokinetic properties of drugs. Total body clearance and volume of distribution of the central compartment are the pharmacokinetic parameters of primary interest. Ideally, phase I studies are started with allometrically scaled and rationalized dose regimens based on pertinent information from pre-clinical animal studies. In reality, prospective and retrospective studies integrating allometric principles in pharmacokinetics frequently result in unsatisfactory results for use in clinical studies. In general allometric scaling of the central volume of distribution is straightforward and in most cases volume can indeed be

predicted with reasonable accuracy. Typically, the exponents in the allometric equation of volume and clearance are approximately 1 and 0.75 respectively.

For clearance, it is advocated that improvements in the prediction are obtained established by inclusion of correction factors for the allometric equation. One approach is that the allometric scaling equation can be extended by incorporation of neoteny. Neoteny refers to retardation of development of portions of the body. Neoteny has been suggested to be associated with the high relative brain weight and with the longer lifespan and maximum lifespan potential in human, compared to other animal species. The incorporation of neoteny might be of value in cases where discrepancies occur in interspecies scaling on the basis of bodyweight. For the prediction of clearance several approaches have been postulated including the product of clearance and maximum lifespan potential and the product or ratio of clearance and brain weight (Boxenbaum, 1984; Lave *et al.*, 1995). The proposed equations are:

$$Cl = \frac{a \cdot BW^b}{MLP} \quad (3)$$

$$Cl = a \cdot BrW^b \cdot BW^c \quad \text{or} \quad Cl = \frac{a \cdot BW^b}{BrW} \quad (4)$$

where *MLP* is the maximum lifespan potential and *BW* is bodyweight and *BrW* is brain-weight. All approaches can be used, albeit that the accuracy of the prediction depends strongly on the selected approach. It has been proposed that each of the approaches be used under specific conditions, which are defined on the basis of the exponent of the allometric equation (Mahmood and Balian, 1996). The analysis of allometric relations has yielded 'rules of thumb' for the use of allometric exponent for clearance:

- $b \leq 0.55$; clearance is underpredicted
- $0.55 \leq b \leq 0.70$; no correction is required, the use of equation will predict clearance most accurately .
- $0.71 \leq b \leq 0.99$; correction for MLP provide more accurate predictions
- $b \geq 1.0$; correction for brain weight provide more accurate predictions
- $b > 1.3$ no accurate prediction of clearance with all approaches

With the introduction of the rule of exponent, drug clearance can be predicted with a higher degree of certainty. However, the specific expressions for allometry have not been explored for endogenous substances or macromolecule drugs. More importantly, the rule of exponent is limited to the prediction of clearance. Subsequent application for the scaling of pharmacodynamic drug characteristics remains unclear.

It can be argued whether neoteny is indeed a key element in the prediction of biological processes across species, since neoteny is purely an empirical phenomenon not associated

with biological pathways determining the fate of drug disposition in man. Therefore, the merit of neoteny seems to be minimal, since no mechanistic explanation is provided for the inclusion of one of the correction factors. The selection of the best model is therefore merely based on mathematical considerations and not on *prior* knowledge on the pathways implicated in drug disposition. In retrospective studies in which both animal and human data are available, the most appropriate model can be selected by taking into consideration model selection criteria (i.e. goodness-of-fit and parameter estimates, etc.). However in the design of dose regimens for first time in human prospective studies, selection of the most appropriate model can not be based on model selection criteria, since no human data are available to optimize the allometric model. Since, neoteny is an empirical phenomenon, the use of correction factors for the design of prospective studies remains unclear (Bonate and Howard, 2000; Mahmood, 2000).

Estimation/prediction of allometric exponents

Although allometric scaling of pharmacokinetic characteristics has been successfully applied to a wide variety of drugs, no consensus has been reached on the existence of an universal scaling factor for the allometric exponent (Reigner *et al.*, 1997; Iavarone *et al.*, 1999). In particular, the discussion is focused on the impact of changes in bodyweight on functional property variables like metabolic rate. Over the years, much work has been published on the scaling of metabolic rates as a function of body size. Among the first papers, Kleiber proposed to scale metabolic rates to the 0.75 power of bodyweight (Kleiber, 1932). Recently, the value of the exponent, 0.75 was confirmed by others (McMahon, 1973; West *et al.*, 1997; Banavar *et al.*, 1999; West *et al.*, 2001). However, the value of the exponent remains controversial since other conflicting values have also been proposed, namely 0.67, which is based on the geometric scaling of the body surface area, is the most apparent value (Heusner, 1982; Dodds *et al.*, 2001). Taking into consideration pharmacokinetic processes and more specifically clearance, the most compelling evidence revealed a most likely value of 0.75 for the allometric exponent factor, albeit that a value of 0.67 might apply for drugs that are eliminated mainly by renal excretion (Bonati *et al.*, 1984; Bjorkman and Redke, 2000; Hu and Hayton, 2001). Under the assumption that for clearance and volume often the exponent values of 0.75 and 1 are used, respectively, the corresponding exponent value for first-order rate constants is -0.25 (Schmidt-Nielsen, 1995). Subsequently, for the half-life time ($t_{1/2}$) exponent value 0.25 is used. Subsequently, the model parameters can be extrapolated from animal to human using allometric techniques:

$$P_{i,man} = P_{i,rat} \cdot \left(\frac{BW_{man}}{BW_{rat}} \right)^b \quad (5)$$

where $P_{i,man}$ is the pharmacokinetic parameter in man, $P_{i,rat}$ is the respective parameter in rat and b is the scaling factor. When parameter P is a rate-constant, the exponent b is -0.25.

The most recent development has been the allometric scaling of the rate constant for biophase distribution in the animal to human extrapolation of the PK-PD properties of the antinociceptive and respiratory depressant effect of buprenorphine. On the basis of equation 5 an estimate of the allometric exponent for the rate constant of biophase distribution was obtained of -0.28 (Yassen *et al.*, 2007).

Physiological pharmacokinetic models

For the majority of the compounds approaches, other than compartmental modeling, are required for the prediction of the time course of the concentration in man. In order to predict the pharmacokinetics properties of compounds, the discipline of physiological pharmacokinetic modeling has been developed. Physiological-based pharmacokinetic (PB-PK) models comprise real physiological compartments in order to describe the complex relationship between drug delivery and drug disposition. In general, the physiological compartments reflect a set of organ and biological matrix compartments which are linked in series or in parallel. The structure of the PB-PK model is derived from the anatomical, biological and physiological structure of the species of interest and is based on underlying physiological processes. Physiological parameters like for instance organ blood flow rates, tissue to blood partitioning and plasma protein binding are incorporated. Three types of PB-PK models can be distinguished (Nestorov, 2003):

- whole body PB-PK models
- partial PB-PK models of isolated body systems
- liver (metabolism) models

Whole-body PB-PK models are extremely useful in the prediction of drug concentrations in humans from data obtained in animal studies, since these type of models represent physiological processes ungoing in biological systems in man more accurately than PK compartmental models (Gerlowski and Jain, 1983; Charnick *et al.*, 1995; Ploeger *et al.*, 2000). For midazolam, it has been shown that PB-PK modeling could predict the disposition of midazolam in surgical patients by scaling physiological parameters from rat to human. More importantly, the PB-PK model was also successfully used to explore characteristics which explain individual differences in drug concentrations (Bjorkman *et al.*, 2001). Physiologically-based modeling constitutes a mechanistic basis for the characterization and prediction of drug disposition and the identification of factors which influence changes in pharmacokinetics. In that sense, PB-PK models portray the physiological functioning of the human body with respect to absorption, distribution, metabolism and elimination processes. The more physiological relevant parameters are integrated in the PB-PK model, the better the model can predict the time course of drug concentration, also at the target site.

In PK-PD modeling, an important determinant step on the causal path is often the distribution to the target site (Danhof *et al.*, 2007). This concerns in particular drugs with an intracellular target (i.e. corticosteroids, anti-cancer drugs) and drugs which act in tissues that are protected by specific barriers (i.e. the central nervous system). With respect to CNS active drugs, their distribution to the central nervous system is significantly influenced when active efflux mechanisms are operative at the blood-brain barrier (BBB). Drug efflux transporters such as P-glycoprotein (P-gP) and other transport carriers which are located at the luminal face of the blood-brain barrier may limit the transport of drugs from blood to the target site in the CNS. For instance, loperamide, a peripherally acting antidiarrheal agent, is a transported substrate for the mouse *MDR1A* P-glycoprotein and its human homologue *MDR1*. Henceforth, in mice lacking *MDR1A* P-gP, loperamide displayed central opioid activity (Schinkel *et al.*, 1996). Furthermore, morphine and fentanyl have also been shown to interact with *MDR1A*. The established interaction between opioids and drug transport mechanisms correlates well with pharmacological opioid activity. In mice lacking P-glycoprotein a significant increase in antinociceptive effect was reported for fentanyl and morphine (Thompson *et al.*, 2000). These results point to the possible role that the drug-transporting P-gP(s) may play in the clinical use of many drugs, especially those with potential targets in the central nervous system. The characterization of drug transport to the biophase in a strict mechanistic and quantitative manner is of great importance for understanding the interaction between drug transporters and their substances, ultimately in relation to kinetics of drug action and their biological effect intensity.

The development of PB-PK models which characterize drug transport characteristics facilitate understanding of the relationship between drug exposure at the biophase and biological effect. Finally, the integration of drug transport characteristics in PB-PK models in a strict mechanistic and quantitative manner may supersede the need for a hypothetical effect-compartment models in order to predict target site exposure (Groenendaal *et al.*, 2007a,b).

PHARMACODYNAMIC MODELS

Receptor theory for the prediction of concentration-effect relationships

An initial requirement for successful allometric scaling is thorough understanding of the biological and/or physiological processes involved in drug action. PK-PD models containing general empirical expressions for quantification of drug action make prediction of kinetics of drug action in man from animal data extremely difficult, if not impossible. Receptor theory provides a theoretical framework for characterization of drug action based on separated expressions to characterize biological processes leading from receptor binding to pharmacological response. The last decade application of more mechanistically-

orientated PK-PD models is appreciated as a valuable and innovative tool with the aim to characterize and predict *in vivo* concentration *versus* effect relationships. As a result, PK-PD modeling is progressing from an empirical descriptive discipline to a more mechanistic discipline, acknowledging the physiological and/or pharmacological background underlying the kinetics of drug effect.

Mechanism-based PK-PD models aim at the separation of drug-related properties from system-related properties. Receptor theory based on Stephenson's concept and the operational model of agonism are different types of approaches which may form the cornerstone for the development of mechanism-based PK-PD models. In theory, pharmacodynamic parameters quantifying the drug-receptor interaction are unique for each drug and independent of species, while the system-related pharmacological characteristics of drug action are the same for each class of drugs, but different among species. By considering separated expressions for drug- and system-related properties, allometric scaling is simplified since only the system-related parameters are implicated in interspecies extrapolation. Consequently, the allometric relationship will be estimated with a higher degree of certainty, yielding more accurate prediction of the concentration *versus* effect relationship in human.

Recently, the pharmacokinetics and pharmacodynamics of the semi-synthetic opioid buprenorphine were successfully scaled from animal to human using a PK-PD model derived from the classical receptor theory. The proposed model contained specific expressions for biophase distribution and receptor association/dissociation kinetics. It was shown that, consistent with the degree of homology of the μ -opioid receptor between rat and human, the rate constants of receptor association and dissociation were identical in both species (drug-specific). The rate constant of biophase equilibration was scaled using an allometric equation (system-dependent) (Yassen *et al.*, 2007). The results show that predictions of pharmacological effects in humans can be obtained on the basis of animal data by allometric scaling of pharmacodynamic parameters. In this respect, an important prerequisite for successful animal to human extrapolation is the use of PK-PD models which make a clear distinction between drug-specific (species-independent) and system-dependent parameters (species-dependent).

Accurate predictions of concentration *versus* effect relationships requires attention for inter- and intra-individual variability. For instance, it was shown that not taking into account inter-individual variability in system-related parameters may result in biased estimates of drug-dependent characteristics like affinity and efficacy (der Graaf and Danhof, 1997). With respect to interspecies extrapolation, variability is a potential source for uncertainty in allometric scaling. Ignoring inter- and intra-individual variability in allometric scaled parameters may lead to biased estimates of pharmacodynamic parameters or allometric coefficient and exponent. An important question is whether the magnitude of inter- and intra-individual variability is different across species. To the best of our knowledge this question has yet not been addressed. Further research is required to explore optimal conditions for allometric scaling of inter-individual variability or whether vari-

ability terms are weight-independent.

The population approach as implemented in NONMEM takes into consideration several sources of variability and offers important advantages above conventional regression analysis. With mixed-effect modeling, the allometric coefficient and exponents are estimated in one step from the pooled data of all species. This approach facilitates the extrapolation and prediction of PK-PD parameters in non-studied species (i.e. man). Furthermore, the approach allows for quantification of inter-individual variability. More importantly, characteristics can be identified which influence the magnitude of variability. In this context, the allometric approach is attractive since different factors can be explored providing detailed information on the functioning of the biological system under physiological and pathological conditions. Moreover, mixed-effect models provide more accurate predictions compared to conventional regression models that do not account for the error structure. In a wider perspective, a mixed-effect model consists of a structural and a stochastic model. Both components of the model should be optimized to facilitate accurate allometric scaling of PK-PD characteristics from animal to man. The better patterns of inter- and intra-individual variability are established in the mixed-effects model, the more the model can be trusted to reflect variability in its predictions, ultimately in relation to extrapolation from animal to human. In this respect, PK-PD models derived from receptor theory can be applied to predict intra- and inter-individual variability on the basis of biological system specific information (i.e. receptor expression).

Dynamical system analysis for prediction of time-dependencies

Application of allometry becomes more complicated when biological systems are involved which evolve over time showing changes in physiological and/or pharmacological behavior, typically resulting in complex effect *versus* time profiles. For example, complex effect *versus* time profiles are observed when homeostatic feedback mechanisms are operative or after tolerance development upon drug exposure. Especially, when multiple sites are implicated it becomes very difficult to disentangle the separated activities. Recently, the incorporation of dynamical system analysis predicted the time course of 5-HT_{1A}-agonists-induced hypothermia in rats in conjunction with their respective corresponding plasma concentrations. The PK-PD model based on physiological principles contained an expression for the control of body temperature via the thermostat and an indirect response model to take into account warming and cooling of the body associated with changes in the set point temperature (Zuideveld *et al.*, 2001). Dynamical system analysis incorporating receptor theory principles provide a theoretical framework for the prediction of pharmacodynamic response in human on the basis of *in vivo* animal studies. More specifically, principles from receptor theory provide unique information on drug characteristics while detailed information on the functioning of the biological system is obtained from dynamical system analysis. This approach has been successfully applied to the prediction of the temperature *versus* time profile of ipsapirone in human using allometric scaled

system-related parameters determined in rat (Zuideveld *et al.*, 2002).

Interspecies scaling may be additionally supported by combining *in vitro* bio-assay data with *in vivo* concentration *versus* effect relationships. This technique had been successfully applied to predict *in vivo* clearance on the basis of *in vitro* metabolism studies. Mechanism-based PK-PD models based on receptor theory have proven to be an innovative tool to establish *in vitro-in vivo* correlations for the affinity constant. For example, on the basis of a simultaneous analysis of the concentration *versus* EEG effect relationships of four benzodiazepines a close *in vitro-in vivo* correlation was found. In a similar fashion, a correlation between *in vitro* GABA-shift and *in vivo* efficacy was established for a series of 6 benzodiazepines and 4 neurosteroids (Visser *et al.*, 2003). These results indicate that on the basis of *in vitro* studies relevant information can be obtained to facilitate the prediction of *in vivo* drug response but also the magnitude of maximal biological effect intensity.

More importantly, close correlations for *in vitro-in vivo* drug characteristics confirm the validity and application of mechanism-based PK-PD models to characterize drug response on the basis of specific and unique expressions reflecting the underlying mechanism of action. This approach is a novel instrument to predict *in vivo* concentration-effect relationships of other compounds on the basis of information obtained in *in vitro* binding studies. Clearly, this facilitates drug development and drug candidate selection, since essential information on the *in vivo* kinetics of drug action is obtained at an early stage of preclinical development. Although, currently efforts are being made to explore methods that can be used to predict metabolic clearance in human using *in vitro* models for metabolism, the role of *in vitro* models to predict drug action is relatively minimal. This underscores the importance of the use of mechanism-based PK-PD models for the prediction of biological effect in human using the combination of *in vitro* data and allometry.

CONCLUSION

This chapter focuses on the animal to human extrapolation of the PK-PD characteristics of drugs. In recent years, PK-PD modeling has progressed from an empirical descriptive discipline into a mechanistic science. Mechanism-based PK-PD models have much improved properties for extrapolation and prediction and therefore constitute a scientific basis for animal to human extrapolation of PK and PD properties of drugs. Physiologically-based pharmacokinetic models appear to be excellent tools to aid in the prediction of human pharmacokinetics on the basis of animal data and therefore considered truly mechanistic pharmacokinetic models.

Parallel to the physiologically-based PK models, mechanism-based PD models containing specific expressions on the causal pathway between drug administration and pharmacological effect are invaluable for the prediction, in a strictly quantitative manner, of drugs effects in man. Specifically, mechanism-based PD models which rely on the sepa-

rate characterization of drug-specific and biological system dependent parameters have proven to be a successful concept in the prediction of pharmacological effects of variety of drugs. Also these type of models may serve as a basis for the prediction of inter- and intra-individual variability. It was shown that with mechanism-based PD models *in vivo* concentration-effect relationships can be predicted in man on the basis of *in vivo* preclinical data. This opens new opportunities in drug development, specifically in drug candidate selection and lead optimization. Thus, mechanism-based PK-PD modeling constitutes a novel scientific basis for the prediction of drug effects in man, with regard to both efficacy and safety, enabling the selection of drug candidates with an optimal efficacy-safety balance.

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**SECTION
II**

**MECHANISM-BASED PK-PD MODELING
OF THE ANTINOCICEPTIVE EFFECT OF
(SEMI-)SYNTHETIC OPIOIDS**

PHARMACOKINETIC-PHARMACODYNAMIC MODELING OF THE ANTINOCICEPTIVE EFFECT OF BUPRENORPHINE AND FENTANYL IN RATS: ROLE OF RECEPTOR EQUILIBRATION KINETICS

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ABSTRACT

The objective of this investigation was to characterize the pharmacokinetic-pharmacodynamic correlation of buprenorphine and fentanyl for the antinociceptive effect in rats. Data on the time course of the antinociceptive effect following intravenous administration of buprenorphine or fentanyl was analyzed in conjunction with plasma concentrations by non-linear mixed effects analysis.

For fentanyl the pharmacokinetics was described on the basis of a two-compartment pharmacokinetic model. For buprenorphine, a three-compartment pharmacokinetic model best described the concentration time course. To explain time-dependencies in pharmacodynamics of buprenorphine and fentanyl a combined effect compartment-receptor binding model was applied. A log-logistic probability distribution model is proposed to account for censored tail flick latencies. The model converged yielding precise estimates of the parameters characterizing hysteresis.

The results show that the onset and offset of the antinociceptive effect of both buprenorphine and fentanyl is mainly determined by biophase distribution. The k_{e0} was 0.024 min^{-1} (95 % CI: $0.018 - 0.030 \text{ min}^{-1}$) and 0.123 min^{-1} (95 % CI: $0.095 - 0.151 \text{ min}^{-1}$) for buprenorphine and fentanyl respectively. On the other hand, part of the hysteresis in the buprenorphine pharmacodynamics could be explained by slow receptor association/dissociation kinetics. The k_{off} was 0.073 min^{-1} (95 % CI: $0.042 - 0.104 \text{ min}^{-1}$) and k_{on} was 0.023 ml/ng/min (95 % CI: $0.013 - 0.033 \text{ ml/ng/min}$). Fentanyl binds instantaneously to the μ -opioid receptor as no reasonable values for k_{on} and k_{off} were obtained with the dynamical receptor model.

The findings of this study show that, in contrast to earlier reports in the literature, the rate-limiting step in the onset and offset of buprenorphine's antinociceptive effect is distribution to the brain.

INTRODUCTION

Buprenorphine is a semi-synthetic opioid synthesized from the precursor thebaine. Several studies have revealed μ -opioid receptor agonistic binding capacity for buprenorphine. More specifically, a study conducted in the spinal dog classified buprenorphine as a partial agonist for the μ -opioid receptor (Martin *et al.*, 1976). The μ -opioid receptor is of specific interest, given its role in the mediation of analgesia (Zhang and Pasternak, 1981; Lutfy *et al.*, 2003).

In principle, partial agonists only produce a sub-maximal response relative to full agonists which display full efficacy. However, the exact behavior of buprenorphine at the μ -opioid receptor in relation to its analgesic effect has not yet been unequivocally clarified. Data from animal studies suggest that buprenorphine-mediated analgesia might be governed by a bell-shaped dose-response curve (Cowan *et al.*, 1977a,b; Dum and Herz, 1981). At the lower dose range a dose-dependent increase in analgesia is observed, while at intermediate doses the response is diminished. At relatively high doses evidence for an inverse dose-response relationship has been obtained in animals. However, the observed pharmacological behavior at high doses can not be explained by partial agonistic activity. Furthermore, the existence of a bell-shaped dose-response relationship in human, remains controversial, based on the results of studies in volunteers and patients. On the basis of studies in humans it has been claimed that ceiling effect, for side effects as respiratory depression, may occur either within the therapeutic analgesic dose range or at doses exceeding the clinically relevant range, reflecting partial agonism (Walsh *et al.*, 1994). In addition, several studies have demonstrated full analgesic efficacy for buprenorphine over a wide dose range (Mok *et al.*, 1981; Watson *et al.*, 1982; Christoph *et al.*, 2005).

Besides its intrinsic activity at the μ -opioid receptor, buprenorphine has another interesting characteristic with respect to μ -opioid receptor binding (Cowan *et al.*, 1977a; Boas and Villiger, 1985). The kinetics of binding to and dissociation from the μ -opioid receptor is slow. The slow receptor binding kinetics at the μ -opioid receptor is reflected by a slow onset and a prolonged duration of effect. The slow receptor kinetics combined with its low intrinsic activity make buprenorphine an attractive compound for the treatment of opioid addiction as an alternative for methadone (Jasinski *et al.*, 1978). However, the slow receptor equilibration kinetics also attributes to the difficulty of naloxone to compete with buprenorphine for the μ -opioid receptor. Consequently, reversal of buprenorphine's effect with naloxone appears to be difficult (Gal, 1989). In contrast, fentanyl binds to and dissociates from the μ -opioid receptor rapidly. Observed hysteresis in concentration-effect data of fentanyl is typically explained by factors related to the blood-brain equilibration (Scott *et al.*, 1991).

Surprisingly, despite the fact that buprenorphine and fentanyl have similar physicochemical properties (high lipophilicity) nobody has addressed the question whether the kinetics of buprenorphine effect is also delayed by biophase distribution. Despite the

increasing progress in receptor pharmacology and clinical pharmacology of opioids little information is available on the *in vivo* kinetics of drug action and more specifically the PK-PD correlation of buprenorphine and fentanyl.

In recent years, there has been an increasing interest in the application of receptor theory in PK-PD modeling with the aim to predict *in vivo* concentration-effect relationships (Van der Graaf and Danhof, 1997). An important feature of these mechanism-based models is the strict distinction between drug- and system-related properties (Van der Graaf *et al.*, 1999; Visser *et al.*, 2002; Zuideveld *et al.*, 2004). A common feature of these models is that rapid equilibration of the drug-receptor complex is assumed. However, some drugs do not bind rapidly with their pharmacological target and therefore the time course of drug effect is influenced by the kinetics of the target equilibration (Shimada *et al.*, 1996).

In this investigation a mechanism-based PK-PD model is proposed which contains specific expressions for the kinetics of the drug-receptor interaction *in vivo*. A specific feature of the model is that it allows separation of the kinetics of biophase distribution from the receptor association/dissociation kinetics to explain time-dependencies in pharmacodynamics. Identification and quantification of the rate-limiting step for kinetics of drug action will improve the understanding of the differences in PK-PD properties between buprenorphine and fentanyl, ultimately, also in relation to the kinetics of the interaction with naloxone. In the investigation, tail flick latency has been used as a response measure. The developed PK-PD model was evaluated and validated over a wide dosing range and by application of several infusion rates. Furthermore, the accuracy and precision of the pharmacokinetic model predictions was assessed by bootstrap analysis and a visual predictive check.

MATERIALS AND METHODS

Animals

Male Wistar rats were used in all experiments. The animals were housed in plastic cages in groups before surgery and individually after surgery. The animals were housed under laboratory standard conditions at constant room temperature (21 °C) and on a 12-h light/dark cycle, with lights turned on at 7:00 am and off at 7:00 pm. Food (RMH-TM; Hope Farms, Woerden, The Netherlands) and acidified water were allowed *ad libitum*. The animals were handled and allowed for acclimation to the experimental environment for ten days prior to the start of the experiment. The protocol was approved by the Ethical Committee on Animal Experimentation of Leiden University.

Surgical Procedure

Surgery was carried out under anesthesia with an intramuscular injection of 0.1 mg/kg medetomidine hydrochloride (Domitor 1 mg/ml; Pfizer, Capelle a/d IJssel, The Netherlands) and 1 mg/kg Ketamine base (Ketalar 50 mg/ml; Parke-Davis, Hoofddorp, The Netherlands).

Two days before the experiment indwelling cannulae were implanted, one in the left femoral artery and one in the right jugular vein. The cannula in the right jugular vein was used for administration of the opioid while the cannula in the left femoral artery was used for serial collection of arterial blood samples. The cannulae were made from pyrogen free, non-sterile polyethylene tubing. One day before surgery cannulae were disinfected in a benzalkoniumchlorid 1 % solution. The venous cannula consisted of 3 cm polyethylene tubing (0.28 mm i.d.; Portex Limited, Kent, United Kingdom) heat-sealed to 9 cm polyethylene tubing (0.58 mm i.d.; Portex Limited, Kent, United Kingdom). The arterial cannula consisted of 3 cm polyethylene tubing (0.28 mm i.d.) heat-sealed to 21 cm polyethylene tubing (0.58 mm i.d.). The cannulae were tunnelled subcutaneously and fixed at the back of the neck with a rubber ring. The skin in the neck and throat was stitched with normal suture. The skin in the groin was closed with wound clips.

In order to prevent clotting and cannula obstruction the cannulae were filled with a 25% (w/v) polyvinylpyrrolidone solution (PVP; Brocacef, Maarssen, The Netherlands) in pyrogen-free physiological saline (B. Braun Melsungen AG, Melsungen, Germany) containing 20 IU/ml heparin (Hospital Pharmacy, Leiden University Medical Center, Leiden, The Netherlands).

Drugs and dosages

Buprenorphine hydrochloride and fentanyl monocitrate were kindly donated by Grünenthal GmbH (Aachen, Germany). Buprenorphine hydrochloride solution was prepared in saline with aid of two drops polysorbate 80 (Hospital Pharmacy, Leiden University Medical Center, Leiden, The Netherlands). To accelerate solubility, the solution was placed in an ultra-sonification bath for 30 min. Fentanyl monocitrate solution was prepared in saline. The doses and concentrations of buprenorphine and fentanyl are expressed as free base.

Measurement of antinociceptive effect

A tail flick analgesia meter (Columbus Instruments, Columbus, Ohio, USA) was used to determine the pain sensitivity in the control and the drug-treated rats (D'Amour and Smith, 1941). Radiant heat was applied using a shutter-controlled lamp as a heat source focused on a spot located 6.5 to 7.5 cm from the tip of the tail. The intensity of the beam was set at a level producing basal latency times between 2.5 and 3.5 s. To prevent tissue injury,

the cut-off time was fixed at 10 s. A digital response time indicator with a resolution of 0.1 s measured the time between activation of the light beam and the tail flick.

Drug analysis

Buprenorphine

Buprenorphine and norbuprenorphine plasma concentrations were determined by HPLC coupled to tandem mass spectrometry (LC/MS/MS). The chromatographic system consisted of an Agilent HP 1100 HPLC system (Agilent, Waldbronn, Germany) coupled to an API 4000 LC/MS/MS system (Applied Biosystems, Darmstadt, Germany). Chromatography was performed on a precolumn (Metaguard Polaris 3 μ m C_{18A} 2 mm, Varian Darmstadt, Germany) guarded Synergi 4 μ m Hydro-RP 80A column 75 mm x 2 mm (Phenomenex, Aschaffenburg, Germany) at 40 °C and a flow rate of 0.8 ml/min.

The mobile phase consisted of water (solvent A) and acetonitrile:tetrahydrofuran (90:10, v/v) (solvent B) both containing 0.1% formic acid. The programme started with 90% A for 1 min followed by a linear gradient from 90% A to 25% A ramped up in 4 min. After two minutes with 25 % A the gradient was switched back to 90 % A in 0.1 min. The system was equilibrated for 3 minutes before injecting the next sample. The total run time was set at 10.1 min. A retention time of 3.3 min for norbuprenorphine and 3.8 min for buprenorphine was found for both analytes and their respective deuterated internal standards.

A plasma volume of 50 μ l was used for the assay of rat samples, standards and quality control samples. All plasma samples (rat samples, standards and quality control samples) were spiked with 1 ng (25 μ l of 4 μ g/100 ml) of the internal standard (²H₄-buprenorphine and ²H₉-norbuprenorphine). After adding 25 μ l concentrated ammonia, the samples were extracted for 15 min by liquid/liquid extraction with 600 μ l tert.-butyl methyl ether (MTBE). After centrifugation at 13200 rpm for 8 minutes, the organic phase was transferred to autosampler vials, evaporated to dryness at 40 °C under a gentle stream of nitrogen and reconstituted in 125 μ l of 0.1% formic acid in acetonitrile:tetrahydrofuran (90:10, v/v). A volume of 50 μ l was injected onto the HPLC column.

For the construction of the calibration curve for buprenorphine and norbuprenorphine the following standard solutions were used: 0.047, 0.092, 0.19, 0.37, 0.73, 1.5, 2.9, 5.9 and 12 ng/ml. The calibration curve was linear in the range from 0.047 to 12 ng/ml for both analytes ($r > 0.999$). The lower limit of quantification (LLOQ) was 0.047 ng/ml for buprenorphine and norbuprenorphine. The accuracy ranged from 99.4 to 102.1 % for buprenorphine and from 96.1 to 101.0 % for norbuprenorphine. The precision for the determination of buprenorphine, expressed as coefficient of variation, ranged from 2.2 to 6.4 % for concentrations between 0.14 and 8.9 ng/ml. The respective values for norbuprenorphine are 2.0 to 3.7 % in the concentration range of 0.14 to 8.7 ng/ml.

Fentanyl

Fentanyl plasma concentrations were analyzed using HPLC coupled to tandem mass spectrometry (LC/MS/MS). The chromatographic system consisted of an Agilent HP 1100 HPLC system (Agilent, Waldbronn, Germany) coupled to an API 3000 LC/MS/MS system (Applied Biosystems, Darmstadt, Germany). Chromatography was performed on a precolumn (Metaguard Polaris 3 μ m C_{18A} 2 mm, Varian Darmstadt, Germany) guarded Atlantis C₁₈ column 3 μ m 100 mm x 2.1 mm (Waters, Eschborn, Germany).

The mobile phase consisted of water (solvent A) and methanol (solvent B) both containing 0.5 % acetic acid. The programme started with 98% A for 2 min followed by a linear gradient from 98% A to 10% A ramped up in 1 min. After three minutes with 10 % A the gradient was switched back to 98 % A in 0.1 min. The system was equilibrated for 2.5 minutes before injecting the next sample. The total run time was set at 9.6 min. A retention time of 6.1 min for fentanyl and its deuterated internal standard.

A plasma volume of 50 μ l was used for the assay of rat samples, standards and quality control samples. All plasma samples (rat samples, standards and quality control samples) were spiked with 0.370 ng (25 μ l of 14.8 ng/ml) of the internal standard (²H₅-fentanyl). After adding 10 μ l concentrated ammonia the samples were extracted for 15 min by liquid/liquid extraction with 600 μ l tert.-butyl methyl ether (MTBE). After centrifugation at 13200 rpm for 8 minutes, the organic phase was transferred to autosampler vials, evaporated to dryness at 40 °C under a gentle stream of nitrogen and reconstituted in 125 μ l of 0.5% acetic acid/methanol (90:10,v/v). A volume of 25 μ l was injected onto the HPLC column.

For the construction of the calibration curve for fentanyl the following standard solutions were used: 0.118, 0.24, 0.47, 0.94, 1.9, 3.8, 7.5, 15, 30, 60 and 120 ng/ml. The calibration curve was linear in the range from 0.118 to 120 ng/ml ($r > 0.999$). The lower limit of quantification (LLOQ) was 0.118 ng/ml. The accuracy ranged from 87.0 to 96.1 % and the precision from 1.9 to 4.0 % for concentrations in the range from 0.4 to 50.2 ng/ml.

Pharmacokinetic-pharmacodynamic experiments

To minimize the influence of circadian rhythms, all experiments started between 9:00 and 9:30 am. Animals were randomly assigned to the treatment groups. Detailed information regarding experimental design is presented in table 1. Before administration of drug or vehicle four consecutive baseline tail flick latencies were obtained in each animal. The measurements were taken at a 15 min interval. The average of the four baseline latencies was taken as the basal latency time.

Upon administration of buprenorphine or vehicle via a zero-order intravenous infusion using an infusion pump (BAS Bioanalytical Systems Inc., West Lafayette, Indiana, USA), tail flick latency was measured at the following pre-defined time-points; dose I: 0, 5, 9, 14, 19,

24, 30, 40, 50, 95, 105, 125, 155 and 185 min after drug administration, dose II: 0, 5, 10, 14, 19, 24, 30, 40, 50, 65, 70, 95, 105, 125, and thereafter every 30 min till 305 min after drug administration, dose III: 0, 5, 10, 14, 19, 24, 30, 40, 50, 95, 105, 125 and thereafter every 30 min till 215 min after drug administration, dose IV: 0, 5, 10, 14, 19, 24, 30, 40, 50, 70, 90 and thereafter every 30 min till 420 min, dose V: 0, 5, 10, 14, 19, 24, 30, 40, 50, 70, 90, 150 and thereafter every 30 min till 510 min after drug administration.

For fentanyl, antinociceptive measurements were performed at; dose I: 0, 3, 7, 13, 17, 23, 33, 45, 75, 105, 150, and 180 min, dose II: 0, 3, 7, 13, 17, 23, 33, 45, 55, 75, 105 and 135 min, dose III: 0, 3, 7, 13, 17, 23, 33, 45, 55, 75, 105, 135, 150, 180 and 210 min, dose IV: 0, 5, 15, 25, 35, 43, 55, 65, 80, 105, 150 and 180 min, dose V: 0, 3, 7, 17, 23, 33, 45, 55, 75, 95, 135, 150, 210 and 240 min after drug administration.

In cases where blood sampling coincided with the tail flick latency measurement, tail flick latency measurement preceded blood sampling to minimize stress for the animals. Before start of the infusion a blank blood sample (100 μ l) was withdrawn. Each blood sample withdrawn was replaced by an equal volume of heparinized 0.9 % saline (20 IU heparin/ml). This procedure has minimal effects on the pharmacokinetics. In a separate study it has been demonstrated that the values of the pharmacokinetic parameters obtained in this manner were identical to those obtained in a separate (pilot) study without replacement of the collected blood (Yassen and Danhof, unpublished observations). Serial arterial blood samples were collected in heparinized microtubes. Plasma (50 μ l) was separated from the blood by centrifugation at 5000 rpm for 15 min and frozen at -20 °C until analysis.

PK-PD modeling procedure

The pharmacokinetic and pharmacodynamic parameters of buprenorphine and fentanyl were estimated using non-linear mixed-effects modeling as implemented in the NONMEM software version V, level 1.1 (Beal and Sheiner, 1999). The population analysis approach, which takes into consideration both intra-animal and inter-animal variability, was undertaken using the first-order conditional estimation method with η - ϵ interaction (FOCE interaction) for pharmacokinetic analysis. All fitting procedures were performed on an IBM-compatible computer (Pentium IV, 1500 MHz) running under Windows NT with the Fortran compiler Compaq Visual Fortran version 6.1. An in-house available S-PLUS 6.0 (Insightful Corp., Seattle, WA, USA) interface to NONMEM version V was used for data processing and management (including automated visual predictive check and bootstrap) and graphical data display.

Pharmacokinetic analysis

In order to determine the basic structural pharmacokinetic model for buprenorphine and fentanyl one-, two- and three-compartment models were tested. Model selection and

identification was based on the likelihood ratio test, pharmacokinetic parameter point estimates and their respective confidence intervals, parameter correlations and goodness-of-fit plots. For the likelihood ratio test, the significance level was set at $\alpha=0.01$, which corresponds with a decrease of 6.6 points, after the inclusion of one parameter, in objective function value (OFV) under the assumption that the difference in OFV between two nested models is χ^2 distributed. The following goodness-of-fit plots were subjected to visual inspection to detect systemic deviations from the model fits: individual observed *versus* population or individual predicted values and weighted residuals *versus* time or population predicted values. On the basis of model selection criteria, two- and three- compartment models were selected for fentanyl and buprenorphine, respectively. The pharmacokinetic analysis for the selected compounds was performed by use of the

Table 1. Experimental design of the study describing the PK-PD relationship of the antinociceptive effect of buprenorphine and fentanyl, with respect to number of animals per treatment group and their corresponding dose normalized for body weight, absolute dose, length of infusion and body weight. Data are presented as mean $\pm$ standard error (SE)

Group	n	Infusion time (min)	Dose (mg/kg)	Absolute Dose (mg)	body weight (kg)
buprenorphine					
I	5	20	0.015	0.0043 $\pm$ 0.0002	0.288 $\pm$ 0.011
II	8	20	0.030	0.0081 $\pm$ 0.0003	0.271 $\pm$ 0.010
III	7	40	0.030	0.0088 $\pm$ 0.0006	0.293 $\pm$ 0.020
IV	8	20	0.060	0.0160 $\pm$ 0.0018	0.267 $\pm$ 0.029
V	7	20	0.10	0.0276 $\pm$ 0.0027	0.276 $\pm$ 0.027
Control [§]	4	20			0.283 $\pm$ 0.011
Control	4	40			0.289 $\pm$ 0.015
fentanyl					
I	8	20	0.020	0.0052 $\pm$ 0.0002	0.262 $\pm$ 0.010
II	8	20	0.025	0.0067 $\pm$ 0.0004	0.268 $\pm$ 0.015
III	8	20	0.035	0.0089 $\pm$ 0.0005	0.254 $\pm$ 0.015
IV	8	40	0.035	0.0095 $\pm$ 0.0007	0.270 $\pm$ 0.019
V	8	20	0.040	0.0106 $\pm$ 0.0008	0.265 $\pm$ 0.021
Control [‡]	4	20			0.278 $\pm$ 0.025
Control	4	40			0.263 $\pm$ 0.018

[§] Control groups received an infusion of saline containing polysorbate 80

[‡] Control groups received an infusion of saline.

ADVAN3 TRANS4 and ADVAN11 TRANS4 subroutines in NONMEM. For example, for fentanyl, the pharmacokinetic parameters, clearance (Cl), the inter-compartmental clearance (Q) and the volumes of distribution of compartments 1 and 2 (V_1 and V_2) were estimated. The stochastic part of the model was selected to describe inter-animal variability in pharmacokinetic parameters and assumed a log-normal distribution of all model parameters over the population. Therefore an exponential distribution model was used to account for inter-animal variability:

$$P_i = P_{tot} \cdot \exp(\eta_i) \quad (1)$$

in which P_i is the individual value of model parameter P , P_{tot} is the typical value (mean population value) of parameter P in the population, and η_i is the normally distributed inter-animal random variable with mean zero and variance ω^2 . The coefficient of variation of the structural model parameters is expressed as percentage of the root mean square of the inter-animal variance term.

Selection of an appropriate residual error model was based on inspection of the goodness-of-fit plots. On this basis a proportional error model was proposed to describe residual error in the plasma drug concentration:

$$C_{obs,ij} = C_{pred,ij} \cdot (1 + \varepsilon_{ij}) \quad (2)$$

in which $C_{obs,ij}$ is the j th observed concentration in the i th individual, $C_{pred,ij}$ is the predicted concentration, and ε_{ij} is the normally distributed residual random variable with mean zero and variance σ^2 . The residual error term contains all the error terms which can not be explained and refers to for example measurement and experimental error (e.g. error in recording sampling times) and structural model misspecification. Individual empirical Bayes estimates of the pharmacokinetic parameters were obtained from the basic pharmacokinetic model and served as input for the pharmacodynamic model.

To refine the stochastic model, correlation between pharmacokinetic parameter estimates was tested by conducting covariance matrix analysis (OMEGA BLOCK option). A significant correlation between two parameters was assumed when the drop in OFV was more than 6.6 points ($p < 0.01$). Finally, explorative graphical analysis was performed to explore relationships between body weight and pharmacokinetic parameters. The following equation was used to model the pharmacokinetic parameters as function of body-weight (BW):

$$P_i = \theta_{INT} + \theta_{SLOPE} \cdot (BW - \text{median } BW) \quad (3)$$

in which P_i is the individual value of model parameter P , θ_{INT} and θ_{SLOPE} are the intercept and slope of model parameter P versus bodyweight relationship, respectively.

To demonstrate the precision and stability of the pharmacokinetic models and to ascertain accurate prediction of concentration-time profiles of fentanyl and buprenorphine,

the final population pharmacokinetic models were subjected to an internal validation (Ette *et al.*, 2003; Food and Drug Administration, 1999). The validation procedure consisted of two components: bootstrap validation procedure and a visual predictive check. For the bootstrap validation procedure, one thousand data sets were generated randomly sampled from the original data set with replacement. Subsequently, the final population PK models were fitted to the bootstrap replicates one at a time. Finally, the mean, standard error, coefficient of variation and 95 % confidence intervals of all model parameters were calculated and compared to parameter values obtained from the original study. To assess the predictive performance of the population PK models, one thousand data sets were simulated from the original data set and the final model parameter estimates. The median outcome and the 2.5 % and 97.5 % quantiles were calculated from the simulated buprenorphine and fentanyl concentrations at the pre-defined time-points.

Mechanism-based PK-PD analysis

In this study, the tail flick latency is used as a measure of the drug response. The mechanism-based model describing the complex relationship between drug concentration and pharmacological effect is displayed in figure 1. The observed hysteresis in concentration-effect data is traditionally explained by incorporation of a link model. In this model distribution to the biophase is characterized as a first-order process, which is believed to constitute a correct representation of the rate-limiting step in the *in vivo* pharmacodynamics.

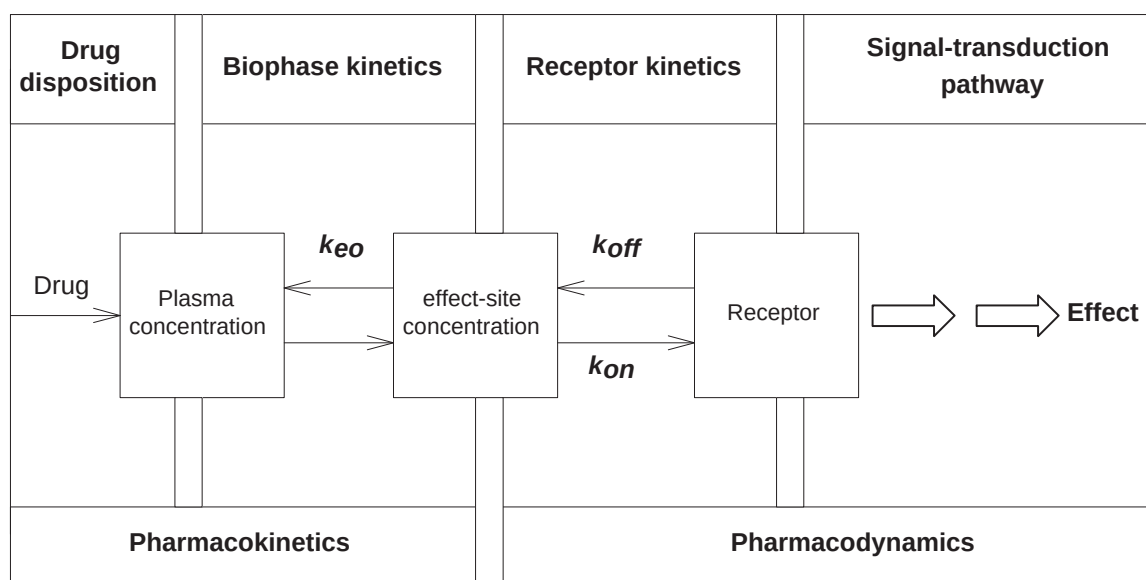


Figure 1. A schematic view of the mechanism-based PK-PD model. The model incorporates different processes to explain time dependencies in pharmacodynamics. The pharmacokinetics describe the disposition of the drug in the plasma and equilibration to the biophase site. At the target site, the drug can bind to the receptor. Upon binding to the receptor, a cascade of transduction processes are activated, ultimately leading to a pharmacological response.

Separation of different biological processes, causing hysteresis (i.e. biophase equilibration, receptor association/dissociation and transduction), frequently results in the inability to obtain unique parameter estimates expressing the respective rate-limiting steps (Tuk *et al.*, 1997, 1998; Cleton *et al.*, 1999). To explain hysteresis on the basis of two biological processes, the availability of a detailed data set including different doses and infusion schemes is required.

Furthermore, with the antinociceptive effect as a pharmacodynamic endpoint the data analysis is complicated by the presence of censored data (tail flick latencies above the cut-off value). To allow for estimation of the effect above the censoring value a maximum likelihood parameter estimation approach was used. This approach requires the specification of a probability distribution for the time at which an animal responds to applied radiant heat. In the statistical literature, several distributions have been proposed to describe time-to-event (also called survival) data; factors such as flexibility and practical implementation (i.e. in NONMEM) suggest the log-logistic and Weibull distributions as suitable candidates (Cox and Oakes, 1984). The log-logistic distribution is characterized by the median time-to-response (prediction) and a shape factor determining its width (Z). The probability of observing a tail flick latency > 10 s is given by the area under the log-logistic curve from 10 s to infinity. So the log likelihood to be maximized is the sum of terms of either:

$$\log P\{\text{latency} = \text{observation}\} = \log(Z) + (Z - 1) \cdot \log\left(\frac{\text{observation}}{\text{prediction}}\right) - 2 \cdot \log\left(1 + \left(\frac{\text{observation}}{\text{prediction}}\right)^Z\right) \quad (4)$$

or

$$\log P\{\text{latency} > \text{cut-off}\} = -\log\left(1 + \left(\frac{\text{observation}}{\text{prediction}}\right)^Z\right) \quad (5)$$

The exposure-response relationships of buprenorphine and fentanyl are quantified using a mechanism-based PK-PD model. This model describes the equilibration to the biophase, where the drug can bind to the μ -opioid receptor. Drug distribution to the site of action (biophase) was characterized on the basis of an effect compartment model (Sheiner *et al.*, 1979). The rate of change of biophase drug concentrations can be described as follows:

$$\frac{dC_e}{dt} = k_{e0} \cdot (C_p - C_e) \quad (6)$$

where k_{e0} is a first-order distribution rate constant describing the rate of change of drug concentration in the effect compartment and C_p represents the plasma concentration and C_e the effect-site concentration. At the site of action, the drug can bind to the μ -opioid

receptor. Following the law of mass action, the rate of drug-receptor binding (dC_e/dt) is proportional to the drug concentration C_e and the free receptor concentration R :

$$\frac{dC_e R}{dt} = k_{\text{on}} \cdot C_e \cdot R - k_{\text{off}} \cdot C_e R \quad (7)$$

in which k_{on} is a second-order rate constant describing the rate of association and k_{off} is a first-order rate constant describing the rate of dissociation of the drug-receptor complex. Under the assumption that the concentration of drug is in excess compared to the free receptor concentration and that the total number of receptors (R_{tot}) is equal to the sum of drug-bound receptors ($C_e R$) and unbound receptors (R), equation 7 can be rearranged into:

$$\frac{dC_e R}{dt} = k_{\text{on}} \cdot C_e \cdot (R_{\text{tot}} - C_e R) - k_{\text{off}} \cdot C_e R \quad (8)$$

The total amount of receptors (R_{tot}) could not be measured *in vivo* and therefore was set to one unity. Receptor binding was directly related to the tail flick latency time according to the following equation:

$$\text{prediction} = \frac{E_0}{1 - C_e R} \quad (9)$$

The concentration-effect data for fentanyl were analyzed by the following model (Sarton *et al.*, 2000):

$$\text{prediction} = E_0 \cdot \left[1 + \left(\frac{C_e}{C_{100}} \right)^y \right] \quad (10)$$

where E_0 is the baseline tail flick latency, C_{100} the effect-site concentration causing 100 % increase in tail flick latency and y a slope parameter. This equation follows from the steady-state solution of equations 8 and 9 in which case k_{on} and k_{off} are not both identifiable ($C_{100} = k_{\text{off}}/k_{\text{on}}$)

RESULTS

Buprenorphine and fentanyl pharmacokinetics

A two compartment model best described the pharmacokinetics of fentanyl, while for buprenorphine a three-compartment model was selected. The observed and population predicted concentration-time courses of buprenorphine and fentanyl are depicted in figure 2 and 3, respectively. All pharmacokinetic parameters were estimated precisely with acceptable coefficient of variance. For buprenorphine the coefficient of variation of the various parameters varied between 2.4 % and 32 %, while for fentanyl the range was between 2.5 % to 19 %. Estimation of inter-animal variability was possible for the following

parameters: Cl , V_1 , V_2 and V_3 of buprenorphine. For fentanyl inter-animal variability was estimated for Cl , V_1 and V_2 .

An overview of the values of the pharmacokinetic parameters and their respective coefficients of variation and inter-animal variability is provided in table 2 and table 3. Covariate analysis revealed a linear relationship between Cl , V_1 , V_2 and V_3 versus bodyweight (BW) and Cl versus bodyweight for buprenorphine and fentanyl, respectively. The equations describing the covariate-pharmacokinetic parameter relationships are shown in table 4. The correlations between the values of inter-animal variability of Cl , V_1 , V_2 and V_3 of buprenorphine and Cl , V_1 , and V_2 of fentanyl were evaluated by using a full omega matrix. Subsequently, the full omega matrix was reduced to a matrix only including significant correlations between parameters. An increase of > 6.6 points in OFV was used to evaluate the significance of any correlation between the parameters after deletion of the respective covariance term. A correlation was observed between $\omega^2 Cl$ and $\omega^2 V_2$ of fentanyl and therefore the covariance of those parameters was added to the final model. The correlation coefficient between the two parameters was 0.70.

Finally, to validate the pharmacokinetic models a bootstrap validation and a visual predictive check were conducted. The final population pharmacokinetic estimates for buprenorphine and fentanyl were nearly identical to the estimates obtained by fitting one thousand data sets to the final population PK models. Also, the estimated inter-

Table 2. Parameter estimates with their respective relative standard error (%) of the final population pharmacokinetic model for buprenorphine and the stability of the parameters using the bootstrap resampling procedure

	Original data set		1000 bootstrap replicates	
	Typical value	IAV (%) [†]	Typical value	IAV (%)
$Cl_{\text{intercept}}$ (ml/min)	14 (2.4)	12 (36)	14 (2.6)	12 (39)
Cl_{slope} (ml/min/kg)	95 (32)		95 (30)	
$V_{1\text{intercept}}$ (ml)	120 (7.6)	60 (61)	113 (7.4)	68 (76)
$V_{1\text{slope}}$ (ml/kg)	2150 (3.9)		2080 (3.9)	
$V_{2\text{intercept}}$ (ml)	300 (20)	19 (41)	306 (16)	18 (44)
$V_{2\text{slope}}$ (ml/kg)	2900 (5.3)		2920 (5.0)	
$V_{3\text{intercept}}$ (ml)	810 (13)	10 (46)	812 (14)	10 (53)
$V_{3\text{slope}}$ (ml/kg)	3900 (31)		3960 (34)	
Q_2 (ml/min)	26 (18)	NE [‡]	27 (20)	NE
Q_3 (ml/min)	7.3 (30)	NE	7.3 (31)	NE
PRE [#] (%)	13 (10)		13 (11)	

[†] inter-animal variability; [‡] not estimated; [#] proportional residual error

Table 3. Parameter estimates with their respective relative standard error (%) of the final population pharmacokinetic model for fentanyl and the stability of the parameters using the bootstrap resampling procedure

	Original data set		1000 bootstrap replicates	
	Typical value	IAV (%)	Typical value	IAV (%)
$Cl_{\text{intercept}}$ (ml/min)	16.4 (2.5)	13 (22)	16.4 (2.9)	13 (23)
Cl_{slope} (ml/min/kg)	135 (19)		127 (22)	
V_1 (ml)	197 (8.3)	43 (30)	196 (8.9)	42 (35)
V_2 (ml)	443 (4.8)	20 (28)	443 (4.6)	19 (28)
Q (ml/min)	14.5 (5.7)	NE	14.5 (5.9)	NE
PRE (%)	20 (9.0)		20 (8.9)	

Fentanyl	
Cl (ml/min/kg)	$= 16.4 + 135 \cdot (BW - 0.263)$
Buprenorphine	
Cl (ml/min/kg)	$= 14 + 95.0 \cdot (BW - 0.281)$
V_1 (ml/kg)	$= 120 + 2150 \cdot (BW - 0.281)$
V_2 (ml/kg)	$= 300 + 2900 \cdot (BW - 0.281)$
V_3 (ml/kg)	$= 810 + 3900 \cdot (BW - 0.281)$

Table 4. Linear regression equations describing the relationship between pharmacokinetic parameters and bodyweight (kg)

animal variability for the final pharmacokinetic parameters was supported by the bootstrap validation (tables 2 and 3). The results of the visual predictive check showed that the population PK models could well predict the time course of buprenorphine and fentanyl concentration after intravenous administration (figures 4 and 5).

Mechanism-based PK-PD model

After start of infusion, the maximal effect for fentanyl was reached after 25 min, while for buprenorphine maximal effect was reached after 50 min. The maximal peak effects obtained were different between buprenorphine and fentanyl, in spite of similarities in the concentration range studied. In the buprenorphine group IV the predicted peak effect was higher than predicted in group V, in spite of a lower dose. For buprenorphine the values of the maximal predicted tail flick latency ($\pm$ SEM) were 4.65 ± 0.11 , 10.03

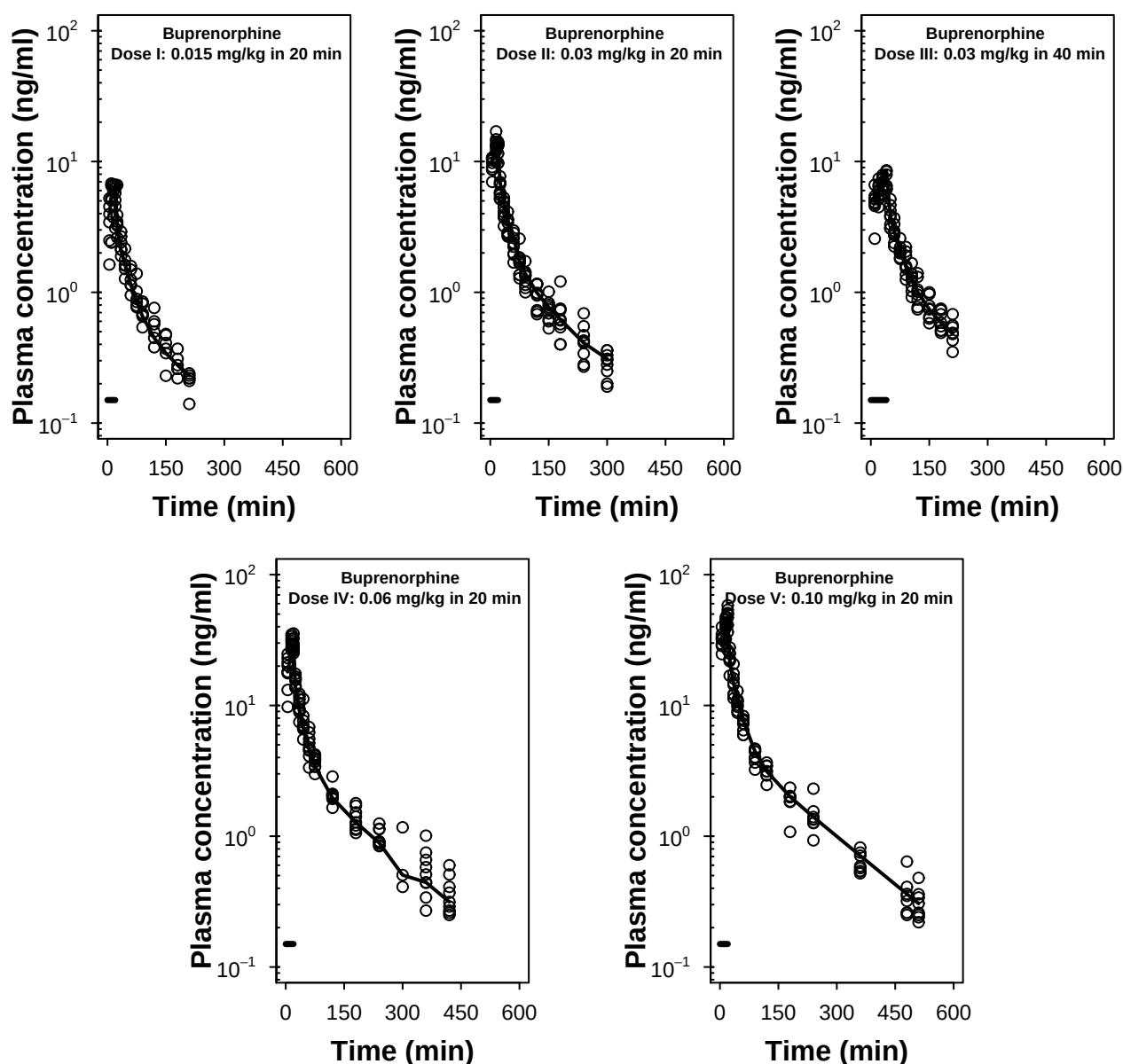


Figure 2. Individual buprenorphine concentration-time profiles for the treatment groups I-V. The observed concentrations (closed circles) and population predictions (thick line) are depicted. The black boxes represent the duration of infusion.

± 0.54 , 5.82 ± 0.90 , 16.36 ± 1.92 and 14.71 ± 1.97 s for dose I-V, respectively. For fentanyl, a dose-related increase in the predicted peak effect was observed. The values of the maximal predicted tail flick latency were 7.26 ± 0.17 , 13.17 ± 2.24 , 15.11 ± 1.7 , 7.95 ± 1.05 , 21.49 ± 3.07 s for dose I-V, respectively. NONMEM analysis revealed that the correlation between the estimates of *in vivo* potency and $E_{\max}$ was highly correlated and consequently the PK-PD models were very unstable. Therefore, the intrinsic activity of buprenorphine and fentanyl could not be estimated. Simplification of the model assuming a linear concentration- or receptor binding-response relationship adequately described the data and led to a more stable model. The predicted tail flick latencies above the cut-off value of 10 s were estimated on the basis of a time-to-event analysis.

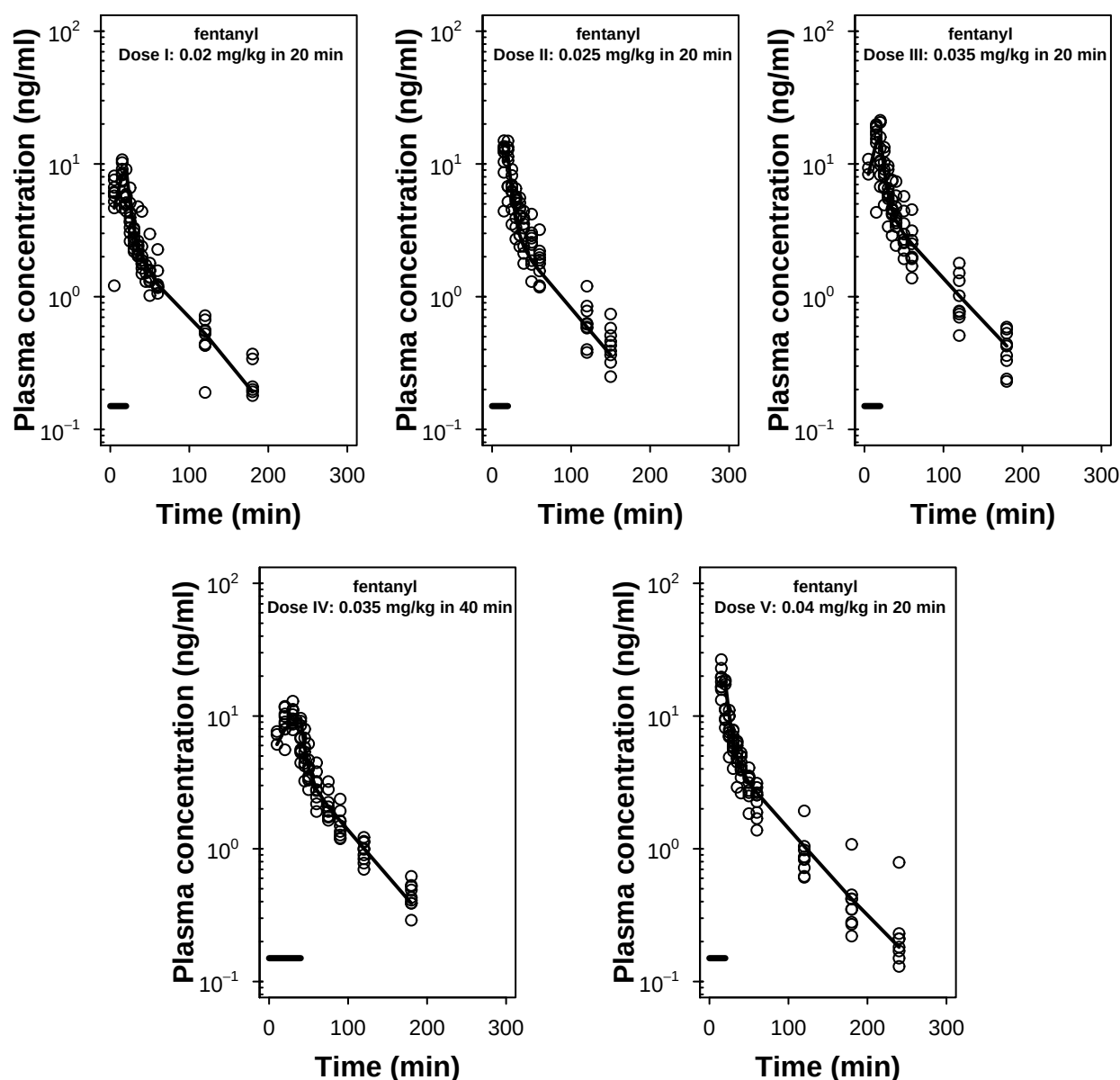


Figure 3. Individual fentanyl concentration-time profiles for the treatment groups I-V. The observed concentrations (closed circles) and population predictions (thick line) are depicted. The black boxes represent the duration of infusion.

The log-logistic and the Weibull probability distribution models were explored to account for censored time to response values. With the Weibull distribution incorporated, the model converged successfully, yielding pharmacodynamic parameters estimates comparable with those obtained with the log-logistic distribution model. However, the latter model was superior to the Weibull model as judged by considerable run-time reduction and fitting performance. For instance, the objective function was -435 *versus* 2000 for the log-logistic and the Weibull model, respectively.

The time course of the antinociceptive effect for both buprenorphine and fentanyl were analyzed using the developed biophase distribution/receptor binding model and an effect compartment model. The buprenorphine *in vivo* data in this study supported

the mechanism-based PK-PD model as indicated by the obtained objective function value (table 5). The population estimates were assessed as well as inter-animal variability. All the model parameters, structural and stochastic, were estimated precisely as indicated in table 6. On the other hand, the fentanyl effect *versus* time data were equally well described with the effect compartment model and the combined biophase equilibration/receptor binding model. Noteworthy, when applying the combined PK-PD model to the fentanyl plasma concentration-tail flick latency data, NONMEM continued iterat-

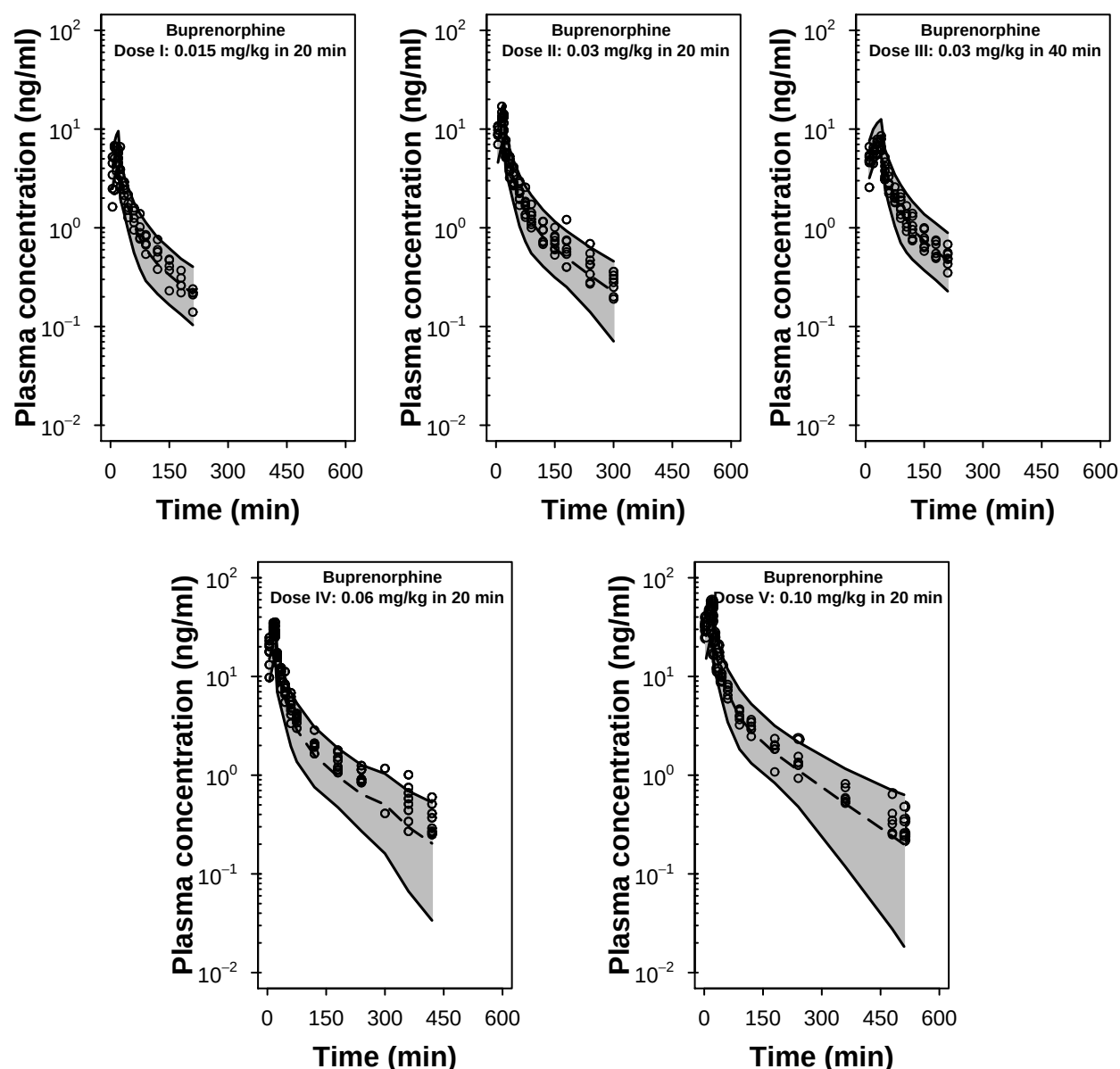


Figure 4. Results of the visual predictive performance of the population three-compartment pharmacokinetic model of buprenorphine for the different treatment groups. The observed concentrations (circles) 2.5 % quantile (lower solid line), median values (dashed line) and 97.5 % quantile (upper solid line) are presented. The influence of bodyweight on the pharmacokinetics of buprenorphine was also taken into account. Bodyweight was simulated for each animal assuming inter-animal variability of bodyweight is 3.5 % (normal distribution) and mean bodyweight is 0.300 kg.

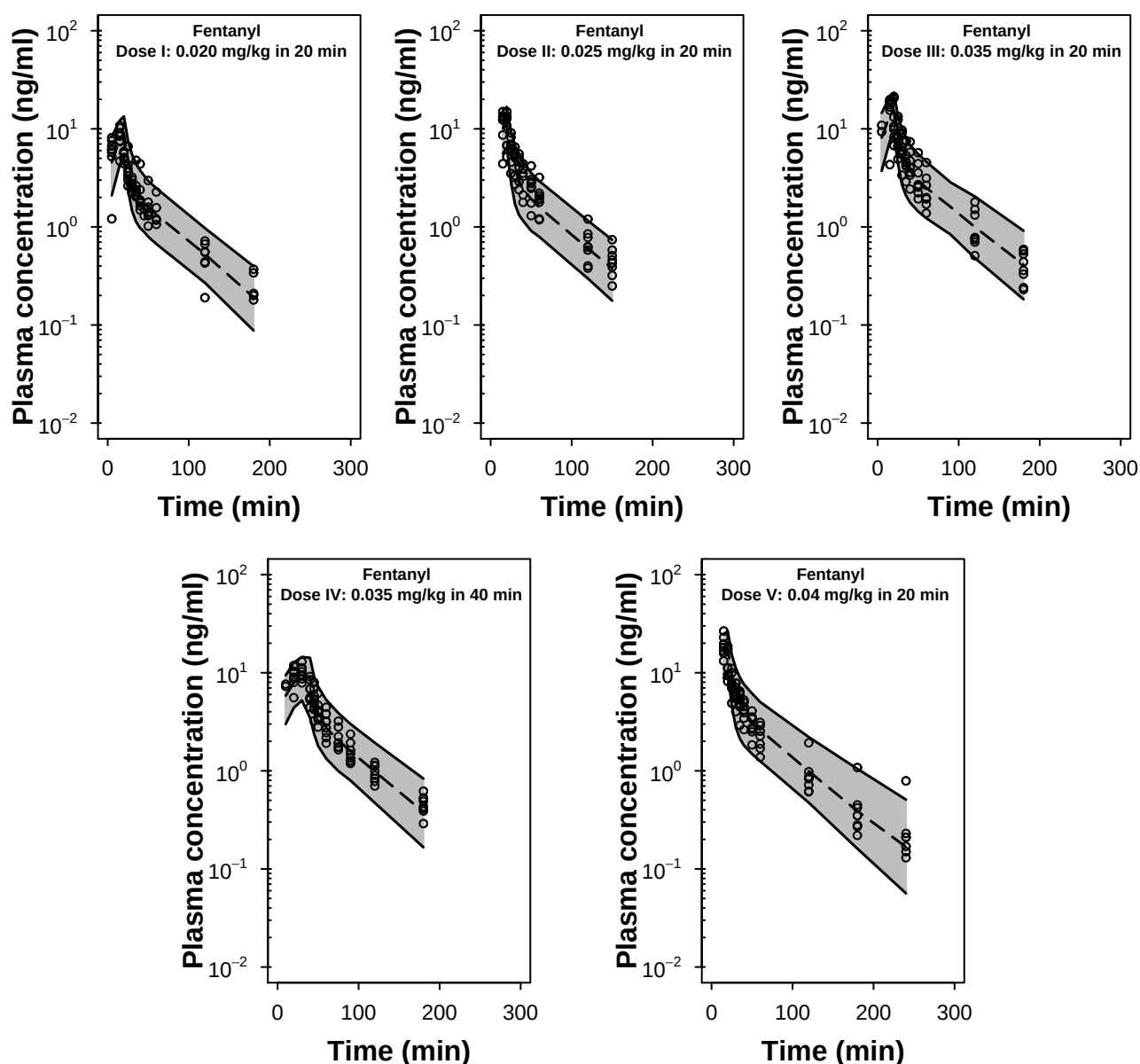


Figure 5. Results of the visual predictive performance of the population two-compartment pharmacokinetic model of fentanyl for the different treatment groups. The observed concentrations (circles) 2.5 % quantile (lower solid line), median values (dashed line) and 97.5 % quantile (upper solid line) are presented. The influence of bodyweight on the pharmacokinetics of fentanyl was also taken into account. Bodyweight was simulated for each animal assuming inter-animal variability of bodyweight is 3.5 % (normal distribution) and mean bodyweight is 0.300 kg.

ing while no significant change in objective function was observed after 50 iterations. During further iteration process, parameter estimates of k_{on} and k_{off} were rising to high values. This implies that fentanyl binding to and dissociation from the μ -opioid receptor occurs rapidly and that the concentration-effect relationship can be characterized under equilibrium conditions. On the other hand, the estimate of k_{e0} was stable and did not change during further iteration. Therefore, information on the PK-PD correlation of fentanyl were obtained with the most parsimonious model, which is the effect compartment model. The pharmacodynamic parameters estimates of fentanyl are presented in table 7.

The combined 'biophase equilibration/receptor binding' model and effect compartment model were able to successfully describe all individual effect *versus* time profiles, yielding estimates of k_{e0} , k_{on} and k_{off} for buprenorphine and of k_{e0} for fentanyl. A summary of the goodness-of-fit of the effect compartment, receptor association/dissociation and combined biophase equilibration-receptor association/dissociation model with respect to the exposure-response relationships of buprenorphine and fentanyl is provided in table 5.

Figure 6 and 7 show the time course of antinociceptive effect of buprenorphine and fentanyl, respectively and figure 7 shows the steady-state receptor binding- and effect-site concentration-antinociceptive effect relationship for both opioids. The population k_{on} and k_{off} were estimated at 0.023 ml/ng/min (95 % CI: 0.013-0.033 ml/ng/min) and 0.073 min⁻¹ (95 % CI: 0.042-0.104 min⁻¹). The biophase equilibration rate constant was 0.024 min⁻¹ (95 % CI: 0.018-0.030 min⁻¹), which corresponds to $t_{1/2,ke0} = 28.6$ min. For fentanyl population k_{e0} was estimated at 0.123 min⁻¹ (95 % CI: 0.095-0.151 min⁻¹), which corresponds to $t_{1/2,ke0} = 5.6$ min. A bootstrap analysis was not conducted for the validation of the population PK-PD models, due to long run times of the respective models in NONMEM.

A visual predictive check was not performed to assess the accuracy of the population PK-PD models, since this validation procedure does not provide additional information on the accuracy of the measured tail flick latency above the value of 10 s. Finally, no significant correlations between the pharmacodynamic parameter estimates or the inter-animal variability terms were observed.

Table 5. The goodness-of-fit, judged by objective function value, of three population PK-PD models containing expressions for the kinetics of onset and offset of buprenorphine and fentanyl. The population PK-PD models incorporate biological processes causing time-dependencies (biophase equilibration, receptor binding kinetics) in the pharmacodynamics of buprenorphine and fentanyl. For buprenorphine hysteresis is explained on the basis of kinetics of target site distribution and receptor association/dissociation kinetics

	objective function value	
	buprenorphine	fentanyl
biophase equilibration model	-283.1	-378.2
receptor binding model	-241.2	-322.4
biophase equilibration/receptor binding model	-523.9	-378.0

	Typical value	IAV (%)
k_{on} (ml/ng/min)	0.0228 (21.9)	NE
k_{off} (min ⁻¹)	0.0731 (21.5)	49 (23)
K_D (ng/ml) [§]	3.20	
k_{e0} (min ⁻¹)	0.0242 (12.2)	45 (29)
E_0 (s)	3.09 (1.4)	7 (36)
Z	14 (7.9)	NE

[§] secondary parameter (k_{off}/k_{on})

Table 7. Population pharmacodynamic estimates and inter-animal variability of fentanyl. For the fixed and random parameter estimates the relative standard error (%) is displayed

	Typical value	IAV (%)
k_{e0} (min ⁻¹)	0.123(11.6)	63 (37)
EC_{50} (ng/ml)	3.51 (7.3)	39 (19)
E_0 (s)	2.79 (1.9)	9 (35)
γ	1.16 (5.3)	26(49)
Z	17.5 (5.9)	NE

DISCUSSION

A population PK-PD model for (semi-)synthetic opioids is proposed, which allows separate characterization of the kinetics of target site distribution and the receptor association/dissociation kinetics as determinants of the time course of the antinociceptive effect. It is shown that for buprenorphine both the target site distribution and the receptor binding kinetics contribute to the observed hysteresis between plasma concentration and effect while for fentanyl, hysteresis is determined by target site distribution only. The pharmacokinetics of buprenorphine and fentanyl were successfully described by a three- and two-compartment model, respectively. All pharmacokinetic parameters, including the estimated stochastic model parameters were estimated precisely, as indicated by the obtained standard errors. Both compounds share similar pharmacokinetic characteristics, with moderate to large steady-state volumes of distribution (0.6 to 1.5 l) and high hepatic clearance values (15 to 20 ml/min for rat weighting 300 g). The high clearance values found for buprenorphine and fentanyl approximate the rat hepatic blood flow and support blood-flow limited clearance of fentanyl and buprenorphine found in human (Bullingham *et al.*, 1980; Stanski, 1987). Finally, PK model validation demonstrated the accurateness and precision of the developed population PK models.

The PK-PD correlation of buprenorphine and fentanyl was determined in the rat using the effect of radiant heat on tail flick withdrawal as a pharmacodynamic endpoint.

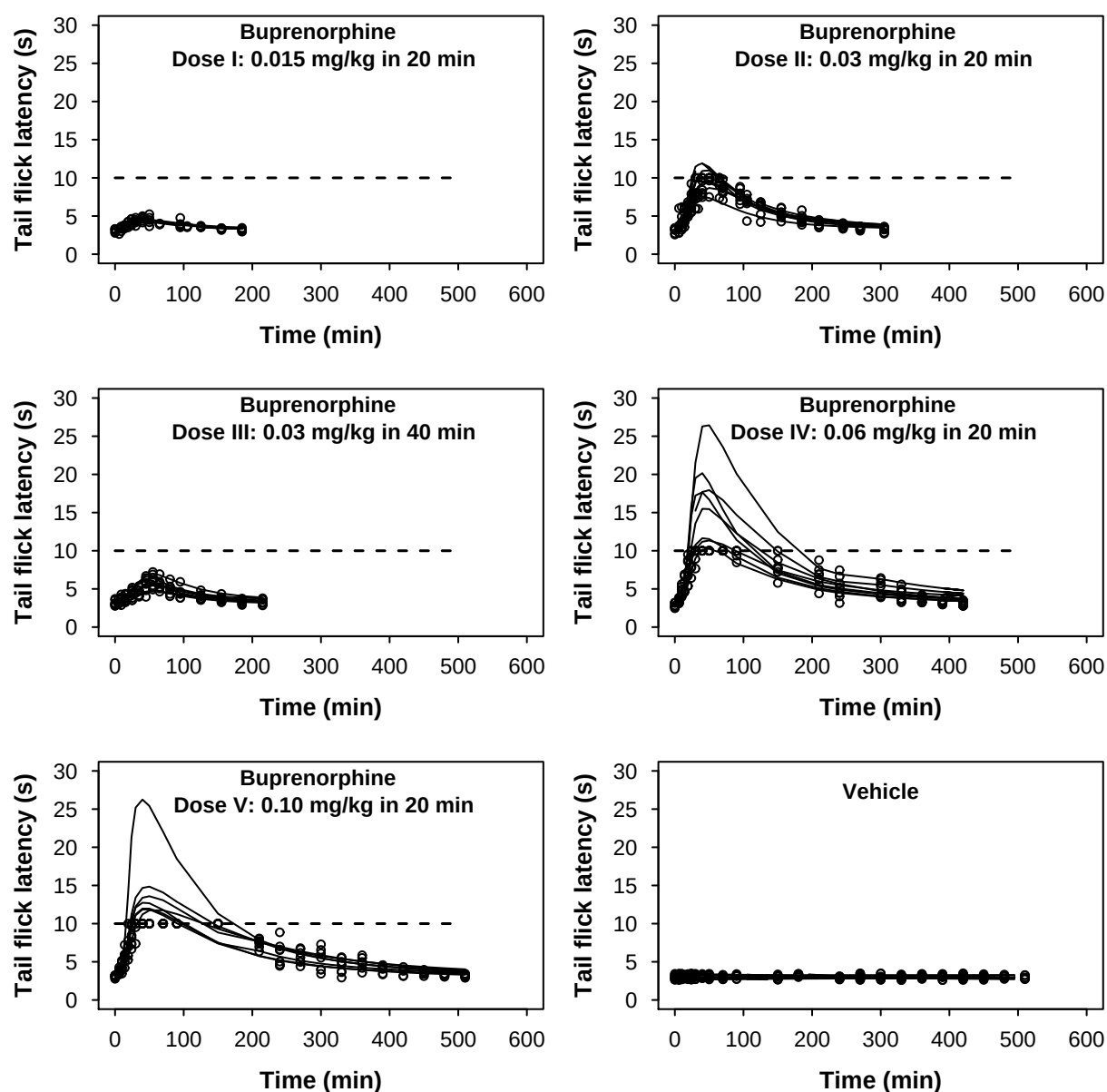


Figure 6. Changes in tail flick latency in time following administration of buprenorphine. For each treatment group (I-V) the observed (circles) and predicted (solid line) time course of antinociceptive effect is shown.

Characterization and prediction of the time course of drug effect was complicated by the presence of censored time to response values, which is an inherent limitation of the applied tail flick rat model. To integrate censored data (latencies above 10 s) in the PK-PD analysis, tail flick latencies were assumed to be log-logistically distributed and a maximum likelihood parameter estimation approach was used. A similar approach had been successfully applied in other studies (Luks *et al.*, 1998; Sarton *et al.*, 2000). An alternative for the log-logistic distribution is the Weibull distribution, which is also often used to describe time-to-event data. From the better performance of the log-logistic distribution in comparison to the Weibull distribution it is concluded that the former better matches the actual distribution of the observed tail flick latencies.

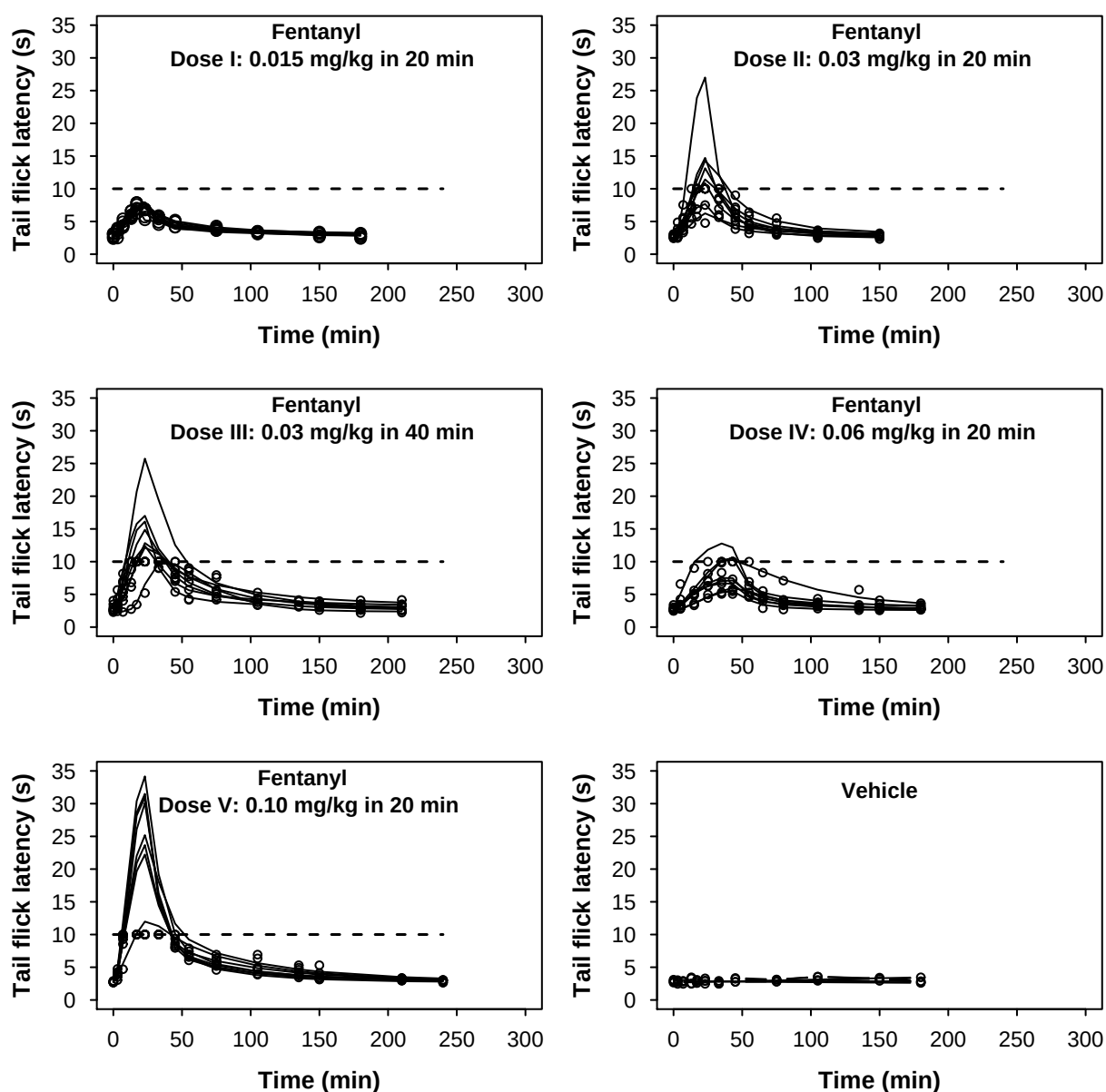


Figure 7. Changes in tail flick latency in time following administration of fentanyl. For each treatment group (I-V) the observed (circles) and predicted (solid line) time course of antinociceptive effect is shown.

The present study provides novel information on the pharmacokinetic-pharmacodynamic relationship of buprenorphine and fentanyl. Considering the time course of drug action, usually a combination of different processes is involved in time delays of the biological effect intensity relative to plasma concentration. However, it is often difficult to extract and discriminate those processes from the available PK-PD data (Jusko *et al.*, 1995; Verotta and Sheiner, 1995; Cleton *et al.*, 1999). In this study, the separation of biophase kinetics from the *in vivo* receptor kinetics is an important feature of the mechanism-based PK-PD model. It is shown that with values of the half-life of biophase equilibration ($t_{1/2,ke0}$) and the receptor dissociation ($t_{1/2,koff}$) of 29 min and 9 min respectively, the rate of onset and offset of the antinociceptive effect is predominantly determined by distribution of

buprenorphine to the effect site, as is also the case with fentanyl. However, in contrast to fentanyl, the contribution of the slow association/dissociation of buprenorphine to the μ -opioid receptor is not negligible. The half-life of biophase equilibration ($t_{1/2,ke0}$) for fentanyl was 5.6 min. These results are consistent with the idea that time-dependencies in fentanyl effect can be attributed to blood-brain concentration equilibration. The value of the half-life for biophase equilibration of fentanyl is remarkably similar to values reported by Scott *et al.* (1991) and Cox *et al.* (1998) who showed that the half-life of blood-brain equilibration is 6.6 and 2.2 min, in rats and human respectively, using EEG effect as pharmacodynamic endpoint. Remarkably, the similarity of these values in human and rat suggests that the rate constant of biophase equilibration of fentanyl is, independent of species, similar for the EEG effect and for antinociception. Due to its high lipophilicity, it is believed that fentanyl penetrates the blood-brain barrier readily (Henthorn *et al.*, 1999). It is reasonable to assume that after blood-brain barrier passage, fentanyl distributes into the brain tissues before it is released to bind to the μ -opioid receptor. Since both compounds are highly lipophilic, it is likely that buprenorphine distributes to the μ -opioid receptor in a similar manner, albeit that the values of the rate constants can be different.

The association/dissociation kinetics of buprenorphine at the μ -opioid receptor have

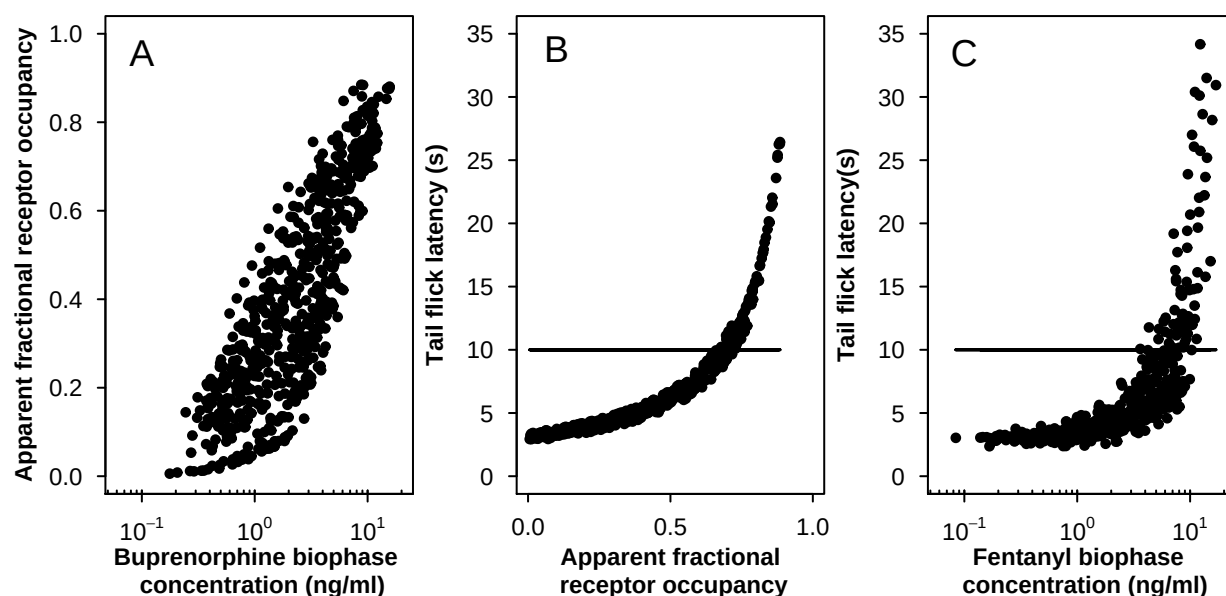


Figure 8. Panel A: *Concentration-receptor occupancy relationship.* The relationship between biophase concentration and receptor occupancy for buprenorphine for all rats. Biophase concentration is depicted on the x-axis on a logarithmic scale and receptor binding, expressed as fractional receptor occupancy, is depicted at the y-axis. The closed circles represent the predicted receptor binding. Panel B: *receptor occupancy-effect relationship.* The receptor occupancy *versus* effect relationship as described by equation 9 for buprenorphine for all rats. The closed circles represent the predicted tail flick latency. Panel C: *fentanyl concentration-effect relationship.* The fentanyl biophase concentration *versus* tail flick latency relationship as described by equation 10. The closed circles represent the predicted tail flick latency. The solid line displays the cut-off tail flick latency time.

also been determined *in vitro* (Villiger and Taylor, 1982; Boas and Villiger, 1985). Based on those receptor binding studies two binding affinity sites for buprenorphine have been identified. Dissociation of buprenorphine was characterized by an initial rapid phase ($t_{1/2, \text{koff}} = 5.6$ min) followed by a slower phase ($t_{1/2, \text{koff}} = 166.4$ min). The estimated *in vivo* dissociation half life for buprenorphine of 9.5 min is in the range of the reported value for the initial rapid phase of the dissociation from the buprenorphine- μ -opioid receptor complex *in vitro*. More important, the estimated *in vivo* equilibration constant K_D for buprenorphine, 3.20 ng/ml, corresponding to 6.85 nM, is in the same range as the *in vitro* dissociation equilibration constant, for which the values of 0.12 nM for the high affinity binding site and 1.38 nM for the low affinity binding site in the spinal cord and 1.0 nM for the binding site in the brain have been reported (Villiger and Taylor, 1982). It should be noted that the estimated *in vivo* K_D is calculated on basis of total plasma concentrations. Correction for the free fractions in plasma will result in an even greater similarity of the K_D values. For buprenorphine, no information on the free fraction is available which can be explained by the physicochemical properties of buprenorphine. Notably, the lipophilicity of buprenorphine complicates accurate measurement of the free fraction (sticking of buprenorphine to the membrane filter).

These results demonstrate the usefulness of this mechanism-based PK-PD model to explore *in vitro-in vivo* K_D correlations. Similar correlations have been reported for calcium channel antagonists using a receptor association/dissociation model (Shimada *et al.*, 1996) and also for A_1 adenosine receptor agonists and GABA $_A$ receptor modulators using a different mechanism-based PK-PD model based on receptor theory (Van der Graaf *et al.*, 1999; Visser *et al.*, 2003). Moreover, the ability to estimate an *in vivo* K_D allows a strict quantitative comparison with the antinociceptive effect of other compounds. An important issue is the potency and intrinsic activity of buprenorphine relative to fentanyl. Interestingly, estimates of *in vivo* potency of buprenorphine and fentanyl are in close agreement and show that both compounds display equi-antinociceptive potency (3.20 versus 3.51 ng/ml). The estimated C_{100} for fentanyl is obtained from equation 10. This equation follows from the steady-state solution of equations 8 and 9 in which case k_{on} and k_{off} are both not identifiable. Under steady-state conditions C_{100} equals $k_{\text{off}}/k_{\text{on}} = K_D$ and therefore K_D and C_{100} can be used to compare *in vivo* potency of buprenorphine and fentanyl.

In addition, also the relative *in vivo* potency of drug and metabolite or drugs exhibiting enantiomeric isoforms can be explored using an integrated mechanism-based PK-PD modeling approach (Zuideveld *et al.*, 2002). For instance, it is postulated that buprenorphine's major metabolite, norbuprenorphine possesses a 50-fold weaker antinociceptive activity than the mother compound (Ohtani *et al.*, 1995). In the present study, the plasma concentrations of buprenorphine's major metabolite, norbuprenorphine, were also measured. However, the norbuprenorphine plasma concentrations were far below the concentration range, reflecting buprenorphine's antinociceptive effect (figure 9). Therefore, it was assumed that the contribution of norbuprenorphine to the overall analgesic effect is

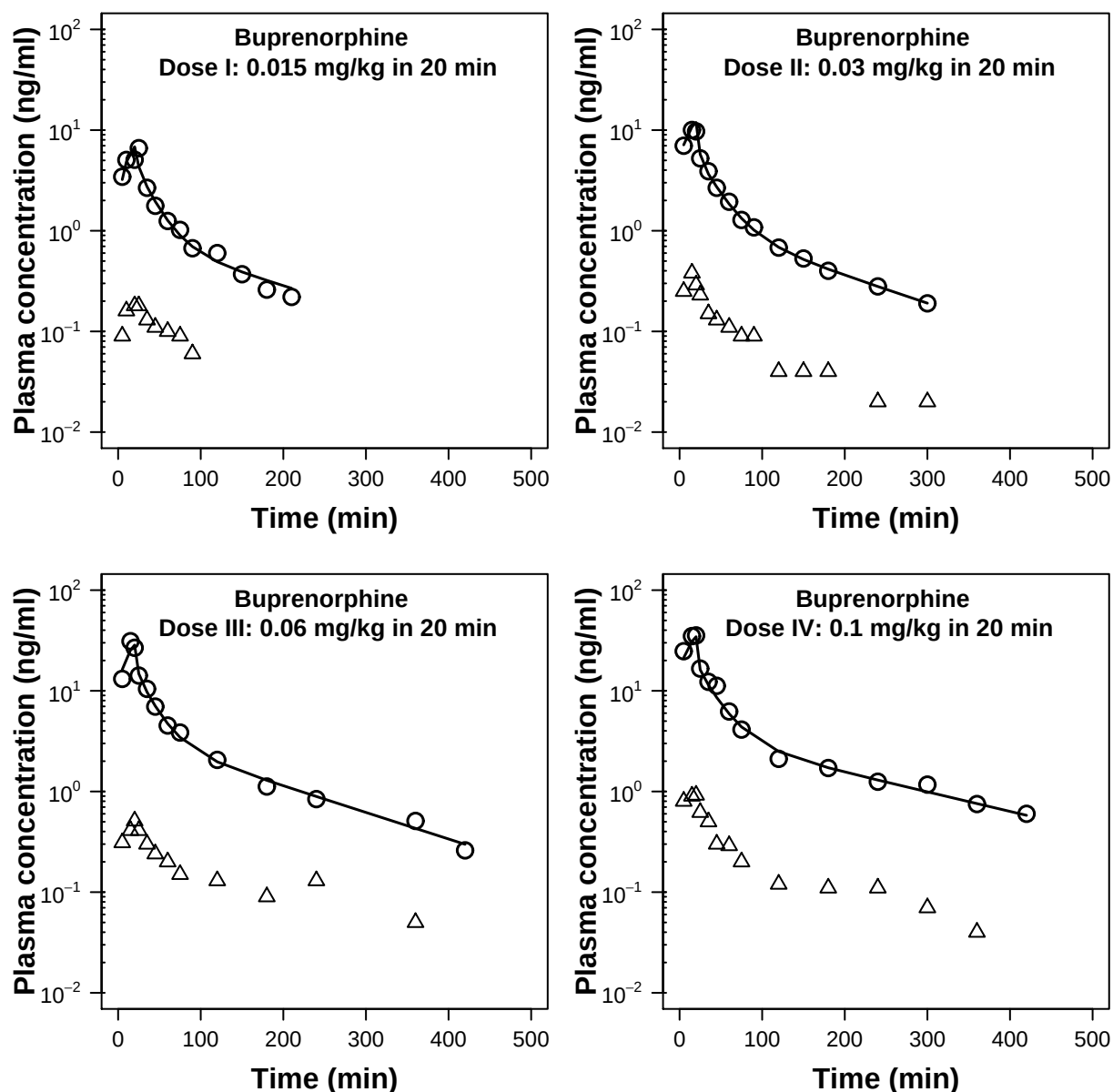


Figure 9. Representative individual concentration *versus* time profiles for buprenorphine and its metabolite norbuprenorphine. The observed buprenorphine concentrations (circles), norbuprenorphine concentrations (triangles) and individual predicted buprenorphine concentrations (solid line) are depicted.

minimal.

An important question is whether buprenorphine acts as a full agonist (i.e. displays full antinociceptive effect). Mechanism-based PK-PD models provide a unique basis to characterize the effects of drugs in terms of *in vivo* potency and intrinsic activity ($E_{\max}$). In recent years this approach has been successfully applied to explore the concentration-effect relationships of several compounds belonging to different drug classes (Van der Graaf *et al.*, 1999; Visser *et al.*, 2002). In the present investigation, maximal antinociceptive effect could not be estimated from the concentration-effect relationships. We relate this to the fact that, in this tail flick rat model stronger stimuli lead to a higher response,

ultimately leading to complete antinociception. Consequently, no $E_{\max}$ is observed and no distinction can be made between partial and full antinociceptive response during analysis. Furthermore, drug efficacy estimation is hampered by the fact that above the cut-off value only a probability distribution-based prediction of the antinociceptive behavior is provided. For some animals receiving the highest buprenorphine dose (0.1 mg/kg), the maximal predicted tail flick latency time is equal to or lower than the maximal predicted tail flick latency time resulted from 0.06 mg/kg administration. This seems consistent with data derived from previous animal studies supporting the concept of ceiling effect for antinociception (Cowan *et al.*, 1977a,b; Dum and Herz, 1981). However, on the basis of the present results no conclusions can be drawn regarding an eventual difference in the intrinsic efficacy of buprenorphine relative to fentanyl.

In conclusion, a mechanism-based PK-PD model has been successfully applied to the antinociceptive effect of buprenorphine and fentanyl. The model was able to separate biophase equilibration and receptor binding kinetics. In this respect it has been shown that the onset and offset of antinociceptive effect of buprenorphine and fentanyl are mainly determined by biophase equilibration. This mechanism-based PK-PD model can be extended for the characterization of buprenorphine's kinetics of action with respect to its respiratory inhibitory effect. From that point of view, the developed mechanism-based PK-PD model may provide a useful tool to gain detailed information on the nature of interaction between buprenorphine and naloxone.

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MECHANISM-BASED PHARMACOKINETIC-PHARMACODYNAMIC MODELING OF THE ANTINOCICEPTIVE EFFECT OF BUPRENORPHINE IN HEALTHY VOLUNTEERS

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ABSTRACT

The objective of this investigation was to characterize the pharmacokinetic-pharmacodynamic (PK-PD) relationship of buprenorphine's antinociceptive effect in healthy volunteers. Data on the time course of the antinociceptive effect following intravenous administration of 0.05-0.6 mg/70 kg of buprenorphine in healthy volunteers was analyzed in conjunction with plasma concentrations by non-linear mixed effects analysis.

A three-compartment pharmacokinetic model best described the concentration time course. Four structurally different pharmacokinetic-pharmacodynamic models were evaluated for their appropriateness to describe the time course of buprenorphine's antinociceptive effect, (1) $E_{\max}$ model with an effect compartment model, (2) "power" model with an effect compartment model, (3) receptor association/dissociation model with a linear transduction function and (4) combined biophase equilibration-receptor association/dissociation model with a linear transduction function. The latter pharmacokinetic-pharmacodynamic model described the time course of effect best and was used to explain time-dependencies in buprenorphine's pharmacodynamics. The model converged, yielding precise estimation of the parameters characterizing hysteresis and the relationship between apparent receptor occupancy and antinociceptive effect.

The rate constant describing biophase equilibration (k_{e0}) was 0.00447 min^{-1} (95 % CI: $0.00299 - 0.00595 \text{ min}^{-1}$). The receptor dissociation rate constant (k_{off}) was 0.0785 min^{-1} (95 % CI: $0.0352 - 0.122 \text{ min}^{-1}$) and k_{on} was 0.0631 ml/ng/min (95 % CI: $0.0390 - 0.0872 \text{ ml/ng/min}$). This is consistent with observations in rats, suggesting that the rate limiting step in the onset and offset of the antinociceptive effect is biophase distribution rather than slow receptor association-dissociation.

In the dose range studied no saturation of receptor occupancy occurred explaining the lack of a ceiling effect for antinociception.

INTRODUCTION

Buprenorphine is a semi-synthetic opioid derived from the morphine precursor thebaine which has been used in clinical practice since 1979 (Budd and Collett, 2003). In patients, buprenorphine is used for treatment of acute and chronic pain using various administration modes, such as intravenous, sublingual and spinal/epidural administration. Several studies have shown that buprenorphine binds to the μ -opioid receptor, producing typical μ -opioid receptor agonist effects like analgesia, sedation, nausea, delayed gastric emptying and respiratory depression.

The first receptor binding studies conducted (Martin *et al.*, 1976) classified buprenorphine as a partial agonist at the μ -opioid receptor. In theory, partial agonists display low intrinsic efficacy at their target receptor relative to full agonists. However, the relationship between the partial agonist nature of buprenorphine and its analgesic effect is not well established. For instance, data from distinct animal studies support partial agonistic activity for buprenorphine-induced antinociception (Cowan *et al.*, 1977; Dum and Herz, 1981). However, at relatively high doses evidence for an inverse U-shaped dose-response relationship has been obtained. In the meantime it has become clear that the shape of dose-analgesic response relationships is dependent on the applied pain model. For instance, in animal models for chronic pain buprenorphine is fully effective over a wide dose range (Christoph *et al.*, 2005). Moreover in a recent mechanism-based PK-PD modeling study a full antinociceptive effect of buprenorphine in rats was observed (Yassen *et al.*, 2005). In addition, several studies have demonstrated full analgesic efficacy for buprenorphine over a wide dose range in volunteers and patients (Budd, 1981; Mok *et al.*, 1981; Watson *et al.*, 1982; Walsh *et al.*, 1994).

Another aspect of buprenorphine's pharmacological profile is the slow receptor association/dissociation kinetics at the μ -opioid receptor (Cowan *et al.*, 1977; Boas and Villiger, 1985). In humans upon parenteral administration the time to the onset of analgesia is 10 to 30 minutes and the duration of analgesic effect is 6 to 8 hours (Downing *et al.*, 1977). The slow onset of analgesic effect complicates optimal dose titration of buprenorphine, while the slow offset of effect offers a clear advantage relative to other opioids, like morphine and fentanyl, as it makes buprenorphine suitable for less frequent daily dosing.

Recently, we have developed a mechanistic PK-PD model allowing separate characterization of biophase equilibration and slow receptor association/dissociation kinetics as determinants of time-dependencies in buprenorphine's antinociceptive effect in rats (Yassen *et al.*, 2005). These investigations showed that contrary to common belief the slow onset and offset of the antinociceptive effect is determined by slow biophase equilibration kinetics, rather than slow receptor association/dissociation kinetics. Furthermore, buprenorphine was shown to act as a full agonist with regard to the antinociceptive effect in rats.

The specific objectives of this study were to characterize the PK-PD correlation of buprenorphine in man. Specifically, the objective was a) to determine whether the same

PK-PD model as previously proposed for rats applies to human situation, b) to estimate the various rate constants that determine the time course of the antinociceptive effect (i.e. biophase equilibration versus receptor association/dissociation kinetics) and c) to determine the *in vivo* concentration-effect relationship for antinociception.

MATERIALS AND METHODS

Subjects

Thirty-four (34) volunteers (17 men, 17 women, aged 18-30 years) were recruited to participate in the investigation after approval was obtained from the Human Ethics Committee and after giving written and oral informed consent. The subjects were healthy and did not have a history of illicit substance abuse. All women used oral contraceptives. The subjects were asked not to eat or drink for at least 6 h prior to the study. Each subject participated once in the study. After arrival in the research unit, an arterial line was placed in the left or right radial artery under local anesthesia (for blood sampling). In the contralateral arm an intravenous line was inserted (for drug infusion).

Study Design

The study had a double-blind, randomized design. There were six treatment levels covering a wide dose range: (1) 4 subjects received 0.05 mg/70 kg iv of buprenorphine, (2) 4 subjects received 0.1 mg/70 kg iv of buprenorphine, (3) 8 subjects received 0.2 mg/70kg iv of buprenorphine, (4) 5 subjects received 0.3 mg/70 kg iv of buprenorphine, (5) 8 subjects received 0.4 mg/kg iv of buprenorphine, (6) 5 subjects received 0.6 mg/70 kg iv of buprenorphine. Eight individuals received placebo treatment. Buprenorphine or placebo was administered over a 30-s period. The local pharmacy performed randomization and prepared 10 ml syringes for buprenorphine prior to the experiment. Buprenorphine (Temgesic, 0.3 mg/ml) was obtained from Reckitt & Coleman Ltd. (London, UK) and was dissolved in 0.9 % NaCl up to a volume of 5 ml.

The Acute Pain Model

Acute pain was induced by an electrical current through two surface electrodes (Red Dot, 3M, London, Ontario, Canada) placed on the skin overlaying the tibial bone (shin bone) of the left leg. The electrodes were attached to a computer interfaced current stimulator (CICS), which was locally designed and constructed. The intensity of the noxious stimulation was increased from 0 mA in steps of 0.5 mA per 1 s. The stimulus train consisted of a square-wave pulse of 0.2 ms duration applied at 10 Hz and had a cutoff at 128 mA. The subjects were instructed to press a button on a control panel when the stimulus became

painful (pain threshold) and when no further increase in stimulus intensity was acceptable (pain tolerance). Upon pressing the pain tolerance button, the stimulus train ended. Since the subjects were naive to pain/analgesia experiments they were trained prior to the actual study for about 1-h in which several stimulus trains were applied. These data were discarded.

After a subsequent resting period, baseline pain variables were obtained. Pain assessment was performed three times prior to drug infusion and at fixed times after drug infusion. The current intensities at which pain threshold and tolerance occurred were automatically collected and stored on the hard disc of a computer for further analysis. In case blood sampling coincided with pain assessment, the pain testing preceded the sampling.

Because pain tolerance is considered more reliable in detecting true opioid-induced analgesic effect, we performed the PK-PD analysis on the pain tolerance data (Luginbuhl *et al.*, 2001).

Determination of Buprenorphine concentrations

Blood samples (10 ml) for the determination of the buprenorphine concentration were collected using lithium heparin vacutainers (Becton Dickinson, UK). After centrifugation, the resultant plasma was transferred into screw-cap polypropylene tubes and stored at $< -20^{\circ}\text{C}$ until analysis. For the determination of buprenorphine concentrations in plasma, a validated GC-MS method was used. To plasma aliquots of 1.0 ml, 1.0 ml 0.5N NaOH, 25 μl internal standard stock solution ($^2\text{H}_4$ -buprenorphine), and 7 ml toluene/diisopropylether (3:1 v/v) were added. After shaking the samples for 20 minutes and centrifugation, the organic phase was transferred to conical glass tubes and stored at about -20°C until derivatization. Samples were evaporated with a stream of nitrogen and the residues were reconstituted in 50 ml of 1 % derivatization reagent in toluene, and 20 ml of 0.2 % triethylamine in toluene. After one minute reaction time at room temperature, the samples were washed with 300 ml water and 2 ml toluene. After the washing step, the organic phase was transferred to glass vials and evaporated to dryness under a very weak stream of nitrogen. The residues were resolved in 25 μl toluene for measurement.

Measurements were performed on a Finnigan MAT 4500 gas chromatography-mass spectrometry (GS-MS) system. The mass spectrometer was operated in the negative ion chemical ionization mode with ammonia as reagent gas. Selected ion monitoring was performed for m/z 708 for buprenorphine and m/z 712 for internal standard. Human plasma used as control for interferences contained no peaks while performing analysis for buprenorphine. Calibration curves were linear ($r > 0.99$) from 25.0 to 10000 pg/ml, using weighted ($1/x^2$) linear regression. The lower limit of quantification (LLOQ) was 25.0 pg/ml using 1 ml of plasma. The overall accuracy and precision for calibration standards and quality controls analysed during the study were well within 11 % at all concentrations. All concentration data presented refer to buprenorphine free base.

Pharmacokinetic-pharmacodynamic modeling procedure

The pharmacokinetic and pharmacodynamic parameters of buprenorphine were estimated using non-linear mixed-effects modeling as implemented in the NONMEM software (Version V, level 1.1) (Beal and Sheiner, 1999). The population analysis approach, which takes into consideration both intra-individual and inter-individual variability, was undertaken using the first-order conditional estimation method with η - ϵ interaction (FOCE interaction). All fitting procedures were performed on an IBM (Armonk, NY, USA)-compatible computer (Pentium IV, 1500 MHz) running under Windows NT with the Microsoft FORTRAN Powerstation 4.0 compiler (Microsoft, Redmond, WA, USA). An in-house available S-PLUS 6.0 (Insightful Corp., Seattle, WA, USA) interface to NONMEM was used for data processing and management (including tools to perform predictive check and non-parametric bootstrap) and graphical data display.

Pharmacokinetic-pharmacodynamic model development strategy

The following method of approach for the development of the PK-PD model was used. In the first approximation the structural part of the model was optimized on the data. In the next stage, inter-individual variability was added to the model parameters and statistical refinement of the random model was applied by means of covariance matrix analysis. In addition, the residual error model was determined. Finally, the relationship between PK-PD model parameters and covariate terms was explored and significant covariates were incorporated in the model (Mandema *et al.*, 1992; Jonsson and Karlsson, 1999). When possible, the physiological relevance of parameter-covariate relationship was considered in the covariate selection. Individual posthoc pharmacokinetic parameter estimates served as input to the pharmacodynamic model. Model selection was based on model parameter estimates and the confidence intervals of the fixed and random parameters, parameter correlations, likelihood ratio test and visual inspection of goodness-of-fit plots. Plots of individual observed *versus* population or individual predicted values and weighted residuals *versus* time or population predicted values were used to detect systemic deviations from the model fits.

Pharmacokinetic data analysis

In order to determine the basic structural pharmacokinetic model, the two- and three-compartment model with first-order elimination was tested. Using the likelihood ratio test, the significance level was set at $\alpha = 0.01$, which corresponds to a reduction of 6.6 units in objective function value (OFV; χ^2 distribution) to discriminate between two nested structural models after inclusion of one additional parameter. Based upon model selection criteria a three-compartment model with first-order elimination from the central compartment was selected to describe the time course of the buprenorphine concentration in

plasma. The model was coded in the ADVAN11 TRANS4 subroutine and allowed the estimation of the parameters Cl , V_1 , V_2 , V_3 , Q_2 and Q_3 . The random part of the model selected to describe inter-individual variability in the pharmacokinetic parameters assumed a log-normal distribution of the model parameters over the population. Therefore an exponential distribution model was used to account for inter-individual variability:

$$P_i = P_{tot} \cdot \exp(\eta_i) \quad (1)$$

in which P_i is the individual value of model parameter P , P_{tot} is the typical value (population value) of parameter P in the population, and η_i is the normally distributed inter-animal random variable with mean zero and variance ω^2 . The coefficient of variation of the structural model parameters is expressed as percentage of the root mean square of the inter-individual variance term. Selection of an appropriate residual error model was based on inspection of the goodness-of-fit plots. Inter-individual variability was added on the model parameters by stepwise addition. A ΔOFV of 6.6 points was required to maintain inter-individual variability on a structural model parameter. Afterwards, the relative importance of each inter-individual variability term was reassessed by deletion of each term one by one from the structural model parameters. An increase of more than 10.8 points (χ^2 distribution, $p < 0.001$, $df=1$) in OFV was regarded as significant for maintenance of inter-individual variability on a model parameter. After establishment of the basic pharmacokinetic model potential correlations between parameters were tested in a covariance matrix analysis in NONMEM (OMEGA BLOCK option). A drop of 6.6 points in OFV indicated the presence of a significant correlation between parameters. The statistical model used to quantify intra-individual variability in buprenorphine plasma concentration had the general form:

$$C_{obs,ij} = C_{pred,ij} \cdot (1 + \varepsilon_{ij}) \quad (2)$$

in which $C_{obs,ij}$ is the j th observed concentration in the i th individual, $C_{pred,ij}$ is the predicted concentration, and ε_{ij} is the normally distributed residual random variable with mean zero and variance ω^2 . The residual error term contains all the error terms which can not be explained and refers to for example measurement and experimental error (e.g. error in recording sampling times) and structural model mis-specification.

Mechanism-based pharmacokinetic-pharmacodynamic analysis

Various structurally different PK-PD models were evaluated for their appropriateness to describe the time course of buprenorphine's antinociceptive effect in healthy volunteers. To this end, PK-PD models were selected and tested on the basis of their ability to discriminate between distinct biological processes causing time-dependencies in the *in vivo* pharmacodynamics. To account for hysteresis between buprenorphine concen-

tration in plasma and antinociceptive effect the following PK-PD models were evaluated: 1) effect compartment model (Sheiner *et al.*, 1979), 2) receptor association/dissociation model (Shimada *et al.*, 1996) and 3) combined biophase equilibration-receptor association/dissociation model (Yassen *et al.*, 2005)

Another important question to be answered is whether ceiling effect for antinociception occurs in the studied dose range. To determine the shape of the function describing the relationship between buprenorphine concentration and antinociceptive effect the *in vivo* concentration-effect relationships were characterized by an effect compartment model in combination with: 1) sigmoid $E_{\max}$ model and 2) "power" pharmacodynamic model (Sarton *et al.*, 2000), which is of the form:

$$E = E_0 \cdot \left(1 + 0.25 \cdot \left(\frac{C_e}{C_{25}} \right)^\gamma \right) \quad (3)$$

where E is the antinociceptive effect, E_0 is the baseline response, C_{25} is the effect-site concentration causing 25 % increase in current for pain tolerance and γ is a shape parameter.

In the final model, the time course of buprenorphine's antinociceptive effect was analyzed using a dynamic receptor model with a linear transduction function. An important feature of this model is that it allows separation of biophase equilibration kinetics from receptor association/dissociation kinetics to account for time-dependencies in the *in vivo* pharmacodynamics. This involves the estimation of the second- and first-order rate constants for receptor association and dissociation in combination with a single rate constant for biophase equilibration. Drug distribution to the site of action (biophase) was characterized on the basis of an effect compartment model (Sheiner *et al.*, 1979). The rate of change of biophase drug concentrations can be described as follows:

$$\frac{dC_e}{dt} = k_{e0} \cdot (C_p - C_e) \quad (4)$$

where k_{e0} is a first-order distribution rate constant describing the rate of change of drug concentration in the effect compartment and C_p represents the plasma concentration and C_e the effect-site concentration.

At the site of action, the drug can bind to the receptor. Following the law of mass action, the rate of drug-receptor binding (dC_eR/dt) is proportional to the drug concentration C_e and the free receptor concentration R :

$$\frac{dC_eR}{dt} = k_{on} \cdot C_e \cdot R - k_{off} \cdot C_eR \quad (5)$$

where k_{on} is a second-order rate constant describing the rate of association and k_{off} is a first-order rate constant describing the rate of dissociation of the drug-receptor complex. Under the assumption that the concentration of drug is in excess compared to the free receptor concentration and that the total number of receptors (R_{tot}) is equal to the sum of drug-bound receptors (C_eR) and unbound receptors (R), equation 5 can be rearranged

into:

$$\frac{dC_e R}{dt} = k_{on} \cdot C_e \cdot (R_{tot} - C_e R) - k_{off} \cdot C_e R \quad (6)$$

The total amount of receptors (R_{tot}) could not be measured *in vivo* and therefore was set to unity. Consequently an apparent relative estimate of *in vivo* fractional receptor binding is obtained. The apparent fractional receptor binding was directly related to the antinociceptive effect according to the following equation:

$$E = \frac{E_0}{1 - C_e R} \quad (7)$$

where E is the antinociceptive effect and E_0 is the baseline antinociceptive response. Note that the steady-state solution of equations 6 and 7 is equal to the "power" model presented in equation 3.

Pharmacokinetic and pharmacokinetic-pharmacodynamic model validation

The predictive performance and precision of the population pharmacokinetic model was evaluated by model validation (Ette *et al.*, 2003). Internal validation was conducted by means of a non-parametric bootstrap technique. Briefly, one thousand bootstrap replicates were generated by sampling randomly from the original data set with replacement. Subsequently, the data sets were fitted one at the time to the final population pharmacokinetic model. The mean, standard error, coefficient of variation and the lower and upper 95 % prediction intervals of the model parameter values were calculated and compared to the typical model parameter values from the original study. Due to long run times of the population PK-PD model in NONMEM, no bootstrap validation was performed.

Sensitivity analysis

To determine the reliability and robustness of an alternative modeling outcome to changes in the pharmacodynamic parameter k_{e0} we have conducted a sensitivity analysis. The parameter k_{e0} was fixed to values ± 50 % in 10 % increments and the objective function values were evaluated and mapped over the range of fixed k_{e0} values selected.

RESULTS

Pharmacokinetics

A three-compartment model with first-order elimination from the central compartment was used to describe the time course of buprenorphine concentration in plasma. The observed buprenorphine concentration *versus* time profiles stratified to dose are displayed

in figure 1. The concentration-time profiles of typical individuals, predicted by the population pharmacokinetic parameter estimates of Cl , V_1 , V_2 , V_3 , Q_2 and Q_3 are shown as solid lines in the respective figures. The population pharmacokinetic estimates of the final model are presented in table 1.

All parameters, including the random model parameters, were estimated precisely with an acceptable coefficient of variation (3.5 % to 36.3 %). Inter-individual variability was low for the parameters Cl and V_3 (17.3 % and 22.9 %, respectively) and relatively high for V_1 , V_2 and Q_2 (58.6 % to 93.4 %). Residual variability was taken into account using a proportional error model and resulted in a prediction error of 14.5 % over the total studied concentration range. Several covariate relationships were explored by visual inspection of the covariate *versus* Bayesian pharmacokinetic parameters estimates and by performance of generalized additive modeling (GAM) analysis. However, in NONMEM no significant improvement of the model fit was detected after inclusion of the covariates in the structural pharmacokinetic model.

Finally, the predictability and stability of the population pharmacokinetic model was evaluated by model validation. The precision and stability of the developed population pharmacokinetic model was explored using internal validation. The final population model estimates for Cl , V_1 , V_2 , V_3 , Q_2 and Q_3 and their respective 95 % confidence intervals were supported by the results of the bootstrap validation (table 1). The estimates of the inter-individual variability of the pharmacokinetic parameters were almost identical to those obtained with the bootstrap validation. The fits of the 1049 bootstrap replicates demonstrated the stability and robustness of the developed population pharmacokinetic model.

Table 1. Parameter estimates with their respective relative standard errors (%) of the final population pharmacokinetic model for buprenorphine and the stability of the parameters using the bootstrap resampling procedure

	Original data set		1049 bootstrap replicates	
	Typical value	IIV (%) [†]	Typical value	IIV (%)
Cl (l/min)	1.45 (3.5)	17.3 (22.7)	1.43 (4.0)	16.9 (22.9)
V_1 (l)	8.39 (14.9)	70.8 (21.0)	8.03 (24.0)	62.8 (51.1)
V_2 (l)	23.3 (20.7)	93.4 (20.5)	23.0 (30.9)	81.9 (50.7)
V_3 (l)	127 (5.9)	22.9 (36.3)	125 (6.7)	21.9 (44.5)
Q_2 (l/min)	1.55 (12.3)	58.6 (19.6)	1.51 (16.5)	51.8 (51.7)
Q_3 (l/min)	0.953 (19.6)	NE [‡]	0.948 (7.1)	NE
PRE [#] (%)	14.5 (18.8)		15.1 (28.6)	

[†] inter-individual variability; [‡] not estimated; [#] proportional residual error

Mechanism-based pharmacokinetic-pharmacodynamic modeling

Baseline values of pain tolerance ($\pm$ SEM) were 18.9 ± 2.8 mA and 17.9 ± 2.6 mA in men and women, respectively. After bolus intravenous administration of buprenorphine the currents needed for pain tolerance significantly increased relative to baseline in both sexes. The maximal peak effect was reached after approximately 70 to 100 min.

In order to demonstrate the appropriateness of the various PK-PD models to characterize the time course of antinociceptive effect in human, model discrimination was performed. The likelihood ratio test was used to evaluate the performance of (1) sigmoid $E_{\max}$ model with effect compartment model, (2) "power" or "Leiden" model (equation 3)

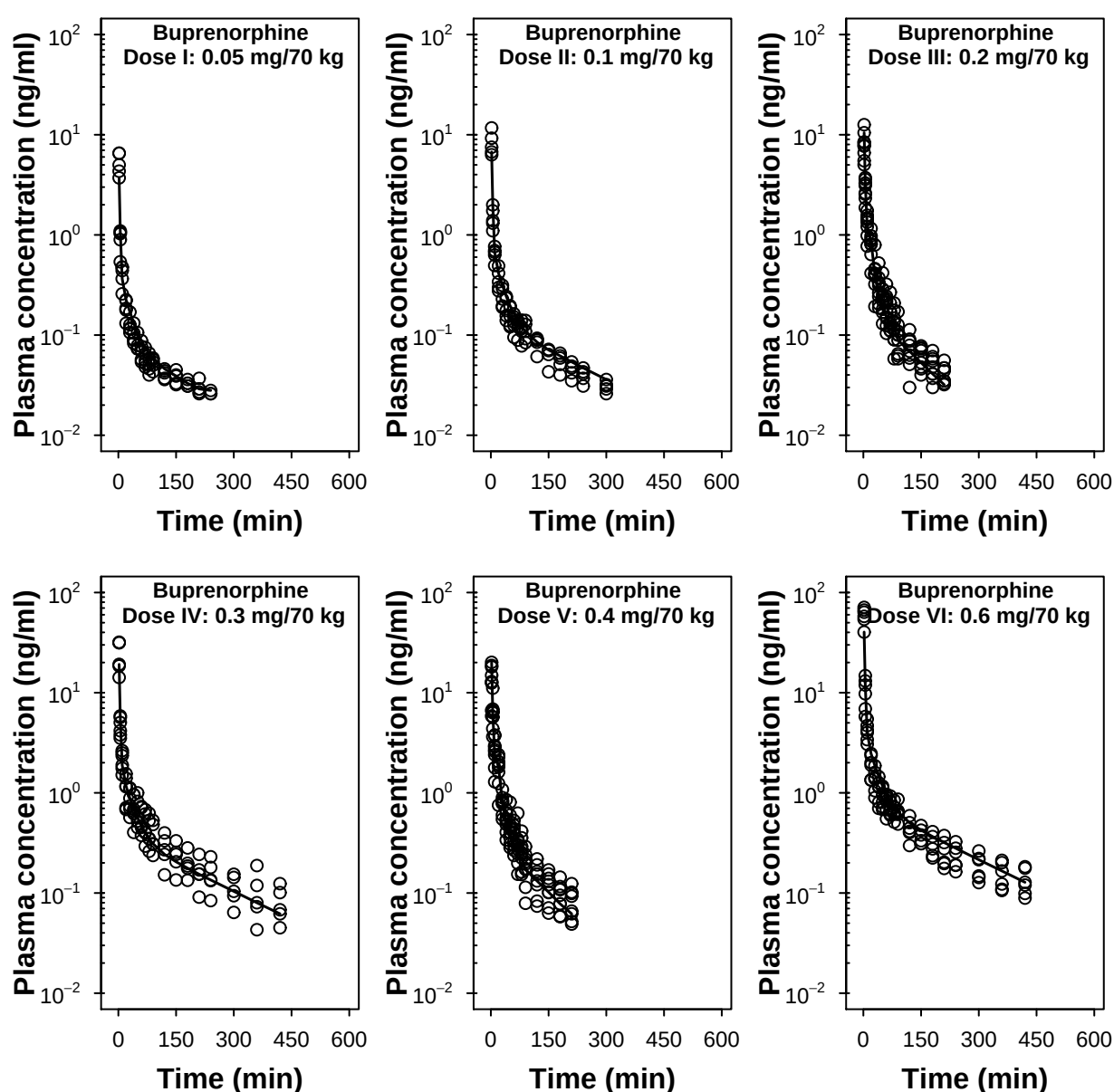


Figure 1. Individual buprenorphine concentration-time profiles for the different treatment groups (0.05–0.6 mg/70 kg). The observed (symbols) and population predictions (solid line) are depicted.

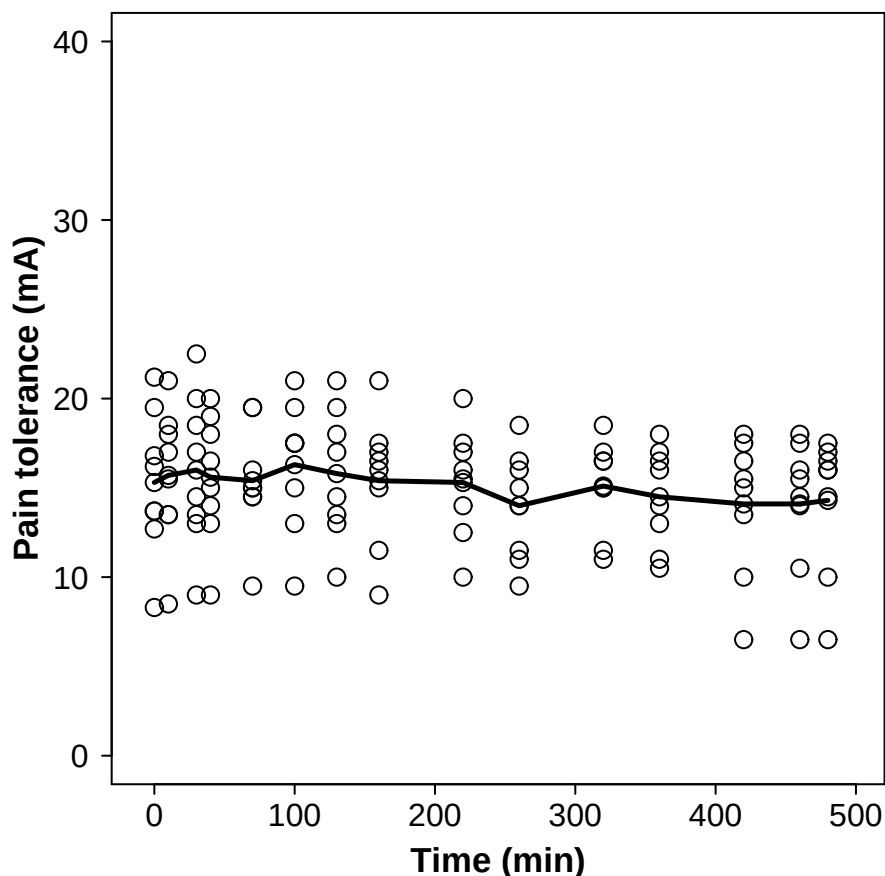


Figure 2. Influence of placebo treatment on the time course of the antinociceptive effect.

with effect compartment model, (3) receptor association/dissociation model and (4) combined biophase equilibration/receptor association/dissociation model including a linear function to describe the relationship between apparent fractional receptor occupancy and antinociceptive effect. The minimum values of the objective function for the PK-PD models (1), (2), (3) and (4) were 3407.2, 3406.4, 3458.3 and 3354.3, respectively. The minimum values of the objective function for the various PK-PD models show that the combined biophase equilibration/receptor binding model described the time course of antinociceptive effect best, yielding estimates for k_{e0} , k_{on} and k_{off} .

The pharmacodynamic parameter estimates are presented in table 2. The placebo antinociceptive response is shown in figure 2. Figure 3 shows the time course of antinociceptive effect for all individuals stratified to the different treatment groups. The antinociceptive responses in typical individuals, predicted on the basis of the population pharmacodynamic parameter estimates are depicted as solid lines. All parameters were estimated precisely, with an acceptable coefficient of variation ($<50\%$). In figure 4, examples of nine individual fits of the antinociceptive effect are given, including the worst, median and best fit determined on the basis of coefficient of variation (R^2) for three dose groups of buprenorphine (0.3 mg/70 kg, 0.4 mg/70 kg and 0.6 mg/70 kg). Figure 5 shows the steady-state receptor binding-antinociceptive effect relationship for buprenorphine. The

population estimates characterizing receptor association/dissociation kinetics were estimated at 0.0631 (95 % CI: 0.0390 - 0.0872) ml/ng/min and 0.0785 (95 % CI: 0.0352 - 0.122) min⁻¹ for the parameters k_{on} and k_{off} , respectively. The rate constant describing biophase equilibration kinetics, k_{e0} , was estimated at 0.00447 (95 % CI: 0.00299 - 0.00595) min⁻¹, which corresponds to $t_{1/2,ke0} = 155$ min *versus* $t_{1/2,koff} = 8.8$ min. The equilibration dissociation constant K_D was calculated from the ratio k_{off}/k_{on} and was 1.24 ng/ml, which corresponds to 2.66 nM.

A sensitivity analysis was performed to assess the impact of changes in the pharmacodynamic parameter k_{e0} on the predictive performance of the combined biophase equilibration/receptor association and dissociation model. The results of the sensitivity analysis are presented in figure 6 and show that the combined biophase equilibration-receptor association/dissociation model is sensitive to changes in the pharmacodynamic parameter k_{e0} . No significant sex dependency was observed for the rate constants of receptor association/dissociation and biophase equilibration. This was tested by inclusion of the covariate sex on the structural pharmacodynamic parameters k_{e0} , k_{on} and k_{off} one at a time (Δ OFV < 1). In addition, pre-treatment pain tolerance values were not significantly different for male and female (Δ OFV < 1).

DISCUSSION

Recently a mechanism-based PK-PD model was proposed to characterize and predict the time course antinociceptive effect of buprenorphine in rats. The model was used to predict buprenorphine brain concentrations, binding of buprenorphine to the μ -opioid receptor in the brain and ultimately the antinociceptive effect. To this end, the PK-PD model incorporated the term k_{e0} to describe biophase equilibration kinetics, the parameters k_{on} and k_{off} to characterize the receptor association/dissociation kinetics of buprenorphine at the μ -opioid receptor and a linear transduction function to describe the relationship between relative receptor occupancy and antinociceptive effect. The present investigation

	Typical value	IIV (%)
k_{on} (ml/ng/min)	0.0631 (19.5)	NE
k_{off} (min ⁻¹)	0.0785 (28.2)	115 (28.0)
K_D (ng/ml) [§]	1.244	
k_{e0} (min ⁻¹)	0.00447 (16.9)	44 (34.4)
E_0 (mA)	16.5 (8.9)	50.0 (25.4)
ARE (mA)	3.7 (34.0)	

Table 2. Population pharmacodynamic estimates and inter-individual variability estimates of buprenorphine. For the fixed and random parameter estimates the relative standard error (%) is displayed

[§] secondary parameter (k_{off}/k_{on}); ^{||} additive residual error.

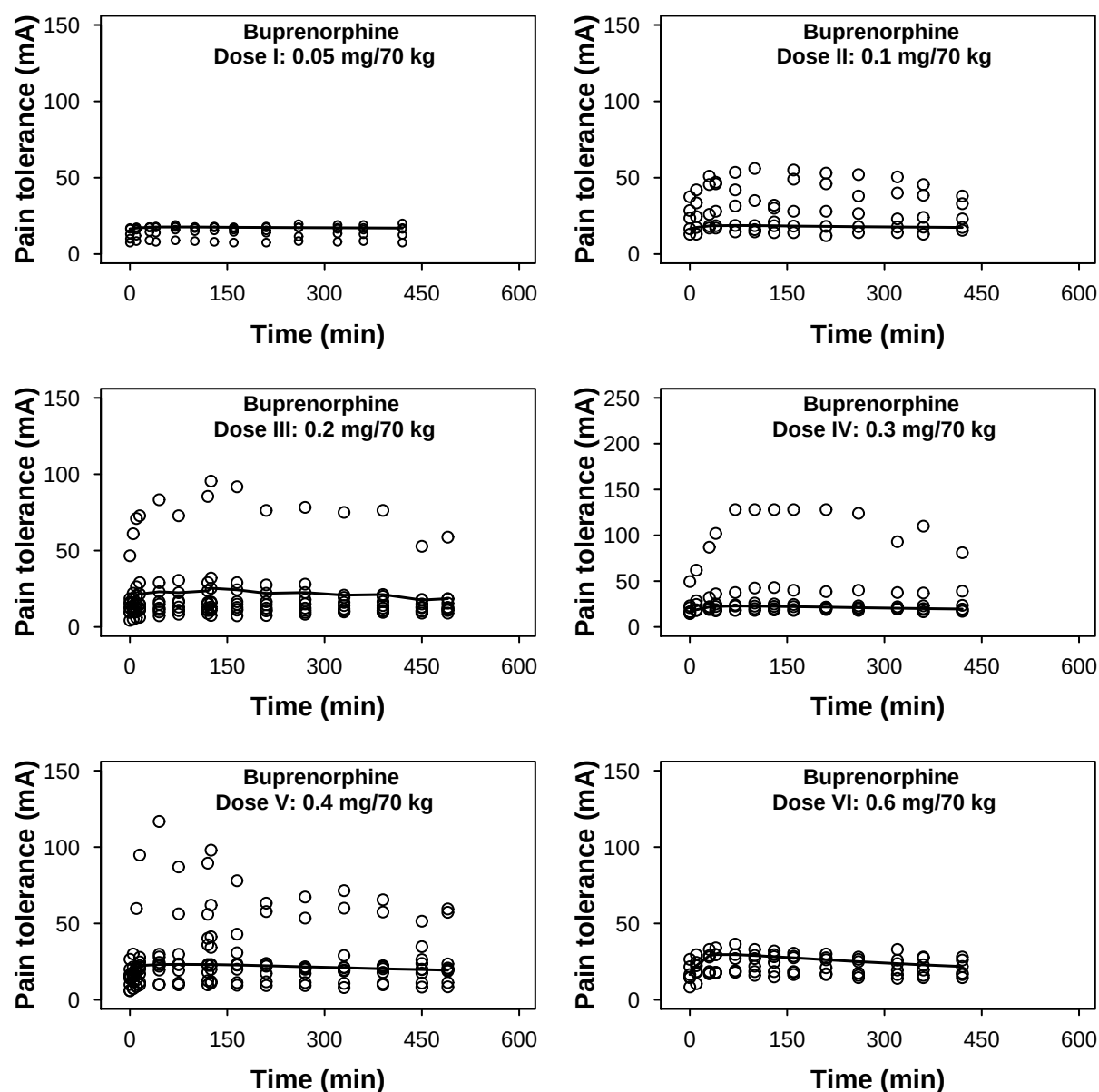


Figure 3. Changes in pain tolerance in time after intravenous administration of buprenorphine. For each treatment group (0.05-0.6 mg/70 kg), the observed (symbols) and mean population predicted (solid line) time course of the antinociceptive effect are shown.

focuses on the PK-PD correlation of buprenorphine in man. This is important since between species both qualitative and quantitative differences may exist between biological systems. In the present investigation, a number of structurally different PK-PD models were evaluated for their usefulness to describe the time course of the antinociceptive effect following administration of buprenorphine. Four different models were tested: (1) sigmoid $E_{\max}$ model with effect compartment model, (2) "power" or "Leiden" model (equation 3) with effect compartment model, (3) receptor association/dissociation model and (4) combined biophase equilibration/receptor association/dissociation model. The combined biophase equilibration-receptor association/dissociation model showed a significantly improved fit compared to an effect compartment model and the receptor associ-

ation/dissociation model. Analysis of the data on the basis of the sigmoid $E_{\max}$ model with effect compartment to the buprenorphine concentration-antinociceptive effect data showed that $E_{\max}$ could not be estimated accurately and precisely (data not shown), indicating that despite the wide dose range tested the maximum antinociceptive response has not been reached in these investigations. Furthermore, analysis of the data with the sigmoid $E_{\max}$ model with effect compartment model showed no improvement of the model fit compared to the power model, confirming that maximum antinociceptive effect has indeed not been achieved in the studied dose range. Since no estimate of $E_{\max}$ could be obtained it was postulated that in the studied dose range no receptor saturation occurred.

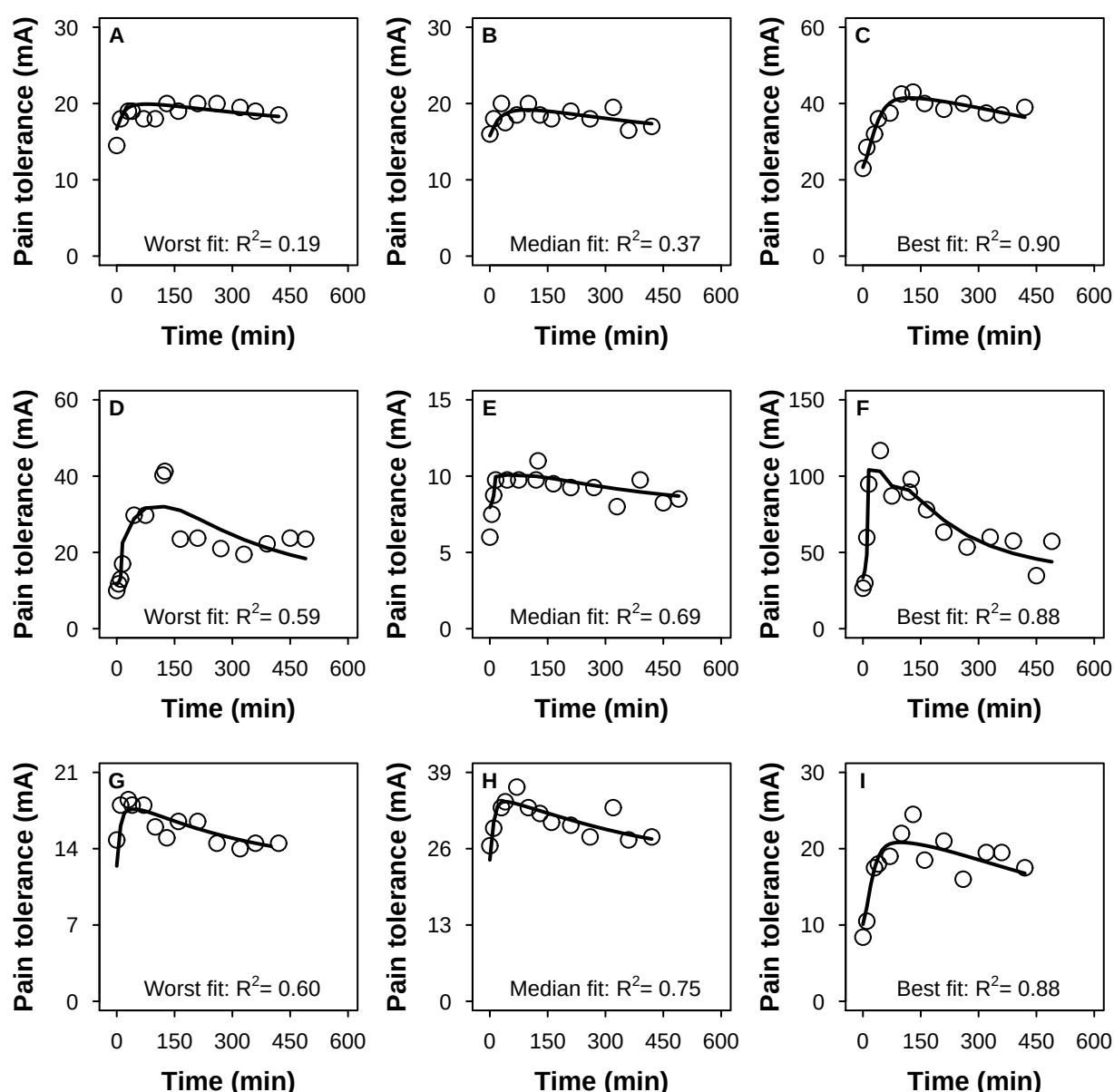


Figure 4. Time courses of antinociceptive effect after buprenorphine administration of 0.3 mg/70 kg (A-C), 0.4 mg/70 kg (D-F) and 0.6 mg/70 kg (G-I) are shown. Worst, median and best data fits between measured (symbols) and individual predicted pain tolerance (solid line) are presented. The goodness-of-fit was assessed by the coefficient of determination (R^2).

This has important implications for the selection of the transducer function in the receptor association/dissociation model or the combined biophase equilibration-receptor association/dissociation model. Specifically, the transducer function describes the relationship between receptor occupancy and the antinociceptive effect. In theory, the transducer function can take any shape. The selection of a transducer function is dependent on the pharmacological behavior, reflected by partial or full agonistic activity, of a drug in the biological system (Black and Leff, 1983; Kenakin, 1997). On the basis of the observed lack of a maximum antinociceptive effect in the present study and the fact that buprenorphine behaves as a partial agonist in receptor assays (Huang *et al.*, 2001) it is plausible to assume a linear transducer function to characterize the apparent receptor binding-effect relationship (Clark, 1937; Ariens, 1954). Upon administration of 0.6 mg/70 kg buprenorphine a predicted maximum of 70 % of the receptors is occupied (figure 5).

Moreover, in the studied dose range the observed buprenorphine concentrations in plasma and predicted concentrations in the biophase are mainly below the estimated K_D

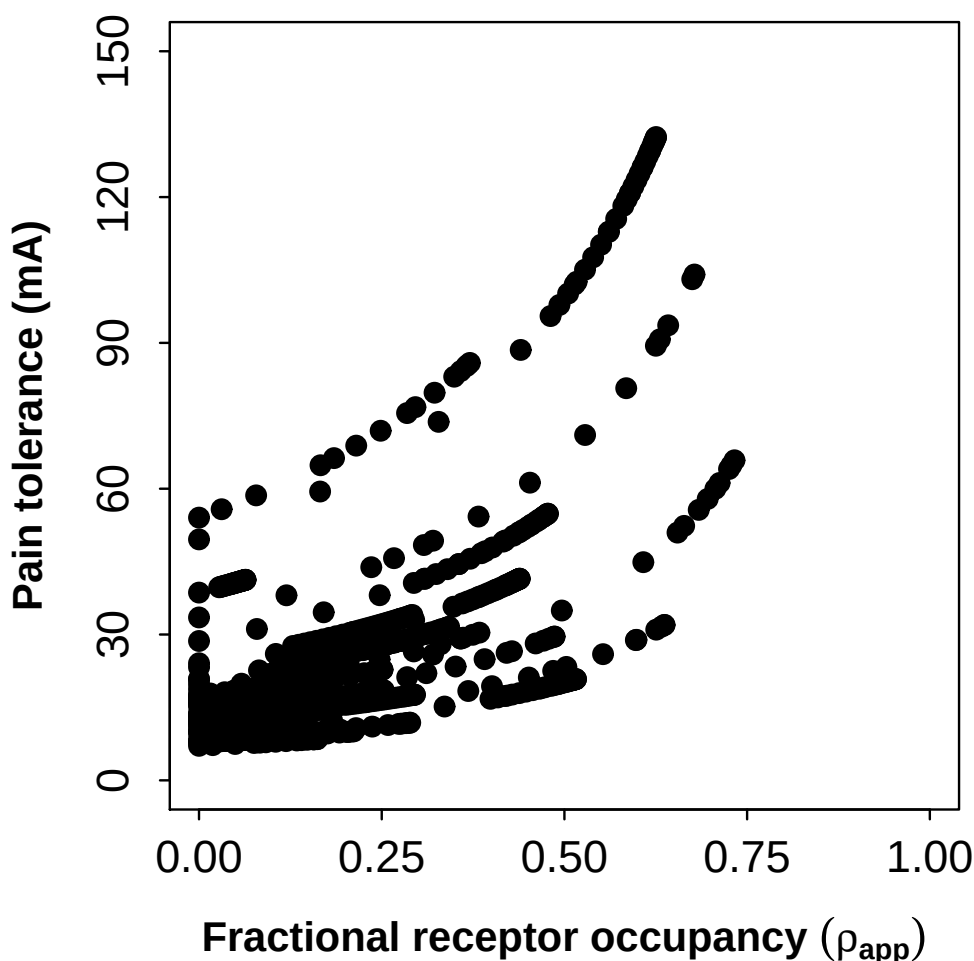


Figure 5. Receptor occupancy-antinociceptive effect relationship of buprenorphine for all individuals. This relation is derived from equation 7. The closed circles represent all individual receptor binding *versus* predicted pain tolerance pairs.

value. Much higher concentrations exceeding the K_D (1.24 ng/ml) are required to achieve maximal receptor binding. Therefore, higher doses are needed to saturate receptor binding (100 % receptor occupancy) and to obtain the maximum antinociceptive effect. On the basis of the combined PK-PD model it was shown that with values of the half-life of biophase equilibration ($t_{1/2,ke0}$) and the receptor dissociation ($t_{1/2,koff}$) of 155 min and 8.8 min, respectively, the rate of onset and offset of the antinociceptive effect is predominantly determined by distribution of buprenorphine to the effect site. Interestingly, the estimated half-life time of biophase equilibration (155 min) in the present study is in the same range as has been reported for buprenorphine-induced analgesia (171 min) (Koppert *et al.*, 2005).

The influence of the pharmacodynamic parameter k_{e0} on the time course of the antinociceptive effect was examined by sensitivity analysis based on the combined biophase equilibration-receptor association/dissociation model (figure 6). The results of the sensitivity analysis showed that the PK-PD modeling outcome is influenced by biophase equilibration, indicating that k_{e0} can be estimated reliably with the present experimental design. This underlines the importance of the application of the combined biophase equilibration-receptor association dissociation model to characterize the time course of buprenorphine's antinociceptive effect. Distribution to the biophase constitutes a biolog-

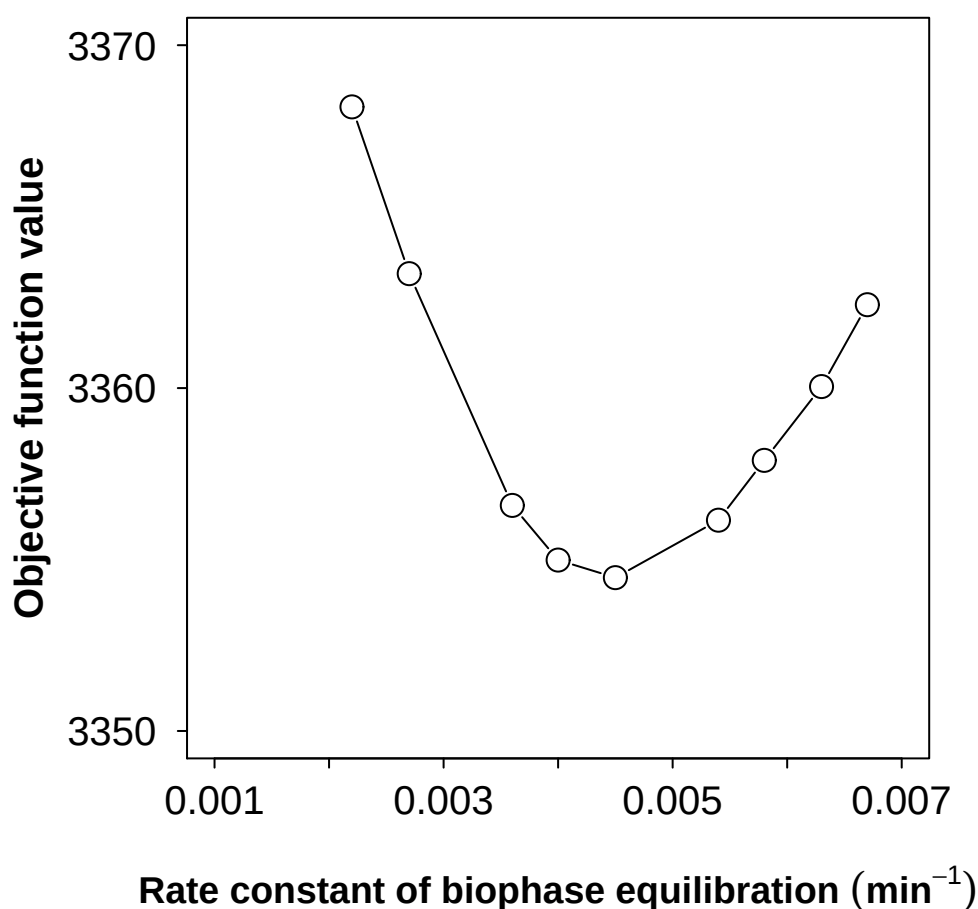


Figure 6. Results of objective function mapping for the pharmacodynamic parameter k_{e0} .

ically plausible representation of the rate-limiting step in onset and offset of buprenorphine's antinociceptive effect. Specifically, buprenorphine is a lipophilic opioid and is, similar to fentanyl, assumed to cross the blood-brain barrier readily (Ohtani *et al.*, 1995). For these drugs the delay in antinociceptive effect is most likely caused by drug distribution within the brain tissue (Ebling *et al.*, 1990; Bouw *et al.*, 2001). The elimination of buprenorphine from the brain is likely to be slow relative to the pharmacokinetics of buprenorphine in plasma, indicating that the elimination of buprenorphine from the brain is the rate-limiting step in terminating the drug's action (figure 7). This is in agreement with results from dedicated drug disposition studies which also show slow elimination of buprenorphine from the brain. Specifically, Pontani *et al.* (1985) reported that after administration of 0.2 mg/kg of buprenorphine in rats the decline of the buprenorphine concentration in the brain was markedly slower than from plasma ($t_{1/2, \text{brain}}$ 2.3 h versus

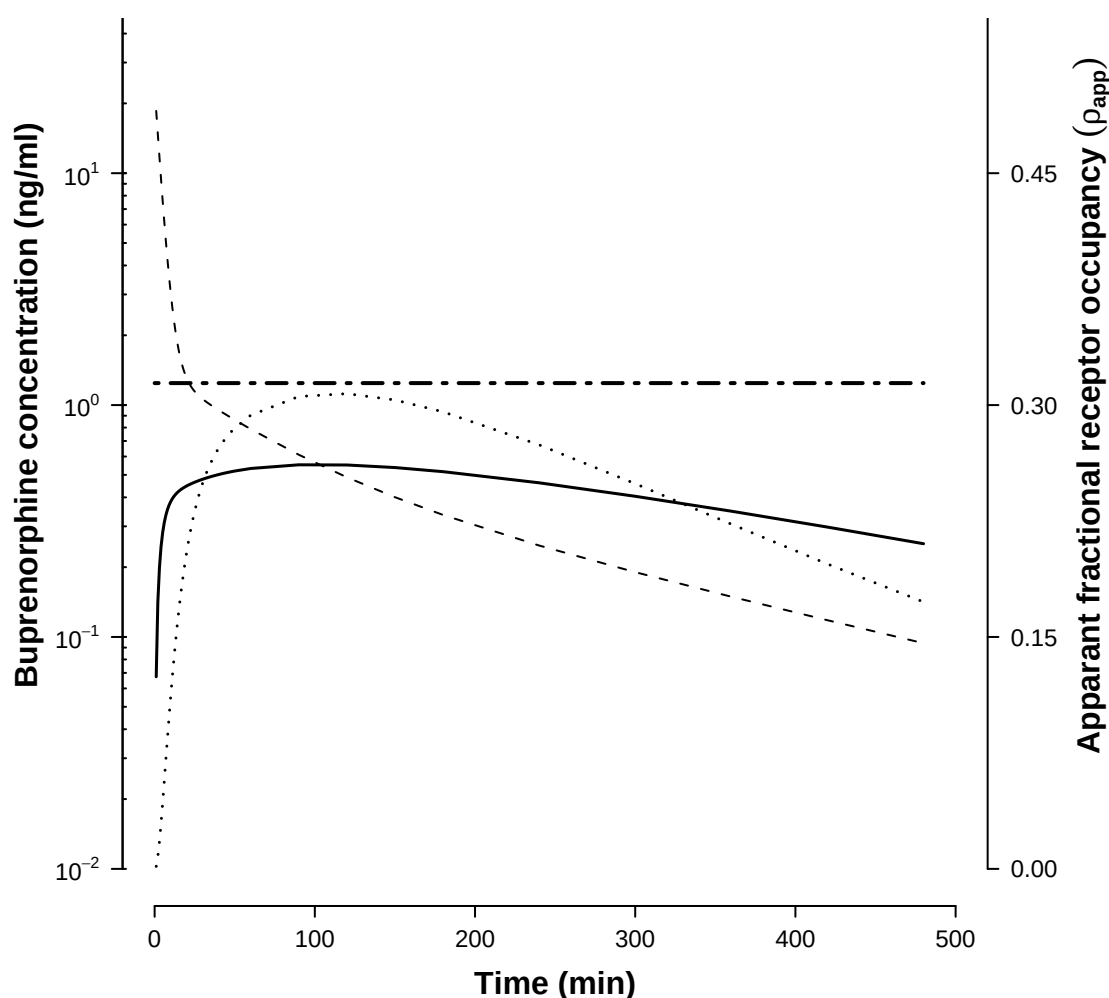


Figure 7. Time course of buprenorphine concentration in plasma (dashed line) and biophase (solid line) in a typical healthy volunteer after intravenous bolus administration of 0.4 mg/70 kg buprenorphine. The dotted line represents the apparent *in vivo* fractional receptor occupancy of buprenorphine. The elimination of buprenorphine from the brain is slower than from plasma. The thick dashed-dotted line represents the K_D value of 1.244 ng/ml. Note that most observed buprenorphine concentrations are below the estimated K_D value (dashed-dotted line).

$t_{1/2, plasma}$ 1.4 hr). In addition, using [^{11}C]-buprenorphine as a positron emission tomography (PET) tracer to characterize buprenorphine disposition characteristics in the brain it was also shown in baboons that the elimination of buprenorphine from brain is slower than from plasma (Shiue *et al.*, 1991).

The onset and offset of the antinociceptive effect, which both determine the observed hysteresis in the time course of the antinociceptive effect, are influenced by different pharmacological mechanisms. This is illustrated in figure 8, where the changes in the time to reach peak effect (T_{max}) with k_{on} (keeping K_D or k_{off}/k_{on} constant) and k_{e0} is depicted. The value of the rate constant k_{on} influences the observed time delay in the antinociceptive effect more than the rate constant for biophase equilibration (panel A). This is in accordance with the observation that transport across the blood-brain barrier is much faster than binding of buprenorphine to the μ -opioid receptor (receptor association kinetics) (Ohtani *et al.*, 1995). On the other hand, biophase equilibration determines the buprenorphine disposition characteristics at the effect-site and influences the biological effect intensity and the duration of the antinociceptive effect (figure 8, panel B).

In mechanism-based PK-PD modeling typically a distinction is made between drug-specific properties and biological system specific properties (Van der Graaf and Danhof, 1997). Drug specific properties comprise receptor affinity and intrinsic efficacy. In principle drug specific properties can be predicted on the basis of information from *in vitro* bio-assays (Van Der Graaf *et al.*, 1997; Visser *et al.*, 2003; Zuideveld *et al.*, 2004). An important question is to what extent the rate constants for receptor association (k_{on}) and

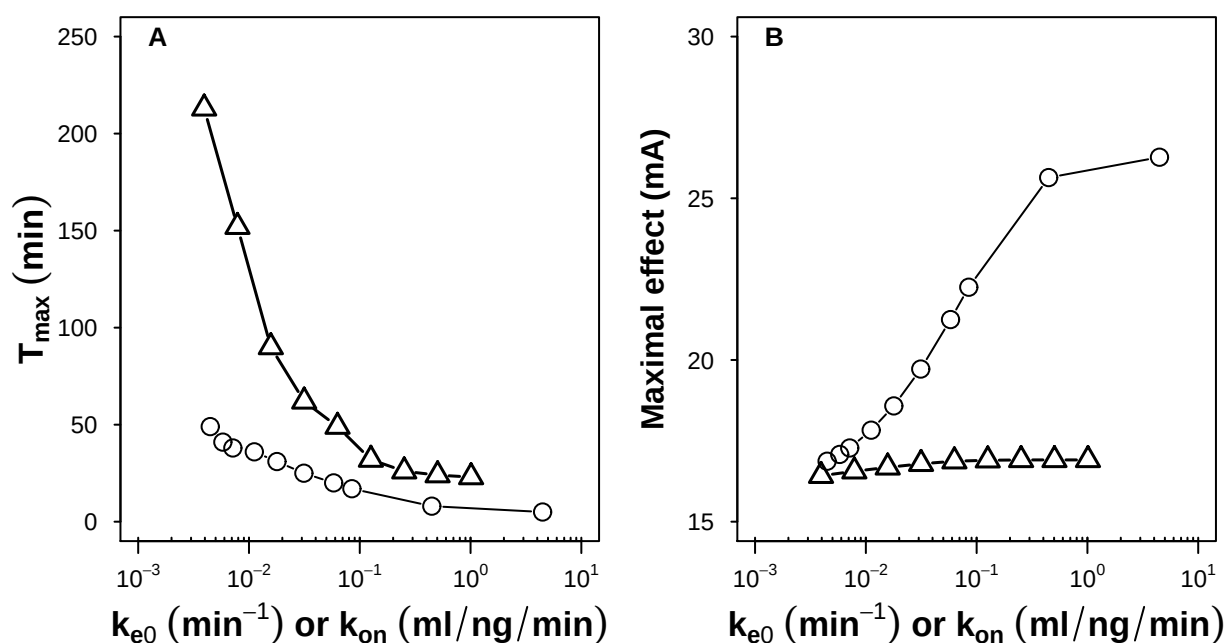


Figure 8. (A) The time to reach peak effect (T_{max}). (B) Time to reach E_{max} . Data simulated with the combined biophase distribution/receptor association/dissociation model with a linear transduction function when k_{on} (solid line) or k_{e0} (dashed line) was varied, keeping K_D (k_{off}/k_{on} constant).

dissociation (k_{off}) could be predicted on the basis of *in vitro* bio-assays. Drug-specific characteristics for buprenorphine, like receptor association/dissociation kinetics have also been investigated *in vitro* (Villiger and Taylor, 1982; Boas and Villiger, 1985). The results from these studies indicate the presence of high-affinity and low-affinity binding sites for buprenorphine in the spinal cord and a low-affinity binding site in the brain. In these *in vitro* experiments, the dissociation of buprenorphine from the μ -opioid receptor was characterized by an initial rapid phase ($t_{1/2, \text{koff}} = 5.6$ min) followed by a slower phase ($t_{1/2, \text{koff}} = 166.4$ min). The estimated *in vivo* dissociation half life for buprenorphine of 8.8 min is in accordance with the reported *in vitro* value for the initial rapid phase of the dissociation of buprenorphine from the μ -opioid receptor.

Interestingly, receptor binding characteristics of buprenorphine have also been investigated *in vivo* using opioid receptor imaging with [^{11}C]-buprenorphine as a positron emission tomography tracer in baboons (Shiue *et al.*, 1991). The dissociation rate constant for buprenorphine was reported to be 0.069 min^{-1} which is equivalent to a $t_{1/2, \text{koff}} = 10$ min, which is again in close agreement with our results. These observations confirm the validity of the PK-PD model and indicate that the rate constants for receptor association and dissociation may be readily predicted from *in vitro* bio-assays. Similar pharmacological characteristics of the *in vivo* kinetics of onset and offset of buprenorphine's antinociceptive effect were observed in rat. In rats, the drug-specific rate constants for receptor association and dissociation were estimated at 0.0228 ml/ng/min and 0.0731 min^{-1} , respectively (Yassen *et al.*, 2005). Thus, in rat and human the kinetics of receptor association and dissociation of buprenorphine are in the same range indicating that the μ -opioid receptor functions in a nearly identical manner in both species (Rothman *et al.*, 1995). On the other hand, the rate constant for biophase equilibration is different between rat ($k_{e0} = 0.0242 \text{ min}^{-1}$) and human ($k_{e0} = 0.00447 \text{ min}^{-1}$). In contrast to receptor association/dissociation drug characteristics, the rate constant for biophase equilibration is dependent on the biological system and may therefore vary among species (e.g. differences in brain distribution characteristics). An intriguing question is whether, taking into consideration allometric scaling laws, biophase equilibration kinetics could be predicted in man on the basis of the estimated rate constant for biophase equilibration from preclinical studies. This will be subject of further investigations in our laboratory.

An important issue is the potency and intrinsic activity of buprenorphine for its antinociceptive effect relative to other opioids like morphine. Using a PK-PD model, the concentration-analgesic effect relationship of morphine has also been characterized (Sarton *et al.*, 2000). On this basis, morphine's potency for acute pain was estimated to be in the range of 32.9 nM to 76.5 nM. The buprenorphine *versus* morphine potency ratio indicate that buprenorphine is 12 to 29 times more potent than morphine for μ -opioid receptor induced antinociception. This is in agreement with the general perception that buprenorphine is 20 to 40 times more potent than morphine (Tigerstedt and Tammisto, 1980; Mok *et al.*, 1981; Wang *et al.*, 1981).

In conclusion, a recently proposed mechanism-based PK-PD model had been success-

fully applied to characterize the time course of antinociceptive effect of buprenorphine in healthy volunteers. The model was able to separate biophase equilibration and receptor association/dissociation properties. PK-PD analysis suggests that time-dependencies in the pharmacodynamics of buprenorphine with respect to antinociception were mainly determined by biophase equilibration kinetics. Similar characteristics for the time course of the antinociceptive effect have been reported for rats. This suggests that this mechanism-based approach constitutes a realistic reflection of the kinetics of buprenorphine action *in vivo*. PK-PD analysis of the *in vivo* concentration-effect relationship showed no ceiling effect for the antinociceptive effect of buprenorphine. In the present analysis maximum predicted receptor occupancy was 70 % in the studied dose range. Higher doses are necessary to saturate receptor binding and to achieve a maximum antinociceptive effect.

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SECTION
III

**MECHANISM-BASED PK-PD MODELING
OF THE RESPIRATORY DEPRESSANT
EFFECT OF (SEMI-)SYNTHETIC OPIOIDS**

MECHANISM-BASED PHARMACOKINETIC-PHARMACODYNAMIC MODELING OF THE RESPIRATORY DEPRESSANT EFFECT OF BUPRENORPHINE AND FENTANYL IN RATS

Ashraf Yassen, Jingmin Kan, Erik Olofsen, Ernst Suidgeest,
Albert Dahan and Meindert Danhof

J. Pharmacol. Exp. Ther. 2006; **319**:682-692.

ABSTRACT

The purpose of this investigation was to develop a mechanism-based PK-PD model to predict the time course of respiratory depression following administration of opioids in rats. The proposed model is based on receptor theory and aims at the separate characterization of biophase distribution and receptor association/dissociation kinetics as determinants of hysteresis between plasma concentration and effect. Individual concentration time courses of buprenorphine and fentanyl were determined in conjunction with continuous monitoring of respiratory depression. Buprenorphine and fentanyl were administered intravenously in various doses.

For buprenorphine hysteresis was best described by a combined biophase distribution-receptor association/dissociation model with a linear transducer function. The values of the parameter estimates of the rate constants for biophase distribution (k_{e0}), receptor association (k_{on}) and dissociation (k_{off}) were 0.0348 min^{-1} (95% CI: $0.0193 - 0.0503 \text{ min}^{-1}$), 0.57 ml/ng/min (95% CI: $0.38 - 0.76 \text{ ml/ng/min}$) and 0.0903 min^{-1} (95% CI $0.035 - 0.196 \text{ min}^{-1}$) respectively. The values of the equilibrium dissociation constant and intrinsic activity were 0.16 ng/ml and 0.48 (95% CI: $0.45 - 0.51$), respectively. The value of the K_D is close to reported estimates of receptor affinity *in vitro* confirming the validity of the mechanism-based PK-PD model.

For fentanyl unrealistically high estimates of the rate constants for receptor association and dissociation were obtained, indicating that hysteresis is caused solely by biophase distribution kinetics. This is consistent with fentanyl's fast receptor association/dissociation kinetics *in vitro*. As a result the mechanism-based PK-PD model of fentanyl could be reduced to a biophase distribution model with fractional sigmoid $E_{\max}$ pharmacodynamic model.

INTRODUCTION

Natural, semi-synthetic and synthetic opioids are widely used in the treatment of pain and for the induction of anesthesia (Joranson *et al.*, 2000; Inturrisi, 2002). Opioids exert their effects through the interaction with specific receptors in the central nervous system (Snyder and Pasternak, 2003). Important progress has been made in the identification of the functionality of several opioid receptor subtypes (Kieffer, 1999). It has been demonstrated that in particular μ -opioid receptors are involved in the opioid-induced analgesia and anesthesia as well as respiratory depression (Romberg *et al.*, 2003; Pasternak, 2004). In recent years several pharmacokinetic-pharmacodynamic (PK-PD) models have been proposed to characterize the time course of the analgesic and anesthetic effects of opioids *in vivo* using a variety of different pharmacodynamic endpoints including quantitative EEG parameters as surrogates for depth of anesthesia (Stanski, 1992; Cox *et al.*, 1998).

In the various investigations there has been a clear trend towards the development of mechanism-based PK-PD models with much-improved properties for extrapolation and prediction. The incorporation of principles from receptor theory has been a key element in this development (Van der Graaf and Danhof, 1997). A specific feature of mechanism-based PK-PD models is the separation between a drug specific part, characterizing the interaction of the drug with the biological system in terms of *in vivo* affinity and intrinsic efficacy, and a biological system specific part characterizing the *in vivo* stimulus-response relationship (Visser *et al.*, 2002; Zuideveld *et al.*, 2004; Jonker *et al.*, 2005). Typically the interaction of the drug with the biological system is described by a hyperbolic function. In contrast, the stimulus-response relationship can in principle take any shape. To date mainly hyperbolic stimulus-response relationships for systems with high receptor reserve and linear stimulus-response relationships for systems with low receptor reserve have been considered (Clark, 1937; Black and Leff, 1983). The investigations on the PK-PD correlations of opioids have shown that in general these operate with a high receptor reserve *in vivo* necessitating the use of hyperbolic stimulus-response relationships (Cox *et al.*, 1998).

At present mechanism-based PK-PD modeling of the effect of opioids on respiration has not been accomplished. This is important since respiratory depression is a serious and potentially life threatening side effect of opioid-induced analgesia (Baxter, 1994; Bailey *et al.*, 2000). Another issue that has received little attention is the modeling of slow receptor association/ dissociation kinetics as a factor causing hysteresis between plasma concentration and effect (Shimada *et al.*, 1996; Yassen *et al.*, 2005, 2006). It is well established that buprenorphine displays slow receptor association/dissociation kinetics *in vitro* as well as *in vivo* causing a slow onset and a long duration of effect (Cowan *et al.*, 1977; Boas and Villiger, 1985). This can be a complicating factor in optimizing the dosing regimen of buprenorphine and in the context of the reversal of opioid-induced respiratory depression with naloxone (Gal, 1989).

The objective of the present investigation was therefore to develop a mechanism-based PK-PD model for the effect of opioids on respiratory depression, with emphasis on the modeling of slow receptor association/dissociation kinetics as a determinant of the time course of the effect. Buprenorphine and fentanyl were chosen as model drugs with slow and fast receptor association/dissociation kinetics respectively. In order to validate the various models we have obtained high-resolution concentration and effect data following the administration of widely different doses of both drugs. In this investigation we apply a novel technique to monitor opioid-induced respiratory depression. In previous investigations opioid-induced respiratory depression has typically been measured using arterial carbon dioxide tension ($P_a\text{CO}_2$) as a surrogate biomarker of minute ventilation (V_i) (Ohtani *et al.*, 1997; Megarbane *et al.*, 2006). However, due to its low sensitivity this technique may well underestimate respiratory depression (Dahan *et al.*, 2005). Therefore, in the present study the effects of buprenorphine and fentanyl on respiration was determined by a novel method in which minute ventilation is measured by whole body plethysmography at a fixed inspired CO_2 concentration of 6.5% (Romberg *et al.*, 2003). Apart from an increased sensitivity, the application this technique offers the advantage of enabling a direct comparison with similar measures obtained in investigations in humans (Dahan and Berkenbosch, 1996).

In this investigation several structurally different models PK-PD models have been evaluated for their ability to describe the time course of the respiratory depression. The most important models tested were: a) a biophase distribution model in combination with a fractional sigmoid E_{max} pharmacodynamic model, b) a receptor/association dissociation model with a linear transduction function and c) a combined biophase distribution receptor association/dissociation model with a linear transduction function.

MATERIALS AND METHODS

Animals

Male Wistar rats, weighting 225-250 g at arrival, were obtained from Charles River BV (Zeist, The Netherlands). The animals were housed in plastic cages in groups before surgery and individually after surgery. The animals were housed under laboratory standard conditions at constant room temperature (21 °C) and on a 12-h light/dark cycle, with lights turned on at 7:00 am and off at 7:00 pm. Food (RMH-TM; Hope Farms, Woerden, The Netherlands) and acidified water were allowed ad libitum. The animals were handled and allowed for acclimation to the experimental environment for ten days prior to the start of the experiment. The protocol was approved by the Ethical Committee on Animal Experimentation of Leiden University.

Surgical procedure

Surgery was carried out under anesthesia with an intramuscular injection of 0.1 mg/kg medetomidine hydrochloride (Domitor 1 mg/ml; Pfizer, Capelle a/d IJssel, The Netherlands) and 1 mg/kg ketamine base (Ketalar 50 mg/ml; Parke-Davis, Hoofddorp, The Netherlands).

Two days before the experiment indwelling cannulae were implanted, one in the left femoral artery and one in the right jugular vein. The cannula in the right jugular vein was used for administration of the opioid while the cannula in the left femoral artery was used for serial collection of arterial blood samples. The cannulae were made from pyrogen free, non-sterile polyethylene tubing. One day before surgery cannulae were disinfected in a benzalkoniumchlorid 1 % solution. The venous cannula consisted of 3 cm polyethylene tubing (0.28 mm i.d.; Portex Limited, Kent, United Kingdom) heat-sealed to 9 cm polyethylene tubing (0.58 mm i.d.; Portex Limited, Kent, United Kingdom). The arterial cannula consisted of 3 cm polyethylene tubing (0.28 mm i.d.) heat-sealed to 21 cm polyethylene tubing (0.58 mm i.d.). Furthermore, a telemetric transmitter (Physiotel implant TA10TA-F40 system, Data Sciences International (DSI), St. Paul, MN, USA) was implanted under the skin in the neck for the measurement of body temperature. The cannulae were tunnelled subcutaneously and fixed at the back of the neck with a rubber ring. The skin in the neck and throat was stitched with normal suture. The skin in the groin was closed with wound clips.

In order to prevent clotting and cannula obstruction the cannulae were filled with a 25 % (w/v) polyvinylpyrrolidone solution (PVP; Brocacef, Maarssen, The Netherlands) in pyrogen-free physiological saline (B. Braun Melsungen AG, Melsungen, Germany) containing 20 IU/ml heparin (Hospital Pharmacy, Leiden University Medical Center, Leiden, The Netherlands).

Drugs and dosages

Buprenorphine hydrochloride and fentanyl monocitrate were kindly donated by Grünenthal GmbH (Aachen, Germany). Buprenorphine hydrochloride solution was prepared in saline with aid of two drops polysorbate 80 (Hospital Pharmacy, Leiden University Medical Center, Leiden, The Netherlands). To accelerate solubility, the solution was placed in an ultra-sonification bath for 30 min. Fentanyl monocitrate solution was prepared in saline. The doses and concentrations of buprenorphine and fentanyl are expressed as free base.

Measurement of respiratory depression

Respiratory depression was determined in unrestrained, conscious animals using whole-body plethysmography for the quantification of ventilation (Model PLY3223, Buxco Elec-

tronics Inc., UK). Briefly, the animals were placed in a plethysmograph, consisting of a measurement chamber and an integrated reference chamber to correct for atmospheric disturbances. Both chambers were connected to a differential pressure transducer (TRD 5700, Buxco Electronics Inc., UK). During the experiment continuous flow of gas was delivered through the measurement chambers. The flow and composition of the gas mixture, consisting of dry air and CO₂, was controlled by mass flow controllers (5850S/BC Mass Flow Controller, Brooks Instruments, The Netherlands) connected to a microprocessor control and read out unit (Model 0152, Brooks Instruments, The Netherlands). O₂ and CO₂ levels in the chambers were monitored continuously using a Datex Multicap gas monitor (Datex-Engstrom, Helsinki, Finland).

In each animal the effects of opioids and vehicle were assessed on ventilation at an inhaled concentration of 6.5 % carbon dioxide on a background of normoxia (20 % oxygen). The inhalation of the gas mixture lasted 5 min to ensure that steady-state ventilation had been reached. Tidal volume (V_T), breathing frequency (RR) and minute ventilation (V_i , where $V_i = V_T \times RR$) were obtained from changes in chamber pressure using a low-pressure differential transducer connected to preamplifier modules (MAX2270, Buxco Electronics Inc., UK). The signals were digitized at a rate of 200 Hz using a CED 1410_{plus} interface (CED, Cambridge, UK). The digitized signals were collected and stored on disk for further off-line analysis. A personal computer running ACQ software (Erik Kruyt, Leiden University Medical Center, Leiden, The Netherlands) integrated the digitized signals to yield a flow signal.

Calibration of the chamber pressure signal was performed dynamically by injection of air into the chamber using a motor-driven 1-ml syringe pump. Minute ventilation was visualized using RRDP software (Erik Olofsen, Leiden University Medical Center) and stored on a breath-to-breath basis. Minute ventilation was averaged over the total number of breaths obtained in one minute and used for PK-PD data analysis. During the experiment body temperature was maintained at 37.5 °C using heating pads. Body temperature was monitored continuously by radiotelemetry (Model RPC-1, Data Sciences International, USA).

Measurement of buprenorphine and fentanyl plasma concentrations

Two different bio-assays were used for the determination of buprenorphine and fentanyl plasma concentration in rats. The details of these assays have been described elsewhere (Yassen *et al.*, 2005). Briefly for buprenorphine, to 50 μ l plasma 25 μ l internal standard (4 mg/100 ml ²H₄-buprenorphine) was added. Subsequently, 25 μ l concentrated ammonia was added and the samples were extracted by liquid/liquid extraction with 600 μ l tert.-butyl methyl ether (MTBE). The chromatographic system consisted of an Agilent HP 1100 HPLC system (Agilent, Waldbronn, Germany) coupled to an API 4000 LC/MS/MS system (Applied Biosystems, Darmstadt, Germany). Chromatography was performed on a precolumn (Metaguard Polaris 3 μ m C_{18A} 2 mm, Varian Darmstadt, Germany) guarded

Synergi 4 μm Hydro-RP 80A column 75 mm x 2 mm (Phenomenex, Aschaffenburg, Germany). The lower limit of quantification (LLOQ) was 0.047 ng/ml for buprenorphine. The accuracy ranged from 99.4 to 102.1 %. The precision expressed as coefficient of variation, ranged from 2.2 to 6.4 % for concentrations between 0.14 and 8.9 ng/ml.

For fentanyl, to 50 μl plasma 25 μl internal standard (14.8 ng/ml $^2\text{H}_5$ -fentanyl) was added. After adding 10 μl concentrated ammonia the samples were extracted by liquid/liquid extraction with 600 μl tert.-butyl methyl ether (MTBE). The chromatographic system consisted of an Agilent HP 1100 HPLC system (Agilent, Waldbronn, Germany) coupled to an API 3000 LC/MS/MS system (Applied Biosystems, Darmstadt, Germany). Chromatography was performed on a precolumn (Metaguard Polaris 3 μ C_{18A} 2 mm, Varian Darmstadt, Germany) guarded Atlantis C₁₈ column 3 μm 100 mm x 2.1 mm (Waters, Eschborn, Germany). The lower limit of quantification (LLOQ) was 0.118 ng/ml. The accuracy ranged from 87.0 to 96.1 % and the precision from 1.9 to 4.0 % for concentrations in the range from 0.4 to 50.2 ng/ml.

Pharmacokinetic-pharmacodynamic experiments

To minimize the influence of circadian rhythms, all experiments started between 9:00 and 9:30 am. Animals were randomly assigned to the treatment groups. Detailed information regarding experimental design and the administered dosages of buprenorphine and fentanyl is presented in table 1.

Before administration of drug or vehicle animals were placed in the measurement chamber for a habituation period of 1 hour. Upon administration of vehicle, buprenorphine, fentanyl or vehicle via a zero order intravenous infusion using an infusion pump (BAS Bioanalytical Systems Inc., West Lafayette, Indiana, USA), minute ventilation was measured at the following pre-defined time-points; 0 (baseline), 10, 25, 40, 55, 80, 95, 120, 150, 180, 240, 300, 360, 420 and 480 min after drug administration. For fentanyl, respiration was measured at; dose I: 0 (baseline), 10, 25, 40, 55 and 70 min, dose II: 0 (baseline), 10, 25, 40, 55, 70, 90 and 120 min, dose III: 0 (baseline), 10, 25, 40, 55, 70, 90 and 120 min, dose IV: 0 (baseline), 10, 25, 40, 55, 70, 90, 120 and 150 min after drug administration.

In cases where blood sampling coincided with the ventilation measurement, ventilation measurement preceded blood sampling to minimize stress for the animals. Serial arterial blood samples were collected in heparinized microtubes. Plasma (50 μl) was separated from the blood by centrifugation at 5000 rpm for 15 min and frozen at -20 °C until analysis.

PK-PD modeling procedure

The pharmacokinetic and pharmacodynamic parameters of buprenorphine and fentanyl were estimated using non-linear mixed-effects modeling as implemented in the NONMEM software version V, level 1.1 (Beal and Sheiner, 1999). The population analysis approach,

Table 1. Experimental design of the study describing the PK-PD relationship of the respiratory depressant effect of buprenorphine and fentanyl, with respect to number of animals per treatment group and their corresponding dose normalized for body weight, absolute dose, length of infusion and body weight. Data are presented as mean $\pm$ standard error (SE)

Group	n	Infusion time (min)	Dose (mg/kg)	Absolute Dose (mg)	body weight (kg)
buprenorphine					
I	8	20	0.025	0.0068 $\pm$ 0.0004	0.270 $\pm$ 0.016
II	8	20	0.05	0.0139 $\pm$ 0.0004	0.277 $\pm$ 0.007
III	8	20	0.1	0.0280 $\pm$ 0.0008	0.280 $\pm$ 0.008
IV	8	20	0.3	0.0816 $\pm$ 0.0045	0.272 $\pm$ 0.015
Control [§]	4	40			0.274 $\pm$ 0.004
fentanyl					
I	8	20	0.03	0.0089 $\pm$ 0.0012	0.298 $\pm$ 0.004
II	8	20	0.06	0.0196 $\pm$ 0.0002	0.327 $\pm$ 0.004
III	8	20	0.08	0.0261 $\pm$ 0.0007	0.326 $\pm$ 0.009
IV	8	20	0.085	0.0257 $\pm$ 0.0007	0.303 $\pm$ 0.007
Control [‡]	4	20			0.294 $\pm$ 0.005

[§] Control groups received an infusion of saline containing polysorbate 80

[‡] Control groups received an infusion of saline.

which takes into consideration both intra-animal and inter-animal variability, was undertaken using the first-order conditional estimation method with η - ϵ interaction (FOCE interaction) for pharmacokinetic-pharmacodynamic analysis. All fitting procedures were performed on an IBM-compatible computer (Pentium IV, 1500 MHz) running under Windows XP with the Fortran compiler Compaq Visual Fortran version 6.1. An in-house available S-PLUS 6.0 (Insightful Corp., Seattle, WA, USA) interface to NONMEM version V was used for data processing and management (including tools to perform non-parametric bootstrap procedures) and graphical data display.

Pharmacokinetic analysis

In order to determine the basic structural pharmacokinetic model for buprenorphine and fentanyl one-, two -and three compartment models were tested. Model selection and identification was based on the likelihood ratio test, pharmacokinetic parameter point estimates and their respective confidence intervals, parameter correlations and goodness-of-fit plots. For the likelihood ratio test, the significance level was set at $\alpha=0.01$, which

corresponds with a decrease of 6.6 points, after the inclusion of one parameter, in objective function value (OFV) under the assumption that the difference in OFV between two nested models is χ^2 distributed. The following goodness-of-fit plots were subjected to visual inspection to detect systemic deviations from the model fits: individual observed *versus* population or individual predicted values and weighted residuals *versus* time or population predicted values. On the basis of model selection criteria, two- and three-compartment models were selected for fentanyl and buprenorphine, respectively. The pharmacokinetic analysis for the selected compounds was performed by use of the ADVAN3 TRANS4 and ADVAN11 TRANS4 subroutines in NONMEM. For example, for fentanyl, the pharmacokinetic parameters, clearance (Cl), the inter-compartmental clearance (Q) and the volumes of distribution of compartments 1 and 2 (V_1 and V_2) were estimated.

The stochastic part of the model was selected to describe inter-animal variability in pharmacokinetic parameters and assumed a log-normal distribution of all model parameters over the population. Therefore an exponential distribution model was used to account for inter-animal variability:

$$P_i = P_{tot} \cdot \exp(\eta_i) \quad (1)$$

in which P_i is the individual value of model parameter P , P_{tot} is the typical value (mean population value) of parameter P in the population, and η_i is the normally distributed inter-animal random variable with mean zero and variance ω^2 . The coefficient of variation of the structural model parameters is expressed as percentage of the root mean square of the inter-animal variance term. Selection of an appropriate residual error model was based on inspection of the goodness-of-fit plots. On this basis a proportional error model was proposed to describe residual error in the plasma drug concentration:

$$C_{obs,ij} = C_{pred,ij} \cdot (1 + \varepsilon_{ij}) \quad (2)$$

in which $C_{obs,ij}$ is the j th observed concentration in the i th individual, $C_{pred,ij}$ is the predicted concentration, and ε_{ij} is the normally distributed residual random variable with mean zero and variance σ^2 . The residual error term contains all the error terms which can not be explained and refers to for example measurement and experimental error (e.g. error in recording sampling times) and structural model mis-specification.

Individual empirical Bayes estimates of the pharmacokinetic parameters were obtained from the basic pharmacokinetic model and served as input for the pharmacodynamic model. Simultaneous analysis of the pharmacokinetic and pharmacodynamic data is computer-intensive. Therefore, the sequential (two stage) approach was preferred above simultaneous PK-PD analysis. Unless the pharmacokinetic model is misspecified, the two-stage approach yield no biased pharmacodynamic parameter estimates (Zhang *et al.*, 2003).

To demonstrate the precision and stability of the pharmacokinetic models and to as-

certain accurate prediction of concentration-time profiles of fentanyl and buprenorphine, the final population pharmacokinetic models were subjected to an internal validation (Ette *et al.*, 2003). The validation procedure consisted of a bootstrap validation procedure. For the bootstrap validation procedure, one thousand data sets were generated randomly sampled from the original data set with replacement. Subsequently, the final population PK models were fitted to the bootstrap replicates one at a time. Finally, the mean, standard error, coefficient of variation and 95 % confidence intervals of all model parameters were calculated and compared to parameter values obtained from the original study. To refine the stochastic model, correlation between pharmacokinetic parameter estimates was tested by conducting covariance matrix analysis (OMEGA BLOCK option). A significant correlation between two parameters was assumed when the drop in OFV was more than 6.6 points ($p < 0.01$). Finally, explorative graphical analysis was performed to explore relationships between body weight and pharmacokinetic parameters.

Mechanism-based PK-PD analysis

In this study minute ventilation is used as a measure of respiratory depression. Various structurally different PK-PD models were tested for their appropriateness to describe the effect of buprenorphine on respiratory depression, including 1) biophase equilibration model with a fractional sigmoid $E_{\max}$ pharmacodynamic model, 2) receptor association/dissociation model with a linear transduction function and 3) combined biophase equilibration-receptor association/dissociation model with a linear transduction function. In the final model the exposure-respiratory depressant effect relationship of buprenorphine is quantified using the combined biophase equilibration-receptor association/dissociation model with a linear transduction function. In this model drug distribution to the site of action (biophase) was characterized on the basis of an effect compartment model (Sheiner *et al.*, 1979). Specifically, the rate of change of biophase drug concentrations is described by the following differential equation:

$$\frac{dC_e}{dt} = k_{e0} \cdot (C_p - C_e) \quad (3)$$

where k_{e0} is a first-order rate constant for distribution in the effect compartment and C_p represents the plasma concentration and C_e the effect-site concentration. At the site of action, the drug can bind to the μ -opioid receptor. Following the law of mass action, the rate of drug-receptor binding reaction is proportional to the concentration of drug C_e and free receptor R :

$$\frac{dC_e R}{dt} = k_{on} \cdot C_e \cdot R - k_{off} \cdot C_e R \quad (4)$$

Under the assumption that the concentration of drug is in excess compared to the free receptor concentration and that the total number of receptors (R_{tot}) is equal to the sum

of drug-bound ($C_e R$) receptors and unbound receptors (R), equation 4 can be rearranged:

$$\frac{d\rho_{\text{app}}}{dt} = k_{\text{on}} \cdot C_e \cdot (1 - \rho_{\text{app}}) - k_{\text{off}} \cdot \rho_{\text{app}} \quad (5)$$

in which ρ_{app} is the apparent fractional receptor occupancy ($C_e R / R_{\text{tot}}$). In the present analysis a linear transduction function was used to characterize the fractional receptor occupancy-respiratory depressant effect relationship:

$$E = E_0 \cdot (1 - \alpha \cdot \rho_{\text{app}}) \quad (6)$$

where E is the ventilatory response, E_0 is the baseline ventilation and α is the intrinsic activity of which the value varies between 0 and 1. In theory, the transduction function may take any shape. Dependent on the behavior of the drug in the biological system a hyperbolic or linear transduction function is selected (Black and Leff, 1983). According to the receptor theory of Clark for partial agonists, the pharmacological response is directly related to the number of receptors occupied by a drug (Clark, 1937). Since buprenorphine has been shown to behave as a partial agonist in receptor assays and *in vivo* investigations a linear function was proposed describing the relationship between receptor occupancy and ventilatory response.

In the final analysis, the exposure-response relationship of fentanyl was described by the biophase distribution model (equation 3) in combination with the fractional sigmoid E_{max} pharmacodynamic model, which is of the form:

$$E = E_0 \cdot \left(1 - \frac{\alpha \cdot C_e^n}{C_e^n + EC_{50}^n} \right) \quad (7)$$

where α is the intrinsic activity and varies between 0 and 1, EC_{50} is the effect-site concentration yielding half-maximal respiratory depression and n a slope parameter. For partial agonists, this equation follows from the steady-state solution of equation 5 and 6. Specifically, for partial agonists, the *in vivo* K_D ($k_{\text{off}}/k_{\text{on}}$) value is equal to EC_{50} . To this end, buprenorphine's *in vivo* estimate of K_D can be used for a direct comparison with fentanyl's *in vivo* potency (EC_{50}).

RESULTS

Pharmacokinetics

A three-compartment linear model best described the time course of buprenorphine concentration in plasma, whereas for fentanyl a two-compartment model was found to appropriately characterize the disposition in plasma. Inter-animal variability could be characterized for the pharmacokinetic parameters of buprenorphine and fentanyl indicated in table 2 and 3. Figure 1 and 2 show the time course of buprenorphine and fentanyl concentra-

tion, respectively. The mean population predicted time course of the drug concentration, calculated on the basis of the values of the population pharmacokinetic parameters, are depicted as solid lines. Proportional error models were used to quantify residual error. No significant parameter-covariate relationships were identified in the present analysis. The population estimates obtained from the final pharmacokinetic models and the stochastic model parameters were very similar to the mean of 1000 bootstrap replicates as indicated in table 2 and 3. The results of the 1000 bootstrap runs showed that the population PK models precisely predict the time course of buprenorphine and fentanyl concentration after intravenous administration.

Table 2. Parameter estimates with their respective relative standard error (%) of the final population pharmacokinetic model for buprenorphine and the stability of the parameters using the bootstrap resampling procedure

	Original data set		1000 bootstrap replicates	
	Typical value	IAV (%) [†]	Typical value	IAV (%)
<i>Cl</i> (ml/min)	26.0 (4.2)	22.6 (29.3)	26.0 (4.2)	21.9 (30.6)
<i>V</i> ₁ (ml)	225 (14.3)	61.2 (57.6)	214 (21.5)	64.6 (62.7)
<i>V</i> ₂ (ml)	559 (12.9)	54.8 (44.3)	558 (16.3)	54.5 (51.9)
<i>V</i> ₃ (ml)	1960 (7.5)	28.1 (51.3)	1966 (8.2)	27.5 (56.8)
<i>Q</i> ₂ (ml/min)	32.9 (10.4)	NE [‡]	34.1 (17.8)	NE
<i>Q</i> ₃ (ml/min)	16.4 (6.1)	NE	16.6 (9.2)	NE
PRE [#] (%)	21.7 (18.0)		17.4 (21.8)	

[†] inter-animal variability; [‡] not estimated; [#] proportional residual error

Table 3. Parameter estimates with their respective relative standard error (%) of the final population pharmacokinetic model for fentanyl and the stability of the parameters using the bootstrap resampling procedure

	Original data set		1000 bootstrap replicates	
	Typical value	IAV (%)	Typical value	IAV (%)
<i>Cl</i> (ml/min)	16.6 (5.3)	28.9 (26.1)	16.6 (5.1)	28.3 (26.2)
<i>V</i> ₁ (ml)	303 (7.6)	40.9 (53.8)	303 (7.7)	40.8 (56.3)
<i>V</i> ₂ (ml)	429 (7.1)	35.1 (31.1)	430 (6.8)	33.7 (31.8)
<i>Q</i> (ml/min)	7.04 (9.8)	45.4 (34.2)	7.07 (9.6)	43.7 (35.1)
PRE (%)	18.2 (23.8)		18.3 (24.6)	

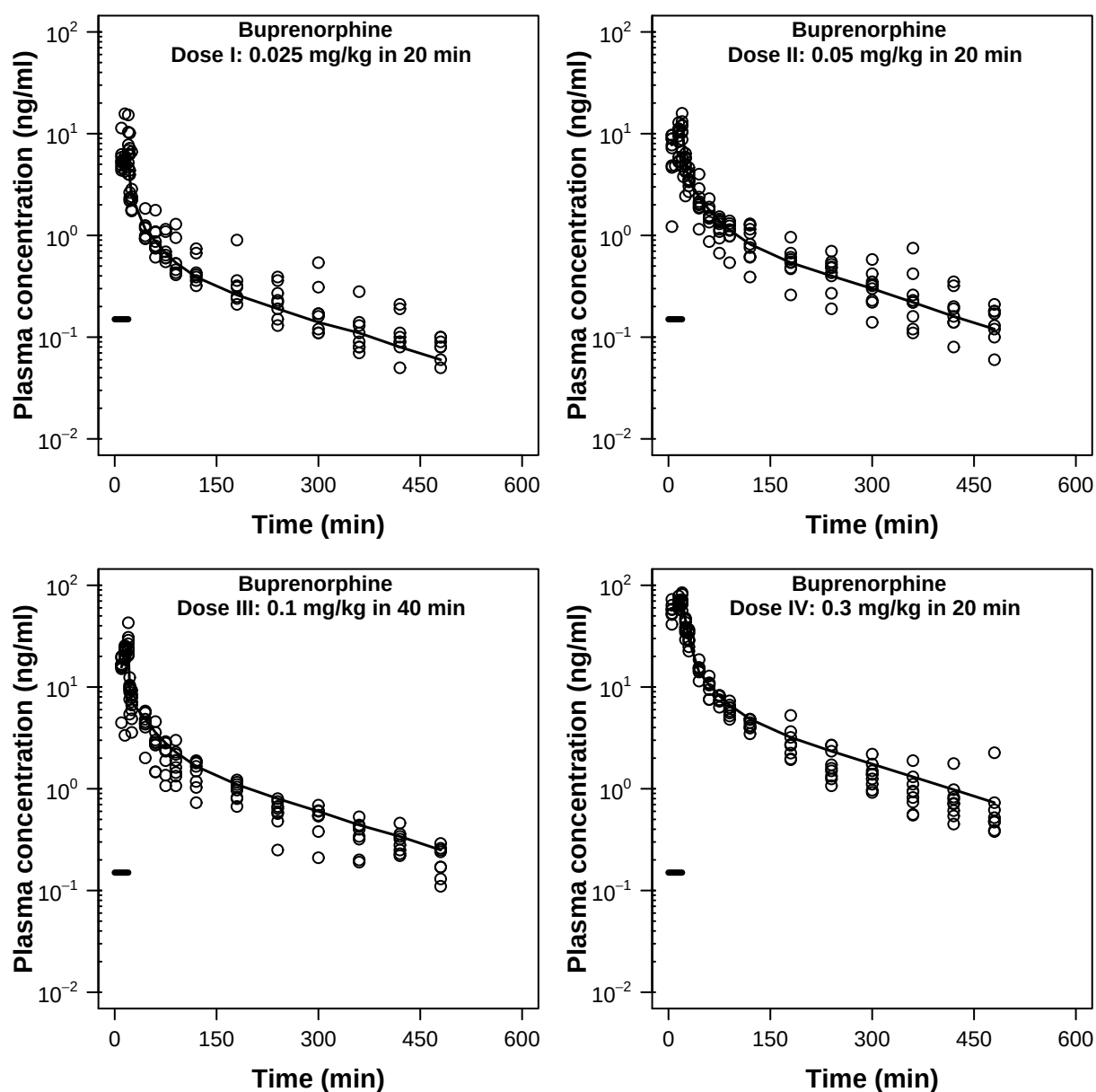


Figure 1. Individual buprenorphine concentration-time profiles for the treatment groups I-IV. The observed concentrations (symbols) and mean population predictions (thick line) are depicted. The black boxes represent the duration of infusion.

Pharmacodynamics

Vehicle treatment had no systematic effect on ventilation over the measurement period. Pre-drug ventilation (SEM) was 60.1 (0.54) ml/min. The lowest observed ventilation after vehicle infusion was 57.6 (0.33) ml/min. For buprenorphine, three structurally different PK-PD models were tested for their appropriateness to characterize the time course of respiratory depression: 1) the biophase equilibration model in combination with the sigmoid $E_{\max}$ pharmacodynamic model, 2) the receptor association/dissociation model and 3) the combined biophase equilibration-receptor association/dissociation model with a linear transduction function. On the basis of the objective function value the biophase

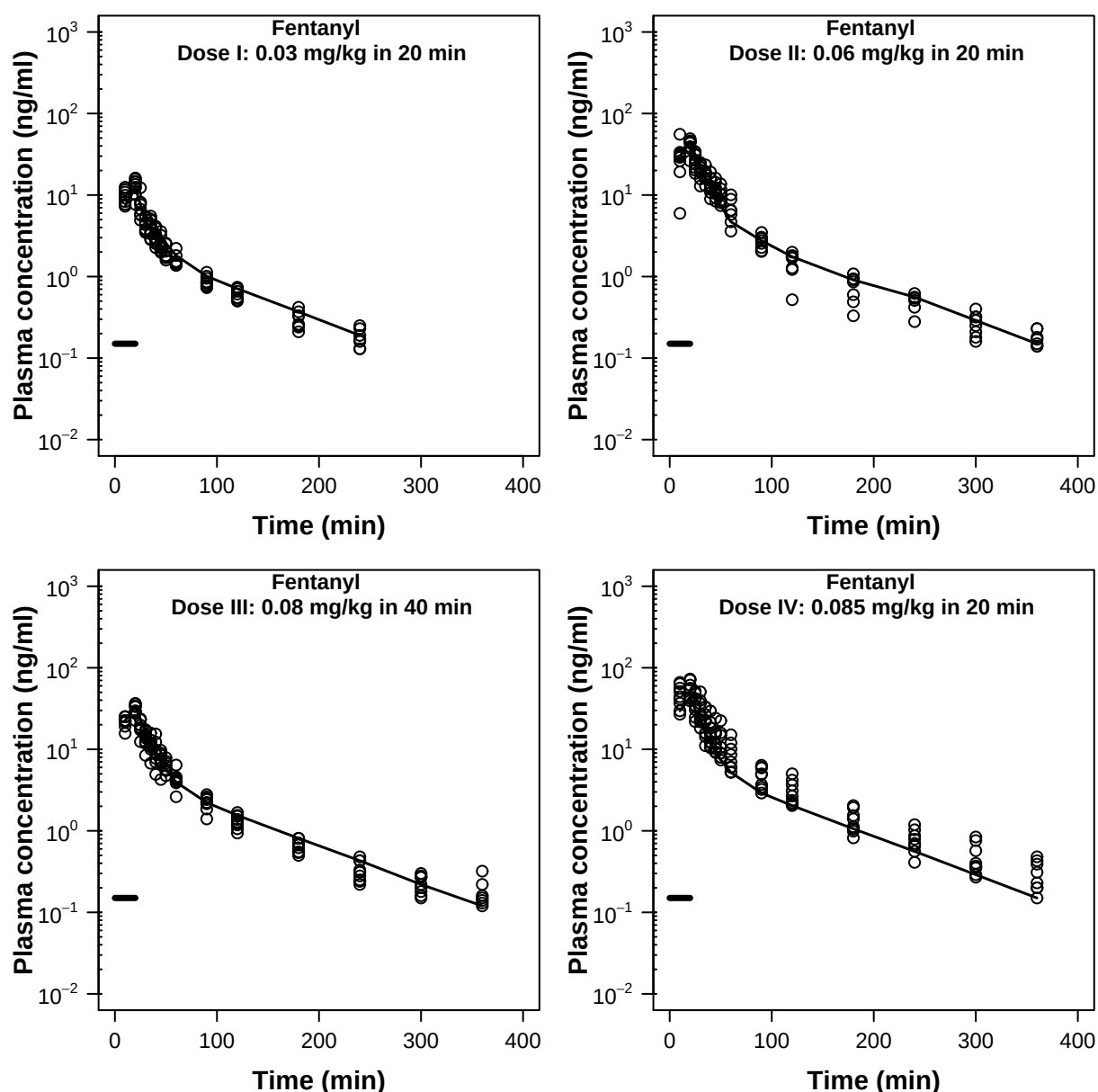


Figure 2. Individual fentanyl concentration-time profiles for the treatment groups I-IV. The observed concentrations (symbols) and mean population predictions (thick line) are depicted. The black boxes represent the duration of infusion.

equilibration receptor model in combination with the fractional sigmoid $E_{\max}$ pharmacodynamic model best described the time course of the respiratory depressant effect following buprenorphine intravenous administration. The objective function values of the various PK-PD models fitted to the concentration *versus* effect data of buprenorphine are shown in table 4.

Although the biophase equilibration model with the fractional sigmoid $E_{\max}$ model best described the time course of the respiratory depressant effect judged on the basis of the objective function value, the combined biophase equilibration-receptor association/dissociation model with a linear transduction function was selected as the final PK-PD model to characterize the time course of respiratory depression. Firstly, the model

is mechanistically plausible, given the slow receptor association/dissociation kinetic and partial agonistic properties of buprenorphine. Secondly, a realistic value of the *in vivo* equilibrium dissociation constant K_D is obtained, which is nearly identical to the K_D value obtained from dedicated *in vitro* receptor binding assays. Finally, the goodness-of-fit plots show a nearly identical description of the time course of the respiratory depressant effect with the two models tested (figure 3).

The changes in observed ventilation following the administration of 0.025 to 0.3 mg/kg buprenorphine are shown in figure 4. The respiratory depressant effect in typical animals, as predicted on the basis of the population pharmacodynamic parameter estimates are depicted as solid lines. The biophase equilibration rate constant was 0.0348 min^{-1} (95 % CI: $0.0193\text{-}0.0503 \text{ min}^{-1}$), which corresponds to $t_{1/2,ke0} = 19.9 \text{ min}$. The typical values for the rate constants of receptor association (k_{on}) and dissociation (k_{off}) kinetics were 0.57 ml/ng/min (95 % CI: $0.38\text{-}0.76 \text{ ml/ng/min}$) and 0.0903 min^{-1} (95 % CI: $0.035\text{-}0.146 \text{ min}^{-1}$), respectively. The half-time of receptor dissociation was 7.7 min ($0.693/k_{off}$) and the equilibrium dissociation constant K_D was 0.16 ng/ml or 0.34 nM (k_{off}/k_{on}). The intrinsic activity of buprenorphine was 0.48 (95 % CI: $0.45 - 0.51$). The pharmacodynamic parameter estimates of buprenorphine are shown in table 5.

For fentanyl, the time course of the respiratory depressant effect was equally well described with the biophase equilibration model with the fractional sigmoid E_{max} pharmacodynamic model and the combined biophase equilibration-receptor association/dissociation model with a linear transduction function (table 4). The typical value estimates of the rate constants characterizing receptor association/dissociation kinetics were very high, suggesting that the receptor association/dissociation kinetics are fast. This is consistent with data obtained from *in vitro* receptor binding studies (Boas and Villiger, 1985). Therefore, the combined biophase equilibration-receptor association/dissociation model can be simplified to the parsimonious biophase equilibration model with the fractional sigmoid E_{max} pharmacodynamic model. The latter model was able to successfully describe the time courses of respiratory depression for all animals, yielding estimates for k_{e0} and EC_{50} . Figure 5 shows the changes in observed ventilation for all individual animals stratified to dose. The respiratory depressant effect in typical animals, predicted on the basis of the population mean pharmacodynamic parameter estimates are depicted as solid lines. In figure 6, examples of six data fits are given, including the best, median and worst fit based on the coefficient of determination (R^2).

The biophase equilibration rate constant was 0.44 min^{-1} (95 % CI: $0.15 - 0.72 \text{ min}^{-1}$), which corresponds to $t_{1/2,ke0} = 1.6 \text{ min}$. The value of k_{e0} obtained with the combined biophase equilibration/receptor association/dissociation model 0.39 min^{-1} , which corresponds to 1.8 min which is nearly identical to the value obtained with the biophase equilibration model. The *in vivo* EC_{50} of fentanyl was 4.83 ng/ml (95 % CI: $3.19 - 6.47 \text{ ng/ml}$). The intrinsic activity of fentanyl was 0.85 (95 % CI: $0.71 - 1.00$). The pharmacodynamic parameter estimates, their respective estimates of inter-animal variability and the corresponding relative standard error are presented in table 6. In the PK-PD analysis, dose

was not a significant covariate of the various parameters, confirming that indeed unique pharmacodynamic parameter estimates have been obtained. The steady-state concentration/receptor occupancy-effect relationships of buprenorphine and fentanyl are depicted in figure 7.

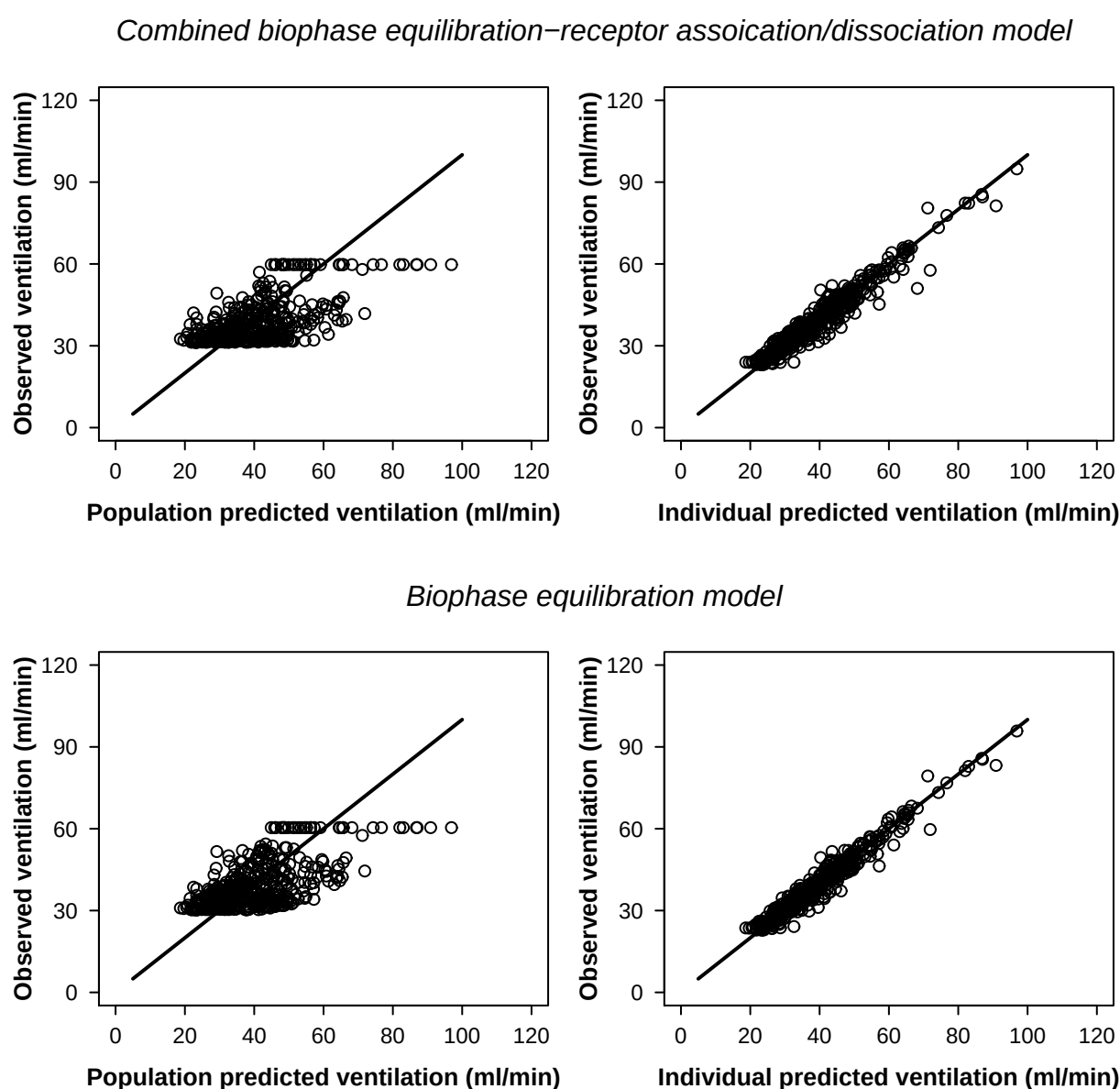


Figure 3. Goodness-of-fit plots for the combined biophase equilibration-receptor association/dissociation model (upper panel) and the sigmoid $E_{\max}$ model with effect compartment (lower panel): observed *versus* population predicted or individual predicted ventilation. The solid line is the identity line.

Table 4. Results of the model discrimination to characterize the time course of respiratory depression following intravenous administration of fentanyl and buprenorphine

	Buprenorphine			Fentanyl		
	Model A [†]	Model B [‡]	Model C ^ˆ	Model A	Model B	Model C
OFV	2581	2598	2594	1246	-	1250
k_{e0} (min ⁻¹)	0.01	-	0.0348	0.44	- [#]	0.39
k_{on} (ml/ng/min)	-	0.0085	0.57	-	-	> 100
k_{off} (min ⁻¹)	-	0.006	0.0903	-	-	> 100

[†] biophase equilibration model with sigmoid E_{max} pharmacodynamic model;

[‡] receptor association/dissociation model with a linear transduction function;

^ˆ combined biophase equilibration-receptor association/dissociation model with a linear transduction function; [#] no successful run

	Typical value	IAV (%)
k_{on} (ml/ng/min)	0.572 (17.1)	NE
k_{off} (min ⁻¹)	0.0903 (31.2)	75.4 (47.2)
K_D (ng/ml) [§]	0.16	-
k_{e0} (min ⁻¹)	0.0348 (22.7)	79.1 (41.7)
E_0 (ml/min)	59.8 (4.0)	20.9 (19.3)
α	0.48 (3.4)	13.6 (31.4)
ARE (ml/min)	3.4 (25.1)	

[§] secondary parameter (k_{off}/k_{on}); ^{||} additive residual error.

Table 5. Population pharmacodynamic estimates and inter-animal variability of buprenorphine. For the fixed and random parameter estimates the relative standard error (%) is displayed**Table 6.** Population pharmacodynamic estimates and inter-animal variability of fentanyl. For the fixed and random parameter estimates the relative standard error (%) is displayed

	Typical value	IAV (%)
k_{e0} (min ⁻¹)	0.44 (33.4)	NE
EC_{50} (ng/ml)	4.83 (17.4)	83.9 (42.8)
E_0 (ml/min)	64.2 (2.5)	11.8 (35.4)
α	0.85 (8.7)	7.6 (39.1)
n	1.15 (15.1)	NE
ARE (ml/min)	5.1 (12.9)	

DISCUSSION

In the present study, minute ventilation was measured as a biomarker for the respiratory depressant effect of buprenorphine and fentanyl in rats. Different PK-PD models were tested for their appropriateness to describe the effect of buprenorphine on respiratory depression. An important model selection criterion is the predictive value of the PK-PD model, enabling the prediction of the time course of respiratory depression beyond scenarios studied in the present investigation. To this end, it is important to develop a mechanism-based PK-PD model characterizing the pharmacodynamic time-dependencies on the basis of specific intermediary processes between pharmacokinetics and pharmacological effect (Danhof *et al.*, 2005).

For buprenorphine, this mainly concerns modeling of the slow receptor association/dissociation kinetics as the key determinant of the antagonism of respiratory depression by naloxone. In this respect, the combined biophase equilibration-receptor association/dissociation model with a linear transduction was proposed. Also, the goodness-of-fit plots for both PK-PD models are nearly identical (figure 3) indicating that both PK-PD models describe the data equally well. Moreover, according to receptor theory the combined biophase equilibration-receptor association/dissociation model with a linear transduction function is a more appropriate model to describe the biological system of partial agonists. The validity of the proposed mechanistic model is supported by the fact that realistic pharmacodynamic parameter estimates (good agreement between *in vitro* and *in vivo* values of k_{on} and k_{off}) are obtained, which underline and confirm buprenorphine's most important pharmacological characteristics (i.e. partial agonism and slow receptor association/dissociation kinetics). Meanwhile, the importance of modeling receptor association-dissociation kinetics has been illustrated in our studies on antagonism of buprenorphine-induced respiratory depression with naloxone (Yassen *et al.*, 2007).

An important issue is the *in vivo* intrinsic activity of buprenorphine for respiratory depression, since this characteristic mainly determines its safety profile. In the present analysis, buprenorphine has a value of 0.48 (95 % CI: 0.45 - 0.51), which was significantly different from 1. This confirms buprenorphine's partial agonistic activity and is consistent with *in vivo* (pre)-clinical data (Cowan *et al.*, 1977; Dahan *et al.*, 2005). Furthermore, there is a good correlation between the estimated *in vivo* K_D value and *in vitro* values. In the present study, the *in vivo* potency of buprenorphine for respiratory depression was 0.16 ng/ml, corresponding to 0.34 nM. Previously, the potency of buprenorphine had been determined in Chinese hamster ovary (CHO) cells, expressing the human μ -opioid receptor, using a [^{35} (S)]-GTP- γ -S-functional binding assay (Huang *et al.*, 2001). The results of that *in vitro* study show that buprenorphine is a potent partial agonist at the μ -opioid receptor with an *in vitro* EC_{50} value of 0.08 nM. In a similar cell culture, the potency of buprenorphine was 0.33 nM (Cassel *et al.*, 2005). The potency of buprenorphine for stimulation of [^{35} (S)]-GTP- γ -S binding to the human neuroblastoma SK-N-SH cells, expressing

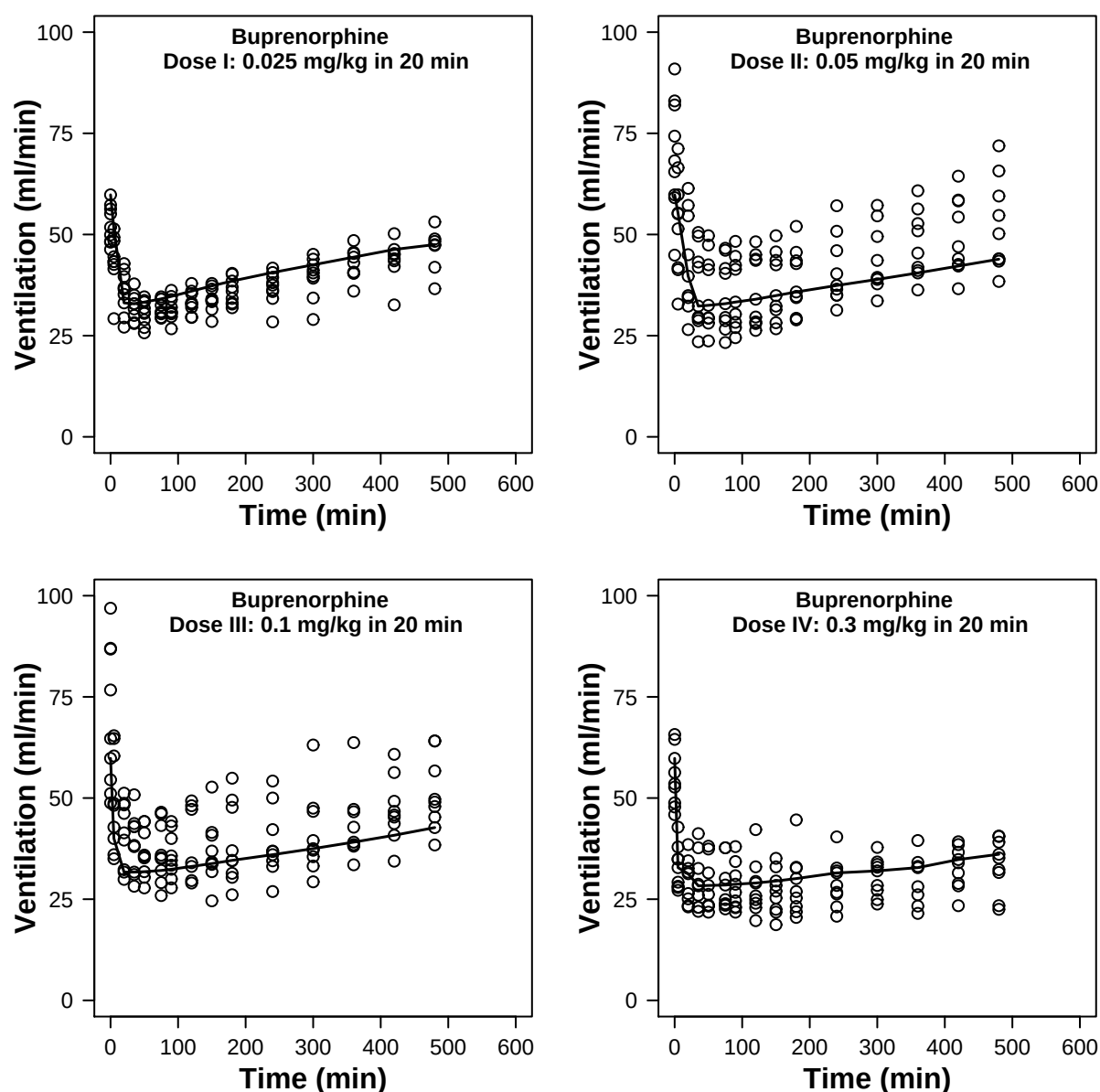


Figure 4. Changes in ventilation in time following administration of buprenorphine. For each treatment group (I-IV) the observed (symbols) and mean population predicted (solid line) time course of the respiratory depressant effect is shown.

the human μ -opioid receptor was estimated at 0.14 nM (Selley *et al.*, 1997). The *in vivo* K_D of buprenorphine is in the same range to these *in vitro* K_D and EC_{50} values, suggesting that buprenorphine's *in vivo* respiratory depressant effect may be predicted on the basis of *in vitro* binding assays.

Finally, the estimated half-time of receptor dissociation kinetics is also consistent with point estimates obtained by other techniques. The half-time of receptor dissociation kinetics was 7.7 min. The dissociation kinetics of buprenorphine from the μ -opioid receptor have been determined in an *in vivo* opioid receptor imaging study using ^{11}C -buprenorphine as a tracer (Shiue *et al.*, 1991). The dissociation rate constant was estimated to be 0.069 min^{-1} which corresponds to $t_{1/2, \text{off}}$ ($0.693/k_{\text{off}}$) of 10 min. In sepa-

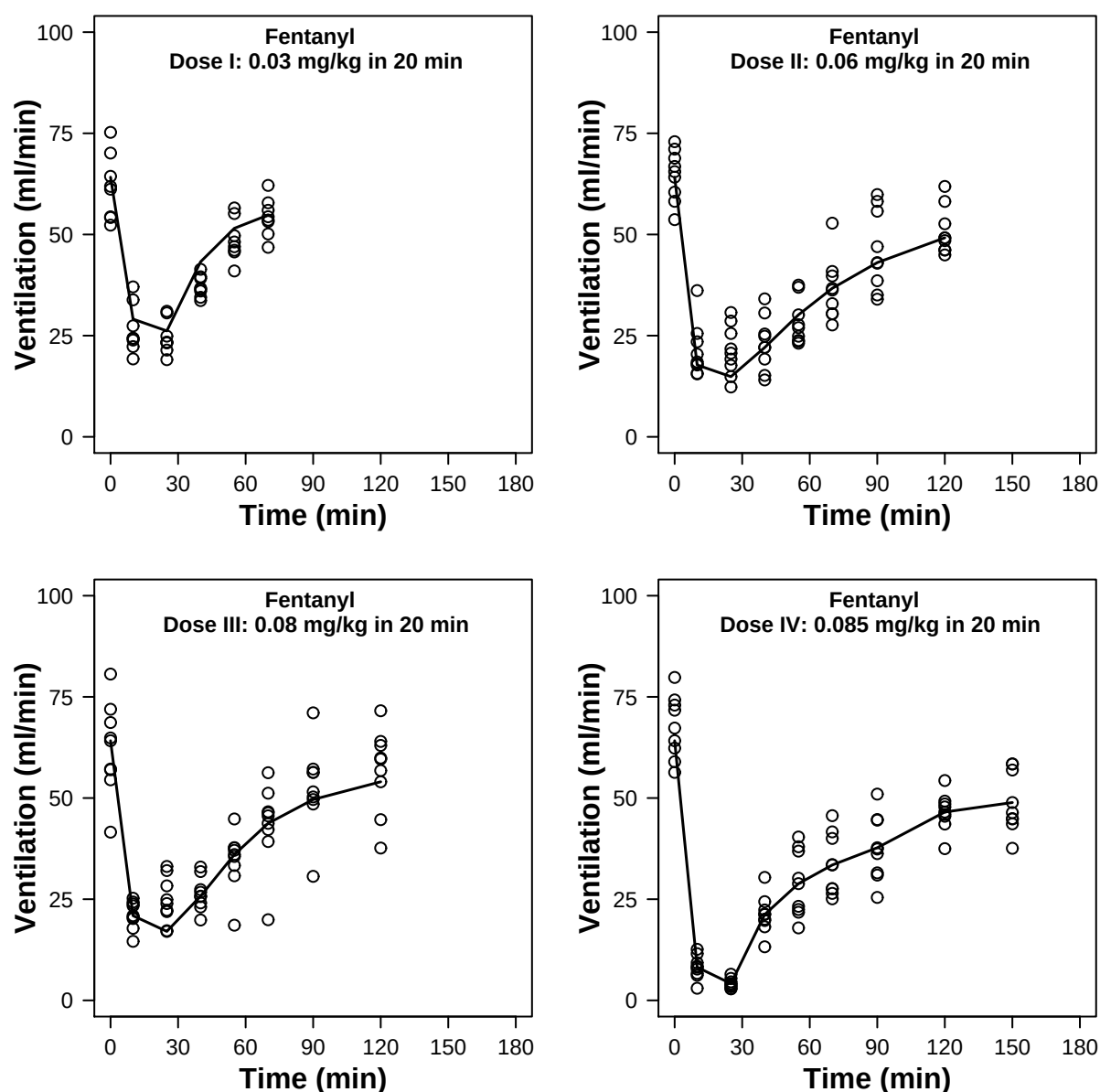


Figure 5. Changes in ventilation in time following administration of fentanyl. For each treatment group (I-IV) the observed (closed circles) and mean population predicted (solid line) time course of the respiratory depressant effect is shown.

rate *in vivo* investigations the receptor dissociation kinetics of buprenorphine from the μ -opioid receptor had been also determined for the antinociceptive effect in rat. The half-time of receptor dissociation was 9.5 min in rat (Yassen *et al.*, 2005). The *in vivo* $t_{1/2, \text{koff}}$ value of 7.7 min obtained in the present investigation is remarkably similar to these *in vivo* $t_{1/2, \text{koff}}$ values. Beside the slow receptor association/dissociation kinetics, biophase equilibration kinetics also contributes to the observed delay in the respiratory depressant effect following the intravenous administration of buprenorphine. Data from animal studies indicate that buprenorphine readily penetrates the blood-brain barrier following intravenous administration (Ohtani *et al.*, 1995). In baboon brain, peak concentration of buprenorphine was achieved after 15 to 20 min following bolus intravenous administra-

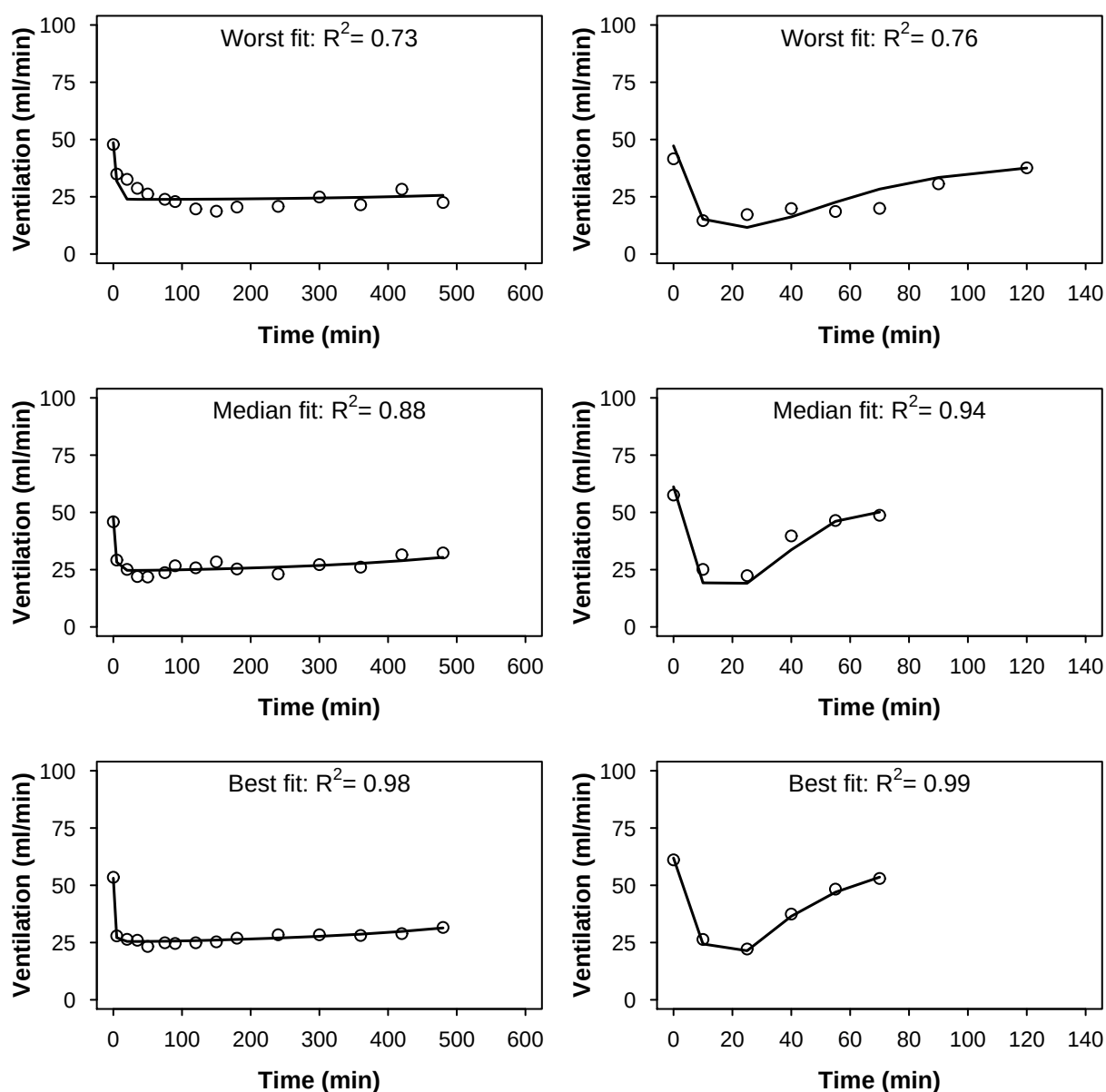


Figure 6. The time course of the respiratory depressant effect following the administration of buprenorphine (left panels) and fentanyl (right panels) is shown. Worst, median, and best data fits between measured (symbols) and individual predicted respiratory depression (solid line) are presented. The goodness of fit was assessed by the coefficient of determination (R^2).

tion of radio-labeled buprenorphine (Galynker *et al.*, 1996). In the present study, after intravenous administration of 0.1 mg/kg buprenorphine, the maximum predicted biophase concentration was reached after 20 to 35 minutes (figure 8) and is in good agreement with the previous reported time frames.

For fentanyl, high values of the rate constants for receptor association and dissociation kinetics were obtained, indicating that hysteresis is caused solely by biophase equilibration kinetics. This is consistent with fentanyl's fast receptor association/dissociation kinetics *in vitro* (Boas and Villiger, 1985). Not surprisingly, the fitting performance of the biophase equilibration model in combination with the fractional sigmoid $E_{\max}$ model is

nearly identical to that of the combined biophase equilibration-receptor association/dissociation model. As a result, in equilibrium (i.e. fast receptor association/dissociation), the combined model can be simplified to the parsimonious biophase equilibration model. Again this is in line with common belief that delay in the CNS effects of fentanyl are attributed to an equilibrium delay between the arterial and biophase concentration of fentanyl (Scott *et al.*, 1991). The half-time of biophase equilibration for fentanyl was 1.6 min. The half-time of biophase equilibration for the fentanyl-induced EEG effect was 2.2 min (Cox *et al.*, 1998) and is in agreement with our value, suggesting that the respiratory depressant and EEG effect are mediated through activation of μ -opioid receptors in the same brain region. Interestingly, for fentanyl the values of rate constant for biophase equilibration obtained with the combined biophase equilibration-receptor association/dissociation model and the biophase equilibration model were nearly identical. This confirms the validity of the combined biophase equilibration-receptor association/dissociation model to obtain an accurate and unique estimate for the rate constant of biophase equilibration and justifies simplification of the combined model to the biophase equilibration model in combination with the fractional sigmoid $E_{\max}$ pharmacodynamic model.

An important feature of the combined biophase distribution-receptor association/ dis-

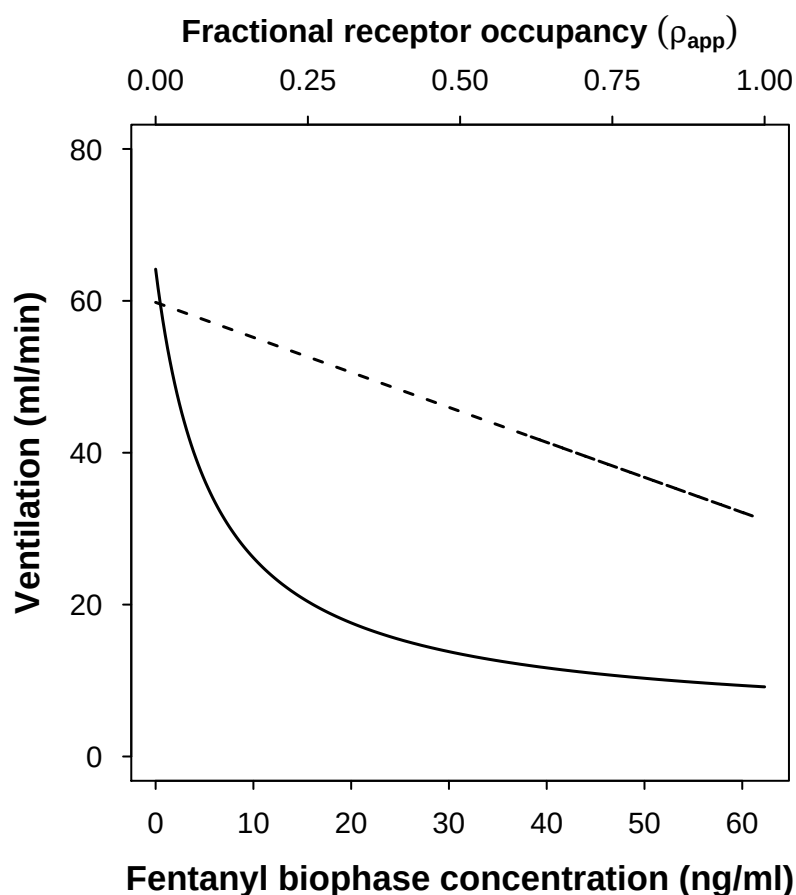


Figure 7. Concentration-effect relationship of fentanyl (solid line) in rat relative to the apparent fractional receptor occupancy-effect relationship of buprenorphine (dashed line).

sociation model is that it is mechanistically plausible. This is particularly important in relation to the prediction of the reversal of respiratory depression by the opioid antagonist naloxone (Gal, 1989). Specifically, the slow *in vivo* receptor association/dissociation kinetics complicate reversal of buprenorphine-induced respiratory depression by naloxone. The combined biophase equilibration-receptor association/dissociation model is that it enables prediction of the complex competitive interaction between buprenorphine and naloxone at the μ -opioid receptor in a strict quantitative manner. This is important since it enables prediction of the time course of the reversal of buprenorphine-induced respiratory depression by naloxone. At present, the modeling of the time course of antagonization of buprenorphine-induced respiratory depression by naloxone is subject of ongoing investigations at our laboratory.

Another intriguing question is whether the combined biophase equilibration-receptor association/dissociation kinetic model, can be applied to predict the respiratory depressant effect in human on the basis of preclinical *in vivo* data. In principle, receptor association/dissociation kinetics are drug-specific and display cross-specie similarity. There is evidence that the μ -opioid receptor functions in a nearly identical manner in rat and human (Rothman *et al.*, 1995). In contrast, biophase distribution kinetics are dependent on the biological system (e.g. differences in brain weight) and can be extrapolated using allometric scaling laws. In a similar fashion, the proposed model could also be used as

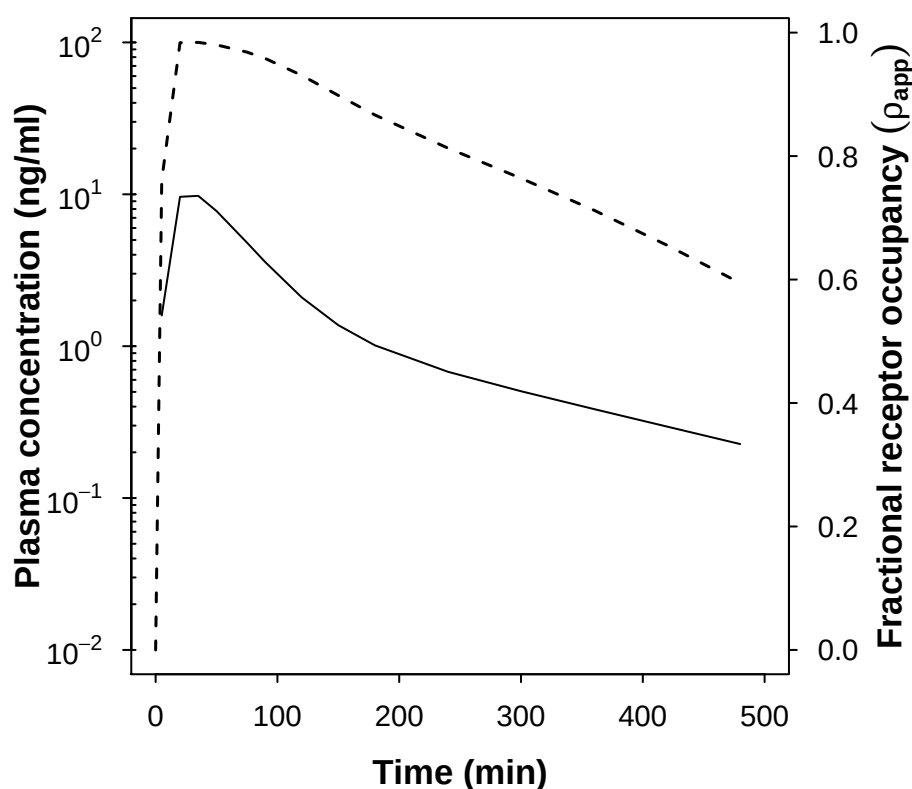


Figure 8. The predicted changes in buprenorphine biophase concentration and apparent fractional receptor occupancy. The solid line is the time course of biophase concentration (left y-axis) and the dashed line is the apparent fractional receptor occupancy in time (right y-axis).

a first step towards the prediction of the contribution of buprenorphine's active metabolite norbuprenorphine to the overall respiratory depressant effect in human. Since norbuprenorphine can not be directly administered to human, its pharmacological and PK-PD properties need to be investigated in a chronically instrumented animal model (whole-body plethysmography). Previously, a mechanism-based PK-PD model for the EEG effect of synthetic opioids in rat had been successfully applied to predict the EEG effect of remifentanyl's metabolite in human (Cox *et al.*, 1999). Currently, extrapolation of the respiratory depressant effect of buprenorphine and the contribution of norbuprenorphine to the overall respiratory depressant effect from rat to human is subject of ongoing research in our laboratory.

In conclusion, a mechanism-based PK-PD model has been successfully applied for characterization of the time course of the respiratory depressant effect of buprenorphine and fentanyl in rats. For buprenorphine, unique and independent estimates for the rate constants of receptor association/dissociation kinetics have been obtained, which are consistent with estimates obtained from the *in vitro* binding studies and *in vivo* receptor imaging techniques. Buprenorphine is a partial agonist for respiratory depressant with an estimated intrinsic activity of 48 %. For fentanyl, the high values for the rate constants of receptor association/dissociation kinetics are in agreement with the fast receptor association/dissociation kinetics *in vitro*. Its intrinsic activity is higher than of buprenorphine and was estimated at 85 % indicating that fentanyl is a full agonist for respiratory depression. These results confirm that the combined biophase equilibration-receptor association/dissociation model constitutes a realistic approach to characterize the respiratory depressant effect of the opioids buprenorphine and fentanyl.

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MECHANISM-BASED PK-PD MODELING OF THE RESPIRATORY DEPRESSANT EFFECT OF BUPRENORPHINE AND FENTANYL IN HEALTHY VOLUNTEERS

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Luc Teppema, Meindert Danhof and Albert Dahan

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ABSTRACT

The objective of this study was to characterize the pharmacokinetic-pharmacodynamic (PK-PD) relationship of buprenorphine and fentanyl for the respiratory depressant effect in healthy volunteers. Data on the time course of the ventilatory response at a fixed $P_{ET}CO_2$ of 50 mmHg and $P_{ET}O_2$ of 110 mmHg following intravenous administration of buprenorphine and fentanyl were obtained from two phase I studies (50 volunteers received buprenorphine: 0.05 - 0.6 mg/70 kg and 24 volunteers received fentanyl: 0.075 - 0.5 mg/70 kg). The PK-PD correlations were analyzed using non-linear mixed effects modeling.

A two- and three compartment pharmacokinetic model characterized the time course of fentanyl and buprenorphine concentration, respectively. Three structurally different PK-PD models were evaluated for their appropriateness to describe the time course of respiratory depression, (1) a biophase distribution model with a fractional sigmoid E_{max} pharmacodynamic model, (2) a receptor association/dissociation model with a linear transduction function and (3) a combined biophase distribution-receptor association/dissociation model with a linear transduction function.

The results show that for fentanyl hysteresis is entirely determined the biophase distribution kinetics, while for buprenorphine hysteresis is caused by a combination of biophase distribution kinetics and receptor association/dissociation kinetics. The half-time of biophase equilibration ($t_{1/2,ke0}$) were 16.4 min and 75.3 min for fentanyl and buprenorphine respectively. In addition for buprenorphine the value of k_{on} was 0.246 ml/ng/min and the value of k_{off} was 0.0102 min^{-1} .

The concentration-effect relationship of buprenorphine was characterized by a ceiling effect at higher concentrations (intrinsic activity $\alpha = 0.56$, 95 % CI: 0.50 - 0.62), while fentanyl displayed full respiratory depressant effect ($\alpha = 0.91$, 95 % CI: 0.19 - 1.62).

INTRODUCTION

Despite decades of effort spent on pharmacologic research and refinements of clinical practice, respiratory depression remains a serious and sometimes life threatening consequence of the treatment of acute and chronic pain (Baxter, 1994; Dahan *et al.*, 1998). The development and use of opioids that produce less respiratory depression is of importance.

Buprenorphine is a semi-synthetic opioid with partial agonistic activity at the μ -opioid receptor (Cowan *et al.*, 1977; Lee *et al.*, 1999; Huang *et al.*, 2001). This *in vitro* binding behavior is clinically reflected by a ceiling or plateau effect for respiratory depression. In a group of healthy volunteers ceiling effect for buprenorphine-induced respiratory depression was reached at doses > 0.1 mg/70 kg following intravenous bolus administration (Dahan *et al.*, 2005). Evidently, this respiratory depressant ceiling effect provides an increased safety margin relative to opioids that display full agonistic activity. Recently, mechanism-based PK-PD models have been developed for the characterization of the time course of the antinociceptive effect in rat and human (Yassen *et al.*, 2005, 2006b) and the respiratory depressant effect in rat (Yassen *et al.*, 2006a). The PK-PD models contain specific expressions for the kinetics of buprenorphine's action at the μ -opioid receptors and aim at the separation of biophase distribution kinetics and slow receptor association/dissociation kinetics (Shimada *et al.*, 1996) as determinants of time-dependencies in buprenorphine's pharmacodynamics. At present little information is available on the PK-PD relationship of buprenorphine with respect to respiratory depression in humans.

The application of a previously proposed mechanism-based PK-PD model is of relevance for the modeling of buprenorphine's slow receptor association/dissociation kinetics at the μ -opioid receptor (Villiger and Taylor, 1982; Boas and Villiger, 1985). Not only do these receptor binding characteristics determine the duration of respiratory depression, the slow receptor association/dissociation kinetics are a complicating factor for the reversibility of buprenorphine-induced respiratory depression by naloxone (Gal, 1989). This mechanism-based PK-PD modeling approach is the basis for clinical trial simulations. The model-based approach may lead to a better understanding of the complex interaction between buprenorphine and naloxone and may be a valuable way to predict reversal of buprenorphine-induced respiratory depression by naloxone.

A specific objective of this study was to characterize the PK-PD relationship of buprenorphine's respiratory depressant effect using the previously developed PK-PD model. Fentanyl was used as comparator compound because of its distinct pharmacological characteristics. In contrast to buprenorphine, fentanyl acts as full agonist at the μ -opioid receptor and binding to and dissociation from the μ -opioid receptor is fast (Boas and Villiger, 1985).

MATERIALS AND METHODS

Subjects

Seventy-four volunteers participated in the investigation after approval was obtained from the local human ethics committee. All subjects were healthy and did not have a history of illicit substance abuse. They were asked to refrain from stimulants and depressant substances for at least 12 h before the study. Each subject participated once in the study. After arrival in the research unit, an arterial line was placed in the left or right radial artery under local anesthesia (for blood sampling). In the contralateral arm an intravenous line was inserted (for drug infusion).

Study Design

The clinical data for this analysis were collected from two randomized double blind phase I pharmacokinetic-pharmacodynamic studies. In the first study the effect of buprenorphine (Reckitt Benckiser Healthcare Ltd, Hull, UK) on the ventilatory response to a fixed end-tidal CO₂ of 50 mmHg (V_{i50}) was assessed at two different doses. In the second study, the subjects were randomly assigned to receive placebo (0.9 % NaCl), buprenorphine or fentanyl (Janssen-Cilag bv, Tilburg, The Netherlands) and their effects on the ventilatory response were assessed at a fixed end-tidal CO₂s of 50 mmHg. The results of the second study have been reported in a descriptive manner in a previous publication (Dahan *et al.*, 2005).

The details of the two study designs are shown in table 1. The highest fentanyl dose (500 μ g/70 kg) was tested only once. After the first subject was dosed with 500 μ g/70 kg, the respiratory effects were so severe (apnea > 5 min and SpO₂ drop below 70 %) that the blinding of this experiment was broken and the decision was made to no longer infuse 500 μ g/70 kg fentanyl. Yet, the 500 μ g/70 kg fentanyl data of this subject were used in the analysis.

Respiration

Apparatus

The subjects were comfortably seated in a hospital bed and breathed through a facemask (Vital Signs, Totowa, NJ). The gas flows were measured with a pneumotachograph connected to a pressure transducer and electronically integrated to yield a volume signal. The volume signal was calibrated with a motor-driven piston pump (stroke volume 1 l, at a frequency of 20/min). Corrections were made for the changes in gas viscosity due to changes in oxygen concentration of the inhaled gas mixtures. The pneumotachograph was connected to a T-piece. One arm of the T-piece received a gas mixture with a flow of

Table 1. Characteristics of two clinical studies analyzed

	Study A		Study B
	Buprenorphine		Fentanyl
Design	Dose finding	Dose escalation	Dose escalation
Dose (mg/70 kg)	0.2, 0.4 mg/70 kg	0.1, 0.3 0.6 mg/70 kg	0.075, 0.15, 0.2 0.3, 0.5 mg/70 kg
Subjects	32	18	24
Age (median and range)(years)	21 (19-30)	20 (18-30)	22 (19-27)
Sex (m/f) [†]	16/16	9/9	12/12
BW [‡] (median and range)(kg)	73 (51-93)	67 (52-92)	73 (54-91)
HT [#] (median and range)(m)	1.77 (1.63-1.96)	1.77 (1.62-1.96)	1.80 (1.63-1.96)

[†] m, male; f, female; [‡] BW, bodyweight; [#] HT, height.

50 l/min from a gas mixing system, consisting of three mass flow controllers (Bronkhorst High Tech BV - F202, Veenendaal, The Netherlands) with which the flow of oxygen, carbon dioxide and nitrogen could be set individually at a desired level. A personal computer provided control signals to the mass-flow controllers so that the composition of the inspired gas mixtures could be adjusted to force end-tidal oxygen and carbon dioxide concentrations ($P_{ET}O_2$ and $P_{ET}CO_2$) to follow a specified pattern in time, independent of the ventilatory response.

The in- and expired $P_{ET}O_2$ and $P_{ET}CO_2$ concentrations and the arterial hemoglobin- $P_{ET}O_2$ saturation (SpO_2) were measured with a Datex Multicap gas monitor (near the mouth) and Datex Satellite Plus pulse oximeter, respectively (Datex-Engstrom, Helsinki, Finland). The gas monitor was calibrated with gas mixtures of known concentration delivered by a gas-mixing pump (W. Stohff, Bochum, Germany). $P_{ET}O_2$, $P_{ET}CO_2$, inspired minute ventilation (V_{i50}) and SpO_2 were collected and stored on disc for further analysis.

Respiration

Hypercapnic ventilation

In the first study (study A), ventilation was measured at a fixed $P_{ET}CO_2$ and $P_{ET}O_2$ of 50 mmHg and 110 mmHg, respectively using the dynamic end-tidal forcing technique (Meert and Vermeirsch, 2005). Prior to drug infusion, control or baseline ventilatory responses were obtained. Next, the drugs were infused. Subsequently, ventilatory responses were obtained at the following times after drug infusion: $t = 15, 75, 140, 180, 240, 300, 360, 420$ and 480 min. All data points were the mean value of the 10 breaths taken after 7-8 min of inhaling the hypercapnic gas mixture. In the second study (study B), we clamped

the $P_{ET}CO_2$ and $P_{ET}O_2$ at 50 mmHg and 110 mmHg, respectively, throughout the studies.

The respiratory studies started after a period of acclimatization to the apparatus and ventilation (at a $P_{ET}CO_2$ of 50 mmHg) had reached a steady-state. Next the drug was infused slowly over 90 s. Subsequently continuous respiratory measurements were obtained for 80 to 90 min, followed by 5 to 10 min measurements at 30 min intervals (until $t = 4$ h after drug infusion) and thereafter at 60 min intervals. In case ventilation returned to baseline values (defined by at least 5 min at or above baseline) prior to the end of the measurement periods the study was ended. In case this did not happen or in case there was no systematic respiratory depressant effect, the study ended seven hours after drug infusion.

Determination of buprenorphine and fentanyl concentrations

Buprenorphine

For the determination of buprenorphine concentrations in plasma, a validated GC-MS method was used (Yassen *et al.*, 2006b). The accuracy and precision for calibration standards were 5.47 % and 1.83 %, respectively at the lower limit of quantification (LLOQ) (25.0 pg/ml) and 0.83 % and 3.79 %, respectively at the upper limit of quantification (ULOQ) of 10000 pg/ml. For the quality controls the accuracy ranged from -1.42 % (75 pg/ml) to 7.74 % (8000 pg/ml). The precision ranged from 1.88 % (8000 pg/ml) to 5.31 % (75 pg/ml). All concentration data presented refer to buprenorphine free base.

Fentanyl

For the determination of fentanyl concentrations in plasma, a validated LC-MS/MS method was used. To plasma aliquots of 1.0 ml, 1.0 ml 500 mM NaOH, 25 μ l internal standard stock solution (2H5-fentanyl), and 6 ml heptane / ethylacetate (1:1 v/v) were added. After shaking the samples for 25 minutes and centrifugation, the organic phase was transferred to conical glass tubes and evaporated to dryness at 50 °C under a very weak stream of nitrogen. The residues were resolved in 100 μ l buffer (20 mM ammonia formate buffer pH 2.8 / methanol, 75:25 v/v). Before injection the samples were centrifuged for 5 min at 4000 rpm. An aliquot of 30 μ l of the sample was injected into the LC-MS/MS system.

The LC-MS/MS analyses were performed on a Finnigan MAT LCQ system (Thermo Electron Corp., Germany). An Ultra IBD column (Fa. Restek, USA) was used for the HPLC separation. The MS was operated in the positive electrospray ionisation mode with nitrogen as sheath and auxiliary gas. Selected reaction monitoring was performed for m/z 188.3, a daughter ion of the mother ion m/z 337.3 of fentanyl. The internal standard was detected at m/z 189.3 the daughter ion of the m/z 343.4 mother ion. Calibration curves were linear ($r > 0.985$) in the measured range between 25.0 to 5000 pg/ml, using weighted ($1/x^2$) linear regression.

The lower limit of quantification (LLOQ) was 25.0 pg/ml using 1 ml of human plasma.

The accuracy and precision for calibration standards were 2.2 % and 3.6 %, respectively at the LLOQ (25.0 pg/ml) and 3.6 % and 5.2 %, respectively at the upper limit of quantification (ULOQ) of 5000 pg/ml. For the quality controls the accuracy ranged from -1.1 % (60 pg/ml) to 4.7 % (4200 pg/ml). The precision ranged from 5.3 % (4200 pg/ml) to 10.5 % (60 pg/ml). All concentration data presented refer to fentanyl free base.

PK-PD data analysis

The pharmacokinetic and pharmacodynamic parameters of fentanyl and buprenorphine were estimated using non-linear mixed-effects modeling as implemented in the NONMEM software (Version V, level 1.1) (Beal and Sheiner, 1999). The population analysis approach, which takes into consideration both intra-individual and inter-individual variability, was undertaken using the first order conditional estimation method with η - ϵ interaction (FOCE interaction).

All fitting procedures were performed on a IBM-compatible computer (Pentium IV, 1500 MHz) running under Windows NT with the Microsoft FORTRAN Powerstation 4.0 compiler. An in-house available S-PLUS 6.0 (Insightful Corp., Seattle, WA, USA) interface to NONMEM was used for data processing and management (including tools to perform predictive check and bootstrap) and graphical data display. Selection criteria during the model development process were based on the goodness-of-fit plots, changes in the objective function value (OFV) and parameter estimates and their respective 95 % CI intervals. Using the likelihood ratio test, the significance was set at $\alpha = 0.01$, which corresponds to a reduction of 6.6 units in objective function value (OFV; χ^2 distribution) to discriminate between two nested structural models after inclusion of one additional parameter.

Pharmacokinetic model

Compartmental PK models were used to describe the time courses of buprenorphine and fentanyl concentration in plasma. The pharmacokinetic parameters were assumed to be log-normally distributed and therefore an exponential distribution model was used to account for inter-individual variability. Proportional error models were used to quantify residual error. Relationships between structural model parameters and covariates were considered during PK model development using the forward inclusion/backward elimination procedure (Mandema *et al.*, 1992; Jonsson and Karlsson, 1999).

Individual Bayesian pharmacokinetic parameter estimates obtained from the final population pharmacokinetic model served as input for pharmacodynamic model to predict fentanyl and buprenorphine concentrations at V_{i50} observation time points (two-stage approach). Due to long run times of the PK-PD models, the two-stage approach was used rather than simultaneous estimation of the pharmacokinetic and pharmacodynamic model parameters.

To ensure that precise estimates of pharmacokinetic parameters are obtained pharmacokinetic model validation was performed (Ette *et al.*, 2003). Internal validation was conducted by means of a non-parametric bootstrap technique.

Mechanism-based PK-PD model

The observed hysteresis in concentration-effect relationships of (synthetic) opioids is traditionally analyzed using an effect compartment link model. Generally, the effect compartment link model, reflects the delay in drug transport from plasma to the site of action. Transport across the blood brain barrier is believed to be a correct representation of the rate-limiting step in *the vivo* pharmacodynamics of opioids.

For buprenorphine however, the observed hysteresis in the concentration-effect relationship may also be explained by the relatively slow receptor association/dissociation kinetics. Therefore, in this study various structurally different PK-PD models were tested for their appropriateness to characterize the time course of buprenorphine -or fentanyl-induced respiratory depression in healthy volunteers: 1) a biophase equilibration model with a fractional sigmoid $E_{\max}$ pharmacodynamic model (Sheiner *et al.*, 1979), 2) a receptor association/dissociation model with a linear transduction function (Shimada *et al.*, 1996) and 3) a combined biophase equilibration-receptor association/dissociation model with a linear transduction function (Yassen *et al.*, 2005).

In the final model, the time course of buprenorphine's respiratory depressant effect was analyzed using the combined biophase equilibration-receptor association/dissociation kinetics model. An important feature of this model is that it allows separate characterization of the biophase equilibration kinetics and receptor association/dissociation kinetics to account for time-dependencies in the *in vivo* pharmacodynamics. This involves the estimation of 1) a first-order rate constant for biophase equilibration and 2) first- and second-order rate constants for receptor association and dissociation kinetics. Drug distribution to the site of action (biophase) was characterized on the basis of an effect compartment model (Sheiner *et al.*, 1979). The rate of change of biophase drug concentrations can be defined as follows:

$$\frac{dC_e}{dt} = k_{e0} \cdot (C_p - C_e) \quad (1)$$

where k_{e0} is a first-order distribution rate constant describing the rate of change of drug concentration in the effect compartment, C_p represents the plasma concentration and C_e the effect-site concentration.

At the site of action, the drug binds to the μ -opioid receptor. It is generally believed that opioids produced their respiratory depressant effect via an action at the μ -opioid receptor gene (OPRM1) product (Dahan *et al.*, 2001). Involvement of other opioid receptors (κ , δ or ORL1 receptors) in opioid-induced respiratory depression is considered unlikely (Ling *et al.*, 1985). Following the law of mass action, the rate of drug-receptor

binding is proportional to the concentrations of drug C_e and free receptor R :

$$\frac{dC_e R}{dt} = k_{\text{on}} \cdot C_e \cdot R - k_{\text{off}} \cdot C_e R \quad (2)$$

Under the assumption that the concentration of drug is in excess compared to the free receptor concentration and that the total number of receptors (R_{tot}) is equal to the sum of drug-bound ($C_e R$) receptors and unbound receptors (R), equation 2 can be rearranged:

$$\frac{d\rho_{\text{app}}}{dt} = k_{\text{on}} \cdot C_e \cdot (1 - \rho_{\text{app}}) - k_{\text{off}} \cdot \rho_{\text{app}} \quad (3)$$

in which ρ_{app} is the apparent fractional receptor occupancy ($C_e R / R_{\text{tot}}$). In the present analysis a linear transduction function was used to characterize the relationship between the fractional receptor occupancy and the respiratory depressant effect relationship:

$$E = E_0 \cdot (1 - \alpha \cdot \rho_{\text{app}}) \quad (4)$$

where E is the ventilatory response, E_0 is the baseline ventilation and α is the intrinsic activity of buprenorphine of which the value varies between 0 and 1. In theory, the transducer function can take any shape. For systems with a high receptor reserve typically a hyperbolic transducer function is used (Black and Leff, 1983). However, for systems with low receptor reserve and for partial agonists (i.e. drugs with a low intrinsic efficacy) a linear transducer function is appropriate (Clark, 1937; Ariens, 1954). In such systems the maximum effect occurs when all receptors are occupied. Since buprenorphine behaves as a partial agonist in receptor assays (Huang *et al.*, 2001), the assumption of a linear relationship between receptor occupancy and respiratory depression is justified.

In the final model the time course of fentanyl's respiratory depressant effect was analyzed using the effect compartment link model with a fractional E_{max} pharmacodynamic model. In this analysis, the fractional sigmoid E_{max} model is of the form:

$$E = E_0 \cdot \left(1 - \frac{\alpha \cdot C_e^n}{C_e^n + EC_{50}^n} \right) \quad (5)$$

where α is the intrinsic activity and varies between 0 and 1, EC_{50} is the effect-site concentration yielding half-maximal respiratory depression and n a slope parameter.

RESULTS

Pharmacokinetics

A three-compartment linear model best described the time course of buprenorphine concentration in plasma, whereas a two-compartment model was found to appropriately characterize the disposition of fentanyl. Inter-individual variability could be characterized

for the pharmacokinetic parameters of buprenorphine and fentanyl (table 2 and 3). No significant parameter-covariate relationships were identified. The population estimates obtained from the final pharmacokinetic models and the stochastic model parameters were very similar to the mean of 1000 bootstrap replicates (table 2 and 3). The results of the 1000 bootstrap runs showed that the population pharmacokinetic model precisely predict the time course of buprenorphine and fentanyl concentration after intravenous administration.

Table 2. Parameter estimates of the final population pharmacokinetic model for buprenorphine and the stability of the parameters using the bootstrap resampling procedure. For the fixed and random parameter estimates the relative standard error (%) is displayed

	Original data set		1000 bootstrap replicates	
	Typical value	IIV (%) [†]	Typical value	IIV (%)
<i>Cl</i> (l/min)	1.55 (2.9)	17.0 (19.7)	1.54 (3.7)	16.5 (21.9)
<i>V</i> ₁ (l)	12.6 (11.8)	70.7 (14.7)	12.2 (17.8)	68.8 (25.1)
<i>V</i> ₂ (l)	26.4 (22.2)	57.6 (33.7)	26.2 (25.1)	55.6 (41.7)
<i>V</i> ₃ (l)	147 (5.5)	20.5 (37.7)	145 (6.7)	20.2 (40.4)
<i>Q</i> ₂ (l/min)	1.56 (16.0)	NE [‡]	1.58 (16.2)	NE
<i>Q</i> ₃ (l/min)	1.28 (11.1)	39.0 (46.0)	1.26 (11.1)	35.9 (57.5)
PRE [#] (%)	15.9 (10.5)		16.0 (12.2)	

[†] inter-individual variability; [‡] not estimated; [#] proportional residual error

Table 3. Parameter estimates of the final population pharmacokinetic model for fentanyl and the stability of the parameters using the bootstrap resampling procedure. For the fixed and random parameter estimates the relative standard error (%) is displayed

	Original data set		1000 bootstrap replicates	
	Typical value	IIV (%)	Typical value	IIV (%)
<i>Cl</i> (l/min)	0.98 (14.9)	67.7 (48.5)	1.0 (16.1)	65.6 (49.7)
<i>V</i> ₁ (l)	19.5 (15.2)	57.4 (35.8)	19.8 (14.6)	55.7 (35.1)
<i>V</i> ₂ (l)	150 (5.5)	NE	148 (6.1)	NE
<i>Q</i> (l/min)	3.51 (11)	NE	3.56 (10.6)	NE
PRE (%)	21.7 (16.6)		21.2 (17.2)	

Mechanism-based PK-PD modeling

Baseline V_{i50} ($\pm$ SEM) were 21.4 ± 4.4 l/min and 24.9 ± 3.5 l/min for fentanyl and buprenorphine respectively. After administration of fentanyl ventilation rapidly decreased and the observed maximum effect was reached after approximately 5 min. For buprenorphine the observed maximum effect was reached at approximately 75 min following intravenous bolus administration. The observed maximum effects were different between fentanyl and buprenorphine. At fentanyl doses > 0.2 mg/70 kg periods of apnea ($V_{i50}=0$ l/min) were observed. In fact, for one individual who received 0.5 mg/70 kg fentanyl severe respiratory depression was observed characterized by a long period of apnea. The overall results for fentanyl showed clearly a dose-dependent increase in respiratory depression. In contrast to fentanyl, following the administration of buprenorphine, in none of the subjects apnea was observed. In the lower dose range (0.05 - 0.1 mg/70 kg) a dose-related decrease in ventilation was seen. At higher doses (> 0.2 mg/70 kg) the dose-response relationship was flattening, indicating that the maximal respiratory depressant effect was reached. A number of structurally different PK-PD models was evaluated for their usefulness to describe the time course of respiratory depression. Three different models were tested (1) a biophase equilibration model in combination with a fractional sigmoid $E_{\max}$ pharmacodynamic model with effect compartment, (2) a receptor association/dissociation model with linear transduction function and (3) a biophase equilibration-receptor association/dissociation model with a linear transduction function. Initially, the time course of respiratory depression for both fentanyl and buprenorphine were analyzed using the combined biophase equilibration-receptor association/dissociation model with a linear transduction function. For buprenorphine, the model discrimination test clearly showed that the combined biophase equilibration-receptor association/dissociation model best described the time course of respiratory depression as indicated by the obtained objective function value (table 4).

Table 4. Results of the model discrimination to characterize the time course of respiratory depression following intravenous administration of buprenorphine and fentanyl

	Buprenorphine			Fentanyl		
	Model A [†]	Model B [‡]	Model C [‡]	Model A	Model B	Model C
OFV	3215	3266	3096	1407	1534	1511
k_{e0} (min ⁻¹)	0.00082	-	0.0092	0.0422	-	0.0333
k_{on} (ml/ng/min)	-	> 100	0.246	-	> 100	> 100
k_{off} (min ⁻¹)	-	< 0.001	0.0102	-	> 100	> 100

[†] biophase equilibration model with sigmoid $E_{\max}$ pharmacodynamic model; [‡] receptor association/dissociation model with a linear transduction function; [‡] combined biophase equilibration-receptor association/dissociation model with a linear transduction function.

In contrast, for fentanyl the biophase equilibration model with a fractional $E_{\max}$ pharmacodynamic model best described the time course of the respiratory depressant effect. No significant parameter correlations ($R < 0.95$) were reported between any of the pharmacodynamic model parameters indicating that unique parameter estimates were obtained. Note that although k_{off} and k_{e0} are first-order rate constants, different time-effect profiles are obtained when the values of both parameters are switched. Thus the biophase equilibration model in combination with the sigmoid $E_{\max}$ pharmacodynamic model and the combined biophase equilibration-receptor association/dissociation model with a linear transduction model were able to successfully describe all individual concentration-effect relationships, yielding estimates of k_{e0} for fentanyl and of k_{e0} , k_{on} and k_{off} for buprenorphine. The pharmacodynamic parameter estimates are presented in table 5 and 6. The respiratory depressant effect in typical individuals, predicted on the basis of the population pharmacodynamic parameter estimates are depicted as solid lines. In figure 1, examples of six data fits are given, including the best, median and worst fit based on the coefficient of determination (R^2).

	Typical value	IIV (%)
k_{on} (ml/ng/min)	0.246 (17.9)	72.1 (26.4)
k_{off} (min^{-1})	0.0102 (23.2)	-
K_D (nM) [§]	0.089	-
k_{e0} (min^{-1})	0.0092 (22.5)	110 (24.1)
E_0 (l/min)	23.9 (3.7)	25.1 (20.9)
α	0.56 (5.6)	35.1 (26.4)
AE (l/min)	1.3 (13.1)	

Table 5. Population pharmacodynamic estimates and inter-individual variability estimates of buprenorphine. For the fixed and random parameter estimates the relative standard error (%) is displayed

[§] secondary parameter ($k_{\text{off}}/k_{\text{on}}$); ^{||} additive residual error.

	Typical value	IIV (%)
k_{e0} (min^{-1})	0.0422 (11.5)	37.8 (32.9)
EC_{50} (ng/l)	1140 (36.9)	18.9 (66.0)
E_0 (l/min)	20.2 (4.9)	26.7 (30.4)
α	0.91 (40.3)	25.4 (45.3)
n	2.68 (74.6)	NE
AE (l/min)	6.1 (22.9)	

Table 6. Population pharmacodynamic estimates and inter-individual variability estimates of fentanyl. For the fixed and random parameter estimates the relative standard error (%) is displayed

For fentanyl, the biophase equilibration rate constant was 0.0422 min^{-1} (95 % CI: $0.0327 - 0.0517 \text{ min}^{-1}$), which corresponds to $t_{1/2,ke0} = 16.4 \text{ min}$. For buprenorphine, the population values of k_{on} and k_{off} were 0.246 ml/ng/min (95 % CI: $0.160 - 0.332 \text{ ml/ng/min}$) and 0.0102 min^{-1} (95 % CI: $0.0056 - 0.0148 \text{ min}^{-1}$), which corresponds to $t_{1/2,koff} = 68 \text{ min}$. The biophase equilibration rate constant was estimated at 0.0092 min^{-1} (95 % CI: $0.0051 - 0.0132 \text{ min}^{-1}$), which corresponds to $t_{1/2,ke0} = 75.3 \text{ min}$. For fentanyl, NONMEM analysis revealed that the values of the parameters k_{on} and k_{off} were rising to high values while the estimate of k_{e0} was stable and did not change during the iteration process. This implies that fentanyl binding to and dissociation from the μ -opioid receptor occurs very rapidly and that the concentration-effect relationship can be characterized under equilibrium conditions using the most parsimonious model which is the sigmoid E_{max} model with effect compartment model. This is confirmed by the fact that for fentanyl the lowest objective function value was obtained with the effect compartment link model. A summary of the fitting performance of the different PK-PD models and their respective parameters estimates is shown in table 4. The steady-state apparent receptor occupancy or fentanyl concentration versus respiratory depressant effect relationship is shown in figure 2.

DISCUSSION

In the present investigation different models have been tested for their ability to characterize the time course of the respiratory depressant effect of buprenorphine and fentanyl. For buprenorphine, the combined biophase equilibration-receptor association/dissociation model with a linear transducer function accurately describes the time course of buprenorphine effect.

Buprenorphine has a value of α of 0.56. For comparison, a value of α of 1 indicates that an opioid displays full respiratory depression. Buprenorphine's intrinsic activity was significantly different from 1 (95 % CI: $0.50 - 0.62$; $p < 0.05$), confirming the partial agonistic activity of buprenorphine *in vivo* with respect to respiratory depression (figure 2). Data from animal and human studies are consistent with our data and confirm ceiling effect for respiratory depression and other opioid-related side effects following parenteral or sublingual administration of buprenorphine (Walsh *et al.*, 1994; Ohtani *et al.*, 1997; Dahan *et al.*, 2005; Meert and Vermeirsch, 2005). This ceiling effect contributes to the safety upon clinical use both upon acute and following prolonged administration even at high dosages (Radbruch, 2003). In addition, the combined biophase equilibration-receptor association/dissociation model yields relevant information on the *in vivo* kinetics of buprenorphine's respiratory depressant action at the μ -opioid receptor.

In contrast to common believe only a part of the hysteresis in the respiratory depressant effect is determined by slow receptor association/dissociation kinetics. The half-time of receptor dissociation ($t_{1/2,koff}$) was 68 min. The receptor dissociation kinetics have also been investigated *in vivo* using μ -opioid receptor imaging with [^{11}C]-buprenorphine as a

PET tracer in baboons (Galynker *et al.*, 1996). In these studies the half-time of receptor dissociation was reported to be 41 min which is in the same range as the estimated *in vivo* dissociation half-life in the present investigation. In addition to the rate constants for receptor dissociation, this investigation has also yielded *in vivo* estimates of the equilibrium dissociation constant K_D . The estimation of the *in vitro-in vivo* correlation of receptor affinity is of considerable interest. The binding properties and potency of buprenorphine have been determined in Chinese hamster ovary (CHO) cells, expressing the human μ -opioid receptor, using a [^{35} S]-GTP- γ -S-functional binding assay (Huang *et al.*, 2001). The results of these *in vitro* studies show that buprenorphine is a potent partial agonist at the

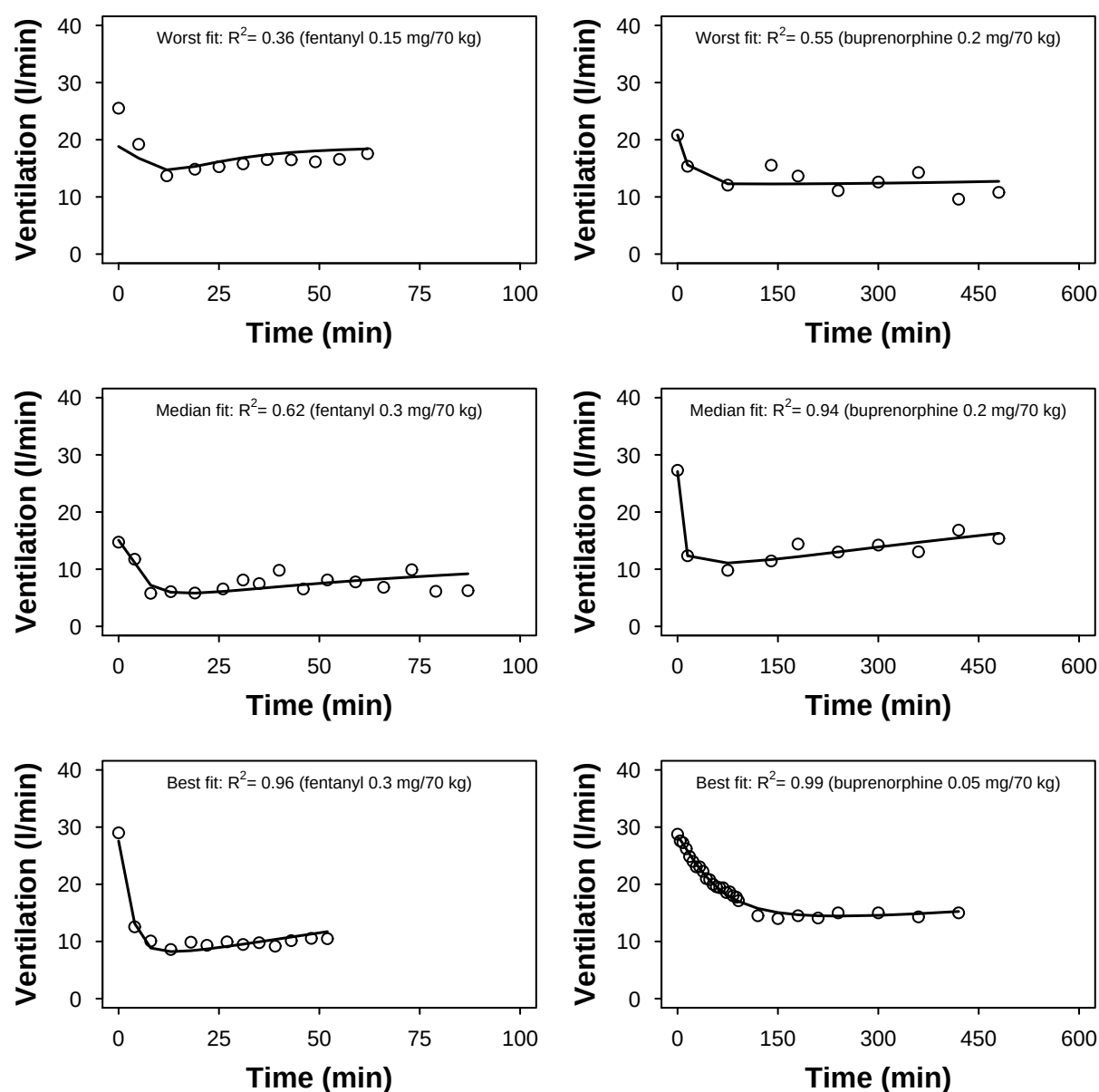


Figure 1. The time course of the respiratory depressant effect following the administration of fentanyl (left panels) and buprenorphine (right panels) is shown. Worst, median and best data fits between measured (symbols) and individual predicted respiratory depression (solid line) are presented. The goodness of fit was assessed by R^2 .

μ -opioid receptor with an *in vitro* K_D value of 0.08 nM. The potency of buprenorphine for stimulation of [^{35} S]GTP-S binding to the human neuroblastoma SK-N-SH cells, expressing the human μ -opioid receptor was estimated at 0.14 nM (Selley *et al.*, 1997). The *in vivo* K_D of buprenorphine of 0.089 nM is in the same range to these *in vitro* K_D and EC_{50} value, confirming the validity of the proposed combined biophase equilibration-receptor association/dissociation model to predict the time course of the respiratory depressant effect of buprenorphine on the basis of *in vitro* binding data.

For fentanyl, the *in vivo* rate constants for receptor association and dissociation were fast relative to the pharmacokinetics in plasma and brain (table 6) indicating that *in vivo* binding to and dissociation from the μ -opioid receptor occurs instantaneously. This is in agreement with fentanyl's relatively fast receptor binding characteristics *in vitro* (Boas and Villiger, 1985). The time course of fentanyl's respiratory depressant effect was characterized using a biophase equilibration model in combination with the fractional sigmoid $E_{\max}$ pharmacodynamic model. The half-time of biophase equilibration ($t_{1/2,ke0}$) was 16.4 min and is higher than the value of 6.6 min which was estimated for the electroencephalographic (EEG) effect of fentanyl in patients (Scott *et al.*, 1991). The respiratory depressant effect occurs in a much lower concentration range than the EEG effect. There is evidence for saturable active transport of fentanyl into the brain at the lower clinical concentration range (2-10 nM), hereby influencing the brain-blood partitioning of fentanyl (Henthorn *et al.*, 1999). Apart from differences in the doses used, alteration of the pH of the blood or the brain due to the measurement of respiratory depression at elevated end-tidal CO_2 levels may also affect the brain distribution kinetics of fentanyl.

Since buprenorphine and fentanyl share similar physicochemical properties (i.e. high lipophilicity) we postulated that biophase equilibration also contributes to the observed hysteresis between buprenorphine plasma concentration and its respective respiratory depressant effect. Although receptor association/dissociation kinetics partly account for the time delay in the respiratory depressant effect, the onset and offset of the respiratory depressant effect is indeed dominated by relatively slow biophase equilibration kinetics. The half-time of biophase equilibration ($t_{1/2,ke0}$) is 75.3 min and is greater than the value of 16.4 min estimated for fentanyl, indicating that the *in vivo* kinetics of buprenorphine action are more delayed than for fentanyl.

Experimental animal studies suggest marked differences in blood-brain partitioning for buprenorphine and fentanyl. After bolus iv administration and during the terminal elimination phase, buprenorphine concentrations in the brain were seven times higher than buprenorphine plasma concentrations (Ohtani *et al.*, 1995). For fentanyl, the brain-blood partitioning coefficient was 4) (Bjorkman *et al.*, 1990). The difference in brain-blood partitioning of the two opioids is in good agreement with their respective lipid solubility (log octanol-water partition coefficient is 4.98 for buprenorphine *versus* 2.3 for fentanyl (Clark's Analysis of Drugs and Poisons, 2006)) and may well explain the difference in half-time of biophase equilibration between buprenorphine and fentanyl. A similar correlation between brain-blood partitioning and biophase equilibration has been shown

for other lipid soluble intravenous anesthetics (Dutta *et al.*, 1998).

In conceptual terms, the biophase equilibration-receptor association/dissociation model with a linear transduction function and the biophase equilibration model with fractional sigmoid $E_{\max}$ pharmacodynamic model are complementary model which can be used to compare the respiratory depressant effect of opioids under dynamic and steady-state conditions. Specifically, for the partial agonist buprenorphine the *in vivo* estimated K_D value ($K_D = k_{\text{off}}/k_{\text{on}}$) is identical to the EC_{50} value, thus enabling a direct comparison to the potency of fentanyl. Likewise the estimated values of α enable a direct comparison of the *in vivo* intrinsic respiratory depressant activity of buprenorphine and fentanyl at the μ -opioid receptor in a strict quantitative manner. The present analysis shows that the intrinsic activity of fentanyl is 0.91 (95 % CI: 0.19 - 1.6) and not significantly different from 1 ($p < 0.05$), indicating that fentanyl displays full respiratory depressant effect (figure 2). The full agonistic activity of fentanyl at the μ -opioid receptor is in agreement with results from other studies and suggest that fentanyl should be administered with caution since at higher doses potentially fatal respiratory depression may occur. In contrast, buprenorphine's intrinsic activity was 0.56 (figure 2) indicating that buprenorphine has a wider margin of safety than fentanyl.

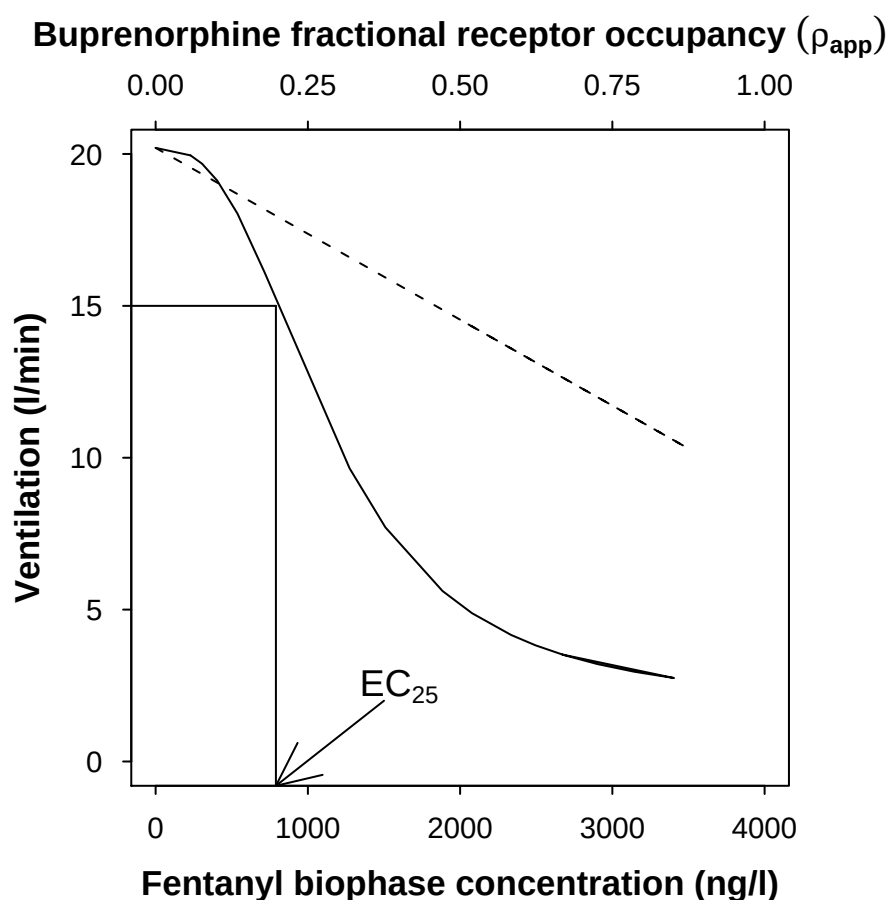


Figure 2. Concentration-effect relationship of fentanyl (solid line) in human relative to the receptor occupancy-effect relationship of buprenorphine (dashed line).

Another important issue is the *in vivo* potency of buprenorphine relative to fentanyl. For partial agonists like buprenorphine the EC_{50} is equal to the *in vivo* estimated K_D . Therefore, the estimates of *in vivo* K_D for buprenorphine and *in vivo* EC_{50} for fentanyl permits a direct quantitative comparison of the *in vivo* potency for respiratory depression. The estimates of *in vivo* potency of fentanyl and buprenorphine are 1140 ng/l and 41.5 ng/l, respectively which correspond to values of 3.39 nM and 0.089 nM, respectively. Since both compounds display different intrinsic activity (56 % for buprenorphine *versus* 91 % for fentanyl) with respect to respiratory depression, an EC_{25} value was calculated for fentanyl to allow for direct comparison with buprenorphine's *in vivo* potency. The calculated EC_{25} for fentanyl is 2.4 nM versus 0.089 nM for the K_D of buprenorphine. The estimates of *in vivo* potency show that with respect to respiratory depression buprenorphine is more potent than fentanyl.

In conclusion, the combined biophase equilibration-receptor association/dissociation model with a linear transducer function and the biophase equilibration model with fractional sigmoid E_{max} model are complementary PK-PD models to characterize the time course of respiratory depression of buprenorphine and fentanyl. The time to onset and the duration of the respiratory depressant effect of fentanyl and buprenorphine was found to be determined by biophase equilibration kinetics. In addition, the prolonged duration of the respiratory depressant effect of buprenorphine, but not fentanyl, was explained by relatively slow receptor dissociation kinetics. PK-PD analysis of the concentration-effect relationships showed ceiling effect for the respiratory depressant effect of buprenorphine, while fentanyl caused apnea.

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PHARMACOKINETIC-PHARMACODYNAMIC MODELING OF THE RESPIRATORY DEPRESSANT EFFECT OF NORBUPRENORPHINE IN RATS

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ABSTRACT

The objective of this investigation was to characterize the PK-PD correlation of buprenorphine's active metabolite norbuprenorphine for the effect on respiration in rats. Following intravenous administration in rats (dose range 0.32 - 1.848 mg) the time course of the concentration in plasma was determined in conjunction with the effect in ventilation as determined with a novel whole-body plethysmography technique.

The pharmacokinetics of norbuprenorphine was best described by a three-compartment PK model with non-linear elimination. A saturable biophase distribution model with a power pharmacodynamic model described the PK-PD relationship best. No saturation of the effect at high concentrations was observed, indicating that norbuprenorphine acts as a full agonist with regard to respiratory depression. Moreover, analysis of the hysteresis on basis of the combined receptor association/dissociation-biophase distribution model yielded high values of the rate constants for receptor association and dissociation, indicating that these processes are not rate-limiting.

In a separate analysis the time course of the plasma concentrations of buprenorphine and norbuprenorphine following administration of both the parent drug and the metabolite were simultaneously analyzed on the basis of a six-compartment PK model with non-linear elimination of norbuprenorphine. This analysis showed that following intravenous administration 10 % of the administered dose of buprenorphine is converted into norbuprenorphine.

By simulation it is shown that following intravenous administration of buprenorphine the concentrations of norbuprenorphine reach values which are well below the values causing an effect on respiration.

INTRODUCTION

Recently, the pharmacokinetic-pharmacodynamic relationship of buprenorphine for the effect on the respiratory response has been studied in rats (Yassen *et al.*, 2006) and in humans (Yassen *et al.*, 2007). In these investigations buprenorphine has been shown to display ceiling respiratory depressant effect, indicating that buprenorphine acts functionally as a partial agonist at the μ -opioid receptor. The *in vivo* behavior correlates well with data obtained from *in vitro* receptor binding assays (Martin *et al.*, 1976; Lee *et al.*, 1999; Lutfy *et al.*, 2003).

Clearly, partial agonistic activity for respiratory depression contributes to the safety profile upon buprenorphine administration even at high doses (Dahan *et al.*, 2006). In the previous investigations, the observed hysteresis between plasma concentration and effect has in part been explained by slow receptor association and dissociation kinetics at the μ -opioid receptor. This pharmacological characteristic is unique for buprenorphine and is not shared by other opioids like morphine and fentanyl (Cowan *et al.*, 1977; Boas and Villiger, 1985). The slow receptor association-dissociation kinetics may be a complicating factor in the reversal of buprenorphine-induced respiratory depression with naloxone. Furthermore, buprenorphine was shown to bind with high affinity to the μ -opioid receptor. Specifically, the estimate of the equilibrium dissociation constant K_D was 0.34 nM and provided a direct estimate of the *in vivo* potency ($K_D = EC_{50}$). In line with its high binding affinity buprenorphine is a potent opioid (Sorge and Sittl, 2004).

Although much of the work on buprenorphine is still focused on the pharmacological and PK-PD properties of the parent drug, consideration should also be given to the role of possible active metabolites. Norbuprenorphine is the N-dealkylated metabolite of buprenorphine (Cone *et al.*, 1984). At present PK-PD modeling of the respiratory depressant effect of norbuprenorphine has not been accomplished. Pertinent questions in this respect are 1) is the potency of norbuprenorphine similar to that of the parent compound, 2) does norbuprenorphine still act as a partial agonist with regard to respiratory depression, 3) do receptor association/dissociation kinetics contribute to the hysteresis between plasma concentration and effect.

Investigations in chronically-instrumented animal models are invaluable in determining the PK-PD correlations of drug metabolites, since in many instances such metabolites can not be directly administered to humans (Breimer and Danhof, 1997). Previously, the pharmacological properties of the major metabolites of remifentanyl and midazolam in man have been successfully predicted on the basis of PK-PD investigations in rats (Cox *et al.*, 1999; Tuk *et al.*, 1999). The objective of this study is to characterize the PK-PD correlation of norbuprenorphine in rat. Specifically, the objective was a) to determine the rate-limiting steps in the time course of norbuprenorphine-induced respiratory depression and b) to characterize the *in vivo* concentration-respiratory depressant effect relationship. Finally, an integrated drug-metabolite population pharmacokinetic model

was developed to explore the contribution of the effect of norbuprenorphine to the observed respiratory depressant effect following intravenous administration of the parent drug.

MATERIALS AND METHODS

Animals

Male Wistar rats, weighting 225-250 g at arrival, were obtained from Charles River BV (Zeist, The Netherlands). The animals were housed in plastic cages in groups before surgery and individually after surgery. The animals were housed under laboratory standard conditions at constant room temperature (21 °C) and on a 12-h light/dark cycle, with lights turned on at 7:00 am and off at 7:00 pm. Food (RMH-TM; Hope Farms, Woerden, The Netherlands) and acidified water were allowed ad libitum. The animals were handled and allowed for acclimation to the experimental environment for ten days prior to the start of the experiment. The protocol was approved by the Ethical Committee on Animal Experimentation of Leiden University.

Surgical procedure

Surgery was carried out under anesthesia with an intramuscular injection of 0.1 mg/kg medetomidine hydrochloride (Domitor 1 mg/ml; Pfizer, Capelle a/d IJssel, The Netherlands) and 1 mg/kg ketamine base (Ketalar 50 mg/ml; Parke-Davis, Hoofddorp, The Netherlands).

Two days before the experiment indwelling cannulae were implanted, one in the left femoral artery and one in the right jugular vein. The cannula in the right jugular vein was used for administration of norbuprenorphine while the cannula in the left femoral artery was used for serial collection of arterial blood samples. The cannulae were made from pyrogen free, non-sterile polyethylene tubing. One day before surgery cannulae were disinfected in a benzalkoniumchlorid 1 % solution. The venous cannula consisted of 3 cm polyethylene tubing (0.28 mm i.d.; Portex Limited, Kent, United Kingdom) heat-sealed to 9 cm polyethylene tubing (0.58 mm i.d.; Portex Limited, Kent, United Kingdom). The arterial cannula consisted of 3 cm polyethylene tubing (0.28 mm i.d.) heat-sealed to 21 cm polyethylene tubing (0.58 mm i.d.). Furthermore, a telemetric transmitter (Physiotel implant TA10TA-F40 system, Data Sciences International (DSI), St. Paul, MN, USA) was implanted under the skin in the neck for the measurement of body temperature. The cannulae were tunnelled subcutaneously and fixed at the back of the neck with a rubber ring. The skin in the neck and throat was stitched with normal suture. The skin in the groin was closed with wound clips.

In order to prevent clotting and cannula obstruction the cannulae were filled with a

25 % (w/v) polyvinylpyrrolidone solution (PVP; Brocacef, Maarssen, The Netherlands) in pyrogen-free physiological saline (B. Braun Melsungen AG, Melsungen, Germany) containing 20 IU/ml heparin (Hospital Pharmacy, Leiden University Medical Center, Leiden, The Netherlands).

Drugs and dosages

Norbuprenorphine hydrochloride was kindly donated by Grünenthal GmbH (Aachen, Germany). Norbuprenorphine hydrochloride solution was prepared in saline. Three doses of norbuprenorphine were tested 0.32, 0.84 and 1.848 mg. All animals were tested once and each treatment group consisted of eight animals. Furthermore, eight animals received vehicle treatment. The doses and concentrations of norbuprenorphine are expressed as free base.

Measurement of respiratory depression

Respiratory depression was determined in unrestrained, conscious animals using whole-body plethysmography for the quantification of ventilation (Model PLY3223, Buxco Electronics Inc., UK). Briefly, the animals were placed in a plethysmograph, consisting of a measurement chamber and an integrated reference chamber to correct for atmospheric disturbances. Both chambers were connected to a differential pressure transducer (TRD 5700, Buxco Electronics Inc., UK). During the experiment continuous flow of gas was delivered through the measurement chambers. The flow and composition of the gas mixture, consisting of dry air and CO₂, was controlled by mass flow controllers (5850S/BC Mass Flow Controller, Brooks Instruments, The Netherlands) connected to a microprocessor control and read out unit (Model 0152, Brooks Instruments, The Netherlands). O₂ and CO₂ levels in the chambers were monitored continuously using a Datex Multicap gas monitor (Datex-Engstrom, Helsinki, Finland).

In each animal the effects of opioids and vehicle were assessed on ventilation at an inhaled concentration of 6.5 % carbon dioxide on a background of normoxia (20 % oxygen). The inhalation of the gas mixture lasted 5 min to ensure that steady-state ventilation had been reached. Tidal volume (V_T), breathing frequency (RR) and minute ventilation (V_i , where $V_i = V_T \times RR$) were obtained from changes in chamber pressure using a low-pressure differential transducer connected to preamplifier modules (MAX2270, Buxco Electronics Inc., UK). The signals were digitized at a rate of 200 Hz using a CED 1410_{plus} interface (CED, Cambridge, UK). The digitized signals were collected and stored on disk for further off-line analysis. A personal computer running ACQ software (Erik Kruyt, Leiden University Medical Center, Leiden, The Netherlands) integrated the digitized signals to yield a flow signal.

Calibration of the chamber pressure signal was performed dynamically by injection of air into the chamber using a motor-driven 1-ml syringe pump. Minute ventilation was vi-

sualized using RRDP software (Erik Olofsen, Leiden University Medical Center) and stored on a breath-to-breath basis. Minute ventilation was averaged over the total number of breaths obtained in one minute and used for PK-PD data analysis. During the experiment body temperature was maintained at 37.5 °C using heating pads. Body temperature was monitored continuously by radiotelemetry (Model RPC-1, Data Sciences International, USA).

Drug analysis

Norbuprenorphine plasma concentrations were determined by HPLC coupled to tandem mass spectrometry (LC/MS/MS) (Yassen *et al.*, 2005). Briefly, to 50 μ l of plasma, 25 μ l of internal standard 4g/100 ml was added. Subsequently, 25 μ l of concentrated ammonia was added and the samples were extracted by liquid/liquid extraction with 600 μ l of methyl tertiary-butyl ether. The chromatographic system consisted of an Agilent HP 1100 high-performance liquid chromatography system (Agilent, Waldbronn, Germany) coupled to an API 4000 liquid chromatography/mass spectrometry/mass spectrometry system (Applied Biosystems, Darmstadt, Germany). Chromatography was performed on a precolumn (Metaguard Polaris, 3 μ m, C_{18A}, 2 mm; Varian, Darmstadt, Germany) guarded Synergi 4 μ m Hydro-RP 80A column, 75 mm x 2 mm (Phenomenex, Aschaffenburg, Germany). The lower limit of quantification was 0.047 ng/ml for norbuprenorphine. The accuracy ranged from 96.1 to 101.0 %. The precision, expressed as coefficient of variation, ranged from 2.0 to 3.7 % for concentrations between 0.14 and 8.7 ng/ml.

Pharmacokinetic-pharmacodynamic experiments

To minimize the influence of circadian rhythms, all experiments started between 9:00 and 9:30 am. Animals were randomly assigned to the treatment groups.

Before administration of norbuprenorphine or vehicle animals were placed in the measurement chamber for a habituation period of 1 hour. Upon administration of norbuprenorphine or vehicle via a constant intravenous rate infusion using an infusion pump (BAS Bioanalytical Systems Inc., West Lafayette, Indiana, USA), minute ventilation was measured during one minute at the following pre-defined time-points; dose I: 0 (baseline), 5, 20, 35, 50, 65, and 90 min, dose II: 0 (baseline), 5, 20, 35, 50, 65, 90, 120, 150 and 180 min, dose III: 0 (baseline), 5, 20, 35, 50, 65, 90, 120, 150, 180 and 240 min after drug administration. Blood samples were collected for each animal at time t= 0 (predose), 10, 20, 25, 35, 40, 45, 50, 60, 75, 90, 120, 150, 180, 240 and 300 (dose II and III) min.

In cases where blood sampling coincided with the ventilation measurement, ventilation measurement preceded blood sampling to minimize stress for the animals. Serial arterial blood samples (100 μ l) were collected in heparinized microtubes. Plasma (50 μ l) was separated from the blood by centrifugation at 5000 rpm for 15 min and frozen at -20 °C until analysis.

PK-PD modeling software

Non-linear mixed effects modeling using the NONMEM software package (version V, level 1.1) (Beal and Sheiner, 1999) was used to characterize the pharmacokinetic-pharmacodynamic relationship of norbuprenorphine in rats. The Fortran compiler Compaq Visual Fortran version 6.1 was used for compilation. The pharmacokinetic and pharmacodynamic parameters were estimated using the first-order conditional estimation (FOCE) method with η - ϵ interaction. Evaluation of NONMEM outputs and graphical analysis were performed using S-PLUS 6.0 (Insightful Corp., Seattle, WA, USA).

Pharmacokinetic analysis of norbuprenorphine

In order to determine the basic structural pharmacokinetic model for norbuprenorphine one-, two- and three compartment models with linear and non-linear elimination were tested. Model selection and identification was based on the likelihood ratio test, pharmacokinetic parameter point estimates and their respective confidence intervals, parameter correlations and goodness-of-fit plots. For the likelihood ratio test, the significance level was set at $\alpha = 0.01$, which corresponds with a decrease of 6.6 points, after the inclusion of one parameter, in objective function value (OFV) under the assumption that the difference in OFV between two nested models is χ^2 distributed. Initially, linear pharmacokinetic models were tested. Thereafter, the individual pharmacokinetic parameter estimates were examined for norbuprenorphine dose dependency. On the basis of model selection criteria, a three-compartment model with concentration-dependent elimination was selected for norbuprenorphine according to:

$$k_{40} = \frac{V_m^{k_{40}} \cdot K_m^{k_{40}}}{C_p + K_m^{k_{40}}} \quad (1)$$

where $V_m^{k_{40}}$ is the maximum elimination rate which is k_{40} at norbuprenorphine plasma concentration $C_p = 0$ and $K_m^{k_{40}}$ is the concentration at which the elimination rate constant is half-minimal. The model algorithm was programmed in ADVAN6 subroutine in NONMEM and was parameterized in terms of rate constants and volume of central distribution (figure 1).

On the basis of the final population PK model, individual estimates of norbuprenorphine concentrations were predicted at pharmacodynamic observation times and served as input for the pharmacodynamic model. This approach is not limited to time constraints (intensive run times) compared to the simultaneous estimation of PK and PD models and is not expected to yield biased pharmacodynamic estimates unless the PK model is misspecified (Zhang *et al.*, 2003).

Pharmacodynamic analysis

To characterize the observed hysteresis in the time course of respiratory depression relative to the plasma concentration various structurally different PK-PD models, incorporating different links models, were evaluated for their appropriateness to characterize the time course of norbuprenorphine's respiratory depressant effect: 1) receptor association-dissociation model in combination with a linear transduction function (Shimada *et al.*, 1996), 2) combined receptor association-dissociation and linear biophase distribution model with a linear transduction function, 3) a linear biophase distribution model with a sigmoid $E_{\max}$ pharmacodynamic model (Sheiner *et al.*, 1979), 4) a linear biophase distribution model with a power pharmacodynamic model (Sarton *et al.*, 2000) and 5) a saturable biophase distribution model with a power pharmacodynamic model. In the final model, the concentration-effect relationship of norbuprenorphine was described using the power pharmacodynamic model. Time-dependencies in norbuprenorphine's pharmacodynamics

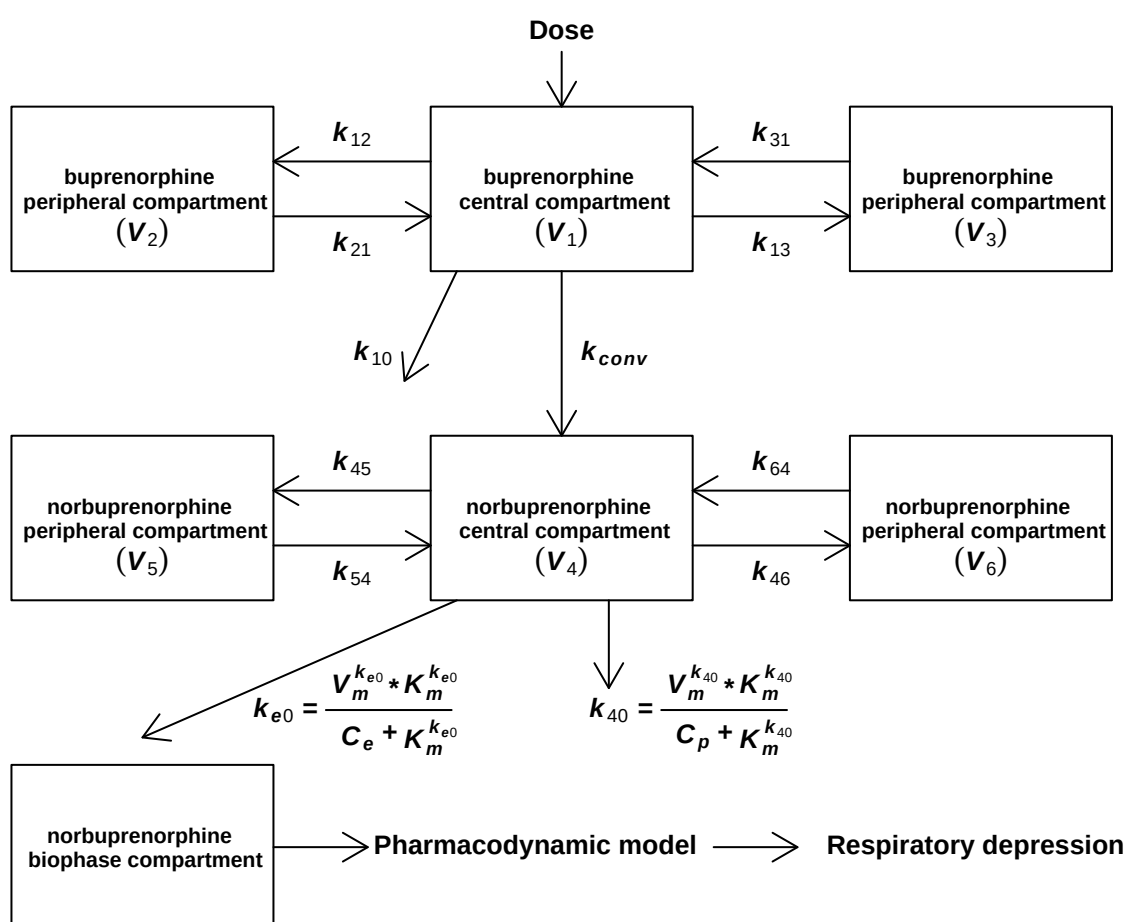


Figure 1. A schematic representation of the population PK-PD model to characterize the observed hysteresis in the time course of norbuprenorphine's respiratory depressant effect relative to plasma concentration.

were explained on the basis of non-linear biophase distribution kinetics:

$$k_{e0} = \frac{V_m^{k_{e0}} \cdot K_m^{k_{e0}}}{C_p + K_m^{k_{e0}}} \quad (2)$$

where C_e is the norbuprenorphine biophase concentration, $V_m^{k_{e0}}$ is the rate constant of biophase distribution k_{e0} at $C_e = 0$, $K_m^{k_{e0}}$ is the concentration at which the rate constant of biophase equilibration k_{e0} is half-minimal. A schematic representation of the population PK-PD model is shown in figure 1. The power pharmacodynamic model is of the form:

$$E = E_0 \cdot \left[1 - 0.5 \cdot \left(\frac{C_e}{EC_{50}} \right)^n \right] \quad (3)$$

where E is the respiratory depressant effect, E_0 is the baseline, EC_{50} is the effect-site concentration causing 50 % decrease in ventilation relative to baseline and n is a shape parameter.

Integrated buprenorphine-norbuprenorphine pharmacokinetic model

To characterize, in a strictly quantitative manner, the conversion of buprenorphine into norbuprenorphine, the data on the time course of the norbuprenorphine concentration from the present study were simultaneously analyzed with data on the concentrations of buprenorphine and norbuprenorphine from separate studies in which buprenorphine was administered at doses ranging from 0.05 mg/kg to 0.3 mg/kg (Yassen *et al.*, 2005, 2006) and at a dose of 1.0 mg/kg (Yassen and Danhof, unpublished data). The concentration-time profiles of buprenorphine and its metabolite norbuprenorphine formed after buprenorphine administration were described using an integrated parent-metabolite pharmacokinetic model with a rate constant k_{conv} characterizing the conversion of buprenorphine to norbuprenorphine (figure 1). This rate constant k_{conv} was subsequently used to calculate the corresponding conversion fraction into norbuprenorphine:

$$F_{conv} = \frac{k_{conv}}{k_{conv} + k_{10}} \quad (4)$$

The stochastic part of the population pharmacokinetic model of norbuprenorphine and the integrated parent-metabolite population PK model was selected to describe inter-animal variability in pharmacokinetic parameters and assumed a log-normal distribution of all model parameters over the population. Therefore an exponential distribution model was used to account for inter-animal variability:

$$P_i = P_{tot} \cdot \exp(\eta_i) \quad (5)$$

in which P_i is the individual value of model parameter P , P_{tot} is the typical value (mean population value) of parameter P in the population, and η_i is the normally distributed inter-animal random variable with mean zero and variance ω^2 . The coefficient of variation of the structural model parameters is expressed as percentage of the root mean square of the inter-animal variance term. Selection of an appropriate residual error model was based on inspection of the goodness-of-fit plots. On this basis a proportional error model was proposed to describe residual error in the plasma drug concentration:

$$C_{obs,ij} = C_{pred,ij} \cdot (1 + \varepsilon_{ij}) \quad (6)$$

in which $C_{obs,ij}$ is the j th observed concentration in the i th individual, $C_{pred,ij}$ is the predicted concentration, and ε_{ij} is the normally distributed residual random variable with mean zero and variance σ^2 . The residual error term contains all the error terms which can not be explained and refers to for example measurement and experimental error (e.g. error in recording sampling times) and structural model mis-specification. To refine the stochastic model, correlation between pharmacokinetic parameter estimates was tested by conducting covariance matrix analysis (OMEGA BLOCK option). A significant correlation between two parameters was assumed when the drop in OFV was more than 6.6 points ($p < 0.01$).

RESULTS

Norbuprenorphine pharmacokinetics

The pharmacokinetics of norbuprenorphine was non-linear as indicated by the non-linear relation between dose and AUC (Figure 2). A three-compartment model with non-linear elimination best described the pharmacokinetics of norbuprenorphine. The rate of elimination decreased with increasing plasma concentrations of norbuprenorphine (figure 2). The observed and mean population predicted concentration-time courses of norbuprenorphine stratified to treatment are shown in figure 3. All pharmacokinetic parameters were estimated precisely with an acceptable coefficient of variance ($< 50\%$) within the range of 10 % to 44 % for the fixed and random effect parameters. Inter-animal variability of the pharmacokinetic parameters $V_m^{k_{40}}$ and k_{31} was 13.4 and 28.1 %. The parameter estimates obtained with the PK model are presented in table 1.

Norbuprenorphine pharmacodynamics

Baseline value of ventilation ($\pm$ SE) were 59.1 ± 10.2 , 58.8 ± 5.03 and 59.8 ± 4.78 ml/min for the doses I-III. After start of infusion, ventilation rapidly decreased and the maximum effect was reached after 20 min. A dose-dependent decrease in ventilation was observed with values ($\pm$ SE) of the minimum ventilation of 30.6 ± 6.22 , 28.5 ± 3.68

	Typical value	IAV (%) [†]
$V_m^{k_{40}}$ min ⁻¹	0.246 (20.1)	13.4 (43.0)
$K_m^{k_{40}}$ (ng/ml)	331 (29.7)	NE [‡]
k_{45} min ⁻¹	0.050 (41.8)	NE
k_{54} (min ⁻¹)	0.002 (44.3)	NE
k_{46} (min ⁻¹)	0.219 (15.8)	NE
k_{64} (min ⁻¹)	0.036 (10.1)	28.1 (34.4)
V_4 (ml)	22.8 (11.9)	NE
PRE [#] (%)	22.8 (11.9)	

Table 1. Population pharmacokinetic estimates and inter-animal variability of norbuprenorphine. For the fixed and random parameter estimates the relative standard error (%) is displayed

[†] inter-animal variability; [‡] not estimated;
[#] proportional residual error

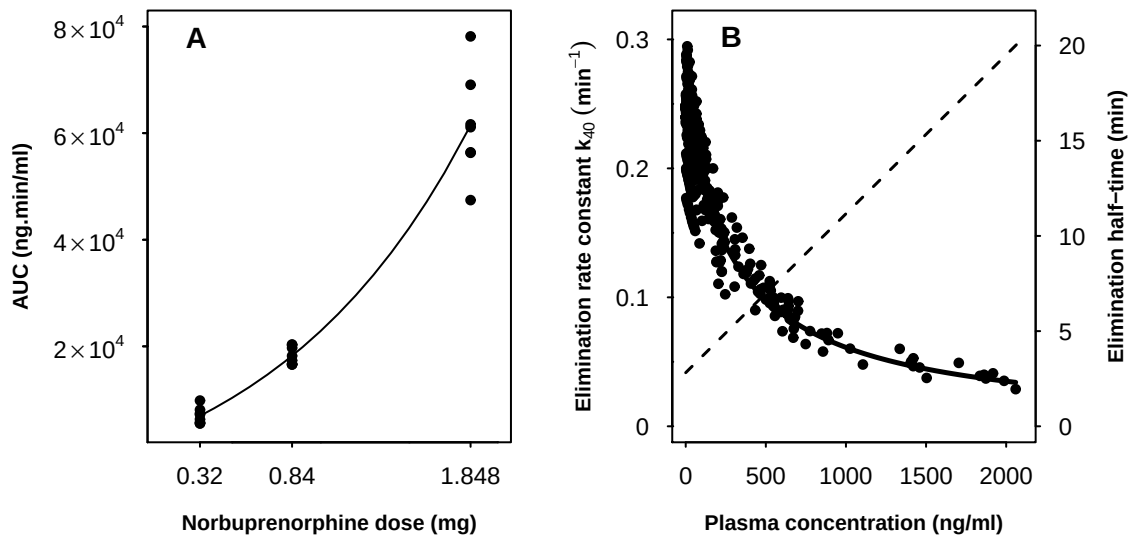


Figure 2. Non-linear pharmacokinetics of norbuprenorphine in plasma illustrated by nonlinear dose-dependent increase in the calculated area under plasma concentration curve (AUC) (panel B). The symbols represent the calculated AUC for the individual animals. In the right panel (B) the predicted elimination rate constant (solid line and left y-axis) and elimination half-life (dashed line and right y-axis) of norbuprenorphine versus plasma concentration are shown.

and 17.5 ± 2.94 ml/min for doses I-III, respectively. In order to demonstrate the appropriateness of the various PK-PD models to characterize the time course of respiratory depression in rats, model discrimination was performed. Application of the receptor association/dissociation and linear biophase distribution model to the data showed that the parameter estimates of the rate constants of receptor association (k_{on}) and dissociation

(k_{off}) were very high. Therefore, the combined biophase distribution-receptor association-dissociation model could be simplified to the biophase distribution model. The fitting performance of the biophase distribution model with the power pharmacodynamic model was equal to the E_{max} pharmacodynamic model, indicating that E_{max} had not been reached yet at 1.848 mg norbuprenorphine as judged by the objective function values (table 2).

Table 2. Results of the model discrimination to characterize the time course of respiratory depression following intravenous administration of norbuprenorphine

	Model A [†]	Model B [‡]	Model C ^ˆ	Model D [#]	Model E [§]
OFV	1300	1099	1030	1029	987
k_{e0} (min^{-1})	-	0.192	0.024	0.023	0.25
k_{on} (ml/ng/min)	> 100	> 100	-	-	-
k_{off} (min^{-1})	> 100	> 100	-	-	-

[†] receptor association/dissociation model with a linear transduction function;

[‡] combined biophase equilibration-receptor association/dissociation model with a linear transduction function; ^ˆ biophase equilibration model with sigmoid E_{max} pharmacodynamic model

combined biophase equilibration-receptor association/dissociation model with a linear transduction function; [#] linear biophase distribution model with a power pharmacodynamic model;

[§] saturable biophase distribution model with a power pharmacodynamic model

The power model with linear biophase equilibration showed a dependence of k_{e0} on norbuprenorphine concentration (figure 4). The power model with non-linear biophase equilibration described the data better than the power model with linear biophase equilibration. We have also tested the power model containing separate expressions for transport to the effect site and non-linear elimination from the brain. Although the objective function value was significantly lower ($p < 0.01$), a significant correlation ($R^2 > 0.95$) was observed between the rate constants characterizing transport to and elimination from the effect site. Thus the saturable biophase distribution model with the power pharmacodynamic model was able to successfully describe the individual respiratory depressant effect-time profiles, yielding estimates of $V_{\text{m}}^{k_{\text{e0}}}$, $K_{\text{m}}^{k_{\text{e0}}}$ and EC_{50} for norbuprenorphine.

Figure 5 shows the observed and mean population predicted effect-time profiles of norbuprenorphine stratified to dose. The typical values of $V_{\text{m}}^{k_{\text{e0}}}$ and $K_{\text{m}}^{k_{\text{e0}}}$ were estimated at 0.113 min^{-1} (95 % CI: $0.0895 - 0.137 \text{ min}^{-1}$) and 35.9 ng/ml (95 % CI: $31.4 - 40.9 \text{ ng/ml}$). The pharmacodynamic parameter estimates are presented in table 3. The theoretically shortest half-life for biophase equilibration was 6 min and at relatively high effect site concentrations the half-life for biophase equilibration is approximately 66 min (figure 4). The *in vivo* potency (EC_{50}) was estimated at 72.8 ng/ml (95 % CI: $40.5 - 105.1 \text{ ng/ml}$).

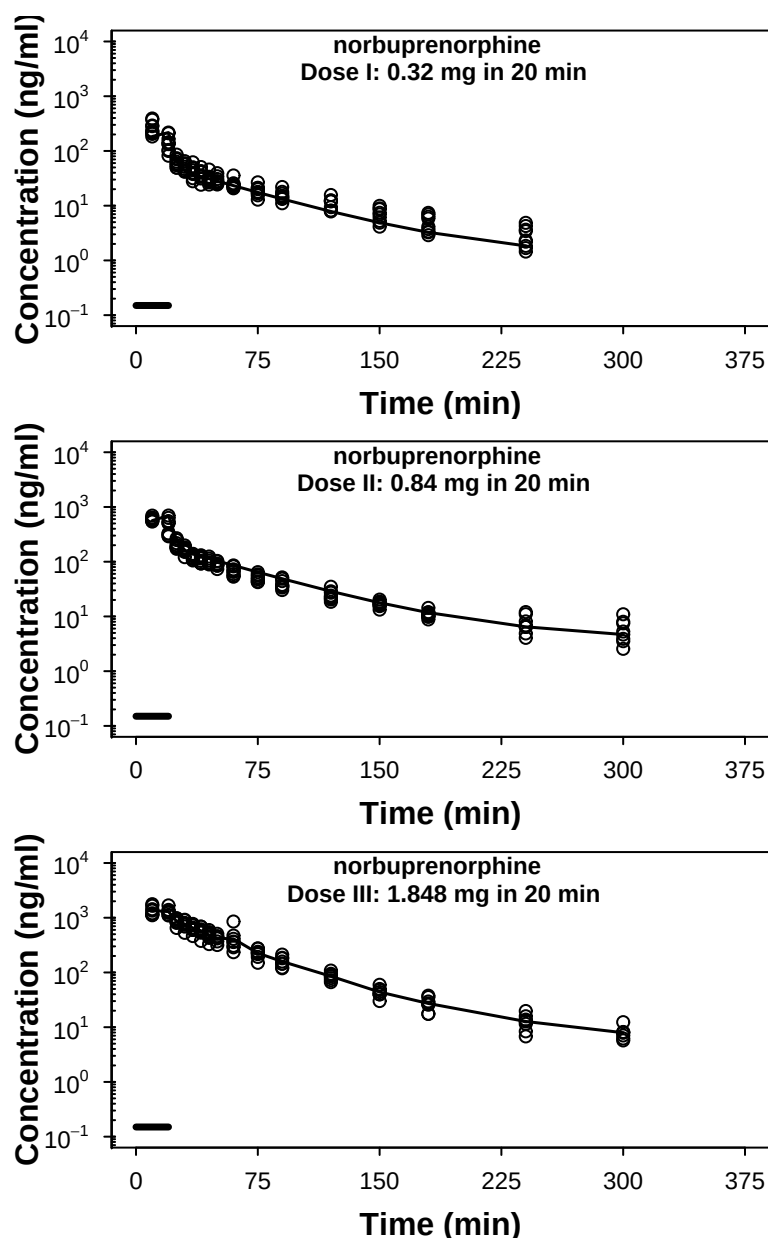


Figure 3. Individual norbuprenorphine concentration-time profiles for the treatment groups I-III. The observed concentrations (symbols) and mean population predictions (thick line) are depicted.

Integrated buprenorphine-norbuprenorphine PK model

The concentration-time profiles of buprenorphine and its metabolite norbuprenorphine, formed after buprenorphine administration and following separate administration of norbuprenorphine were best described by a six-compartment model that consisted of two three-compartment blocks that described the pharmacokinetics of buprenorphine, norbuprenorphine and the formation of norbuprenorphine. A schematic representation of the population PK model is shown in figure 1. Unique and precise pharmacokinetic parameters are obtained with an acceptable coefficient of variance ($< 50\%$) within the range of 6.0 % to 27.8 % for the structural PK parameter estimates. Inter-animal variability

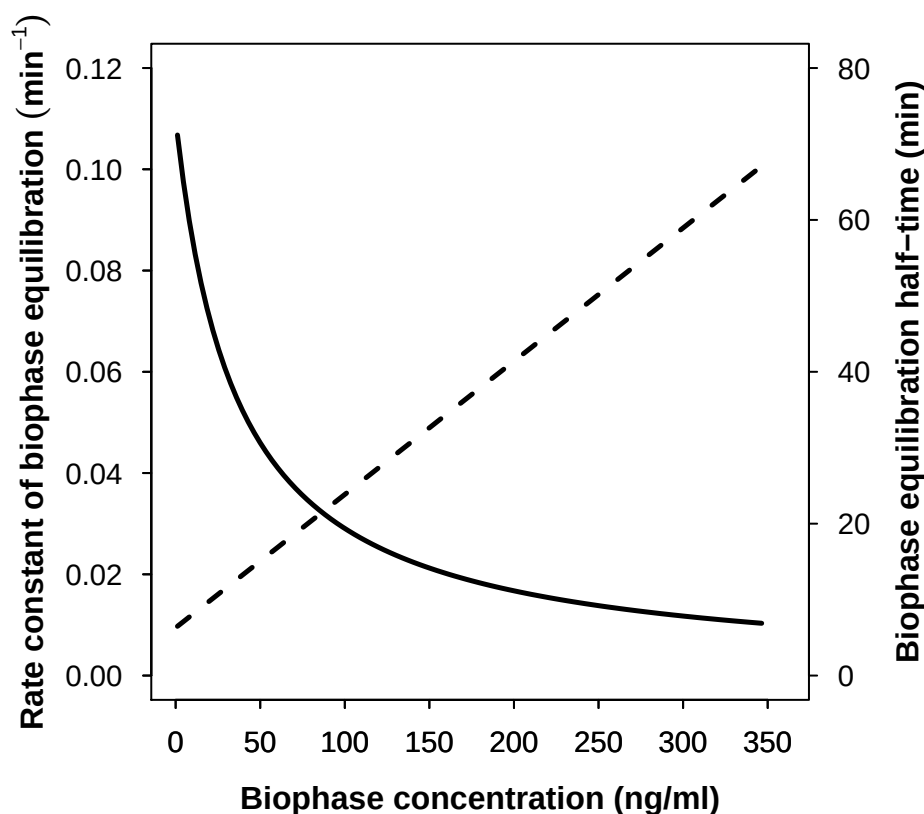


Figure 4. Non-linear pharmacodynamics of norbuprenorphine illustrated by non-linear dose-dependent biophase equilibration kinetics. The predicted biophase equilibration rate constant (solid line and left y-axis) and biophase equilibration half-life (dashed line and right y-axis) of norbuprenorphine versus biophase concentration are shown.

was estimated for k_{13} , k_{31} , V_1 , k_{conv} , $V_m^{k_{40}}$ and k_{64} . The parameter estimates of the integrated drug-metabolite population PK model are presented in table 4. The fraction of norbuprenorphine formed following the administration of buprenorphine was calculated at 10.2 %. Representative simultaneous pharmacokinetics fits of buprenorphine and norbuprenorphine of six animals are shown in figure 6. The PK parameters of buprenorphine

	Typical value	IAV (%)
$V_m^{k_{e0}}$ (min ⁻¹)	0.113 (10.6)	NE
$K_m^{k_{e0}}$ (ng/ml)	35.9 (6.9)	NE
EC_{50} (ng/ml)	72.8 (22.7)	98.2 (40.5)
E_0 (ml/min)	59 (2.4)	7.9 (44.8)
n	0.14 (11.4)	NE
ARE (ml/min)	4.2 (12.0)	

^{||} additive residual error.

Table 3. Population pharmacodynamic estimates and inter-animal variability of norbuprenorphine. For the fixed and random parameter estimates the relative standard error (%) is displayed

and norbuprenorphine obtained using the parent-metabolite population PK model were used to assess the contribution of norbuprenorphine to the overall respiratory depressant effect following the administration of buprenorphine over a wide dose range (0.01 - 30 mg/kg intravenous infusion over 20 min) assuming a zero interaction. The predicted time courses of norbuprenorphine concentration and respiratory depression are shown in figure 7. Only following the administration of high dose 30 mg/kg buprenorphine the peak concentration exceeds that of the estimated EC_{50} value for norbuprenorphine of 72.8 ng/ml. The lower buprenorphine doses yield maximum norbuprenorphine concentrations which are much lower than the EC_{50} value.

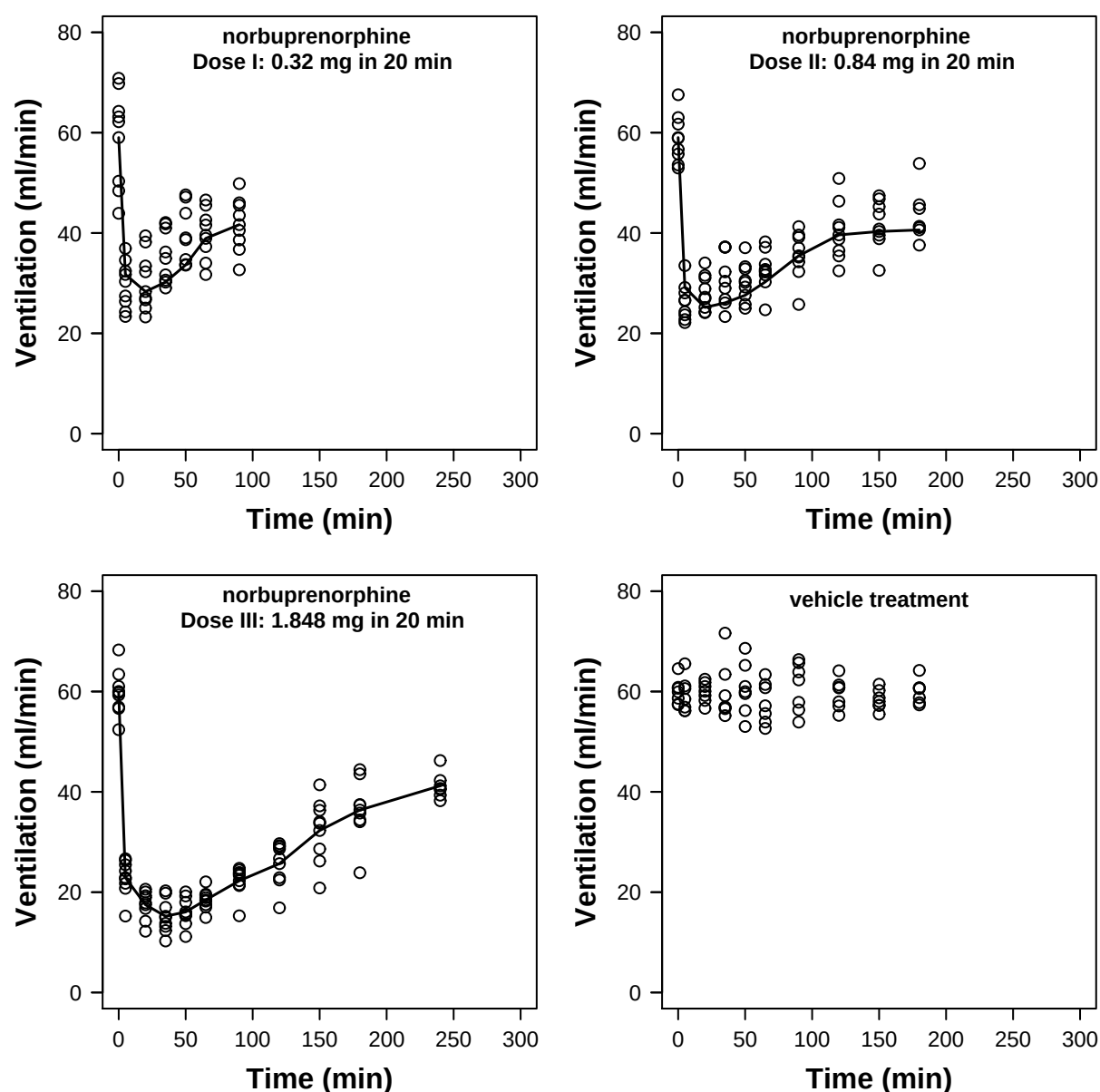


Figure 5. Changes in ventilation in time following administration of norbuprenorphine. For each treatment group (I-III) the observed (closed circles) and mean population predicted (solid line) time course of respiratory depression effect is shown.

	Typical value	IAV (%) [†]
buprenorphine		
k_{10} (min ⁻¹)	0.149 (11.1)	NE
k_{12} (min ⁻¹)	0.221 (18.4)	NE
k_{21} (min ⁻¹)	0.082 (10.6)	NE
k_{13} (min ⁻¹)	0.113 (12.4)	29.0 (25.0)
k_{31} (min ⁻¹)	0.009 (6.0)	16.2 (54.4)
V_1 (ml)	184 (11.1)	23.2 (27.4)
PRE (%)	23.7 (15.1)	
norbuprenorphine		
k_{conv} (min ⁻¹)	0.017 (17.0)	35.5 (37.5)
$V_m^{k_{40}}$ (min ⁻¹)	0.254 (12.4)	46.9 (33.1)
$K_m^{k_{40}}$ (ng/ml)	169 (21.1)	NE
k_{45} (min ⁻¹)	0.049 (27.8)	NE
k_{54} (min ⁻¹)	0.003 (21.3)	NE
k_{46} (min ⁻¹)	0.263 (11.8)	NE
k_{64} (min ⁻¹)	0.036 (9.1)	29.2 (45.0)
V_4 (ml)	205 (8.2)	NE
PRE (%)	24.3 (8.2)	

Table 4. Population PK estimates and inter-animal variability of the final integrated buprenorphine-norbuprenorphine population pharmacokinetic model. For the fixed and random parameter estimates the relative standard error (%) is displayed

DISCUSSION

The present investigation focuses on the PK-PD correlation of the respiratory depressant effect of norbuprenorphine, the N-dealkylated metabolite of buprenorphine. In this respect, the use of chronically-instrumented animal models to establish the PK-PD properties of norbuprenorphine is of importance, since metabolites can often not be administered separately to humans (Breimer and Danhof, 1997). The PK-PD correlation of the respiratory effect of norbuprenorphine has been studied previously (Ohtani *et al.*, 1997). It was shown that norbuprenorphine produces a dose-dependent increase in respiratory depression. However important questions with respect to the kinetics of onset and offset of norbuprenorphine's respiratory depressant effect and its contribution to the overall effect remained unanswered in that study. Despite the similarities in chemical structures, the *a priori* assumption that the observed hysteresis in the concentration-effect relationship of norbuprenorphine can also be explained by both biophase distribution and slow

receptor association-dissociation kinetics is not justified without further PK-PD model evaluation and discrimination. For norbuprenorphine, high values of the rate constants for receptor association-dissociation kinetics were obtained (table 2), indicating that receptor binding kinetics is fast and thus not rate-limiting. Evidently, buprenorphine and norbuprenorphine differ in their *in vivo* μ -opioid receptor association-dissociation kinetics. This is consistent with results from *in vitro* binding studies which show that norbuprenorphine binding to the μ -opioid receptor is more rapid and reversible compared to buprenorphine (Megarbane *et al.*, 2006).

An important issue is the *in vivo* intrinsic activity and potency of norbuprenorphine for respiratory depression. In the present analysis, norbuprenorphine's maximum respiratory depressant effect could not be estimated accurately and precisely on the basis of the biophase distribution model with the sigmoid $E_{\max}$ pharmacodynamic model. No clear maximum was observed in the ventilatory response data, indicating that the typical estimate of *in vivo* intrinsic activity is close to 1, a situation which previously has also been encountered with fentanyl (Yassen *et al.*, 2006). Thus, norbuprenorphine acts as a full agonist at the μ -opioid receptor displaying full respiratory depression. This is confirmed by the observation that the concentration-effect relationship was equally well described with a power pharmacodynamic model and a sigmoid $E_{\max}$ pharmacodynamic model, with a value of the intrinsic activity α of 1 (table 2). Norbuprenorphine's *in vivo* full agonistic activity at the μ -opioid receptor is confirmed by preclinical data from dedicated *in vitro* and *in vivo* (binding) studies (Huang *et al.*, 2001; Lutfy *et al.*, 2003). The *in vivo* potency (EC_{50}) of norbuprenorphine was estimated at 72.8 ng/ml which corresponds to 175 nM. The *in vivo* potency of buprenorphine in rats for respiratory depression was 0.88 nM and shows that norbuprenorphine is approximately 200-fold less potent than buprenorphine for respiratory depression. The binding properties and potency of norbuprenorphine have also been determined in Chinese hamster ovary (CHO) cells, expressing the μ -opioid receptor, using a [^{35} S]-GTP- γ -S functional binding assay (Huang *et al.*, 2001). The results of these *in vitro* studies show that norbuprenorphine is a full agonist at the μ -opioid receptor with an *in vitro* EC_{50} value of 1.5 nM. It should be noted that the *in vivo* EC_{50} is estimated on the basis of total plasma concentrations. Correction for the free fraction in plasma will result in a close similarity to the *in vitro* EC_{50} value.

PK-PD data analysis on the basis of the biophase distribution model with the power pharmacodynamic model revealed a dose-dependent decrease in k_{e0} , indicating saturable biophase distribution kinetics. This may be related to the involvement of active efflux transport mechanisms at the blood-brain barrier. ATP-binding cassette (ABC) transporters are increasingly recognized to be important for drug disposition and response of CNS drugs (Silverman, 1999; Schinkel and Jonker, 2003). For morphine, the role of active transporters like P-glycoprotein (PgP) in brain drug disposition has been well established (Xie *et al.*, 1999). For instance, it has been shown that inhibition of P-glycoprotein leads to higher concentrations of morphine in the brain and has been proven to be associated with an enhanced analgesic effect (Letrent *et al.*, 1999; Thompson *et al.*, 2000). To the

best of our knowledge it is not known whether norbuprenorphine is a substrate for P-glycoprotein or any other active efflux transport systems. Further research is warranted to address the role of active efflux transporters in norbuprenorphine's *in vivo* pharmacological effects.

An important issue is whether norbuprenorphine contributes to the observed respiratory depressant effect following administration of the parent drug buprenorphine. In the present study, an integrated population pharmacokinetic model was developed for the

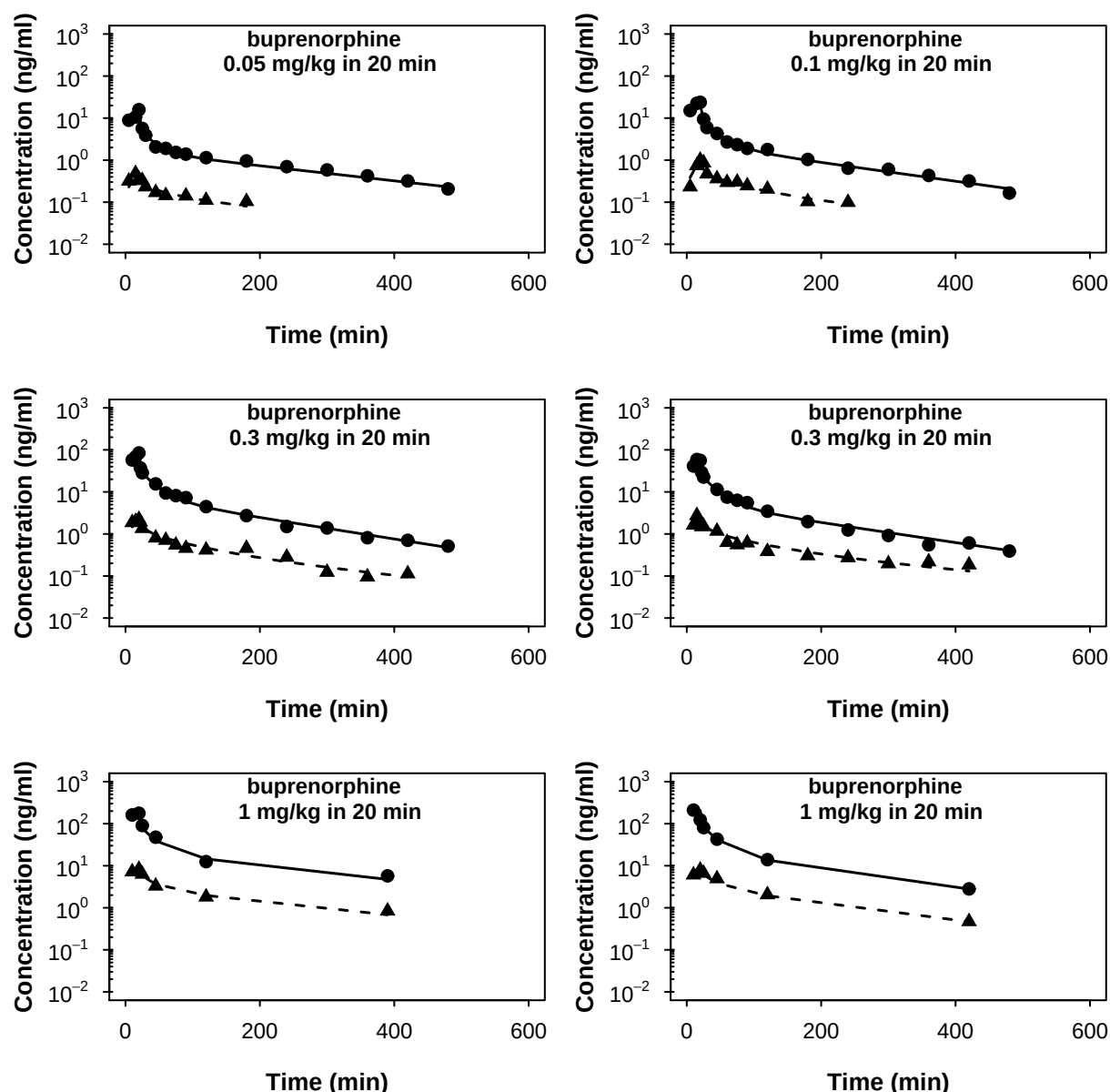


Figure 6. Representative fits of the time course of buprenorphine and norbuprenorphine concentration of six animals following the intravenous administration of buprenorphine. The symbols are the measured buprenorphine (circles) and norbuprenorphine (triangles) concentrations in plasma. The solid and dashed lines are the respective individual predicted time courses of buprenorphine and norbuprenorphine concentration obtained with the integrated drug-metabolite population pharmacokinetic model.

conversion of buprenorphine into norbuprenorphine. Pharmacokinetic analysis revealed that the elimination of norbuprenorphine is non-linear. The disposition of buprenorphine and norbuprenorphine in rats has been studied previously (Ohtani *et al.*, 1994; Gopal *et al.*, 2002). In one study (Gopal *et al.*, 2002), the pharmacokinetics of buprenorphine and norbuprenorphine were simultaneously analyzed after buprenorphine administration at a wide dose range of 0.1 - 30 mg/kg. Interestingly, it was shown, in contrast to the present data, that the pharmacokinetics of norbuprenorphine was not dose-dependent. It should be noted, however, that the measured norbuprenorphine concentrations in their study following the intravenous administration of 30 mg/kg buprenorphine or 1.0 mg/kg norbuprenorphine were lower than the measured norbuprenorphine concentrations in the present study upon intravenous administration of 0.32, 0.84 or 1.848 mg norbuprenorphine, suggesting that high doses of norbuprenorphine (> 0.84 mg) should be administered in order to identify non-linear pharmacokinetic behavior. On the other hand in the same study by Gopal *et al.* (2002) it was shown that buprenorphine displays dose-dependent pharmacokinetics, while in the present study linear pharmacokinetics is assumed for buprenorphine. Again, this is due the fact that the present dose range in which the disposition of buprenorphine was studied did not allow to identify dose-dependent pharmacokinetics. In view of the present results and those obtained by Gopal *et al.* (2002) it appears that the outcomes are not contradictory, but rather complementary. Nonetheless, concentration-dependent elimination of norbuprenorphine may explain the observation that the mean steady-state norbuprenorphine plasma concentration is comparable to or even exceeds the concentration of buprenorphine following chronic sublingual administration in humans (Kuhlman *et al.*, 1998).

Computer simulations with the drug-metabolite pharmacokinetic model showed that the contribution of norbuprenorphine to the overall respiratory depressant effect in rats is minimal within a wide dose range. This is on the assumption of a zero-interaction between buprenorphine and norbuprenorphine. The pharmacokinetic interaction between buprenorphine and norbuprenorphine which has been previously described is not taken into account. In the present drug-metabolite pharmacokinetic model the buprenorphine and norbuprenorphine concentrations following the administration of > 3.0 mg/kg buprenorphine are slightly overestimated (data not shown) in the terminal elimination phase compared to concentrations observed by Gopal *et al.* (2002). Taking into account norbuprenorphine's low *in vivo* potency, it is expected that the implication of buprenorphine's non-linear pharmacokinetics on the predicted contribution of norbuprenorphine's respiratory depressant effect is minimal, also considering that a only a minor fraction of buprenorphine is converted into norbuprenorphine.

In this respect it is important that both buprenorphine and norbuprenorphine act at the μ -opioid receptor. As a consequence the interaction between the two compounds is likely to be competitive. On theoretical grounds competitive interactions are never synergistic. Moreover, competitive interactions are readily predicted on the basis of the relative target affinities Jonker *et al.* (2005). However it cannot be excluded that the pharmacody-

namic interaction is indeed more complex, also in the light that in addition to the μ also the δ -opioid receptor subtype may be involved in the respiratory depressant effect (Su *et al.*, 1998; Gengo *et al.*, 2003).

The implications for dosing of buprenorphine in human are not known, especially upon chronic use. An important utility of the integrated parent-metabolite population

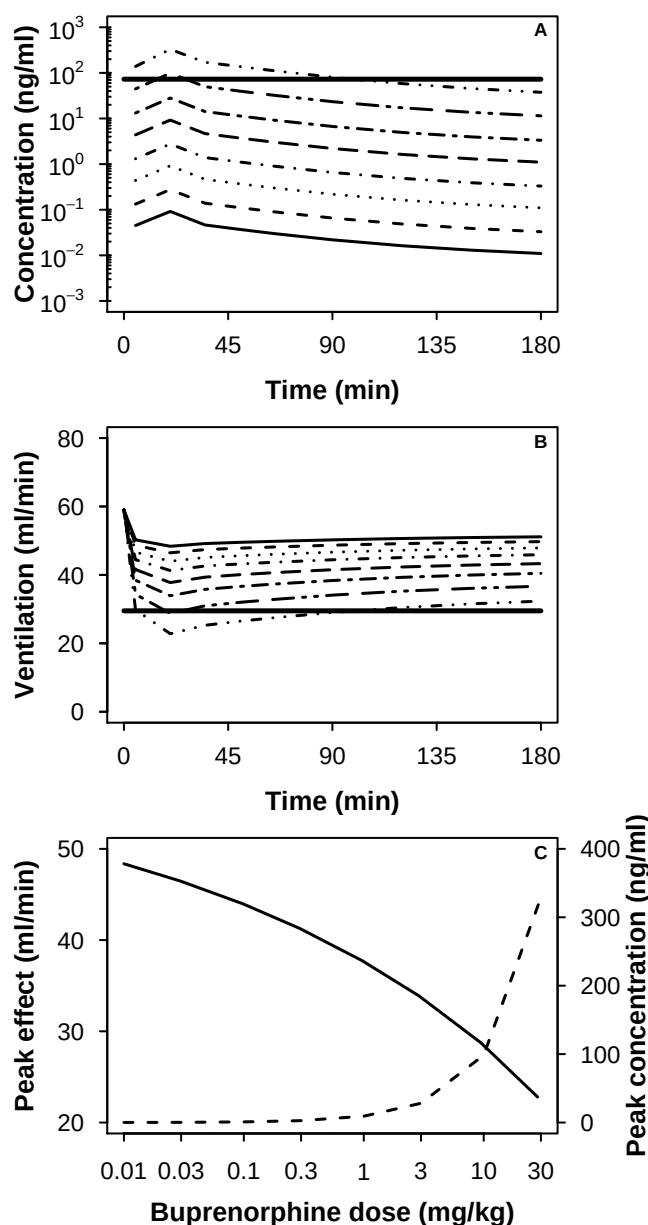


Figure 7. Simulation of the contribution of norbuprenorphine to the overall respiratory depressant effect following the administration of buprenorphine over a wide dose range (0.01 - 30 mg/kg). In the upper panel (A), the predicted time courses of norbuprenorphine is shown. In the middle panel (B), its respective predicted respiratory depressant effect is displayed. In the lower panel (C), the dose versus peak norbuprenorphine plasma concentration (dashed line and right y-axis) and the dose versus maximum respiratory depressant effect (solid line and left y-axis). The predicted time courses of norbuprenorphine concentration and respiratory effect were obtained on the basis of the population pharmacokinetic parameter estimates obtained with the integrated drug-metabolite PK model and the final population pharmacodynamic parameters of norbuprenorphine.

PK-PD model for further investigations is the prediction of the concentration-effect relationship of norbuprenorphine in human. There is evidence that allometric models are accurate predictors for animal to human pharmacokinetic extrapolation of renally excreted drugs or high hepatic extraction drugs (i.e. buprenorphine) (Holford, 1996; West *et al.*, 1997). With respect to the pharmacodynamics, the μ -opioid receptor displays a degree of homology between rats and humans (Rothman *et al.*, 1995). Previously, a close correlation has been established between the *in vitro* receptor affinity and the *in vivo* potency for the EEG effect for synthetic opioids. In addition, it was shown that *in vivo* potency obtained in rats correlates nicely with the *in vivo* potency obtained in humans (Cox, 1997). Whether a similar correlation exists for the respiratory depressant or analgesic effect remains yet unanswered. This will be subject of further investigation in our laboratory.

In conclusion, the pharmacodynamics of the metabolite norbuprenorphine is distinctly different from the pharmacodynamics of the parent compound buprenorphine, both with regard to the receptor association/dissociation kinetics, the *in vivo* potency and the intrinsic efficacy for the respiratory depressant effect. Following intravenous administration of buprenorphine, only a small fraction of buprenorphine is converted into norbuprenorphine. The values of these norbuprenorphine concentrations are well below the values causing an effect on respiration. Therefore, norbuprenorphine does not contribute to the overall respiratory depressant effect of buprenorphine. This is in line with the experience from clinical use of buprenorphine in patients.

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**SECTION
IV**

**APPLICATION OF THE COMBINED
BIOPHASE DISTRIBUTION-RECEPTOR
ASSOCIATION/DISSOCIATION MODEL
IN THE PK-PD ANALYSIS OF BUPRENORPHINE**

PHARMACOKINETIC-PHARMACODYNAMIC MODELING OF THE EFFECTIVENESS AND SAFETY OF BUPRENORPHINE AND FENTANYL IN RATS

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ABSTRACT

Respiratory depression is serious and potentially life-threatening side-effect of opioid therapy. The objective of this investigation was to characterize the relationship between buprenorphine or fentanyl exposure and the effectiveness and safety outcome in rats. Data on the time course of the antinociceptive and respiratory depressant effect were analyzed on the basis of population logistic regression PK-PD models using non-linear mixed effects modeling software (NONMEM).

The pharmacokinetics of buprenorphine and fentanyl were described by a three- and two-compartment model, respectively. A logistic regression model (linear logit model) was used to characterize the relationship between drug exposure and the binary effectiveness and safety outcome. For buprenorphine, the odds ratios (OR) were 28.5 (95 % CI: 6.9 - 50.1) and 2.10 (95 % CI: 0.71 - 3.49) for the antinociceptive and respiratory depressant effect, respectively. For fentanyl these odds ratios were 3.03 (95 % CI: 1.87 - 4.21) and 2.54 (95 % CI: 1.26 - 3.82), respectively. The calculated safety index ($OR_{\text{antinociception}}/OR_{\text{respiratory depression}}$) for fentanyl of 1.20 suggests that fentanyl has a low safety margin, implicating that fentanyl needs to be titrated with caution. For buprenorphine the safety index is 13.54 suggesting that buprenorphine is a relatively safe opioid.

INTRODUCTION

Worldwide millions of people suffer from severe (uncontrolled) and sometimes incurable pain and are dependent on the daily intake of analgesic drugs to relief their pain. Opioid analgesics are among the most effective agents currently available for treating post-operative and chronic (malignant) pain. Apart from their analgesic actions, all opioids produce side effects (Bowdle, 1998; Gan, 2006). Although most of these side effects are mild in nature and have little clinical relevance, respiratory depression is a potentially life-threatening complication of opioid therapy (Baxter, 1994; Bailey *et al.*, 2000). Therefore, careful titration of the opioid dose to the desired analgesic effect is essential to avoid respiratory depression (Lejus *et al.*, 1996). This applies particularly to potent opioid analgesics. Fentanyl is a μ -opioid agonist which is frequently used both in anesthesia and for the treatment of acute and chronic pain (Scott *et al.*, 1991; Modesti *et al.*, 2006; Waara-Wolleat *et al.*, 2006).

In pharmacological terms, fentanyl is a high-efficacy full agonist at the μ -opioid receptor with steep concentration-response curves (Cox *et al.*, 1998; Woodhouse and Mather, 2000). Due to the relatively narrow therapeutic window of fentanyl the risk of severe fatal respiratory depression is rather large. As a result fentanyl dosing needs to be carefully titrated to the optimal analgesic effect (Dahan *et al.*, 2005). To facilitate fentanyl dosing, advanced drug delivery systems, aiming at an improved selectivity of action have been introduced. Slow release preparations and transdermal patches for opioids are examples of drug delivery systems which have been developed with the aim to optimize opioid delivery and to reduced side effects (Skaer, 2006).

Another approach towards the reduction of side effects is the use of compounds with a more favorable effectiveness-safety balance. In this respect it is of interest that in theory, partial agonists can have a greater selectivity of action than full agonists. Considering the potential lethality of opioid-induced respiratory depression, partial agonists at the μ -opioid receptor provide potential advantages over full agonists, in terms of the effectiveness-safety balance for the treatment of pain. Buprenorphine is a semi-synthetic opioid, which has been in clinical use for the treatment of acute and chronic pain since 1979 (Budd, 2003). Recently, it has been demonstrated that buprenorphine behaves as a partial agonist with respect to respiratory depression both in rats (Yassen *et al.*, 2005) and in humans (Yassen *et al.*, 2006a), consistent with its partial agonistic behavior in *in vitro* bioassays (Huang *et al.*, 2001). An important consequence of the observed 'ceiling effect' is that an overdose of buprenorphine is less likely to cause severe and potentially life-threatening respiratory depression. Interestingly, for the antinociceptive effect, buprenorphine displays no ceiling effect in the same concentration range in which maximum respiratory depression is elicited, indicating that within the therapeutic concentration range buprenorphine acts as a full agonists with regard to the antinociceptive effect (Yassen *et al.*, 2005, 2006b). Differences in receptor expression in the distinct brain

tissues can explain why buprenorphine behaves as partial agonist for one effect (respiratory depression, in brain tissue with no receptor reserve) and as a full agonist for another effect (antinociception, in brain tissue with receptor reserve) (der Graaf *et al.*, 1999).

The objective of this study was to characterize the effectiveness and safety of buprenorphine and fentanyl in rats using non-linear mixed effects logistic regression analysis. Therapeutic utility functions (Graham *et al.*, 2002; Jonsson and Karlsson, 2005; Sheiner and Melmon, 1978; Zanderigo *et al.*, 2006) were constructed to assess the effectiveness/safety balances of buprenorphine and fentanyl.

MATERIALS AND METHODS

Animal data for the PK-PD analysis of the safety and effectiveness of buprenorphine and fentanyl were obtained from two previously published studies on the PK-PD relationships of buprenorphine and fentanyl for the antinociceptive (Yassen *et al.*, 2005) and respiratory depressant effect (Yassen *et al.*, 2006a). The protocols of the investigations were approved by the Ethical Committee on Animal Experimentation of Leiden University.

Briefly, the experimental details of the investigations were as follows. Male Wistar rats (Charles River BV, Zeist, The Netherlands), weighting initially 225 - 250 g, were used in the studies. Two days before the start of the experiment, indwelling cannulae were implanted in the right femoral artery for the serial collection of blood samples and in the right jugular vein for drug administration. Surgical procedures were performed under anesthesia with an intramuscular injection of 0.1 mg/kg medetomidine hydrochloride (Domitor, 1 mg/ml, Pfizer, Capelle a/d IJssel, The Netherlands) and 1 mg/kg Ketamine base (Ketalar 50 mg/ml, Parke-Davis, Hoofddorp, The Netherlands).

Different doses of buprenorphine (0.025 to 0.1 mg/kg) and fentanyl (0.015 -0.04 mg/kg) were tested to characterize the concentration-antinociceptive effect relationships. For respiratory depression, buprenorphine and fentanyl were tested in the dose ranges 0.025 - 0.3 mg/kg and 0.03 - 0.085 mg/kg, respectively. Buprenorphine and fentanyl were administered via a zero-order intravenous infusion (infusion duration 20 - 40 min). Antinociception or respiratory depression were measured at pre-defined time points. Serial arterial blood samples were collected at fixed time-points. 50 μ l plasma was separated from the blood by centrifugation and the plasma samples were stored at -20 °C prior to analysis.

Measurement of antinociception

A tail flick analgesia meter (Columbus Instruments, Columbus, Ohio, USA) was used to determine the pain sensitivity in the control and the drug-treated rats (D'Amour and Smith, 1941). Radiant heat was applied using a shutter-controlled lamp as a heat source focused on a spot located 6.5 to 7.5 cm from the tip of the tail. The intensity of the beam was set at a level producing basal latency times between 2.5 and 3.5 s. To prevent tissue injury,

the cut-off time was fixed at 10 s. A digital response time indicator with a resolution of 0.1 s measured the time between activation of the light beam and the tail flick.

Measurement of respiratory depression

Respiratory depression was determined in unrestrained, conscious animals using whole-body plethysmography for the quantification of ventilation (Model PLY3223, Buxco Electronics Inc.,UK). Briefly, the animals were placed in a plethysmograph, consisting of a measurement chamber and an integrated reference chamber to correct for atmospheric disturbances. Both chambers were connected to a differential pressure transducer (TRD5700, Buxco Electronics Inc.,UK). In each animal the effects of opioids and vehicle on ventilation were assessed at an inhaled concentration of 6.5 % carbon dioxide on a background of normoxia (20 % oxygen). The inhalation of the gas mixture lasted 5 min to ensure that steady-state ventilation had been reached. Tidal volume (V_T), breathing frequency (RR) and minute ventilation (V_i , where $V_i = V_T \times \text{RR}$) were obtained from changes in chamber pressure using a low-pressure differential transducer connected to preamplifier modules (MAX2270, Buxco Electronics Inc.,UK). During the experiment body temperature was maintained at 37.5 °C using heating pads. Body temperature was monitored continuously by radiotelemetry (Model RPC-1, Data Sciences International, USA).

Determination of Buprenorphine and Fentanyl Concentrations

Two different chemical assays were used for the determination of buprenorphine and fentanyl plasma concentration in rats. The details of these assays have been described elsewhere (Yassen *et al.*, 2005). Briefly for buprenorphine, to 50 μl plasma 25 μl internal standard (4 mg/100 ml $^2\text{H}_4$ -buprenorphine) was added. Subsequently, 25 μl concentrated ammonia was added and the samples were extracted by liquid/liquid extraction with 600 μl tert.-butyl methyl ether (MTBE). The chromatographic system consisted of an Agilent HP 1100 HPLC system (Agilent, Waldbronn, Germany) coupled to an API 4000 LC/MS/MS system (Applied Biosystems, Darmstadt, Germany). Chromatography was performed on a precolumn (Metaguard Polaris 3 μm C_{18A} 2 mm, Varian Darmstadt, Germany) guarded Synergi 4 μm Hydro-RP 80A column 75 mm x 2 mm (Phenomenex, Aschaffenburg, Germany). The lower limit of quantification (LLOQ) was 0.047 ng/ml for buprenorphine. The accuracy ranged from 99.4 to 102.1 %. The precision expressed as coefficient of variation, ranged from 2.2 to 6.4 % for concentrations between 0.14 and 8.9 ng/ml.

For fentanyl, to 50 μl plasma 25 μl internal standard (14.8 ng/ml $^2\text{H}_5$ -fentanyl) was added. After adding 10 μl concentrated ammonia the samples were extracted by liquid/liquid extraction with 600 μl tert.-butyl methyl ether (MTBE). The chromatographic system consisted of an Agilent HP 1100 HPLC system (Agilent, Waldbronn, Germany) coupled to an API 3000 LC/MS/MS system (Applied Biosystems, Darmstadt, Germany). Chromatography was performed on a precolumn (Metaguard Polaris 3 μm C_{18A} 2 mm, Var-

ian Darmstadt, Germany) guarded Atlantis C₁₈ column 3 μ m 100 mm x 2.1 mm (Waters, Eschborn, Germany). The lower limit of quantification (LLOQ) was 0.118 ng/ml. The accuracy ranged from 87.0 to 96.1 % and the precision from 1.9 to 4.0 % for concentrations in the range from 0.4 to 50.2 ng/ml.

PK-PD modeling procedure

The pharmacokinetic and pharmacodynamic parameters of fentanyl and buprenorphine were estimated using non-linear mixed-effects modeling as implemented in the NONMEM software (Version V, level 1.1) (Beal and Sheiner, 1999). For pharmacokinetic analysis, a population analysis approach using the first order conditional estimation method with η - ϵ interaction (FOCE interaction) was undertaken. For logistic regression pharmacodynamic analysis, the maximum likelihood estimation algorithm in NONMEM with the Laplacian method (FOCE) and the likelihood option was used to estimate the probability of a certain event occurring. All fitting procedures were performed on an IBM-compatible computer (Pentium IV, 1500 MHz) running under Windows XP with the Fortran compiler Compaq Visual Fortran version 6.1. An in-house available S-PLUS 6.0 (Insightful Corp., Seattle, WA, USA) interface to NONMEM was used for data processing and management (including tools to perform non-parametric bootstrap) and graphical data display. Model comparisons were based on the objective function values in NONMEM using the likelihood ratio test. The significance level was set at $\alpha=0.01$, which corresponds to a reduction of 6.6 units in objective function value (OFV; χ^2 distribution) to discriminate between two nested structural models after inclusion of one additional parameter. Furthermore, the goodness-of-fit of the pharmacodynamic models was evaluated by simulating values of the effectiveness and safety outcome from the raw data. The simulations were repeated a total of a 100 times. The proportions of observed and simulated scores were plotted *versus* the 25th, 50th (median), 75th quantiles of the pooled opioid biophase concentrations.

Pharmacokinetic analysis

One, two and three-compartment linear models were evaluated to characterize the time course of buprenorphine and fentanyl concentration in plasma. The pharmacokinetic parameters were assumed to be log-normally distributed and therefore an exponential distribution model was used to account for inter-animal variability. Proportional error models were used to quantify residual error. Individual Bayesian pharmacokinetic parameter estimates obtained from the final population pharmacokinetic model served as input for pharmacodynamic model to predict fentanyl and buprenorphine concentrations at observation scores time points (two-stage approach). The extent of Bayesian shrinkage was evaluated for each parameter in the final population pharmacokinetic models of

buprenorphine and fentanyl by (Mould *et al.*):

$$\text{Shrinkage} = 1 - \frac{SD_{\eta \text{ PK parameter}}}{\omega_{\text{PK parameter}}} \quad (1)$$

in which the SD is the standard deviation of the individual estimates of η for each pharmacokinetic parameter and $\omega_{\text{PK parameter}}$ is the estimate of the standard deviation of the estimated population variance.

To quantify the precision of the pharmacokinetic parameters estimates a non-parametric bootstrap was performed. Briefly, one thousand bootstrap replicates were generated by sampling randomly from the original data set with replacement. Subsequently, the data sets were fitted one at the time to the final population PK model. The mean, standard error, coefficient of variation and the lower and upper 95 % prediction intervals of the model parameter values were calculated and compared to the typical model parameter values from the original study.

Pharmacodynamic data analysis

The effectiveness (antinociception) and safety (respiratory depression) variable were modeled as dichotomous category variables. The following transformation statements were applied to convert the continuous data into logical two-valued data:

- If Antinociceptive Effect = 10 s, then observation score is 1 (a yes response; complete antinociception) else 0 (a no response; no antinociception)
- If Respiratory depressant Effect $\geq$ 50 %, then observation score is 1 (a yes response; severe respiratory depression) else 0 (a no response; no respiratory depression)

Logistic regression analysis was performed to determine the relationship between opioid concentration at the biophase (C_e) and its respective antinociceptive or respiratory depressant effect in rats. The opioid biophase concentration was predicted using a standard effect-compartment model:

$$\frac{dC_e}{dt} = k_{e0} \cdot (C_p - C_e) \quad (2)$$

in which C_p represents the opioid plasma concentration, C_e the opioid concentration at the biophase and k_{e0} the first-order rate constant for biophase distribution. A linear logit model was fitted to relate the probability of having complete antinociception or respiratory depression to drug exposure at the biophase:

$$P_{(Y_{ij}=1)} = \frac{1}{1 + e^{-f(C_e)}} \quad (3)$$

The logit transformation ensures that the probability P will fall between 0 and 1, $f(C_e)$ is a linear function of drug exposure:

$$f(C_e) = \alpha + \beta \cdot C_e \quad (4)$$

in which α and β are the intercept and slope of the regression equation and C_e is the drug plasma concentration. Incorporation of individual random effects, η with mean zero and variance ω^2 , was tried by assuming exponential distributions for the regression parameters (α and β). For each concentration-effect relationship, the odds ratio was calculated using the exponent of the estimated slope β (e^β). The odds ratio is defined as the change in the estimated odds of the outcome when the plasma concentration increases by 1 unit. Bootstrap analysis was performed with the final logistic regression pharmacodynamic models to construct the 95 % confidence intervals of the calculated odds ratios.

Finally, an overall ordinal utility function was constructed to evaluate the effectiveness/safety balance of buprenorphine and fentanyl as a function of drug exposure. The utility function was determined by the sum of the weighted probabilities of an efficacious and safe outcome. The effectiveness and safety variables were weighted equally. For the desirable antinociceptive effect the weight factor was positive and for respiratory depression the factor was negative. The effectiveness/safety balance, characterized by the utility function was thus expressed as $\text{Pr}\{\text{effectiveness}\} - \text{Pr}\{\text{safety}\}$, where $\text{Pr}\{x\}$ is the probability of outcome x (Cullberg *et al.*, 2005). A negative value of the utility function indicates that the respiratory depressant effect dominates and a positive value suggests that the desired antinociceptive effect dominates.

RESULTS

Pharmacokinetics

A three-compartment model adequately described the time course of the concentration profiles of buprenorphine after intravenous administration in rats. Inter-individual variability was estimated for all pharmacokinetic parameters except for Q_3 . The parameter estimates with their corresponding standard error (SE) are summarized in table 1. For fentanyl, a two-compartment pharmacokinetic model with inter-animal variability on all pharmacokinetic parameters best described the disposition in plasma. The parameter estimates with their corresponding standard error (SE) are summarized in table 2. Estimates of shrinkage were low and ranged from 0.02 (Cl) to 0.19 (V_1) for buprenorphine and from 0.03 (Cl) to 0.12 (V_2) for fentanyl, suggesting that the individual pharmacokinetic parameter estimates of buprenorphine and fentanyl are robust. For comparison, values of shrinkage close to 1 indicate poor individual estimates of the pharmacokinetic parameters. The goodness-of-fit plots (population predicted/individual predicted *versus* observed concentrations, population predicted concentrations *versus* weighted residuals

for population predictions (WRES) and time *versus* weighted residuals for population predictions (figure 1 and 2) indicated that the pharmacokinetic models adequately described the concentrations of buprenorphine and fentanyl. Finally, bootstrap analysis showed that the 95 % CI of the pharmacokinetic parameter estimates and their random effect variables, except for $\omega^2 V_2$ of buprenorphine, did not overlap their respective null values (table 1 and 2).

Table 1. Parameter estimates of the final population pharmacokinetic model for buprenorphine and the stability of the parameters using the bootstrap resampling procedure. For the fixed and random parameter estimates the standard error is displayed

	Original data set		1000 bootstrap replicates	
	Typical value	ω^2 †	Typical value	ω^2
Cl (ml/min)	18 (0.87)	0.128 (0.01)	18 (0.97)	0.125 (0.02)
V_1 (ml)	110 (16.6)	0.936 (0.30)	108 (21.6)	0.914 (0.33)
V_2 (ml)	370 (29.4)	0.274 (0.08)	400 (41)	0.353 (0.20)
V_3 (ml)	1200 (97.6)	0.337 (0.07)	1181 (140)	0.239 (0.09)
Q_2 (ml/min)	30 (2.0)	NE ‡	30.3 (2.3)	NE
Q_3 (ml/min)	11 (0.73)	0.172 (0.04)	10.2 (1.2)	0.128 (0.09)
σ^2 #	0.032 (0.005)		0.033 (0.006)	

† inter-animal variability ($\sqrt{\omega^2} \cdot 100$ %); ‡ not estimated; # proportional residual error ($\sqrt{\sigma^2} \cdot 100$ %)

Table 2. Parameter estimates of the final population pharmacokinetic model for fentanyl and the stability of the parameters using the bootstrap resampling procedure. For the fixed and random parameter estimates the standard error is displayed

	Original data set		1000 bootstrap replicates	
	Typical value	ω^2	Typical value	ω^2
Cl (ml/min)	17 (0.5)	0.060 (0.01)	16.6 (0.49)	0.058 (0.01)
V_1 (ml)	250 (16.2)	0.227 (0.05)	255 (16.4)	0.224 (0.05)
V_2 (ml)	440 (18.3)	0.080 (0.02)	444 (18.1)	0.077 (0.02)
Q (ml/min)	9.7 (0.70)	0.224 (0.05)	9.7 (0.71)	0.216 (0.05)
σ^2	0.034 (0.005)		0.034 (0.005)	

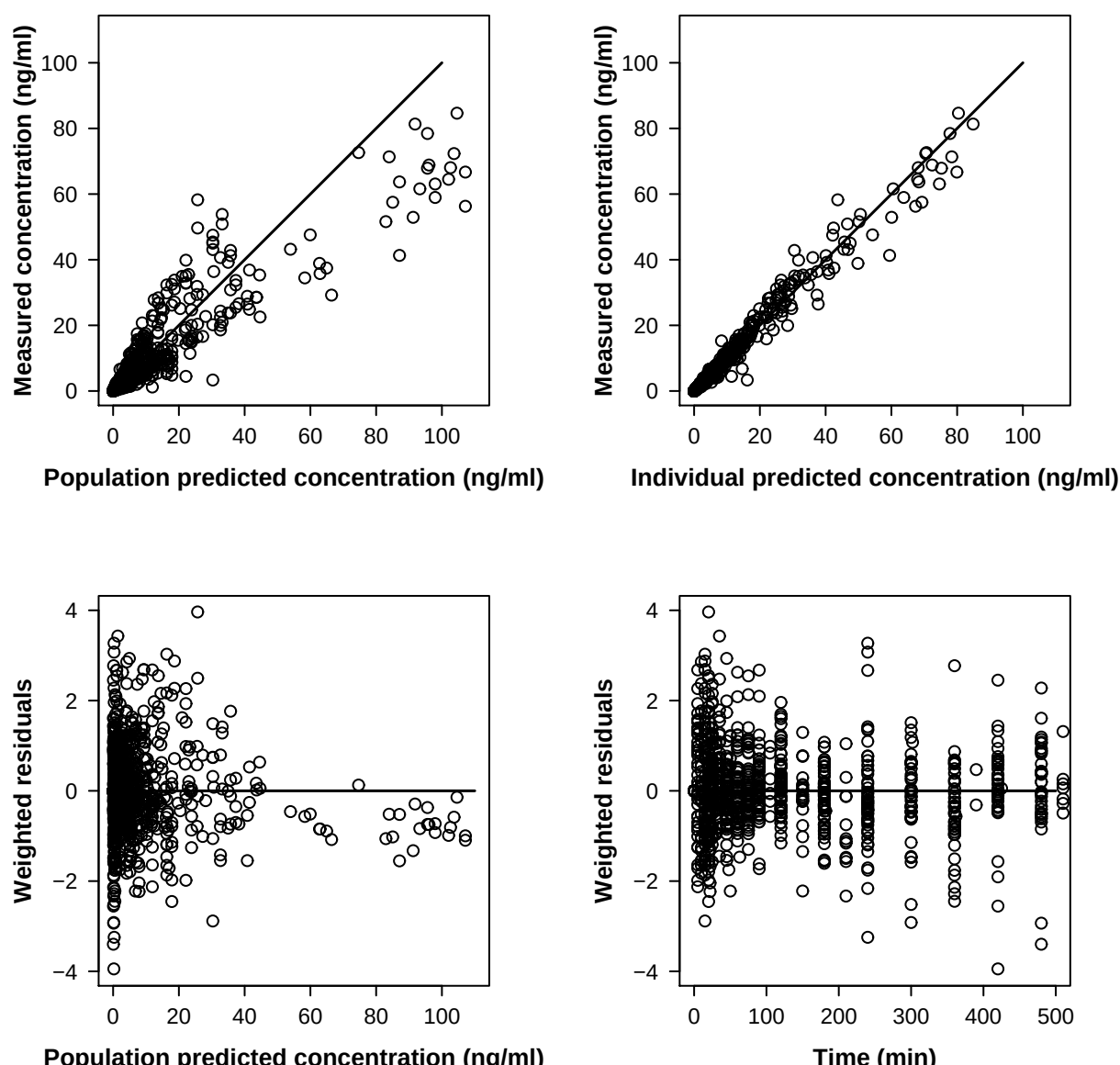


Figure 1. Goodness-of-fit plots for the final population pharmacokinetic model of buprenorphine.

Pharmacodynamics

After transformation of the continuous data into categories, 615 (35 animals) and 444 (32 animals) observations of response/no response scores to complete antinociception and severe respiratory depression, respectively, were available for analysis of the pharmacodynamic effect of buprenorphine in rats. For fentanyl, 486 (40 animals) and 216 (32 animals) observations were included for pharmacodynamic analysis for the antinociceptive and respiratory depressant effect, respectively. Population logistic regression PD models were used to predict the probability of a response to complete antinociception or severe respiratory depression to plasma drug exposure.

For the antinociceptive effect of buprenorphine, the values of parameters characterizing the linear logistic regression model were -13.3 (95 % CI: -16.08 - -10.52) for the intercept α and 3.35 (95 % CI: 2.59 - 4.11) for the slope β of the regression equation.

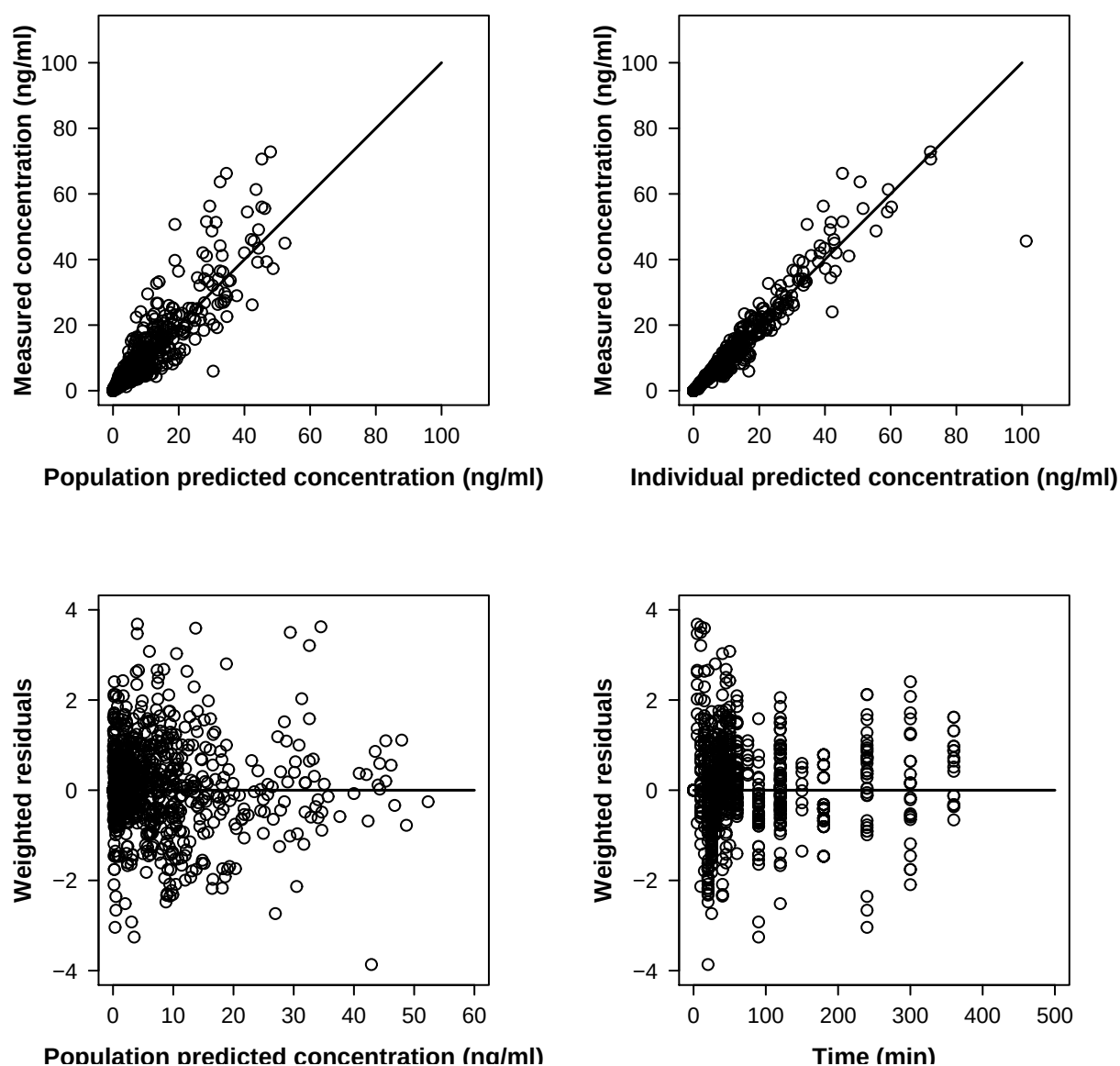


Figure 2. Goodness-of-fit plots for the final population pharmacokinetic model of fentanyl.

The odds ratio of complete antinociception occurring is equal to $e^{3.35}$, corresponding to an odds ratio of 28.5 (95 % CI: 6.9 - 50.1). The odds ratio is statistically different from 1, indicating that the probability of complete antinociception occurring is dependent on the buprenorphine plasma concentration. Inter-animal variability, ω^2 was estimated for β and was 0.130 (95 % CI: 0.08 - 0.181). For respiratory depression, the typical values estimates for the intercept (α) and slope (β) of the regression equation were -5.22 (95 % CI: -6.54 - -3.90) and 0.744 (95 % CI: 0.083 - 1.405), respectively. The odds ratio of severe respiratory depression occurring is equal to $e^{0.774}$, corresponding to an odds ratio of 2.10 (95 % CI: 0.71 - 3.49). The odds ratio is not statistically different from 1, indicating that the probability of observing severe respiratory depression does not change within the studied buprenorphine biophase concentration range. Inter-animal variability, ω^2 was estimated for β and was 1.82 (95 % CI: 0.57 - 3.07). The odds ratio estimates of buprenorphine obtained from the logistic regression analysis are shown in table 3. Goodness-of-fit was

evaluated by the objective function value (OFV) and by simulating the raw data from the final pharmacodynamic logistic regression model. The proportion of the simulated values of the outcome scores for buprenorphine agreed well with the observed scores as can be seen in figure 3.

For fentanyl, the values of the parameters α and β were -8.77 (95 % CI: -11.49 - -6.05) and 1.11 (95 % CI: 0.726 - 1.494), respectively. The odds ratio for the antinociceptive effect was 3.03 (95 % CI: 1.87 - 4.21) and was significantly different from 1 ($p < 0.05$). Inter-animal variability, ω^2 was estimated for and was 0.0839 (95 % CI: -0.01 - 0.177). For the respiratory depressant effect the values of the logistic regression parameters and were -5.86 (95 % CI: -8.17 - -3.55) and 0.930 (95 % CI: 0.430 - 1.430). The odds ratio for the occurrence of severe respiratory depression was 2.54 (95 % CI: 1.26 - 3.82). Inter-animal variability, ω^2 was estimated for β and was 0.193 (95 % CI: 0.085 - 0.300). The odds ratio estimates of fentanyl obtained from the logistic regression analysis are shown in table 3. The goodness-of-fit plots for fentanyl are shown in figure 4. . The goodness-of-fit plots for fentanyl are shown in figure 4. In the final analysis, the half-time of biophase distribution was included in the final model. Although its value is not significantly different from zero, its inclusion significantly improved the fits as judged by a significant drop (> 6.6 points) in objective function value.

Finally, a therapeutic overall utility function was constructed for the effectiveness/safety balance of buprenorphine and fentanyl. In figure 5, it is shown that in the measured concentration range, the utility function of buprenorphine is positive. This indicates that the antinociceptive effectiveness of buprenorphine exceeds the risk of the occurrence of respiratory depression. For fentanyl, the utility function is negative in the measured concentration range, indicating that fentanyl produced more respiratory depression than buprenorphine. At relatively high concentrations the utility function is close to zero for fentanyl and buprenorphine, suggesting that the probability of a desired and side effect are equal.

Table 3. Odds ratio and half-time of biophase distribution ($t_{1/2,ke0}$) estimates (95 % CI) obtained from the logistic regression pharmacodynamic analysis for buprenorphine and fentanyl

	Buprenorphine	Fentanyl
	$t_{1/2,ke0}$ (min)	
Antinociception	86.4 (71.3 - 101.5)	6.08 (4.43 - 7.73)
Respiratory depression	109 (49 - 169)	1.97 (-0.32 - 4.26)
	Odds ratio	
Antinociception	28.5 (6.9 - 50.1)	3.03 (1.87 - 4.21)
Respiratory depression	2.10 (0.71 - 3.49)	2.54 (1.26 - 3.82)

DISCUSSION

Population PK-PD models of buprenorphine and fentanyl, administered intravenously with plasma concentration as the exposure variable and the response to complete antinociception or severe respiratory depression as a binary outcome variable were developed to evaluate the effectiveness and safety of these opioids in rats.

The assignment of observation scores 0 and 1 to the pharmacodynamic safety outcome to indicate "no" or "severe" respiratory depression, respectively, was arbitrarily chosen and is a matter of discussion. Despite its severity, there is no clear and unifying definition for respiratory depression in the literature (Ko *et al.*, 2003). Partly this is related to the difficulty of measuring opioid-induced respiratory depression in a reliable and strictly quantitative manner. Although various biomarkers for respiratory depression have been proposed, including the measurement of respiratory rate (Grace *et al.*, 1995) and arterial $P_a\text{CO}_2$ values (Jacobson *et al.*, 1989), many of these biomarkers are rather subjective and only provide a qualitative assessment of respiratory depression (Gustafsson *et al.*, 1982). Furthermore, many of the techniques used to characterize respiratory depression, may well underestimate the respiratory depressant effect of opioids (Dahan *et al.*, 2005). In contrast, minute ventilation measured by whole-body plethysmography or the dynamic end-tidal forcing technique has been shown to be an objective, reproducible and sensitive measure of the respiratory effects of opioids (Dahan *et al.*, 1990). More importantly, minute ventilation as a biomarker of respiratory depression has been shown to reflect the functioning of various opioids at the μ -opioid receptor in a strictly quantitative manner (Romberg *et al.*, 2003; Yassen *et al.*, 2006a). Since measurement of minute ventilation allows for a quantitative assessment of respiratory depression, we defined severe respiratory depression as a decrease in ventilation of more than 50 % relative to baseline.

For the antinociceptive effect, reaching the cut-off tail flick latency time is frequently used to indicate that complete antinociception is reached (Brady and Holtzman, 1982). In the present investigation, therefore, the same definition was used to indicate complete antinociception. PK-PD analysis revealed marked difference in the kinetics of onset and offset for buprenorphine *versus* fentanyl. The biophase distribution of buprenorphine was significantly slower than for fentanyl. On the basis of the physico-chemical properties (buprenorphine being more lipophilic than fentanyl) (Yassen *et al.*, 2007b) it can be expected that buprenorphine displays slower biophase distribution. Furthermore, buprenorphine's slow receptor association/dissociation kinetics contributes to the delay in the onset of buprenorphine's analgesic and respiratory depressant effect. Previously it was shown that the delay in buprenorphine's antinociceptive and respiratory depressant effect is mainly caused by biophase distribution rather than slow receptor association/dissociation kinetics (Yassen *et al.*, 2005, 2006a).

Beside the kinetics of the opioid action, the PK-PD analysis yielded important information on the effectiveness and safety outcome of fentanyl and buprenorphine. The odds

ratios which had been calculated for the estimated probability of a complete antinociceptive or severe respiratory depressant event can be used as a measure of pharmacological effect size. For fentanyl, the calculated odds ratios for the antinociceptive and respiratory depressant effect were 3.03 (95 % CI: 1.87 - 4.21) and 2.54 (95 % CI: 1.26 - 3.82), respectively, suggesting that increasing the fentanyl concentration with 1 ng/ml, there is a 3.03 and 2.54 greater chance of having complete antinociception or respiratory depression, respectively. In terms of probabilities rather than changes in odds, the estimated risk of severe respiratory depression increased from 10.5 % to 22.9 % when the concentration was increased from 4 to 5 ng/ml. In addition, the probability of achieving complete antinociception increased 0.4 % from 1.2 %.

The estimated values of the odds ratio for antinociception (3.03) and respiratory depression (2.54) are nearly identical and suggest that fentanyl displays almost no *in vivo* tissue selectivity. Accordingly, the ratio of the two OR's (antinociception/respiratory depression) is equal to 1.20 (3.03/2.54) for fentanyl. This ratio can be used as a safety index and suggests that fentanyl displays no selectivity of action. For buprenorphine, the odds ratios were 28.5 (95 % CI: 6.9 - 50.1) and 2.10 (95 % CI: 0.71 - 3.49) for the antinociceptive and respiratory depressant effect, respectively. The large difference in calculated odds ratio's for the antinociceptive and respiratory depressant effect indicates that buprenorphine's displays a varying degree of tissue selectivity. The safety index is equal to 13.54 (28.5/2.10) and suggests a favorable selectivity of action. This provides further evidence, in contrast to fentanyl, for the relative safety of buprenorphine when administered in therapeutic analgesic doses. In various receptor binding studies, buprenorphine has been shown to act as a partial agonist at the μ -opioid receptor (Huang *et al.*, 2001). Partial agonists typically require complete receptor occupancy for maximal response (Clark, 1937). For the respiratory depressant effect, the *in vitro* partial agonist activity correlates well its *in vivo* respiratory depressant effect, characterized by ceiling at higher concentrations (Walsh *et al.*, 1994; Dahan *et al.*, 2006).

Buprenorphine and fentanyl are μ -opioid agonists with distinct pharmacological properties. Since fentanyl behaves as full agonist its *in vivo* potency for respiratory depression and antinociception are determined by both the affinity and the intrinsic efficacy at the μ -opioid receptor. In contrast, for the partial agonist buprenorphine the *in vivo* potency is determined solely by its affinity for the μ -opioid receptor (Kenakin, 1997). These differences between efficacy- and affinity-driven agonism may have major implications for the therapeutic utility of opioids. Therapeutic utility is a measure of the effectiveness of drugs. Given this measure, one can speak of increasing or decreasing utility and thereby explain pharmacological behavior of a drug in terms of attempts to optimize pharmacotherapy. As recently discussed by Cullberg *et al.* (2005) the most adequate representation of drug's effectiveness/safety balances with a utility function remains a matter of debate. In the present study, the therapeutic utility function is equal to the sum of the weighted probabilities of an effective (antinociception) and a safety (respiratory depression) outcome. The effect measures, antinociception and respiratory depression,

were weighted equally and this seems justified, given that respiratory depression is a potentially life-threatening side effect with an acute time course. Furthermore, for the construction of the utility function the continuous antinociceptive and respiratory depressant continuous effect variables were transformed into dichotomous data. This type of data transformation will entail loss of pharmacodynamic information. Especially the kinetics of onset and offset of the opioids effect can be estimated less accurately. However, the kinetics of onset and offset of buprenorphine's antinociceptive and respiratory

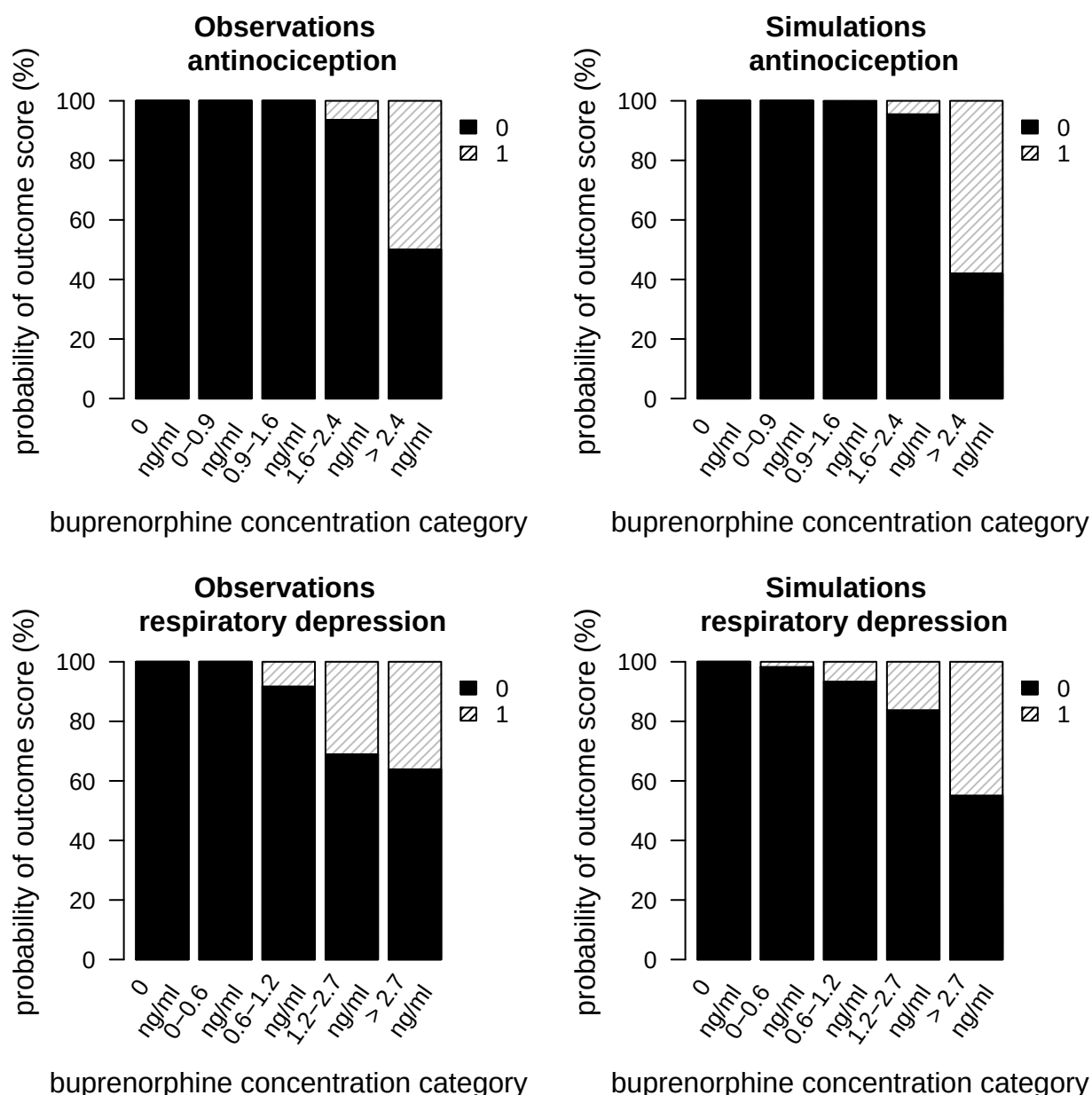


Figure 3. Goodness-of-fit plots of the logistic regression model for buprenorphine. Observed (left panels) and simulated (right panels) values of the buprenorphine's effectiveness (upper panels) and safety (lower panels) outcome for the different concentration categories. Simulations were repeated in total a 100 times. The concentrations were divided in the following categories: scores at $C_e=0$, between 0 and 25th quantiles, 25th-50th quantiles, 50th-75th quantiles, > 75th quantiles.

depressant effect have been characterized previously (Yassen *et al.*, 2005, 2006a). Using a continuous measure of respiratory depression and antinociception one can still construct utility functions. However, using this approach it requires a clear definition of an $E_{\max}$ value for respiratory depression and antinociception. For antinociception no $E_{\max}$ could be identified in the studied dose range. As a consequence the therapeutic utility of buprenorphine can be overestimated when the continuous variables were used for the construction of a utility function. Therefore, the continuous data were transformed into

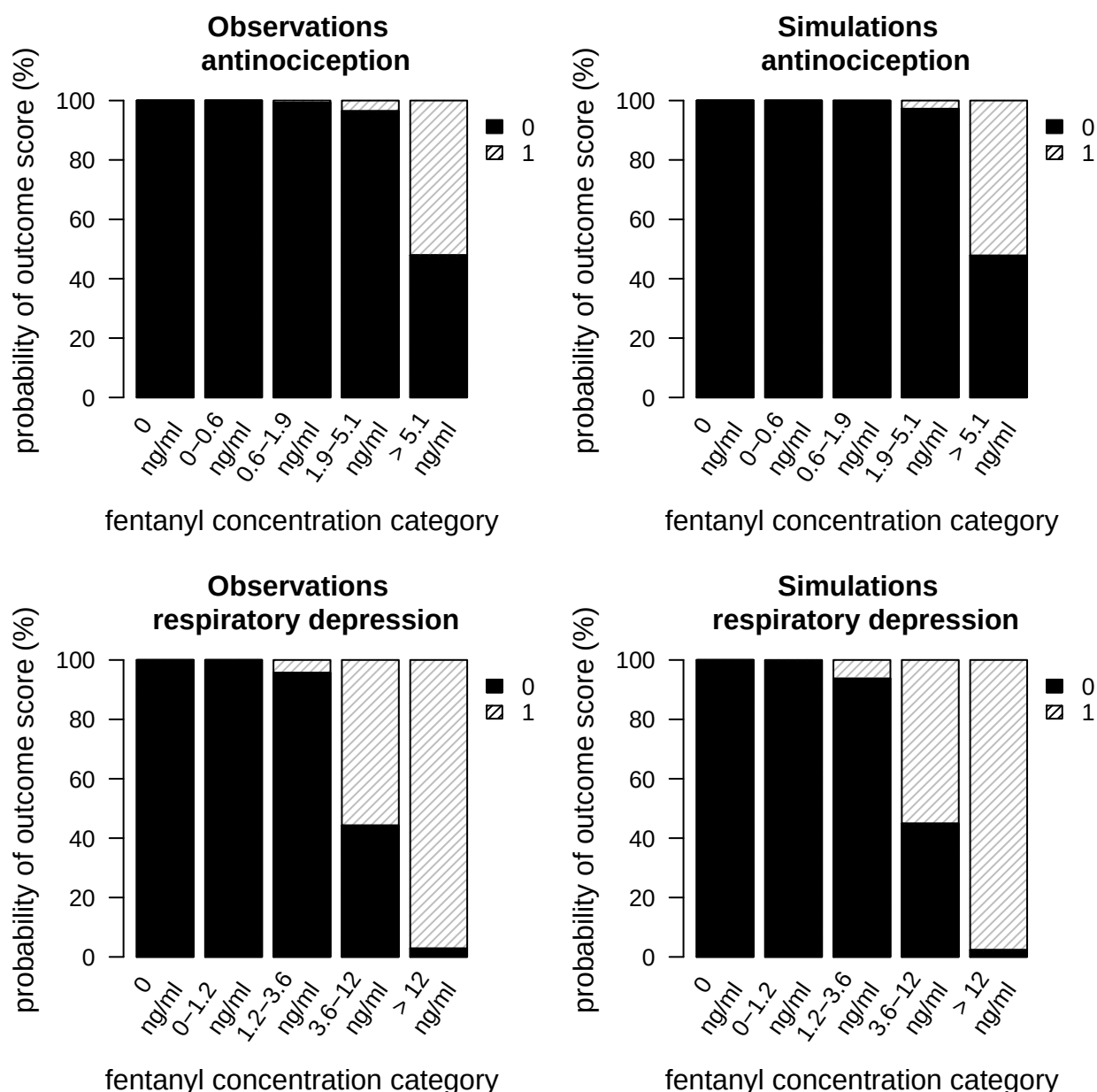


Figure 4. Goodness-of-fit plots of the logistic regression model for fentanyl. Observed (left panels) and simulated (right panels) values of the buprenorphine's effectiveness (upper panels) and safety (lower panels) outcome for the different concentration categories. Simulations were repeated in total a 100 times. The concentrations were divided in the following categories: scores at $C_e=0$, between 0 and 25th quantiles, 25th-50th quantiles, 50th -75th quantiles, > 75th quantiles.

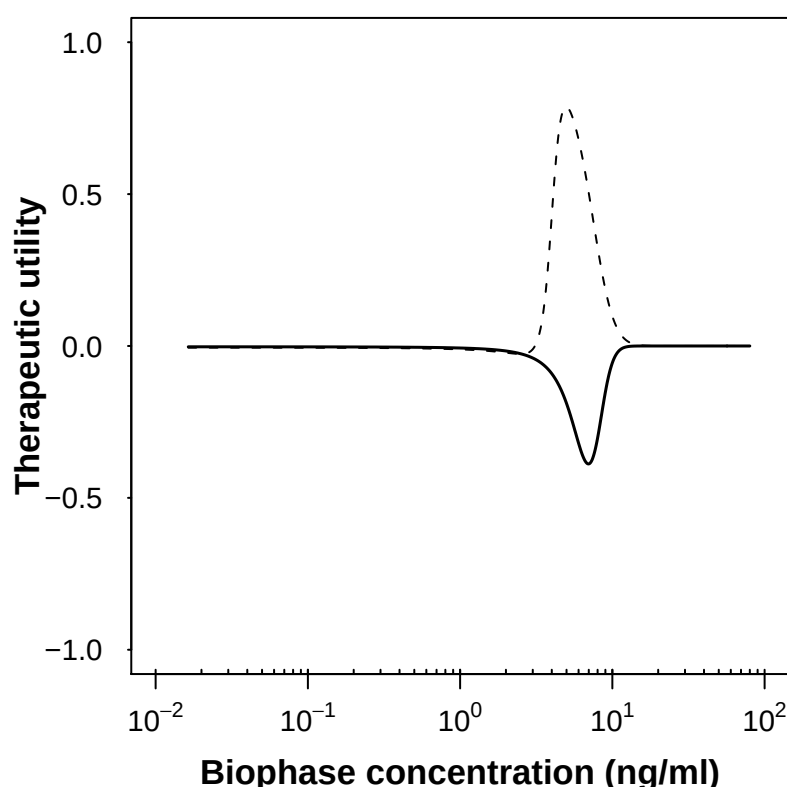


Figure 5. Estimated therapeutic utility function of buprenorphine (dashed line) or fentanyl (solid line) treatment as a function of its respective biophase exposure. The therapeutic utility function is defined as Probability of antinociception - Probability of respiratory depression.

dichotomous data enabling the definition of clear cut-offs.

An intriguing question remains whether the calculated safety margins and therapeutic utilities of buprenorphine and fentanyl are similar in rats and humans. The calculated therapeutic utility is determined by the opioid's affinity and intrinsic effectiveness at the μ -opioid receptor. In theory, these two pharmacological terms are drug-specific parameters, both of which are expected to be similar between man and rat for the μ -opioid receptor (high degree of receptor homology). Recently, it was shown that indeed the drug-specific parameters of buprenorphine are similar in rats and humans for both the antinociceptive and respiratory depressant effect (Yassen *et al.*, 2007a). It is therefore advocated that the calculated therapeutic utility for buprenorphine, in line with its clinical experience, is similar in rats and humans. Since fentanyl is a full μ -opioid agonist for both antinociception and respiratory depression, producing maximal pharmacological responses in rats and humans, it is expected that the therapeutic utility of fentanyl is not significantly different between these two species. On the basis of the calculated therapeutic utility of fentanyl in rats it can be concluded that fentanyl has a narrow therapeutic window in man (low safety index) requiring fentanyl to be administered cautiously to patients. In fact, a series of recent case-reports on fentanyl-induced severe respiratory depression and death in patients confirm fentanyl's low safety margin underscoring the importance of careful dose titration of high-efficacy opioids like fentanyl (Regnard and

Pelham, 2003). For buprenorphine, the utility function also approaches zero at relatively high concentration upon acute exposure. It should be noted, however, that this is inherent to the definition of the occurrence of respiratory depression in the present study. Previously it has been shown that the maximum respiratory depressant effect of buprenorphine is approximately 48 to 55 % *versus* 90 to 100 % for fentanyl, suggesting that although the utility function becomes zero, the absolute degree of respiratory depression is less for buprenorphine than for fentanyl (Yassen *et al.*, 2006a, 2007b). Furthermore, the calculated safety index indicates that buprenorphine has a wider therapeutic window, allowing for the administration of relatively high doses of buprenorphine. Reported safety incidences related to buprenorphine are low and deaths due to buprenorphine misuse are rare. This makes buprenorphine a particularly safe opioid with an increased margin of safety (Sporer, 2004).

As evaluation of drug safety becomes increasingly more important in contemporary drug development, there will be a clear shift towards the application of PK-PD modeling to predict drug safety outcomes. As more mechanistic biomarkers become available to assess drug safety, this gives rise to the development of more sophisticated and mechanistic PK-PD models to assess drug efficacy/safety balances. Clearly, this also influences the selection and structure of therapeutic utility functions to assess the effectiveness/safety balances of drugs under development. To this end, utility function will become less descriptive and more predictive. Also, the potential utility of mechanistic biomarkers to facilitate drug development may lead to better techniques to assess the safety in humans more in line with those used in animals, yielding more accurate animal to human efficacy/safety extrapolation in an early stage of drug development.

In conclusion, logistic regression pharmacodynamic models were developed to predict the safety and effectiveness outcome of buprenorphine and fentanyl in rats. Buprenorphine has been shown to display ceiling in respiratory effects but not in analgesia upon acute administration. This explains the relatively high safety index and indicates that the ceiling respiratory effect contributes to the safety profile of buprenorphine. For fentanyl a narrow therapeutic window has been observed, complicating fentanyl dose titration to the optimum analgesic effect without the occurrence of severe respiratory depression. The modeling of the efficacy/safety balances is a first quantitative assessment of the efficacy/safety of opioids and these results are in good agreement with the general perception on the safety issues of buprenorphine and fentanyl, underscoring the importance of the development of PK-PD models to quantitatively assess the rationality of pharmacotherapeutic utilities.

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ANIMAL-TO-HUMAN EXTRAPOLATION OF THE PHARMACOKINETIC AND PHARMACODYNAMIC PROPERTIES OF BUPRENORPHINE

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ABSTRACT

This investigation describes the interspecies scaling of the pharmacokinetics and pharmacodynamics of buprenorphine. Data on the time course of the antinociceptive and the respiratory depressant effect in rats and in humans were simultaneously analyzed on the basis of a mechanism-based pharmacokinetic-pharmacodynamic model.

An allometric three-compartment pharmacokinetic model described the time course of the concentration in plasma. The value of the allometric coefficient for clearance was 35.2 ml/min (relative standard error RSE = 5.6 %) and the value of the allometric exponent 0.76 (RSE: 5.61 %). The predictive value of the model was demonstrated by the successful prediction of the pharmacokinetics in adults.

A combined biophase distribution-receptor association/dissociation model with a linear transduction function described hysteresis between plasma concentration and effect. The values of the drug-specific pharmacodynamic parameters were identical in rats in humans. For the respiratory depressant effect the values of k_{on} and k_{off} were 0.23 ml/ng/min (RSE = 15.8%) and 0.014 min⁻¹ (RSE = 27.7 %) respectively and the value of K_D was 0.13 nM. The value of the intrinsic activity was 0.52 (RSE = 3.4 %). For the antinociceptive effect the values of k_{on} and k_{off} were 0.015 ml/ng/min (RSE = 18.3 %) and 0.053 min⁻¹ (RSE = 23.1 %) respectively. The value of K_D was 7.5 nM. An allometric equation described the scaling of the system-specific parameter k_{e0} . The value of the allometric coefficient for k_{e0} was 0.0303 min⁻¹ (RSE = 11.3 %) and the value of the exponent -0.28 (RSE = 9.6 %).

The different values of the drug specific pharmacodynamic parameters are consistent with the different μ -opioid receptor subtypes involved in the antinociceptive and respiratory depressant effect.

INTRODUCTION

In the selection of drug candidates interspecies extrapolation is a valuable tool, especially for dose selection for first time in human studies. Various techniques are available to for interspecies extrapolation of drug disposition characteristics, including allometric modeling and physiologically-based pharmacokinetic modeling (PB-PK) (Mahmood, 1999; Nestorov, 2003). Despite the increasing awareness of the need to develop innovative tools to predict pharmacological effects of drug candidates in man little to no progress had been made in the animal to human scaling of PK-PD properties.

Typically, PK-PD models link drug concentration to pharmacological effect in an empirical manner thereby ignoring the biological basis of the PK-PD correlation. This may explain the high failure rate of prediction of PK-PD properties in man. In principle, mechanism-based PK-PD models, which are derived from physiological and biological knowledge, are more likely to possess improved properties for prediction and extrapolation (Van der Graaf and Danhof, 1997). A key component of this class of PK-PD models is the clear distinction between drug-specific and biological system-specific pharmacodynamic parameters (Visser *et al.*, 2002; Zuideveld *et al.*, 2004). In theory, the values of the drug-specific parameters are unique to the drug. As a result the values are identical across species. They can therefore be used for the prediction of the pharmacological effect in various species, given a high degree of homology of receptors in the respective species (Rothman *et al.*, 1995). On the other hand, the values of the system-specific parameters are different between species. The values of these pharmacodynamic parameters need to be scaled from animal to human using allometry in order to predict the pharmacological effect appropriately.

Recently, a mechanism-based PK-PD model was proposed to characterize the time course of the antinociceptive and respiratory depressant effect of buprenorphine in rats and humans (Yassen *et al.*, 2005, 2006b). The PK-PD model is derived from receptor theory and contains specific expressions for the *in vivo* receptor association/dissociation kinetics of buprenorphine at the μ -opioid receptor. An important aspect is the scaling of the slow receptor association/dissociation kinetics and the biophase equilibration of buprenorphine *in vivo*. Referring to the key feature of mechanism-based PK-PD models, we postulate that the rate constants of receptor association/dissociation kinetics are species-independent parameters and the rate constant of biophase equilibration is a biological system-dependent parameter.

The objective of this study is to investigate the animal to human scaling of the PK-PD properties of buprenorphine with respect to both the antinociceptive and respiratory depressant effect.

MATERIALS AND METHODS

Animal and human data for this PK-PD analysis were collected from two preclinical PK-PD studies in rats and two phase I PK-PD studies in humans. Eighty-three healthy volunteers and 47 animals received intravenous buprenorphine administration. In each study, repeated measures of buprenorphine concentrations and respiratory depressant or antinociceptive effect were taken for PK-PD evaluation. For the animal studies, the protocols were approved by the Ethical Committee on Animal Experimentation of Leiden University. For the phase I clinical studies, approval from the local human ethics committee was obtained and written consent was obtained from each participant. The PK-PD analysis of the 4 studies has been published separately (Yassen *et al.*, 2005, 2006a,b, 2007). The designs of the studies are summarized in table 1.

Table 1. Characteristics of four (pre)-clinical pharmacokinetic-pharmacodynamic studies analyzed

	Rat	Human
Respiratory depression		
Dose [†]	0.025, 0.05 0.1 and 0.3 mg/kg	0.05, 0.1, 0.2, 0.3, 0.4 and 0.6 mg/70 kg
Mode of administration	20 min i.v. infusion	bolus i.v. infusion
No. Subjects/animals	32	50
weight (kg) [‡]	0.275 (0.240-0.320)	70 (51-93)
Antinociception		
Dose	0.015 and 0.03 mg/kg	0.05, 0.1, 0.2, 0.3, 0.4 and 0.6 mg/70 kg
Mode of administration	20 or 40 min i.v. infusion	bolus i.v. infusion
No. Subjects/animals	15	33
weight (kg)	0.288 (0.263-0.319)	70 (51-85)

[†] doses are weight corrected; [‡] presented as median (range)

Quantification of respiratory depression

Respiratory depression was determined in unrestrained, conscious animals using whole-body plethysmography (Model PLY3223, Buxco Electronics Inc., UK) for the quantification of ventilation (Yassen *et al.*, 2006a). In each animal the effects of opioids and vehicle on ventilation were assessed at an inhaled concentration of 6.5 % carbon dioxide on a background of normoxia (20 % oxygen). The inhalation of the gas mixture lasted 5 min to

ensure that steady-state ventilation had been reached. In the healthy volunteers ventilation was measured at a fixed $P_{ET}CO_2$ and $P_{ET}O_2$ of 50 mmHg and 100 mmHg, respectively using a dynamic end-tidal forcing technique (Dahan *et al.*, 1990).

Quantification of antinociception

A tail flick analgesia meter (Columbus Instruments, Columbus, Ohio, USA) was used to determine the pain sensitivity in the buprenorphine-treated rats (Yassen *et al.*, 2005). Radiant heat was applied using a shutter-controlled lamp as a heat source. To prevent tissue injury, the cut-off time was fixed at 10 s. In humans, acute pain was induced by an electrical current through two surface electrodes (Red Dot, 3M, London, Ontario, Canada) placed on the skin overlaying the tibial bone (shin bone) of the left leg (Yassen *et al.*, 2006b). The intensity of the noxious stimulation was increased from 0 mA in steps of 0.5 mA per 1 s. The stimulus train consisted of a square-wave pulse of 0.2 ms duration applied at 10 Hz and had a cutoff at 128 mA.

Determination of Buprenorphine Concentrations

For the determination of buprenorphine in rats, a validated LC/MS/MS method was used. The details of the assay have been described elsewhere (Yassen *et al.*, 2005). The lower limit of quantification (LLOQ) was 0.047 ng/ml for buprenorphine. The accuracy ranged from 99.4 to 102.1 %. The precision expressed as coefficient of variation, ranged from 2.2 to 6.4 % for concentrations between 0.14 and 8.9 ng/ml. For the determination of buprenorphine concentrations in human plasma, a validated GC-MS method was used (Yassen *et al.*, 2006b). The accuracy and precision for calibration standards were 5.47 % and 1.83 %, respectively at the LLOQ (0.025 ng/ml) and 0.83 % and 3.79 %, respectively at the upper limit of quantification (ULOQ) of 10 ng/ml. For the quality controls the accuracy ranged from -1.42 % (0.075 ng/ml) to 7.74 % (8 ng/ml). The precision ranged from 1.88 % (8 ng/ml) to 5.31 % (0.075 ng/ml). All concentration data presented refer to buprenorphine free base.

PK-PD data analysis

The pharmacokinetic and pharmacodynamic parameters of buprenorphine and where applicable the coefficients and exponents of the power equations were estimated using non-linear mixed-effects modeling as implemented in the NONMEM software (Version V, level 1.1) (Beal and Sheiner, 1999). The population analysis approach, taking into consideration both intra-individual and inter-individual variability, was undertaken using the first order conditional estimation method with η - ϵ interaction (FOCE interaction). All fitting and simulation procedures were performed on an IBM-compatible computer (Pentium IV, 1500 MHz) running under Windows XP with the Microsoft FORTRAN Powerstation 4.0 compiler.

An in-house available S-PLUS 6.0 (Insightful Corp., Seattle, WA, USA) interface to NONMEM was used for data processing, management and graphical data display.

Pharmacokinetic data analysis

For the simultaneous analysis of the PK in rat and human a three-compartment model was selected. Data collected from the two studies on the antinociceptive effect in rat and human were used for the development of the population pharmacokinetic model. The final data set for the development of the population PK model contained 458 concentrations from rats and 539 concentrations from volunteers. The model was coded in the ADVAN11 TRANS4 subroutine and allowed the estimation of the parameters Cl , V_1 , V_2 , V_3 , Q_2 and Q_3 . The structural population pharmacokinetic parameters were defined as a function of bodyweight (WT) according to the equation (Boxenbaum, 1982):

$$P_{tot} = a \cdot WT^b \quad (1)$$

where P_{tot} is the pharmacokinetic parameter of interest, a is the coefficient and b is the exponent of the power function. The stochastic model assumed log-normal distribution of the pharmacokinetic parameters over the population. Therefore an exponential distribution model was used to account for species-dependent inter-individual variability:

$$P_{i, \text{rat}} = P_{tot} \cdot \exp(\eta_{i, \text{rat}}) \quad \text{or} \quad P_{i, \text{human}} = P_{tot} \cdot \exp(\eta_{i, \text{human}}) \quad (2)$$

where $P_{i, \text{rat}}$ and $P_{i, \text{human}}$ are the individual values of model parameter P in rats or humans, respectively, P_{tot} is the typical value (mean population value) of parameter P in the population, and η_i is the normally distributed inter-individual random variable with mean zero and variance ω^2 . The coefficient of variation of the structural model parameters is expressed as percentage of the mean root square of the inter-individual variance term. Selection of an appropriate residual error model was based on inspection of the goodness-of-fit plots. Inter-individual variability was added on the model parameters by stepwise addition. A ΔOFV of 6.6 points (χ^2 distribution, $p < 0.01$, $\text{df}=1$) was required to maintain inter-individual variability on a structural model parameter. Afterwards, the relative importance of each inter-individual variability term was reassessed by deletion of each term one by one from the structural model parameters. An increase of more than 10.8 points (χ^2 distribution, $p < 0.001$, $\text{df}=1$) in OFV was regarded as significant for maintenance of inter-individual variability on a model parameter. The statistical model used to quantify residual variability in buprenorphine plasma concentration had the general form:

$$C_{obs, ij} = C_{pred, ij} \cdot (1 + \varepsilon_{ij}) \quad (3)$$

where $C_{obs, ij}$ is the j th observed concentration in the i th individual, $C_{pred, ij}$ is the predicted concentration, and ε_{ij} is the normally distributed residual random variable with mean

zero and variance σ^2 . To correct for differences in residual error between rat and human a species-dependent residual error term was estimated.

Predictive performance of the pharmacokinetic model for interspecies allometric scaling

Pharmacokinetic data obtained from the animal and human studies on the respiratory depressant effect were used to support the utility of the developed population pharmacokinetic model for animal to human extrapolation. To explore whether the predicted time course of buprenorphine concentration on the basis of developed population pharmacokinetic model follows the general allometric scaling laws, the time course of buprenorphine concentration in humans was predicted on the basis of the pharmacokinetic parameter estimates obtained from rats (Yassen *et al.*, 2006a). From these pharmacokinetic estimates and their respective variances 500 data sets were simulated for each dose group in humans. The median outcome and the 2.5 % and 97.5 % quantiles were calculated from the simulated buprenorphine concentrations at predefined time points. Within a dose group, the observed buprenorphine plasma concentration in humans (Yassen *et al.*, 2007) was compared with the simulated median and the 95 % predicted intervals of the simulated time course of buprenorphine concentration. Rat to human pharmacokinetic predictions were performed on the basis of the following allometric equation:

$$P_{\text{human}} = P_{\text{rat}} \cdot \left(\frac{WT_{\text{human}}}{WT_{\text{rat}}} \right)^b \quad (4)$$

in which P_{human} and P_{rat} are the pharmacokinetic parameters in humans and rats respectively and b is the allometric exponent which is fixed at 0.75 for functional flow parameters (Cl , Q_2 and Q_3) and 1 for structural volume parameters (V_1 , V_2 and V_3) (Holford, 1996; West *et al.*, 1997).

Mechanism-based PK-PD analysis

A previously proposed mechanism-based PK-PD model was used to characterize the time course of the respiratory depressant and antinociceptive effect. A specific feature of this model is that it contains separate expressions for the characterization of biophase equilibration and receptor association/dissociation kinetics as determinants of hysteresis between plasma concentration and effect. Within the context of interspecies scaling this is important, since the first-order rate constant for biophase distribution is a system-specific variable, of which the value can differ between species. In contrast, the second and first-order rate constants for receptor association/dissociation are assumed to display cross-species similarity.

Drug distribution to the site of action (biophase) was characterized on the basis of an effect compartment model (Sheiner *et al.*, 1979). The rate of change of biophase drug

concentrations can be defined as follows:

$$\frac{dC_e}{dt} = k_{e0} \cdot (C_p - C_e) \quad (5)$$

where k_{e0} is a first-order distribution rate constant describing the rate of distribution between plasma and a hypothetical effect compartment, C_p represents the plasma concentration and C_e the effect-site concentration. The rate constant for biophase equilibration is related to bodyweight on the basis of the following equation:

$$k_{e0} = a \cdot WT^b \quad (6)$$

where k_{e0} , the rate constant for biophase equilibration, is a function of bodyweight (WT), a the coefficient and b is the exponent of the power equation. At the site of action, the rate of drug-receptor binding is proportional to the concentrations of drug C_e and free receptor R :

$$\frac{dC_e R}{dt} = k_{on} \cdot C_e \cdot R - k_{off} \cdot C_e R \quad (7)$$

Under the assumption that the concentration of drug is in excess compared to the free receptor concentration and that the total number of receptors (R_{tot}) is equal to the sum of drug-bound ($C_e R$) receptors and unbound receptors (R), equation 7 can be rearranged:

$$\frac{d\rho_{app}}{dt} = k_{on} \cdot C_e \cdot (1 - \rho_{app}) - k_{off} \cdot \rho_{app} \quad (8)$$

in which ρ_{app} is the apparent fractional receptor occupancy ($C_e R / R_{tot}$). In the present analysis a linear transduction function was used to characterize the relationship between the fractional receptor occupancy and the pharmacological effect intensity. For the respiratory depressant effect this relationship was described by the following equation:

$$E = E_0 \cdot (1 - \alpha \cdot \rho_{app}) \quad (9)$$

and for antinociception:

$$E = \frac{E_0}{1 - C_e R} \quad (10)$$

where E is the pharmacological response, E_0 is the baseline response and α is the intrinsic activity of buprenorphine of which the value varies between 0 and 1. Pharmacodynamic model evaluation was based on the likelihood ratio test. This test was performed to determine whether the measured pharmacodynamic endpoint and specie were statistical significant covariates for the pharmacodynamic parameter estimates of k_{on} , k_{off} , k_{e0} and α . Model selection is done by comparing the difference in objective function values

between the full model (with the covariate under investigation) and reduced model (without covariate) with the critical value from the χ^2 distribution is equal to 6.6 ($p < 0.01$, $df=1$). If the difference in the 2 objective function values exceeds the χ^2 critical value, the null hypothesis is rejected and it is concluded that the covariate has a significant effect. In the PK-PD analysis, residual variability was assumed to be log-normally distributed. Residual variability was described using an additive error model.

RESULTS

Pharmacokinetic modeling

Simultaneous analysis of plasma concentration time profiles of buprenorphine in rat and human on the basis of the three-compartment pharmacokinetic model was successful, enabling precise estimation of the allometric coefficients (i.e. the standardized values of volume and clearance for a subject with a bodyweight of 1 kg) and the allometric exponents. For example, for a typical healthy subject with weight 70 kg the estimates of clearance (Cl) and central volume of distribution (V_1) are 888 ml/min (53.3 l/hr) and 19.8 l, respectively. The population pharmacokinetic parameter estimates, the corresponding estimated inter-individual variability terms per specie and their respective relative standard errors are shown in table 2. The goodness-of-fit plots are displayed in figure 1. To explore the use of allometry for the prediction of the time course of buprenorphine concentration in humans on the basis of the pharmacokinetic data obtained in rats, an external predictive performance test was conducted. The results of the external method validation are shown in figure 2. The time course of buprenorphine concentration in humans was accurately predicted using allometry.

Pharmacodynamic modeling

The combined biophase equilibration-receptor association/dissociation model was successfully applied to the data on the time course of the respiratory depressant and antinociceptive effect in rats and humans. The equilibrium dissociation constant can be directly calculated from the ratio of k_{off} to k_{on} . For the respiratory depressant effect the calculated value of the equilibrium dissociation constant was 0.06 ng/ml, which corresponds to 0.13 nM. For buprenorphine's antinociceptive effect, the value of the equilibrium dissociation constant K_D was 3.5 ng/ml or 7.5 nM (k_{off}/k_{on}). The power equation (equation 6) was used to scale the rate constant of biophase equilibration from animal to human, yielding estimates for the allometric coefficient a and exponent b . For a typical human with weight 70 kg the estimate of k_{e0} is 0.0092 min^{-1} or $t_{1/2,ke0}$ is 75.2 min and for a rat weighting 300 g the value for biophase equilibration is 0.042 min^{-1} which corresponds to a half-time of biophase equilibration of 16.3 min. The pharmacodynamic parameter

Table 2. Mean population pharmacokinetic parameter estimates of buprenorphine with their respective relative standard errors (%) obtained with the final allometric pharmacokinetic model

	Allometric coefficient	Allometric exponent
Cl (ml/min)	35.2 (5.6)	0.76 (5.61)
V_1 (ml)	381 (11)	0.93 (3.43)
V_2 (ml)	1100 (6)	1.07 (1.42)
V_3 (ml)	3300 (11)	1.33 (6.18)
Q_2 (ml/min)	71.9 (8.8)	0.87 (2.73)
Q_3 (ml/min)	23.1 (6.5)	0.89 (3.54)
Inter-individual variability		
ωCl_{rat} (%)	15.1 (56.8)	NE [‡]
ωCl_{human} (%)	14.4 (66.8)	NE
$\omega V_{1\text{human}}$ (%)	24.9 (30.2)	NE
Residual variability [#]		
σ_{rat} (%)	18.9 (21.2)	
σ_{human} (%)	21.1 (14.2)	

[‡] not estimated; [#] proportional residual error

estimates, their respective estimates of inter-individual variability and the corresponding relative standard errors are presented in table 3. The goodness-of-fit plots for the combined biophase equilibration-receptor association model are shown in figure 3 and 4. Representative individual fits for the antinociceptive and respiratory depressant effect in rat and human are shown in figure 5 and 6. Likelihood ratio tests were performed to test whether 1) the drug-specific parameters are dependent on the measured pharmacological effect (respiratory depression and antinociception) and specie (rat and human), 2) the system-dependent parameter k_{e0} is different for antinociception and respiratory depression. The objective function values of the various scenarios are presented in table 4.

DISCUSSION

In this publication we report the interspecies scaling of the pharmacokinetic and pharmacodynamic characteristics of buprenorphine. A three-compartmental pharmacokinetic

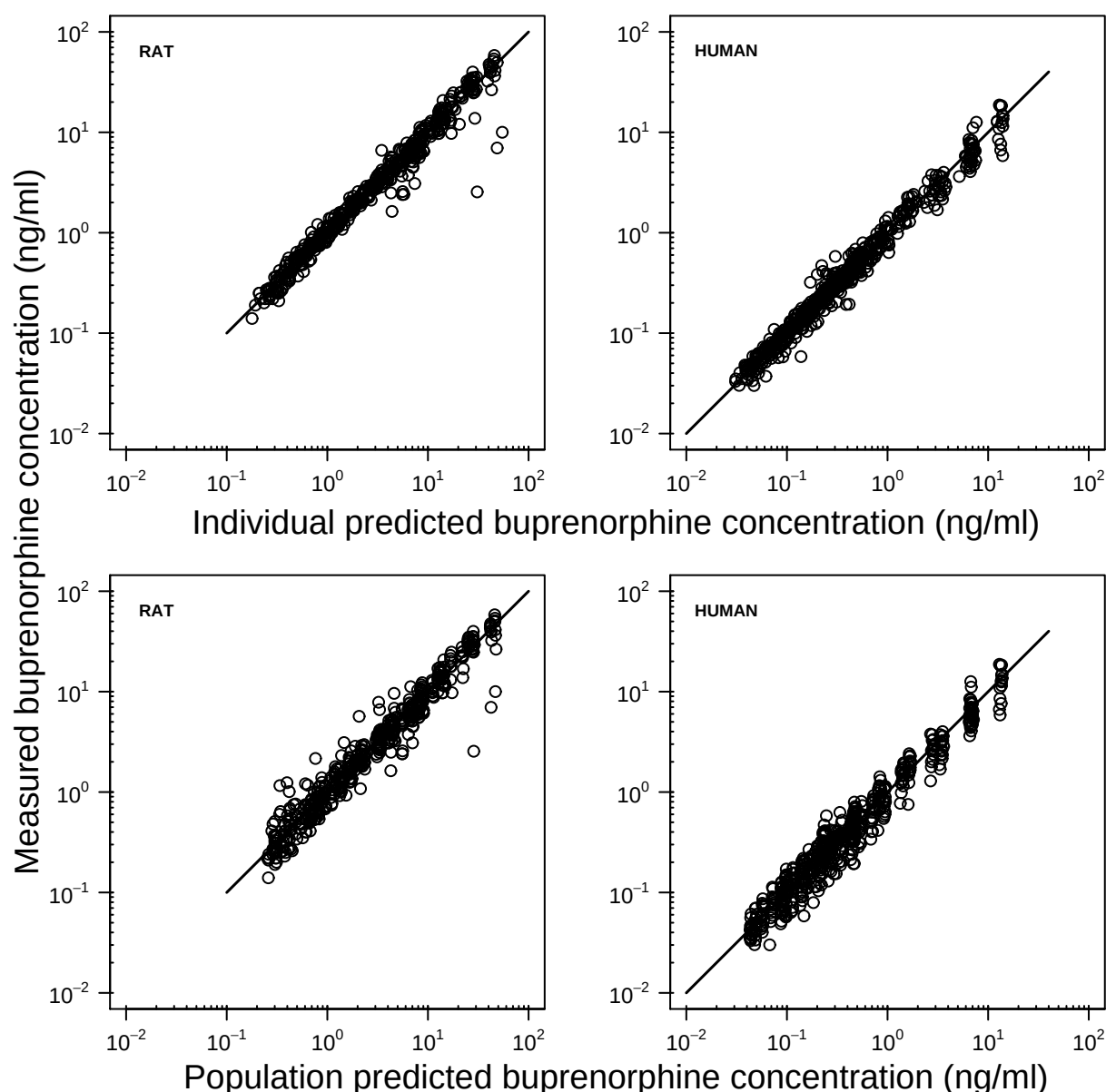


Figure 1. Goodness-of-fit plots for the pharmacokinetics of buprenorphine in rats and humans: observed *versus* individual predicted or population predicted buprenorphine concentration by the three-compartment pharmacokinetic model. The solid line is the identity line.

model was successfully fitted to the time course of buprenorphine concentration in rats and humans. A power model was used to describe the relationship of the pharmacokinetic parameters to bodyweight. This pharmacokinetic model yielded precise estimation of the coefficients and exponents of the power function. The applied expression for animal to human extrapolation is structurally identical to the general allometric equation which is frequently used to relate pharmacokinetic processes (i.e. drug distribution and elimination) to bodyweight (Holford, 1996). Also, the estimated values of the exponents of the power function are in close agreement with the values which had been previously proposed to scale volume and metabolic processes (West *et al.*, 1997). In fact, the conduction of allometric scaling (equation 4) to predict the time course of buprenorphine

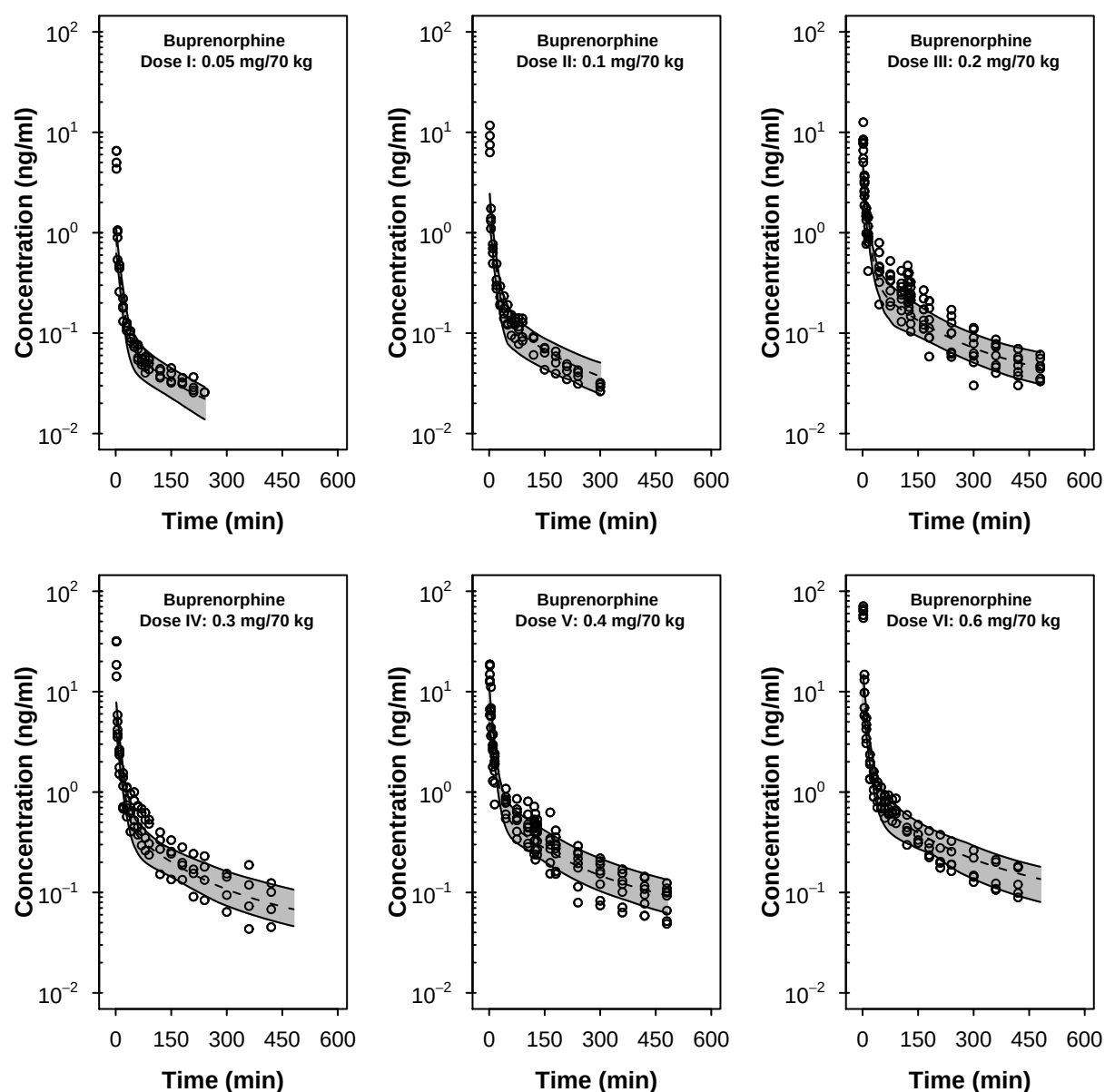


Figure 2. Prediction of the time course of buprenorphine in humans on the basis of pharmacokinetic parameter estimates obtained in rats. The measured buprenorphine concentrations (symbols) were compared with the medians (dotted lines) and the 95 % confidence intervals (solid lines) of the predicted time course of buprenorphine concentration for each individual.

concentration in humans on the basis of pharmacokinetic parameters obtained from rats yielded nearly identical accurate predictions of the time course of buprenorphine as with the developed population pharmacokinetic model. This confirms the utility of the population pharmacokinetic model for animal to human extrapolation and also suggests that allometric scaling can be adequately applied for the prediction of the pharmacokinetics of buprenorphine in humans from rat data. In principle, for the animal to human extrapolation, various different techniques can be utilized 1) allometric scaling and 2) physiologically-based pharmacokinetic modeling (Mahmood, 1999; Nestorov, 2003). The latter approach is based on the physiological principles of drug disposition. However,

Table 3. Mean population pharmacodynamic parameter estimates with the relative standard error (%) of buprenorphine obtained with the combined biophase distribution-receptor association/dissociation model

	Typical value	IIV (%) [‡]
Drug-specific parameters for respiratory depression		
k_{on} (ml/ng/min)	0.23 (15.8)	97 (22)
k_{off} (min ⁻¹)	0.014 (27.7)	166 (21)
α	0.52 (3.4)	28 (23)
Drug-specific parameters for antinociception		
k_{on} (ml/ng/min)	0.015 (18.3)	82 (33)
k_{off} (min ⁻¹)	0.053 (23.1)	120 (27)
System-dependent parameter k_{e0}		
a (min ⁻¹)	0.0303 (11.3)	NE
b	-0.28 (9.6)	NE
Baseline parameters respiratory depression		
$E_{0,\text{rat}}$ (ml/min)	60.2 (3.8)	5.8 (39)
$E_{0,\text{human}}$ (l/min)	23.9 (3.7)	45 (27)
Baseline parameters antinociception		
$E_{0,\text{rat}}$ (s)	3.1 (2.0)	21 (19)
$E_{0,\text{human}}$ (mA)	16.9 (1.4)	25 (21)
Residual variability [#]		
Respiratory depression		
σ_{rat} (ml/min)	3.2 (18.5)	
σ_{human} (l/min)	1.1 (13.3)	
Antinociception		
σ_{rat} (s)	0.35 (25.7)	
σ_{human} (mA)	3.5 (36.9)	

[‡] inter-individual variability; [#] additive residual error

pharmacokinetic modeling of the system physiology is computer-intensive and its use is limited due to experimental constraints (i.e. measurement of various physiological or biochemical parameters). Therefore, frequently allometric scaling is applied. Buprenorphine is a high extraction ratio drug and its clearance is largely determined by the hepatic blood flow (Bullingham *et al.*, 1980). Generally, for high-extraction-ratio drugs, compart-

mental allometric scaling appears to provide satisfactory prediction of drug disposition in man (Knibbe *et al.*, 2005). The high cross-species similarities in buprenorphine's pharmacokinetics, with the strong dependency of clearance of hepatic blood flow, may explain the accurate and precise pharmacokinetic estimates and their respective allometric exponents obtained with the population pharmacokinetic model on the basis of two species.

A mechanism-based PK-PD model was applied to characterize the time course of the respiratory depressant and antinociceptive effect of buprenorphine. An important feature of this model is the strict distinction between drug-specific and biological system-specific parameters to describe the time course of the drug effect. Within the context of inter-species scaling this is important. Interestingly, the rate constants for receptor association and dissociation kinetics were identical in rats and humans, indicating quantitative similarities in receptor binding kinetics of buprenorphine in rat and man. This observation provides further support for the concept that buprenorphine- μ -opioid receptor binding is independent of specie. The values of the rate constants for receptor association/dissociation kinetics are different for respiratory depression and antinociception. The *in vivo* K_D ($k_{\text{off}}/k_{\text{on}}$) value was 0.13 nM and 7.55 nM for respiratory depression and

Table 4. The objective function values of the different PK-PD modeling scenarios evaluated to characterize the time course of respiratory depression and antinociception in rat and human

PK-PD modeling scenarios	Objective function value
Final PK-PD model. Drug-specific parameters k_{on} and k_{off} are:	
• dependent of the pharmacological effect	11200
• independent of specie	
System-specific parameter k_{e0} is:	
• independent of the pharmacological effect	
Final PK-PD model with modification where:	
Drug-specific parameters k_{on} and k_{off} are:	
• independent of the pharmacological effect	11580
Drug-specific parameters k_{on} and k_{off} are:	
• dependent of specie	11580
System-specific parameter k_{e0} is:	
• dependent of the pharmacological effect	11200

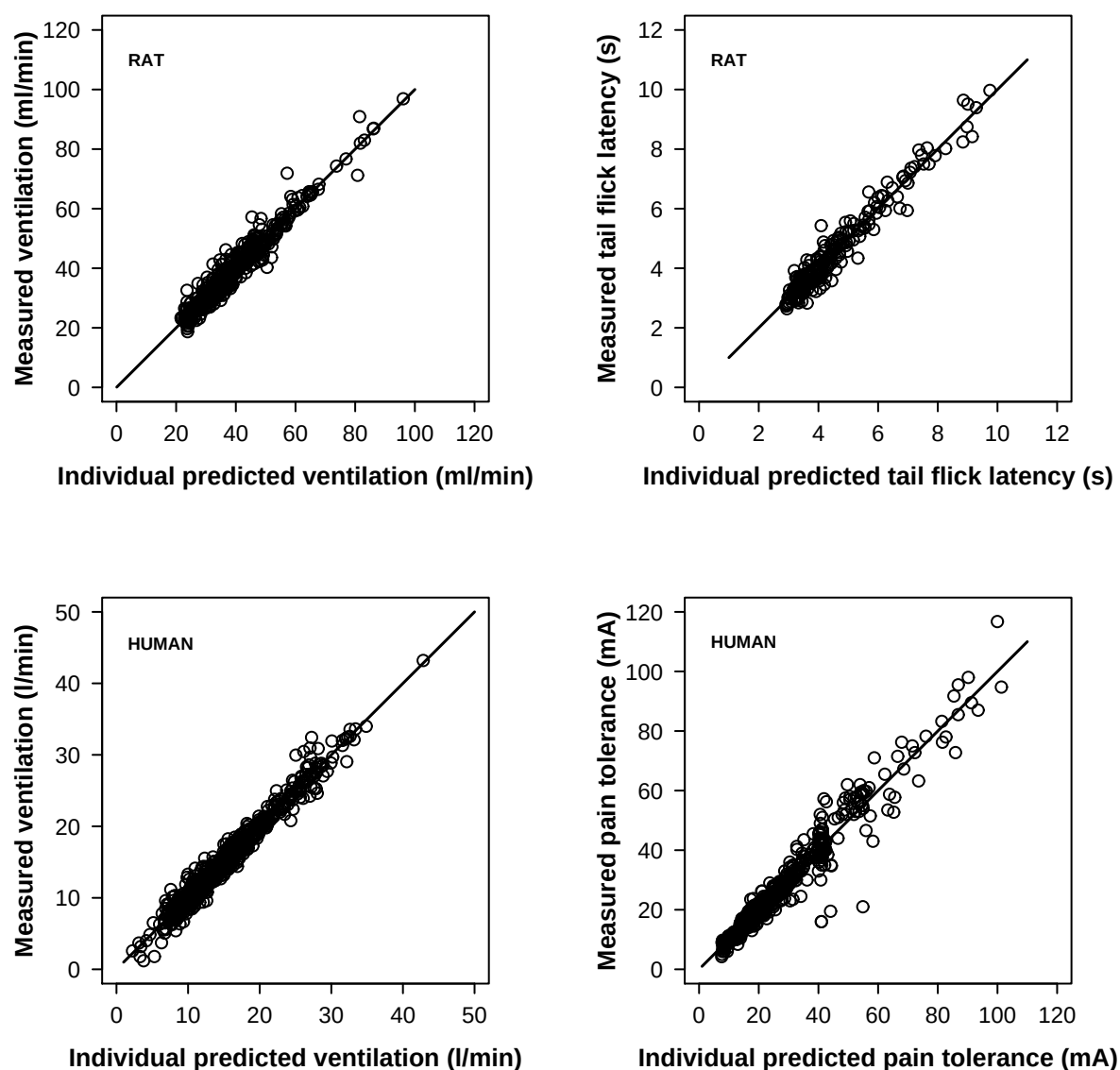


Figure 3. Goodness of fit plots for the effects of buprenorphine on antinociception and respiratory depression in rat and human: observed *versus* individual predicted by the combined biophase equilibration-receptor association/dissociation model. The solid line is the identity line.

antinociception, respectively. This suggests that the differential pharmacological effects of buprenorphine are mediated via distinct μ -opioid receptor subtypes in the central nervous system (CNS). The *in vivo* function of the μ -opioid receptor has been found to be diverse. Specifically, there exists subtypes of the μ -opioid receptor, μ_1 and μ_2 -opioid receptor subtypes, which are involved in the differential effects of opioids (Pasternak, 2001; Pan *et al.*, 2005). For instance, the μ_1 -opioid receptor has been shown to be involved in the analgesic effect and the μ_2 -opioid receptor in respiratory depression (Ling *et al.*, 1985). For buprenorphine, high and low affinity binding sites have been identified in the CNS (Boas and Villiger, 1985). The estimated *in vivo* K_D values for respiratory depression (0.13 nM) and antinociception (7.55 nM) are consistent with the presence of high and

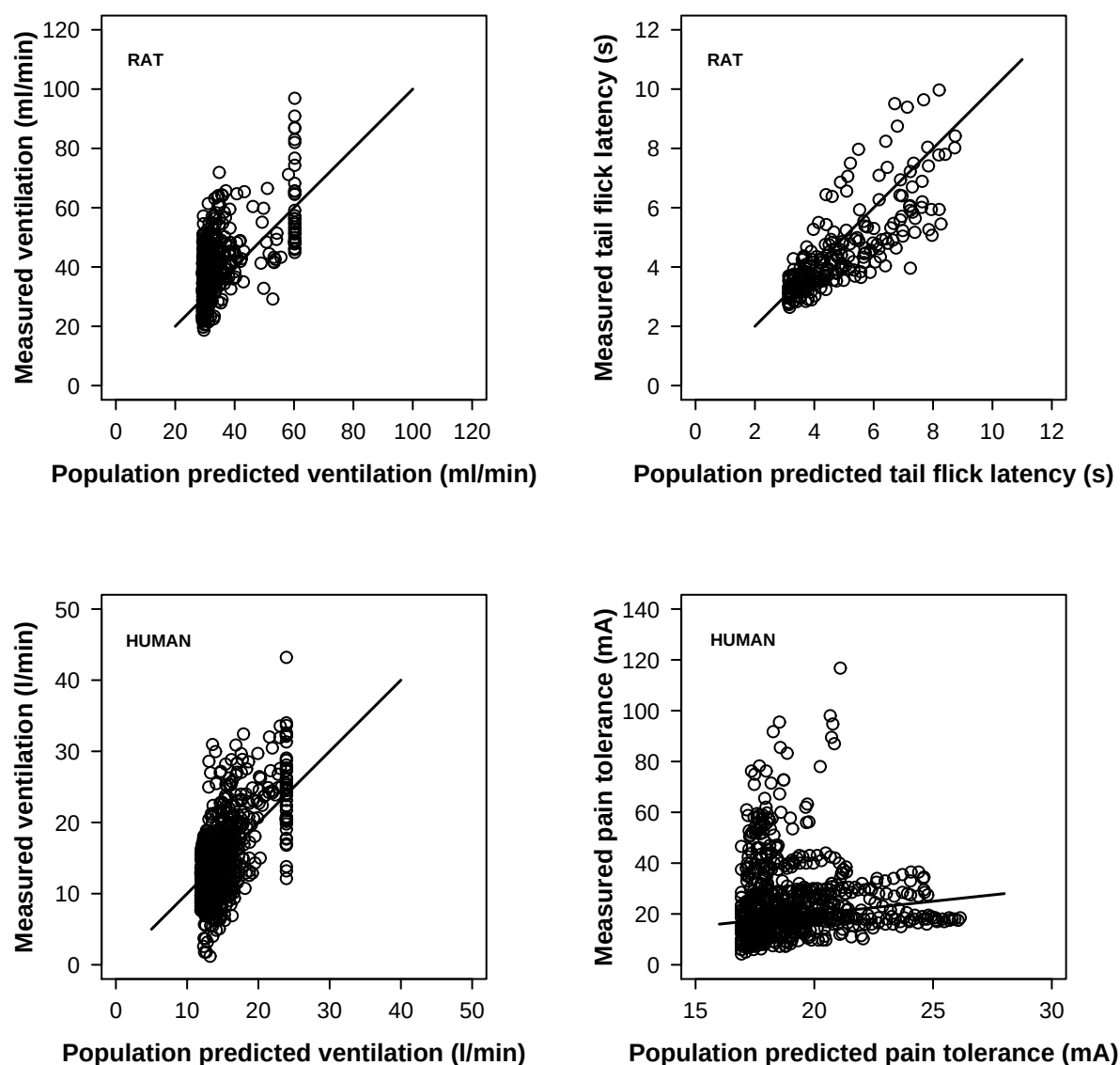


Figure 4. Goodness of fit plots for the effects of buprenorphine on antinociception and respiratory depression in rat and human: observed *versus* population predicted by the combined biophase equilibration-receptor association/dissociation model. The solid line is the identity line.

low affinity μ -opioid binding sites, most likely representing the μ_2 and μ_1 -opioid receptor subtype, respectively.

In the present analysis, the intrinsic activity (α) of buprenorphine for the respiratory depressant effect was not significantly different, on the basis of the likelihood ratio test, between rats and humans. Buprenorphine has a value of α of 0.52 (95 % CI: 0.48-0.55), which is significantly different from 1 ($p < 0.05$). For comparison, a value of α of 1 indicates that an opioid displays full respiratory depression. Clearly, buprenorphine acts as a partial agonist for respiratory depression in both species. Typically, partial agonists like buprenorphine operate in biological systems with a low receptor reserve. In such biological systems, 100 % receptor occupancy is required to elicit a maximal drug

response (Clark, 1937). In that case the maximum respiratory depressant effect is determined by buprenorphine's intrinsic efficacy, which is strictly a drug-specific parameter (Furchgott, 1967). Not surprisingly, buprenorphine's intrinsic efficacy for respiratory depression is identical in rat and man, since the maximum respiratory depressant effect is equal in both species. For the antinociceptive effect of buprenorphine in rat and man, no maximum effect was detected in the studied dose range. PK-PD data analysis showed that higher doses are required to achieve 100 % receptor occupancy in order to obtain the maximum observable antinociceptive effect (Yassen *et al.*, 2006b).

Buprenorphine is a highly lipophilic drug and readily crosses the blood-brain bar-

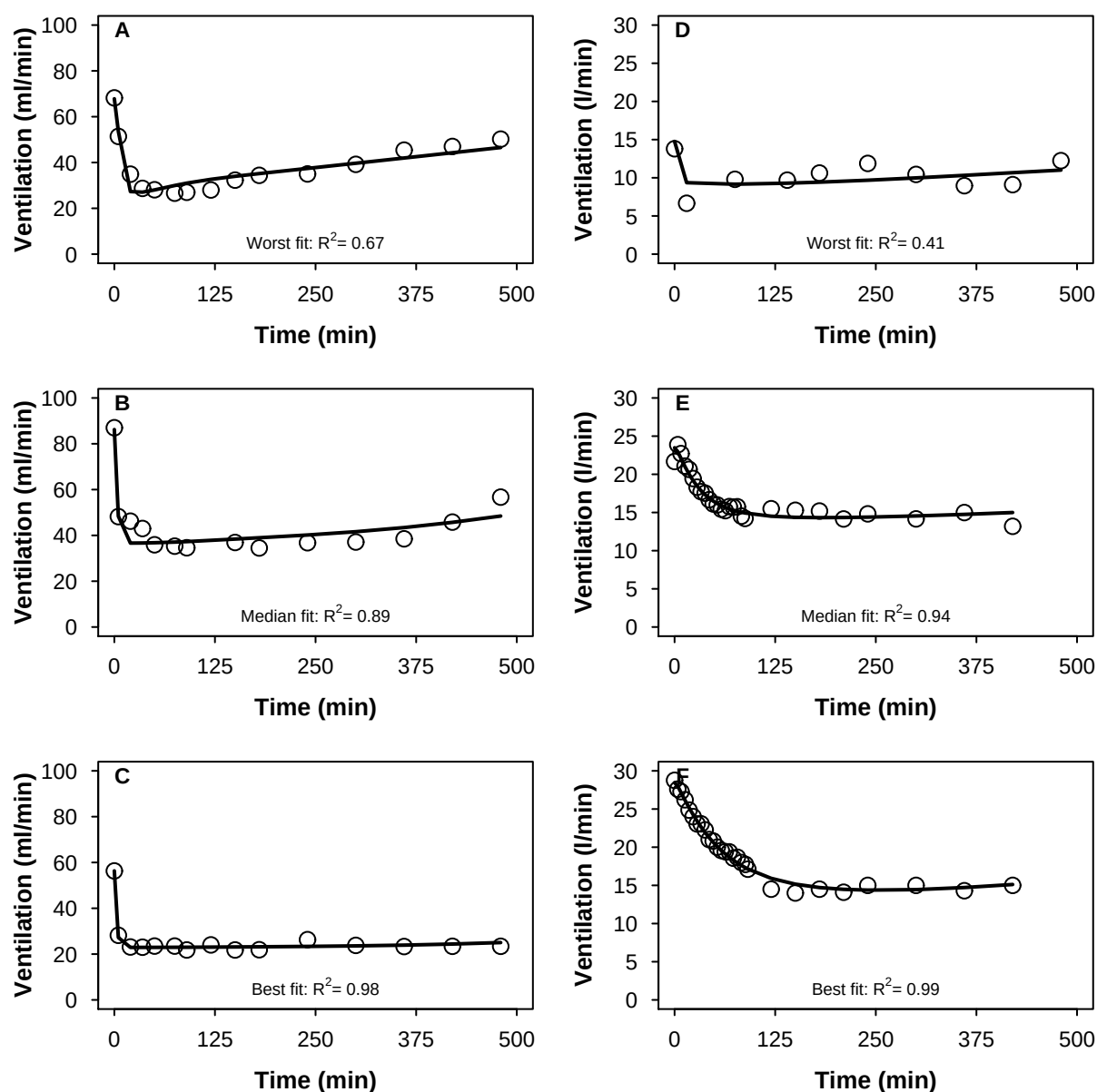


Figure 5. Time courses of the antinociceptive effect following buprenorphine administration in rats (A-C) and humans (D-F) are shown. Worst, median and best data fits between measured (symbols) and individual predicted respiratory depression are presented. The goodness of fit was assessed by the coefficient of determination (R^2).

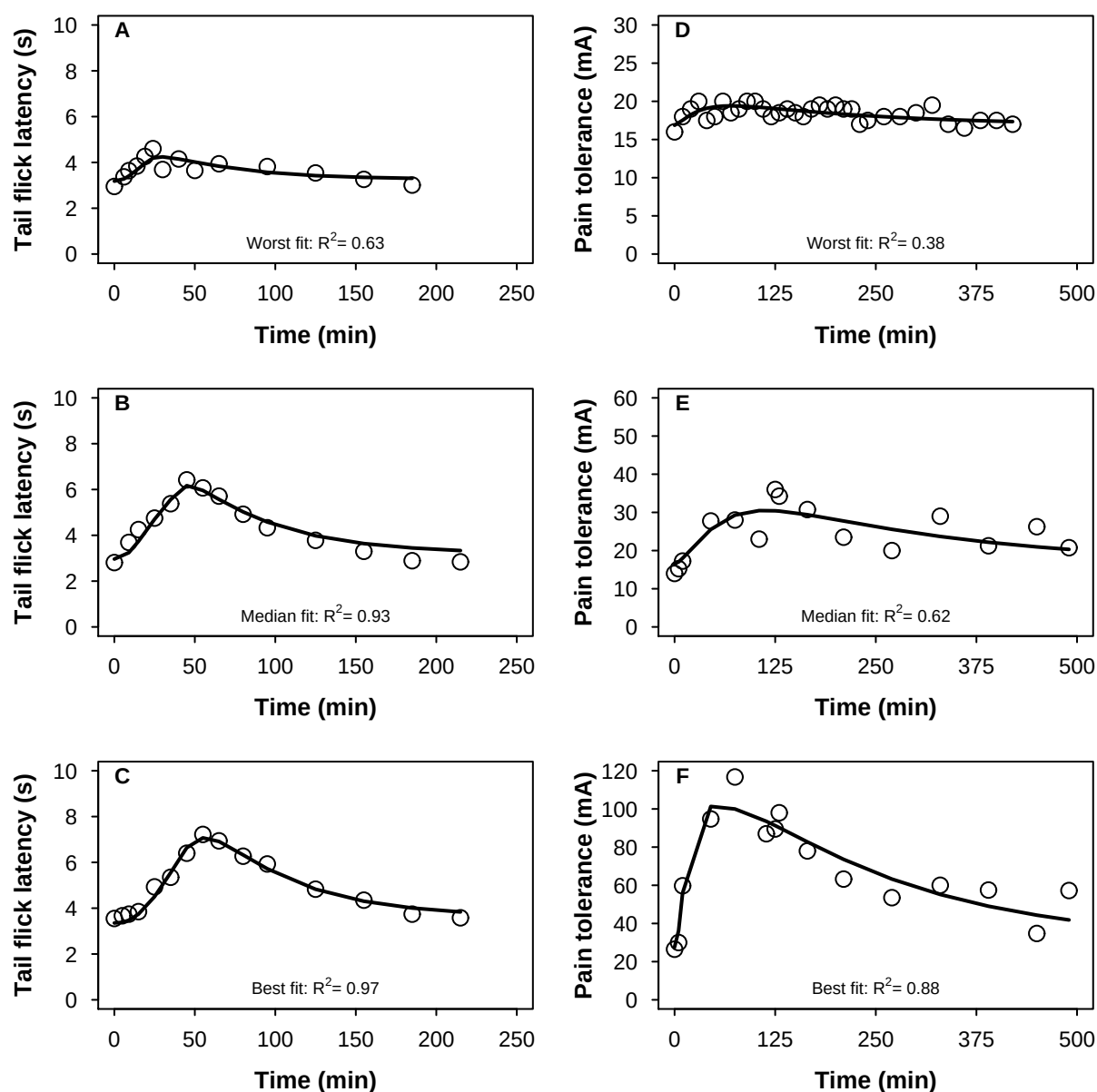


Figure 6. Time courses of the respiratory depressant effect following buprenorphine administration in rats (A-C) and human (D-F) are shown. Worst, median and best data fits between measured (symbols) and individual predicted respiratory depression are presented. The goodness of fit was assessed by the coefficient of determination (R^2).

rier (Ohtani *et al.*, 1995). It is reasonable to assume that after blood-brain barrier passage, buprenorphine is distributed within brain tissue before it is released to bind to the μ -opioid receptor. In fact, the distribution kinetics of buprenorphine to the various regions in the brain has been shown to be different (Galynker *et al.*, 1996). The observed hysteresis in the concentration-effect relationship can be therefore partly explained by buprenorphine distribution within the brain tissue rather than buprenorphine transport across the blood brain barrier. In principle, distribution kinetics are dependent on bodyweight and thus differ between rat and human. To this end, the rate constant for biophase equilibration is assumed to be a biological system-dependent parameter which may be scaled

from animal to human using allometry. The allometric exponent b was -0.28 (95 % CI: $-0.32 - -0.22$) and is not significantly different from the value of -0.25 which is generally accepted for allometric model in which functional flow and structural volume parameters are assumed to 0.75 and 1, respectively, indicating that a good prediction of biophase equilibration kinetics is obtained in both species using allometry. Interestingly, the value of biophase equilibration rate constant is similar for respiratory depression and antinociception, suggesting that buprenorphine's pharmacological effects are mediated via distinct μ -opioid receptor subtypes in the same brain region. Albeit μ -opioid receptors are localized throughout the central nervous system, a high density of μ -opioid receptor are predominantly found in the thalamus and the brainstem (Sprenger *et al.*, 2005). In specific areas of the brain stem, lateral medulla and pons, the neuronal pathways involved in the control of pain transmission are similar to the pathways involved in the ventilatory control (Nattie, 1995). These findings may explain the similarity found in the rate constants of biophase equilibration for the antinociceptive and respiratory depressant effect of buprenorphine.

In conclusion, a previously proposed mechanism-based PK-PD model had been successfully applied to predict the time course of buprenorphine's respiratory depressant and antinociceptive effect in human on the basis of preclinical data. The PK-PD model is based on receptor theory and allows for separate characterization of biophase equilibration and receptor association/dissociation kinetics as determinants of hysteresis between plasma concentration and effect. An important feature of the PK-PD model is the clear distinction between drug-specific and system-dependent parameters. In this respect, it was shown that the rate constants of receptor association/dissociation kinetics are species-independent and biophase equilibration is system-dependent. The allometric exponent for the system-dependent parameter k_{e0} was nearly identical to the value of -0.25 which is generally accepted in biology for the interspecies scaling of physiological processes characterized by rate constants. This indicates that the proposed PK-PD model constitutes a realistic approach to characterize the PK-PD correlation of buprenorphine in rat and human using one model. The strict distinction between drug-and system dependent parameters is a key feature of PK-PD models with improved properties for extrapolation and prediction. In this respect, the development of mechanistic PK-PD models is of importance, as these PK-PD models allow more accurate predictions of pharmacological effects in human on the basis of preclinical *in vivo* data.

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MECHANISM-BASED
PHARMACOKINETIC-PHARMACODYNAMIC MODELING OF THE
REVERSAL OF BUPRENORPHINE-INDUCED RESPIRATORY
DEPRESSION BY NALOXONE:
AN EXPERIMENTAL STUDY IN HEALTHY VOLUNTEERS

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ABSTRACT

Respiratory depression is a potentially life-threatening side-effect of opioid therapy. It has been postulated that the difficulty of reversing buprenorphine-induced respiratory depression is caused by slow receptor association-dissociation kinetics at the μ -opioid receptor. The aim of this study was to characterize the pharmacodynamic interaction between buprenorphine and naloxone in healthy volunteers.

A competitive pharmacodynamic interaction model is proposed to describe and predict the time course of naloxone-induced reversal of respiratory depression. The model was identified on data from an adaptive naloxone dose selection trial following the intravenous administration of 0.2 mg/70 kg or 0.4 mg/70 kg buprenorphine. The pharmacokinetics of naloxone and buprenorphine were best described by a two- and three compartment model, respectively.

A combined biophase equilibration-receptor association-dissociation model described the competitive interaction between buprenorphine and naloxone at the μ -opioid receptor. For buprenorphine, the values of the rate constants of receptor association (k_{on}) and dissociation (k_{off}) were 0.203 ml/ng/min and 0.0172 min⁻¹, respectively. The value of K_D was 0.18 nM. The half-time of biophase equilibration was 173 min. These estimates of the pharmacodynamic parameter estimates are similar to values obtained in the absence of naloxone co-administration. For naloxone the $t_{1/2,ke0}$ was 6.5 min.

Due to the slow receptor association-dissociation kinetics of buprenorphine, in combination with the fast elimination kinetics of naloxone, naloxone is best administered as a continuous infusion for reversal of buprenorphine-induced respiratory depression.

INTRODUCTION

Opioids are commonly used for the treatment of acute and chronic pain. Despite their analgesic effectiveness, respiratory depression remains the chief hazard of opioid agonists (Baxter, 1994; Dahan *et al.*, 1998). Since respiratory depression is often unpredictable and shows considerable variability across patients, fast and complete reversal of opioid-induced respiratory depression is required. Clinically, the opioid antagonist naloxone is commonly used to treat and to prevent the recurrence of profound respiratory depression (Davies *et al.*, 1980; Taylor *et al.*, 2005).

The intensity and duration of the respiratory depressant effect have been shown to depend on the pharmacological characteristics and the dose of the administered opioid (Zuurmond *et al.*, 2002; Dahan *et al.*, 2005). Along the same line, the reversal of opioid-induced respiratory depression depends also on the pharmacological properties of the opioid. In addition to the receptor affinity, this concerns in particular also the kinetics of receptor association and dissociation. In recent years research has improved our understanding of the factors which determine the time course of opioid-induced respiratory depression (Yassen *et al.*, 2006, 2007b). This is important for the dosing of naloxone in order to achieve and maintain complete reversal of opioid-induced respiratory depression. This concerns the opioid buprenorphine in particular (Cowan *et al.*, 1977; Gal, 1989).

Recently, a mechanism-based PK-PD model has been proposed for the effects of buprenorphine on respiration in humans and rats (Yassen *et al.*, 2006, 2007b). The main outcome of the PK-PD analysis is that buprenorphine is a partial agonist displaying a ceiling effect for respiratory depression. Moreover, it was shown that, in addition to the biophase distribution kinetics, the slow receptor association and dissociation kinetics is a major determinant of the slow onset and long duration of the respiratory depressant effect. The observed slow *in vivo* receptor association-dissociation kinetics is consistent with observations in *in vitro* receptor binding studies (Kosterlitz *et al.*, 1975; Villiger and Taylor, 1982; Boas and Villiger, 1985). It has been postulated that the slow receptor binding kinetics contributes to the difficulty of reversing buprenorphine-induced respiratory depression (Gal, 1989).

The objective of this study was to characterize the *in vivo* pharmacodynamic interaction between buprenorphine and naloxone in healthy volunteers using minute ventilation as a biomarker for respiratory depression. This ventilatory effect parameter provides a continuous, reproducible and clinically relevant measure of the drug response. In this investigation, high-resolution concentration and effect data were obtained on the reversal of buprenorphine-induced respiratory depression following the administration of widely different doses of naloxone.

MATERIALS AND METHODS

Study design

Forty-four male and female subjects (age range 18-30 yrs) were enrolled in this placebo-controlled adaptive naloxone dose design study. All subjects were healthy and did not have a history of illicit drug use or mental disease. All women were taking oral contraceptives. Subjects were asked to have a normal night of sleep and not to eat or drink for at least 6 h prior to the study. After arrival in the research unit, an arterial line, for blood sampling, was placed in the left or right radial artery under local anesthesia. In the contra-lateral arm two intravenous lines were inserted for infusion of buprenorphine and naloxone, respectively. After a 30-min resting period, a face mask (Vital Signs, Totowa, NJ) was placed and respiratory measurements started. Breathing was stimulated by adding carbon dioxide to the inhaled gas such that the end-expiratory $P_{ET}CO_2$ was maintained at 7 kPa (see below). When on-line analysis revealed that breathing had stabilized, buprenorphine was infused (time $t = 0$ min). Two doses of buprenorphine were tested 0.2 mg and 0.4 mg/70 kg. All subjects received half of the buprenorphine dose at $t = 0$ min, followed by a continuous infusion for 1-h of the remaining dose (total dose = 0.2 or 0.4 mg in 60 min). At $t = 30$ min, naloxone was given, one half as a bolus and the remaining half infused over 30 min. The total naloxone doses tested were: 0, 1, 2, 3, 4, 5, 6, and 7 mg (table 1). Finally, eight subjects (4 male/4 female) received 0.2 mg buprenorphine. At $t=30$ min a bolus injection of 3.0 mg naloxone and a subsequent continuous infusion at a rate of 4.0 mg/hr during 90 min was given. All buprenorphine and naloxone doses are per 70 kg body weight. All bolus infusions were given over 90 seconds (table 1). The results of a part of this study have been reported in a descriptive manner in a previous publication (van Dorp *et al.*, 2006). For this study approval of the protocol was obtained from the Human Medical Ethics Committee (CME, Leiden University Medical Center). Oral and written informed consent was obtained from all subjects.

Measurement of ventilation

In- and expired gas flows were measured with a pneumotachograph connected to a pressure transducer and electronically integrated to yield a volume signal. The volume signal was calibrated with a motor-driven piston pump (stroke volume 1000 ml, at a frequency of 20/min). The pneumotachograph was connected to a T-piece. One arm of the T-piece received a gas mixture with a flow of 45 l/min from a gas mixing system, consisting of three mass flow controllers (Bronkhorst High Tech BV, Veenendaal, The Netherlands) with which the flow of O_2 , CO_2 and N_2 could be set individually at a desired value. A Personal Computer provided control signals to the mass-flow controllers so that the composition of the inspired gas mixtures could be adjusted to force end-tidal oxygen and carbon dioxide concentrations ($P_{ET}O_2$ and $P_{ET}CO_2$) to follow a specified pattern in time,

Table 1. Characteristics of pharmacokinetic-pharmacodynamic studies analyzed

	buprenorphine 0.2 mg/70 kg	buprenorphine 0.4 mg/70 kg
Mode of buprenorphine administration	half buprenorphine dose bolus injection over 90-s followed by half buprenorphine dose over 60 min infusion	
Naloxone dose tested	0, 0.5, 1, 2, 3, 4, 5, 6, 7 mg/70 kg	0, 0.5, 2, 3, 4, 5 and 6 mg /70 kg
Mode of naloxone administration	half naloxone dose bolus injection over 90-s followed by half naloxone dose over 30-min infusion	
placebo/naloxone	6/16	5/9
male/female	11/11	7/7
age (year)	21 (18-30)	25 (18-30)
weight (kg)	70.5 (58-88)	73.6 (57-93)
height (m)	1.80 (1.61-1.94)	1.80 (1.68 - 1.97)

[§] Control groups received an infusion of saline containing polysorbate 80

[‡] Control groups received an infusion of saline.

independent of the ventilatory response (i.e., Dynamic End-tidal Forcing). In this study, end-tidal P_{ETCO_2} was clamped at 7 kPa (53 Torr) throughout the measurements, while end-tidal P_{ETO_2} was maintained at a normoxic value of 14.5 kPa (109 Torr). The O_2 and CO_2 concentrations and the arterial Hb- O_2 saturation (SpO_2) were measured with a Datex Multicap gas monitor near the mouth (Datex-Engstrom, Helsinki, Finland) and a Masimo pulse oximeter (using a finger probe), respectively. The gas monitor was calibrated with gas mixtures of known concentration delivered by a gas-mixing pump (W. Stohff, Bochum, Germany). P_{ETCO_2} , P_{ETO_2} , inspired minute ventilation (V_i) and SpO_2 were collected on a breath-to-breath basis and stored on disc for further analysis.

Measurement of buprenorphine and naloxone concentration

Blood samples (10 ml) for the determination of buprenorphine and naloxone were collected using lithium heparin vacutainers (Becton Dickinson, UK). After centrifugation, the resultant plasma was transferred into a screw-cap polypropylene tube and stored at $<-20^\circ C$ until analysis. Samples were withdrawn prior to the iv administration, and at 2, 5, 10, 20, 30, 32, 35, 40, 50, 60, 62, 65, 70, 80, 90, 120, 150, 180, 240, 300, 360, 420 and 480 minutes after the start of the buprenorphine administration, respectively. For the determination of buprenorphine concentrations in plasma, a validated GC-MS method was used (Yassen *et al.*, 2007b). The accuracy and precision for calibration standards were 5.47 % and 1.83 %, respectively at the LLOQ (25.0 pg/ml) and 0.83 % and 3.79 %, respectively at the upper limit of quantification (ULOQ) of 10000 pg/ml. For the quality controls

the accuracy ranged from -1.42 % (75 pg/ml) to 7.74 % (8000 pg/ml). The precision ranged from 1.88 % (8000 pg/ml) to 5.31 % (75 pg/ml). All concentration data presented refer to buprenorphine free base.

For the determination of naloxone concentrations in plasma, a validated HPLC method using electrochemical (EC) detection was applied. The assay method involves a liquid-liquid extraction of human plasma samples followed by chromatographic separation on a RP18 column under isocratic conditions and EC detection. To 500 μ l of plasma, 10 μ l of internal standard solution (Naltrexone 5.00 μ g/ml), 250 μ l of a solution of 53 g of sodium carbonate, in 447 g HPLC-water at pH 10 (85 % phosphoric acid), 2.0 ml dichloromethane, and 4.5 ml diethyl ether were added. After shaking for 15 min at room temperature and centrifugation at 4000 rpm for 10 minutes at 10 °C, the organic layer was transferred into 200 μ l of a solution of 0.2 g of 85 % phosphoric acid in 1000 g HPLC-water. After a second shaking and centrifugation procedure, the organic layer was discarded and an aliquot of 30 μ l of the residual solution was injected into the chromatographic system, after one hour in a SPE-box. The HPLC-ECD detection was performed using a Shimadzu LC-10AD pump, a Gilson Abimed 234 injector, a Shimadzu CTO-10A column oven and an ESA Coulochem II detector. Integration was performed using the Waters Millenium integration system. The mobile phase consisted of a mixture of 15 g ammonium acetate in 3600 g HPLC-water, 50 g acetonitrile, and 100 g methanol at pH=3.0 (phosphoric acid 85 %). The column flow was 1 ml/min at column oven temperature of 50 °C. The calibration range of the method was fixed to 1.00-100 ng/ml with a lower limit of quantification of 1.00 ng/ml. The linearity within the calibration range was 0.99982 (R^2 of the standard curve). The accuracy of the method was better than 100 ± 2 %; the precision was found to be 5 %. All concentration values refer to free naloxone base.

PK-PD data analysis

PK-PD modeling was performed with NONMEM V software (level 1.1) (Beal and Sheiner, 1999). Using the population approach, first-level and second-level random effects are taken into account as well as the interaction between both levels of random effects (FOCE interaction). All fitting procedures were performed on an IBM-compatible computer (Pentium IV, 1500 MHz) running under Windows XP with the Fortran compiler Compaq Visual Fortran version 6.1. An in-house available S-PLUS 6.0 (Insightful Corp., Seattle, WA, USA) interface to NONMEM was used for data processing and management (including tools to perform predictive check and bootstrap) and graphical data display. Selection criteria during the model development process were based on the goodness-of-fit plots, changes in the objective function value (OFV) and parameter estimates and their respective 95 % CI intervals. Using the likelihood ratio test, the significance was set at $\alpha = 0.01$, which corresponds to a reduction of 6.6 units in objective function value (OFV; χ^2 distribution) to discriminate between two nested structural models after inclusion of one additional parameter.

Pharmacokinetic models

For buprenorphine and naloxone linear compartment pharmacokinetic models were considered to describe the time course of buprenorphine and naloxone concentration. The pharmacokinetic parameters were assumed to be log-normally distributed and therefore an exponential distribution model was used to account for inter-individual variability. Proportional error models were used to quantify residual error. Relationships between structural model parameters and covariates were considered during PK model development using the forward inclusion/backward elimination procedure (Mandema *et al.*, 1992). Briefly, explorative graphical analysis and general additive model (GAM) analysis, available in the S-PLUS based model building aid Xpose version 3.01 (Jonsson and Karlsson, 1999), were used to explore the relationship between the structural PK model parameters of buprenorphine and naloxone and the covariates sex, age, dose, bodyweight and height. Also the co-administration of naloxone was explored to influence the pharmacokinetics of buprenorphine. Significant parameter-covariate relationships were selected in the final PK model on the basis of the likelihood ratio test which was set at the 5 % significance level in the forward inclusion step and at the 1 % significance level in backward elimination step. Individual Bayesian pharmacokinetic parameter estimates obtained from the final population pharmacokinetic models served as input for pharmacodynamic model to predict buprenorphine and naloxone concentrations at V_{i50} observation time points (two-stage approach). Due to long run times of the PK-PD models, the two-stage approach was used rather than simultaneous estimation of the pharmacokinetic and pharmacodynamic model parameters.

To ensure that precise estimates of PK parameters are obtained pharmacokinetic model validation was performed (Ette *et al.*, 2003). Internal validation was conducted by means of a non-parametric bootstrap technique. Briefly, one thousand bootstrap replicates were generated by sampling randomly from the original data set with replacement. Subsequently, the data sets were fitted one at the time to the final population PK model. The mean, standard error, coefficient of variation and the lower and upper 95 % prediction intervals of the model parameter values were calculated and compared to the typical model parameter values from the original study.

Pharmacodynamic model

The pharmacodynamic interaction between buprenorphine and naloxone was characterized using a competitive interaction PK-PD model. This model is based on a previously proposed PK-PD model for the characterization of the time course of buprenorphine-induced respiratory depression (Yassen *et al.*, 2007b). Briefly, the PK-PD model contains specific expressions for the *in vivo* kinetics of buprenorphine action at the μ -opioid receptor and aims at separate characterization of the biophase distribution and the (slow) receptor association-dissociation kinetics as determinants of the time-dependencies in

buprenorphine's pharmacodynamics. For the interaction, a competitive interaction PK-PD model was used to predict buprenorphine and naloxone biophase concentrations, competitive binding of buprenorphine and naloxone to the μ -opioid receptor and ultimately the reversal of the respiratory depressant effect. Buprenorphine and naloxone distribution from plasma (C_p) to the site of action was characterized on the basis of a biophase distribution model. The rate of change of biophase buprenorphine ($C_{e,bup}$) and naloxone ($C_{e,nal}$) concentration is governed by the rate constant k_{e0} and can be described as follows:

$$\frac{dC_{e,bup}}{dt} = k_{e0,bup} \cdot (C_{p,bup} - C_{e,bup}) \quad (1)$$

$$\frac{dC_{e,nal}}{dt} = k_{e0,nal} \cdot (C_{p,nal} - C_{e,nal}) \quad (2)$$

At the site of action, buprenorphine and naloxone competitively bind to the μ -opioid receptor. Following the law of mass action, the rate of drug-receptor binding is proportional to the concentration of drug C_e and free receptor R :

$$\frac{dC_{e,bup}R}{dt} = k_{on,bup} \cdot C_{e,bup} \cdot R - k_{off,bup} \cdot C_{e,bup}R \quad (3)$$

$$\frac{dC_{e,nal}R}{dt} = k_{on,nal} \cdot C_{e,nal} \cdot R - k_{off,nal} \cdot C_{e,nal}R \quad (4)$$

where k_{on} and k_{off} are the respective second- and first-order rate constants of receptor association and dissociation and C_eR is the concentration of the drug-receptor complex. The total receptor concentration R_{tot} is given by the sum of the free receptor R and the drug-receptor complexes C_eR . Therefore:

$$R_{tot} = R + C_{e,bup}R + C_{e,nal}R \quad (5)$$

Under the assumption that the concentration of drug is in excess compared to the free receptor concentration R equations 3 and 4 can be rearranged:

$$\frac{d\rho_{app,bup}}{dt} = k_{on,bup} \cdot C_{e,bup} \cdot (1 - \rho_{app,bup} - \rho_{app,nal}) - k_{off,bup} \cdot \rho_{app,bup} \quad (6)$$

$$\frac{d\rho_{app,nal}}{dt} = k_{on,nal} \cdot C_{e,nal} \cdot (1 - \rho_{app,bup} - \rho_{app,nal}) - k_{off,nal} \cdot \rho_{app,nal} \quad (7)$$

in which ρ_{app} is the apparent fractional receptor occupancy (C_eR/R_{tot}) of buprenorphine and naloxone respectively. At equilibrium, the rate of naloxone- μ -opioid receptor association equals the rate of naloxone- μ -opioid receptor dissociation and the equilibrium condition for naloxone becomes:

$$\rho_{\text{app,nal}} = \frac{(1 - \rho_{\text{app,nal}}) \cdot C_{e,\text{nal}}}{\left(\frac{k_{\text{off,nal}}}{k_{\text{on,nal}}}\right) + C_{e,\text{nal}}} \quad (8)$$

Substituting for $\rho_{\text{app,nal}}$ from equation 8, the rate of change in buprenorphine- μ -opioid receptor complex becomes:

$$\frac{d\rho_{\text{app,bup}}}{dt} = k_{\text{on,bup}} \cdot C_{e,\text{bup}} \cdot \left[(1 - \rho_{\text{app,bup}}) \cdot \left(1 - \frac{C_{e,\text{nal}}}{K_{D,\text{nal}} + C_{e,\text{nal}}} \right) \right] - k_{\text{off,bup}} \cdot \rho_{\text{app,bup}} \quad (9)$$

in which $K_{D,\text{nal}}$ is the equilibrium dissociation constant of naloxone and is equal to $k_{\text{off,nal}}/k_{\text{on,nal}}$. In the present analysis, a linear transduction function was used to characterize the fractional receptor occupancy-respiratory depressant effect relationship (Yassen *et al.*, 2007b):

$$E = E_0 \cdot (1 - \alpha \cdot \rho_{\text{app,bup}}) \quad (10)$$

where E is the ventilatory response, E_0 is the baseline ventilation and α is the intrinsic activity of which the value varies between 0 and 1.

RESULTS

Pharmacokinetics

A three- and two-compartment pharmacokinetic model was found to appropriately characterize the time course of buprenorphine and naloxone concentration respectively. Inter-individual variability could be characterized for the pharmacokinetic parameters of buprenorphine and naloxone indicated in table 2 and 3. The administration of naloxone did not alter buprenorphine disposition as tested by inclusion of naloxone as a covariate for the various pharmacokinetic parameters of buprenorphine. In addition bodyweight or other demographic variables were also not a significant covariate for the pharmacokinetics of buprenorphine and naloxone. Some diagnostic plots for the final pharmacokinetic models of naloxone and buprenorphine are shown in figure 1. Although for low concentrations naloxone, a deviation is observed from the population predicted versus observed concentration plot (figure 1), no systematic trends were shown in the population or individual predictions versus the observations. Table 2 and 3 list the results of the bootstrap procedures, presented as mean and coefficient of variation (% CV). The mean values of the bootstrap procedure were comparable to the parameter estimates from the original data sets, underscoring the precision and robustness of the pharmacokinetic models for buprenorphine and naloxone.

Table 2. Mean population pharmacokinetic parameter estimates and relative standard errors (RSE) of buprenorphine obtained with the final three-compartment pharmacokinetic model and the stability of the respective PK model using the bootstrap resampling procedure.

	Original data set		1000 bootstrap replicates	
	Typical value	RSE (%) [†]	Typical value	RSE (%)
<i>Cl</i> (l/min)	1.43	3.0	1.42	2.9
<i>V</i> ₁ (l)	5.71	6.4	5.72	6.4
<i>V</i> ₂ (l)	22.1	11.3	22.2	10.7
<i>V</i> ₃ (l)	135	5.9	135	5.8
<i>Q</i> ₂ (l/min)	1.45	9.4	1.44	9.7
<i>Q</i> ₃ (l/min)	1.00	5.7	1.00	5.8
ωCl (%)	19.1	25.6	18.8	25.8
ωV_1 (%)	18.4	70.7	18.7	66.8
ωV_3 (%)	35.2	31.3	34.7	30.8
ωQ_2 (%)	36.5	51.2	35.6	47.1
ωQ_3 (%)	28.1	29.2	27.7	28.7
PRE [#] (%)	13.5	18.2	13.6	18.2

[†] relative standard error; [#] proportional residual error

Pharmacodynamics

Goodness-of-fit analysis

The buprenorphine-naloxone interaction model with biophase distribution and slow receptor association/dissociation kinetics successfully characterized the reversal of buprenorphine-induced respiratory depression after naloxone administration. Diagnostic plots for the final pharmacodynamic interaction model are shown in figure 2 and did not reveal any substantial model mis-specification. The estimates of the structural pharmacodynamic model parameters are listed in table 4. The observed time course of the ventilatory response for all individuals stratified to naloxone and buprenorphine dose treatment groups are shown in figure 3 (buprenorphine 0.2 mg/70 kg) and figure 4 (buprenorphine 0.4 mg/70 kg). The mean population predicted time course of the ventilatory response, calculated on the basis of the values of the population pharmacodynamic parameters of naloxone and buprenorphine, are depicted as solid lines. In figure 5, examples of nine data fits are given, including the best, median and worst fit based on the coefficient of determination (R^2).

Table 3. Mean population pharmacokinetic parameter estimates and relative standard errors (RSE) of naloxone obtained with the final two-compartment pharmacokinetic model and the stability of the respective PK model using the bootstrap resampling procedure.

	Original data set		1000 bootstrap replicates	
	Typical value	RSE (%)	Typical value	RSE (%)
Cl (l/min)	3.45	5.3	3.44	5.1
V_1 (l)	12.1	11.8	12.2	12.0
V_2 (l)	102	5.4	101.7	5.6
Q (l/min)	4.74	13.1	4.79	12.9
ωCl (%)	25.3	22.5	24.9	23.9
ωV_2 (%)	19.2	36.1	18.8	37.8
ωQ (%)	45.8	67.6	46.7	58.5
PRE (%)	16.4	14.2	16.3	14.4

Pharmacodynamic parameter estimates

Placebo treatment had no significant effect on the reversal of respiratory depression during the measurement period (figure 3). The estimated baseline (pre-drug) ventilation was 24.7 ± 4.8 l/min. A biophase distribution model was incorporated to describe the distribution of buprenorphine and naloxone to and within the brain. On the basis of the first-order rate constant k_{e0bup} , the half-time of biophase distribution was 173 min for buprenorphine. The population values of the rate constants for receptor association k_{on} and dissociation k_{off} were 0.203 ml/mg/min (95 % CI: 0.132 - 0.274 ml/mg/min) and 0.0172 min^{-1} (95 % CI: 0.0080 - 0.0264 min^{-1}), which corresponds to $t_{1/2,ke0} = 40.3$ min. The half-time of receptor dissociation is shorter than the half-time of biophase distribution, indicating that the distribution of buprenorphine to and from the brain is the rate-limiting step in the onset and offset of the respiratory depressant effect. The equilibrium dissociation constant K_{Dbup} was 84.7 ng/l corresponding to 0.18 nM. The intrinsic activity of buprenorphine was 0.67 (95 % CI: 0.60 - 0.75). A value of 1 for the intrinsic activity indicates full agonistic activity. The estimated value of intrinsic activity of 0.67 shows that buprenorphine acts as a partial agonist for respiratory depression.

For naloxone, the rate constants of receptor association/dissociation kinetics were assumed to be fast relative to the pharmacokinetics in plasma and brain. Therefore the biophase distribution model was applied to explain the time delay in the kinetics of onset of reversal by naloxone. The rate constant of biophase distribution k_{e0nal} is 0.106 min^{-1} (95 % CI: 0.09 - 0.12 min^{-1}), which corresponds to a half-time of 6.8 min, suggesting a relatively fast onset of naloxone's action to reverse respiratory depression. The K_D of naloxone is 1.87 ng/ml (95 % CI: 1.06 - 2.68 ng/ml), corresponding to 5.7 nM.

Predictive performance of the PK-PD interaction model

To test the appropriateness of the developed PK-PD interaction model to predict reversal of respiratory depression after any type of naloxone administration, external validation was performed with additional data obtained from healthy volunteers that received naloxone (3 mg/70 kg as a bolus followed by a continuous infusion at a rate of 4 mg/70kg/hr) during 90 min. Figure 6 shows that the PK-PD interaction accurately predicted the time course of respiratory depression upon prolonged administration of naloxone and underlines the importance of the interaction PK-PD model in designing dosage regimens for complete and persistent reversal of buprenorphine-induced respiratory depression.

Table 4. Population pharmacodynamic estimates and their inter-individual variability estimates of buprenorphine and naloxone obtained from the pharmacodynamic interaction model. For the fixed and random parameter estimates the relative standard error (RSE) is displayed.

	Typical value	RSE (%)	IIV (%) [†]	RSE (%)
Buprenorphine				
k_{on} (ml/ng/min)	0.203	17.9	86.3	30.5
k_{off} (min ⁻¹)	0.0172	27.4	96.5	27.8
k_{e0} (min ⁻¹)	0.00404	15.8	83.5	24.9
E_0 (l/min)	24.0	2.4	21.8	19.3
α	0.67	5.9	24.8	32.6
Naloxone				
k_{e0} (min ⁻¹)	0.106	7.5	NE [‡]	NE
$K_{D,nal}$ (ng/ml)	1.87	21.1	89.4	41.4
ARE (l/min)	1.5	11.7		

[†] inter-individual variability; [‡] not estimated; ^{||} additive residual error.

DISCUSSION

In the present investigation, a competitive interaction PK-PD model is proposed to characterize the *in vivo* pharmacodynamic interaction between buprenorphine and naloxone in healthy volunteers. The respiratory effects of buprenorphine and naloxone were assessed by the dynamic end-tidal forcing technique (Dahan *et al.*, 1990). Drug-induced changes in minute ventilation were used as a biomarker for the respiratory depressant effect. Another frequently used surrogate biomarker for respiratory depression, partial arterial CO₂ pressure ($P_a\text{CO}_2$), has previously been shown to underestimate the respiratory depressant effect of buprenorphine (Dahan *et al.*, 2005). The lack of measurement sensitivity makes

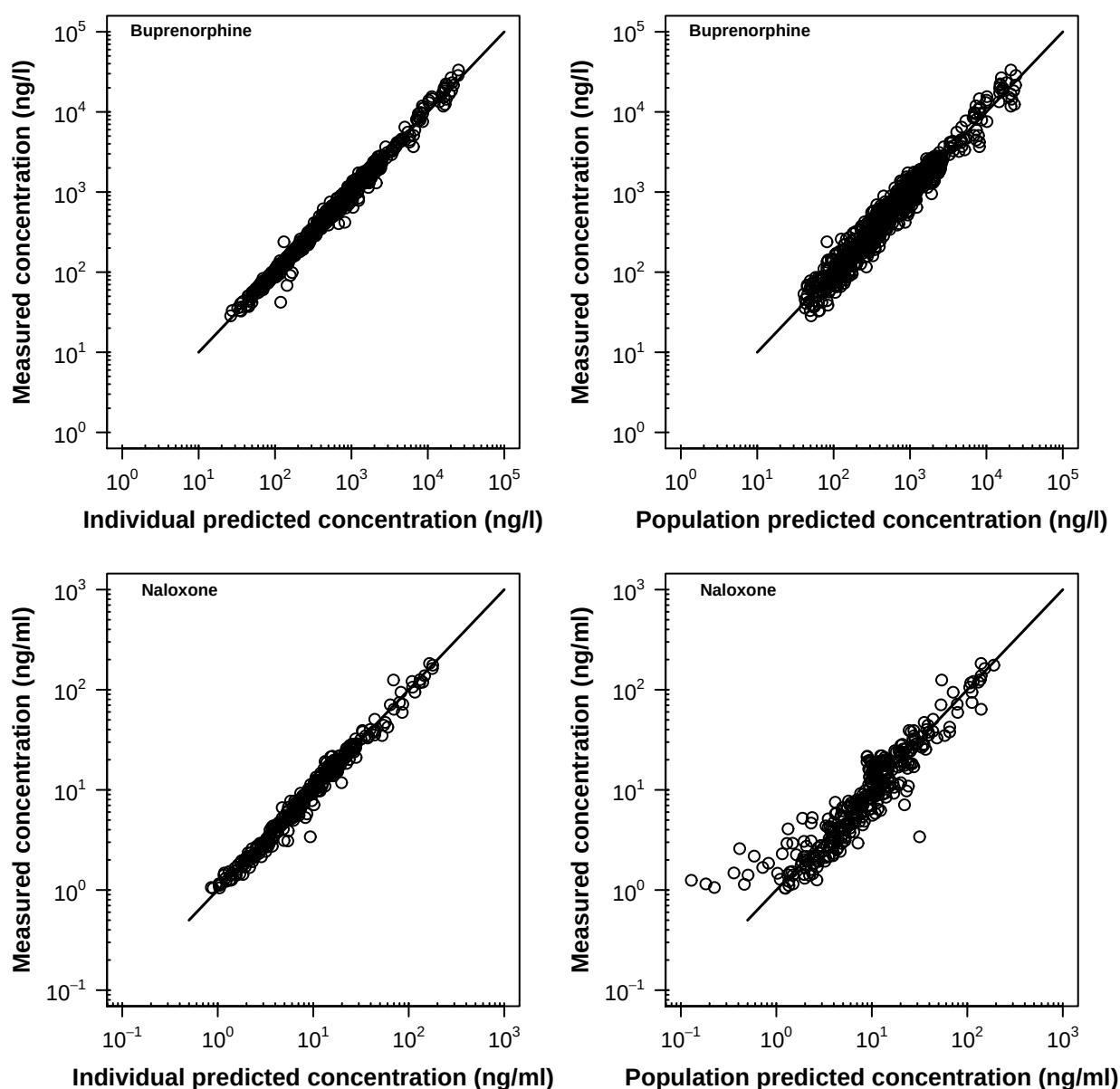


Figure 1. Goodness of fit plots for the pharmacokinetics of buprenorphine and naloxone in human: observed *versus* individual predicted or population predicted buprenorphine or naloxone concentration by the three- and two-compartment pharmacokinetic model, respectively. The solid line is the identity line.

$P_a\text{CO}_2$ not a suitable candidate biomarker to study the effects of naloxone on the reversal of buprenorphine-induced respiratory depression. Recently, minute ventilation was used as biomarker to characterize the respiratory depressant effect of buprenorphine (Yassen *et al.*, 2007b). In this respect, minute ventilation has been shown to be an objective, reproducible and continuous measure of the respiratory depressant effect which closely correlated with the *in vivo* receptor binding kinetics and intrinsic activity of buprenorphine at the μ -opioid receptor.

The competitive interaction PK-PD model yields relevant information on the *in vivo* kinetics of buprenorphine's respiratory depressant action also with regard to the interac-

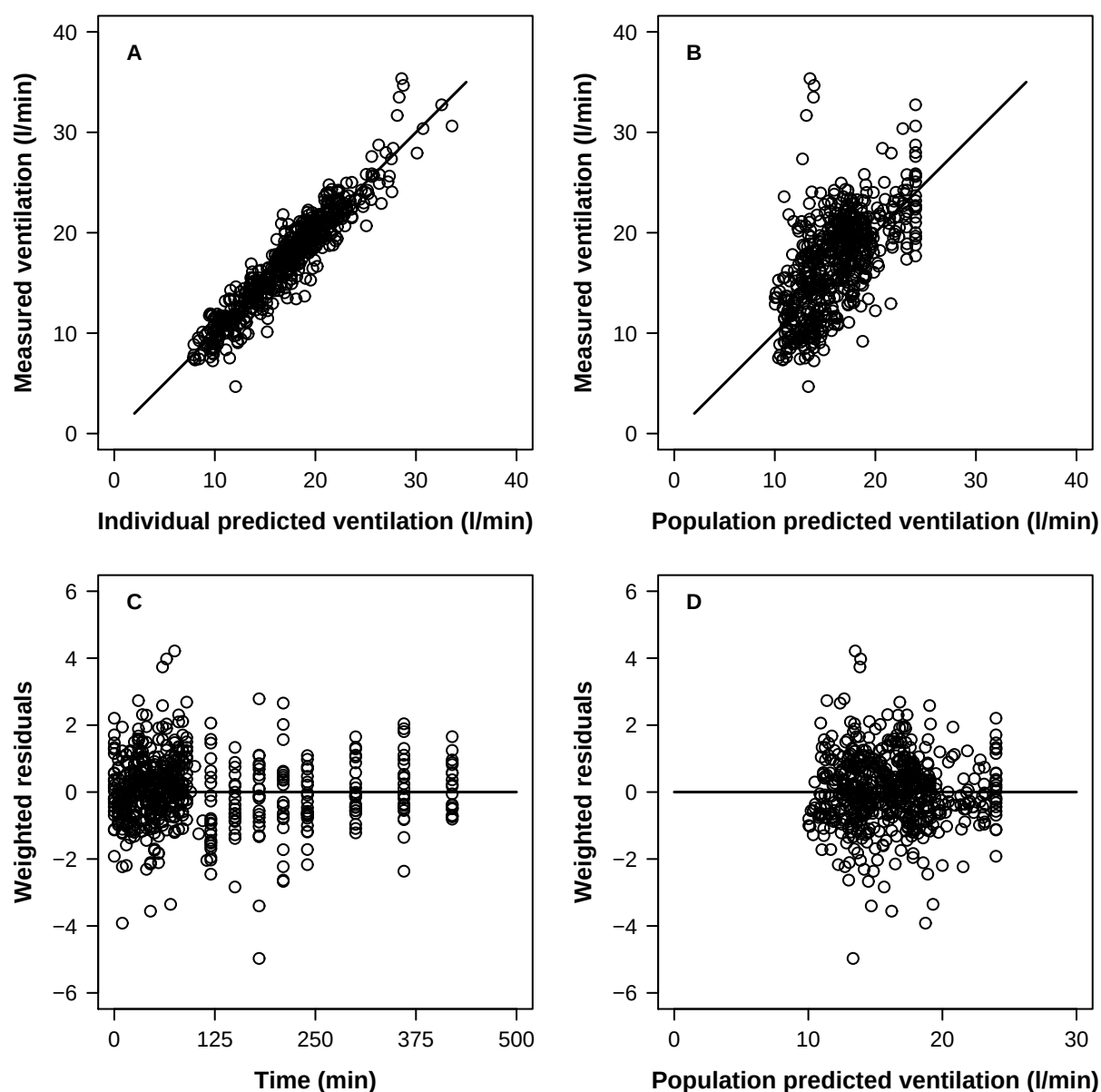


Figure 2. Goodness of fit for the pharmacodynamic interaction model: observed *versus* individual predicted (A) or population predicted (B) ventilatory response. The solid line is the identity line. The weighted residuals *versus* time (C) or population predicted effect (D) are randomly distributed around the zero-line indicating no systemic deviations of the model outcome.

tion with the competitive opioid-antagonist naloxone. For buprenorphine, the half-time of biophase distribution is 173 min in the presence of naloxone. Interestingly, a similar estimate for the half-time of biophase distribution was previously obtained for buprenorphine's antinociceptive and respiratory depressant effect (Yassen *et al.*, 2007a). This indicates that buprenorphine distribution within the brain tissue is not influenced by naloxone disposition characteristics at the site of action. Besides the biophase equilibration kinetics, slow receptor association/dissociation kinetics contributes to the observed hysteresis. The half-time of receptor dissociation ($t_{1/2, \text{koff}}$) for buprenorphine was 40.3 min, which is in the same range of the value of 49.5 min, which was previously estimated for

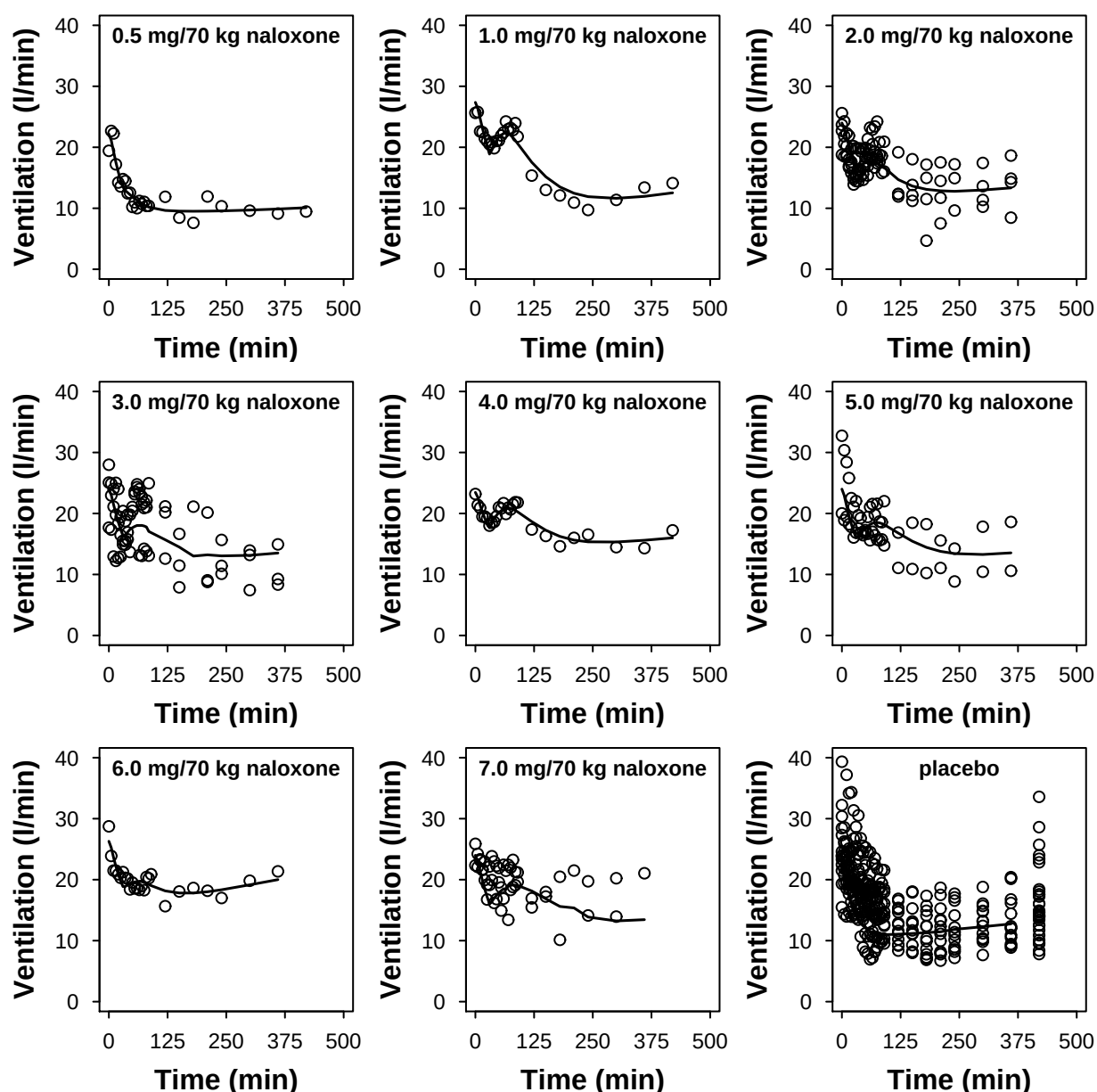


Figure 3. Individual observed respiratory effect-time profiles for the different naloxone dose treatment groups. The observed concentrations (closed circles) and population predictions (thick line) are depicted. All individuals received 0.2 mg/70 kg buprenorphine followed by naloxone. The time courses of respiratory depression of individuals that received 0.2 or 0.4 mg/70 kg buprenorphine followed by placebo were pooled in one graph.

buprenorphine's respiratory depressant effect, without the co-administration of naloxone, in rats and humans. Independent of the model-based approach, the half-time of receptor dissociation was also determined in an *in vivo* receptor binding assay using a PET receptor imaging technique (Galynker *et al.*, 1996). The estimate of half-time of receptor dissociation of 41 min obtained from the PET study is nearly identical to the value of 40.3 min reported in the present study. This investigation has also yielded an *in vivo* estimate of the equilibrium dissociation constant K_D for buprenorphine. The $K_{D\text{bup}}$ ($k_{\text{off}}/k_{\text{on}}$) was 84.7 ng/l corresponding to 0.18 nM and is in the same range to the estimate of the *in*

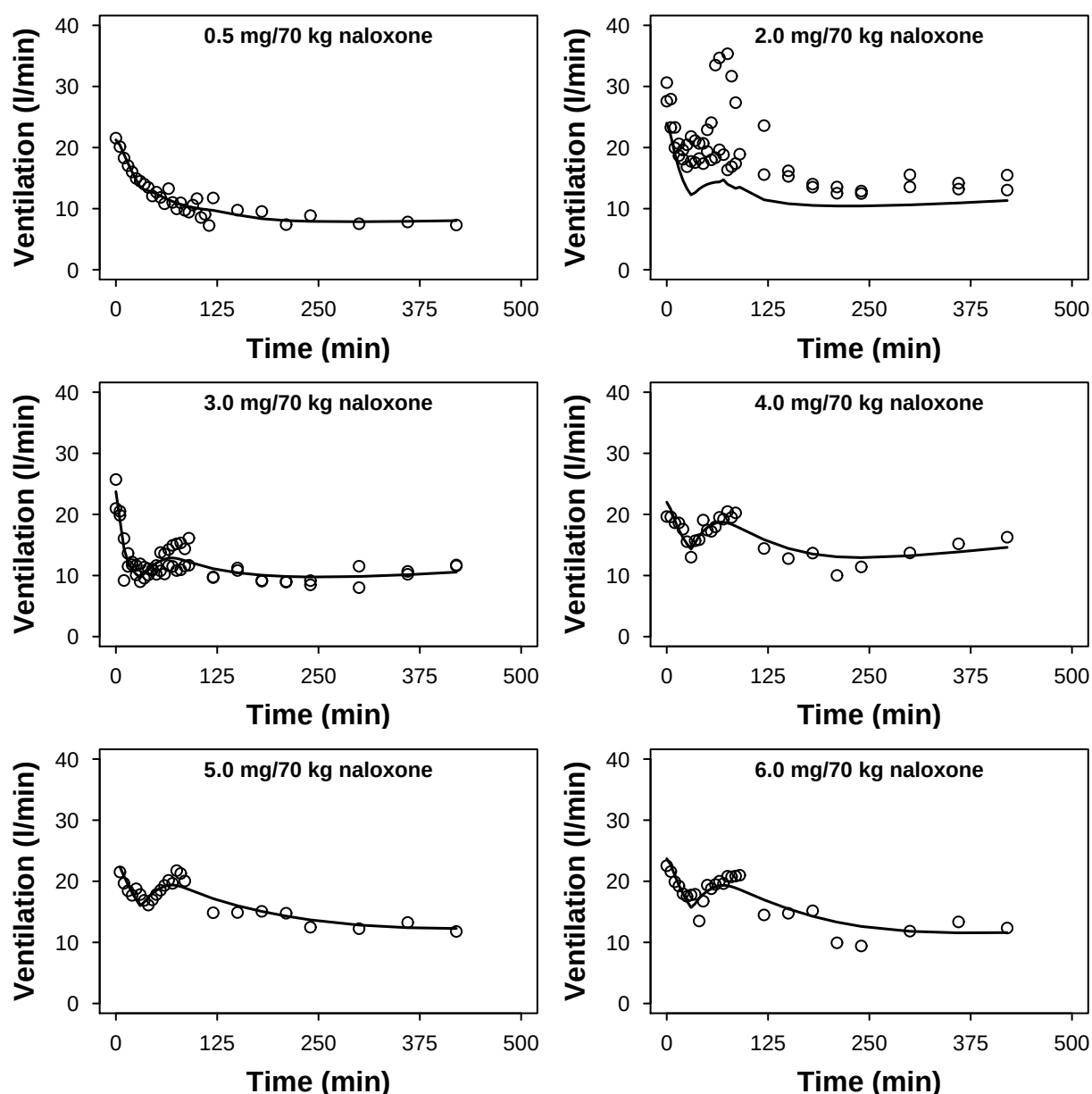


Figure 4. Individual observed respiratory effect-time profiles for the different naloxone dose treatment groups. The observed concentrations (closed circles) and population predictions (thick line) are depicted. All individuals received 0.4 mg/70 kg buprenorphine followed by naloxone.

vivo $K_{D_{bup}}$ of 0.13 nM reported in a previous study on the respiratory depressant effect of buprenorphine (Yassen *et al.*, 2007b). The fact that the values of the rate constants of biophase equilibration and receptor association/dissociation in the two studies are nearly identical indicates that their estimates are unique and independent of the administration of naloxone.

For naloxone, the receptor association/dissociation kinetics has been assumed to be fast. This is consistent with naloxone's fast receptor association/dissociation kinetics *in vitro*. For instance, the *in vitro* half-time of dissociation of the naloxone- μ -opioid receptor complex was estimated to be 0.82 min using CHO-K1 cells transfected with the human μ -opioid receptor (Cassel *et al.*, 2005). Following intravenous administration, the estimated

elimination half-life time ($t_{1/2} = 0.693/\beta$) of naloxone from plasma was 33 min in healthy volunteers. Clearly, the receptor dissociation kinetics are relatively fast to the pharmacokinetics in plasma and therefore not rate-limiting in the kinetics of naloxone's action at the μ -opioid receptor. The incorporation of a biophase distribution model suggests that there is a time delay between naloxone concentration and the pharmacological effect. The half-time of biophase equilibration is ($t_{1/2,ke0}$) 6.5 min. The short half-time of biophase distribution indicates that naloxone is rapidly distributed to and from the brain site of action (Weinstein *et al.*, 1973; Ngai *et al.*, 1976), with the concentration-time profile being a good reflection of the kinetics at the site of action. Not surprisingly, apart from

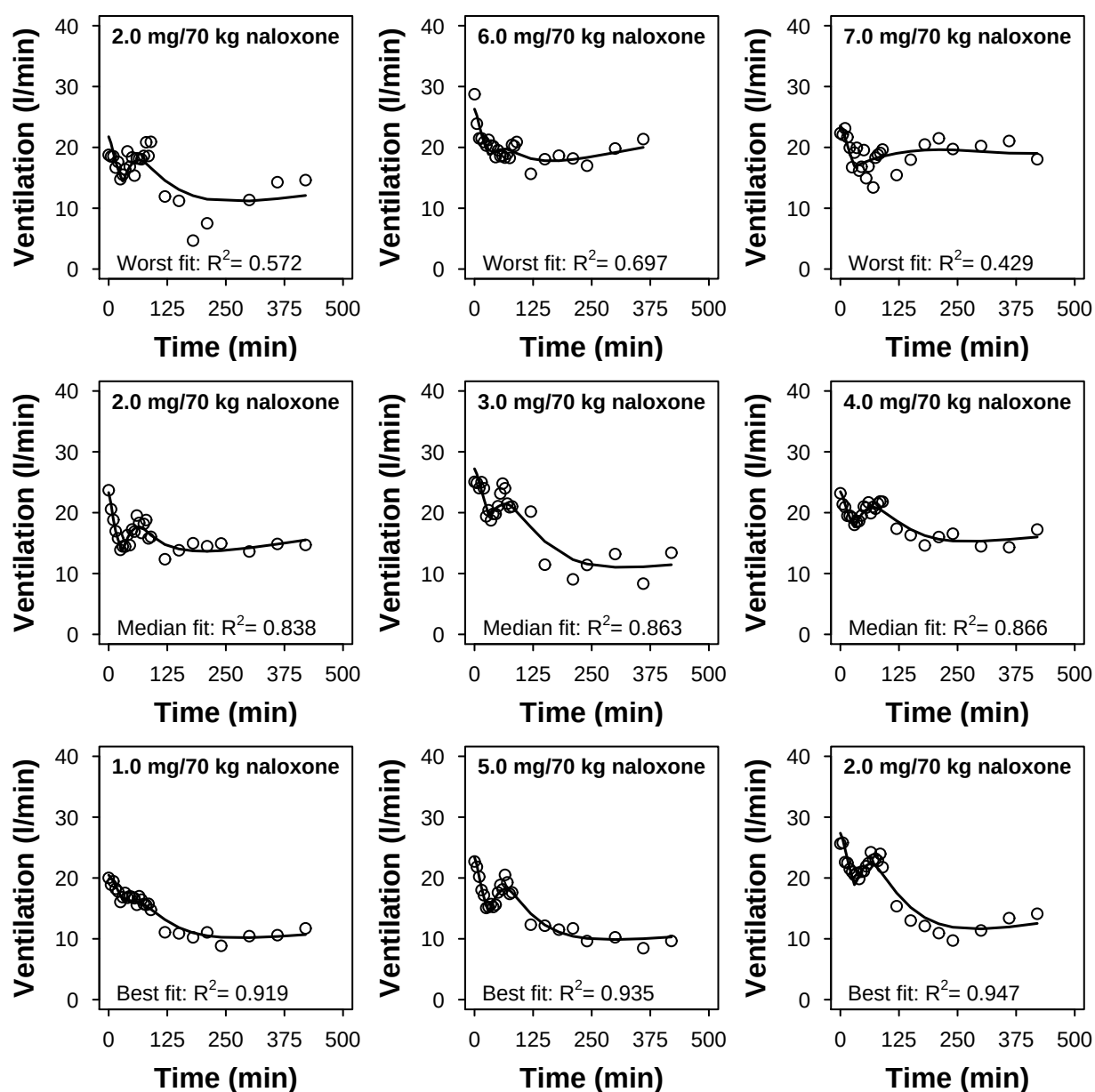


Figure 5. The time course of the respiratory depressant effect following the administration of buprenorphine is shown. Best (lower panel), median (middle panel), and worst (upper panel) data fits between measured (symbols) and individual predicted respiratory depression (solid line) are presented. The goodness of fit was assessed by the coefficient of determination (R^2).

buprenorphine's slow receptor association/dissociation kinetics *in vivo*, the short half-time of naloxone elimination from the brain appear to complicate rapid and complete reversal of buprenorphine-induced respiratory depression.

The competitive interaction PK-PD model constitutes a clinically relevant basis for the design of naloxone dose regimens with the aim to completely reverse buprenorphine-induced respiratory depression. In line with previous clinical experiences with buprenorphine (Bowdle, 2003; Mehta *et al.*, 2005), clinical trial simulations show the difficulty of naloxone, when administered as a clinically common or even higher bolus dose, to completely reverse respiratory depression (figure 7). Furthermore, the simulations revealed that complete reversal is possible, depending on the mode of naloxone administration. Specifically, it was shown that in principle complete reversal of buprenorphine-induced

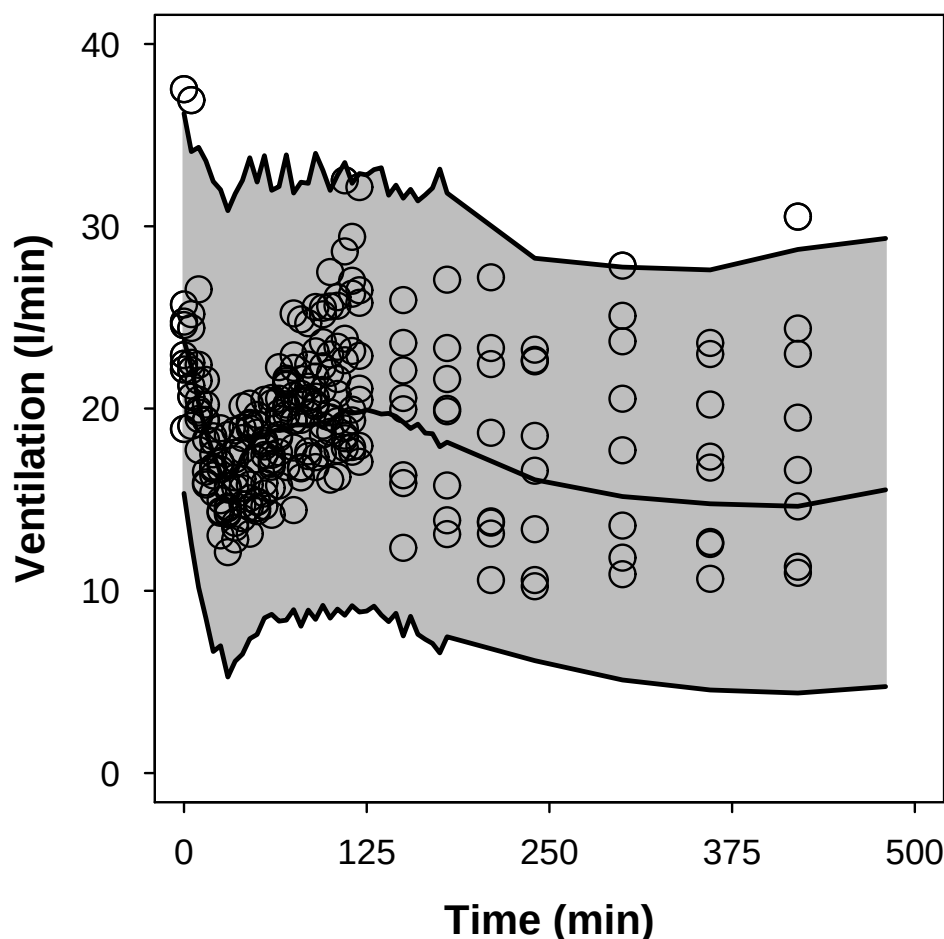


Figure 6. Predictability and accuracy of the PK-PD model outcome of the reversibility of buprenorphine-induced respiratory depression with a more prolonged infusion of naloxone. Buprenorphine 0.2 mg was administered. After 30 min a bolus injection (90 s) of 3 mg naloxone was given followed by a continuous infusion of 4 mg/70/hr during 90 min. The lower and upper dashed lines represent the 2.5 and 97.5 % percentiles of the simulated time courses of respiratory depression ($n = 250$), respectively. The solid line is the simulated median response. The symbols are the actual observed time courses of respiratory depression of eight healthy volunteers, which received a similar treatment as in the simulation scenario.

respiratory depression is feasible with a continuous infusion of naloxone. In the present investigation, experimental data confirm the simulation outcomes and reveal almost complete reversal of respiratory depression following the administration of 2 to 4 mg/70kg/hr naloxone. However, upon termination of the naloxone infusion there was a rapid re-

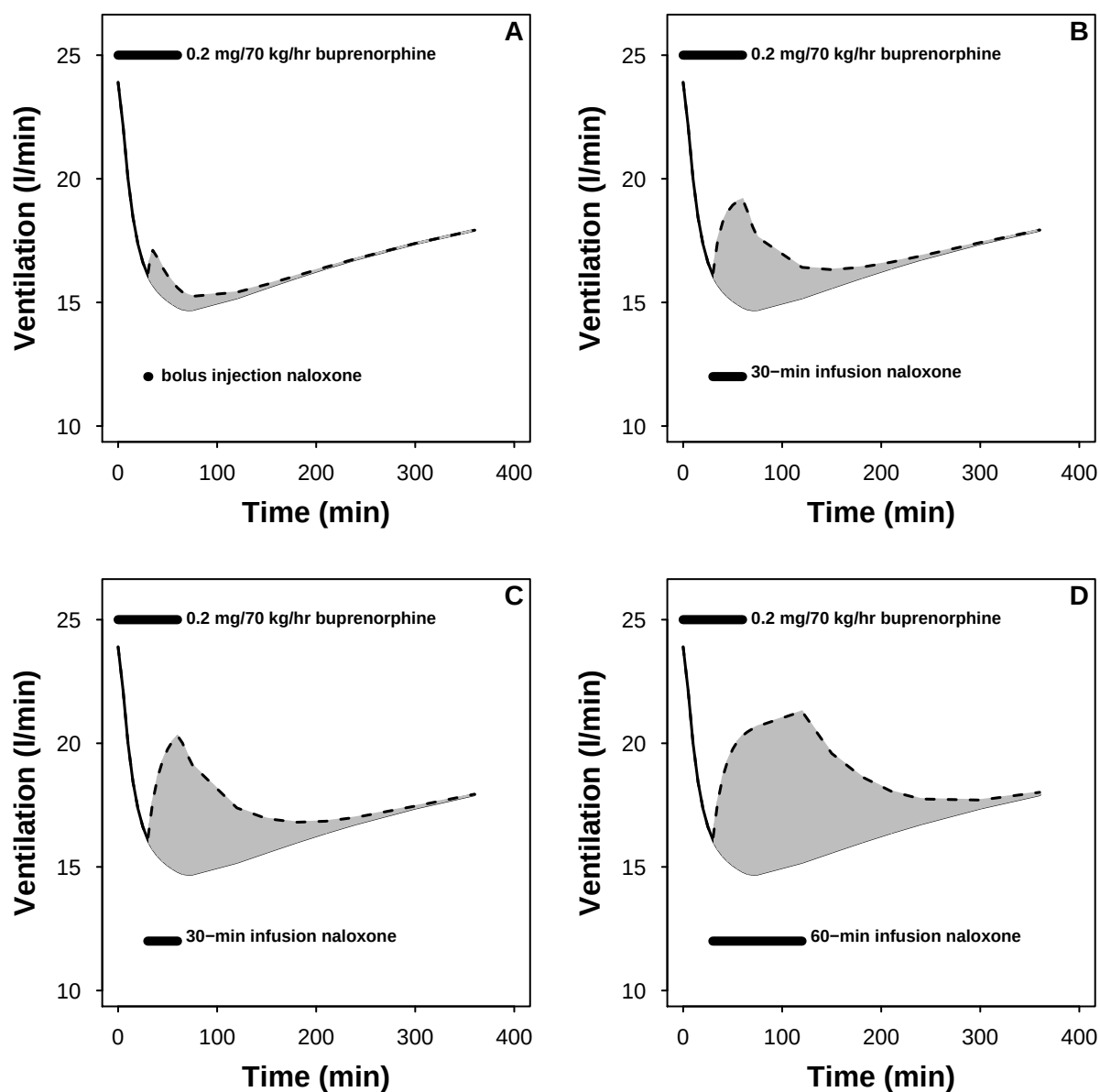


Figure 7. Simulation of the reversal of buprenorphine-induced respiratory depression at various doses of naloxone. The individuals received 0.2 mg/70 kg buprenorphine. Half of the dose was given over 90-s, the remainder over 59 min. Thirty minutes after the buprenorphine bolus, the subjects were given naloxone. The naloxone doses given were (A) 0.8 mg/70 kg as a bolus injection, (B) 2 mg/70 kg, half of the naloxone dose was given over 2 min, the remainder over 28 min (C) same like (B) 4 mg/70 kg and (D) 4 mg/70 kg, half of the naloxone dose was given over 2 min, the remainder over 88 min. The solid line is the control (no naloxone) time course of respiratory depression. The dashed lines are the time course of respiratory depression following the administration of naloxone. The black boxes represent the duration of infusion of buprenorphine (upper box) and naloxone (lower box).

currence of respiratory depression, most likely related to the combination of the rapid wash-out of naloxone from the brain and buprenorphine's slow elimination kinetics. This implies that naloxone should be administered continuously over a longer time interval to prevent the recurrence of respiratory depression. Simulations, on the basis of the competitive interaction PK-PD model, suggest that continuous infusion of naloxone at a rate of 4 mg/70kg/hr rather than at a rate of 2 mg/70 kg/hr is indicated to prevent the recurrence of respiratory depression (figure 8).

Recently, it was shown that continuous infusion of naloxone at a rate exceeding 4 mg/70 kg/hr is not recommended as it may lead to a decrease in the effectiveness of buprenorphine reversal (bell-shaped dose-response curve) (van Dorp *et al.*, 2006). In the present study the prediction of the reversal of respiratory depression following the administration of naloxone doses of more than 4 mg/hr was poorer than for the lower naloxone doses (figure 5). This suggests that indeed a different type of mechanism-based PK-PD model is needed to accurately predict the reversal of respiratory depression following the administration of naloxone doses of more than 4.0 mg/hr. Also the results presented in figure 3 suggest that the administration of higher doses naloxone than 4 mg/hr yield less reversal, indicating the presence of a bell-shaped dose-response curve.

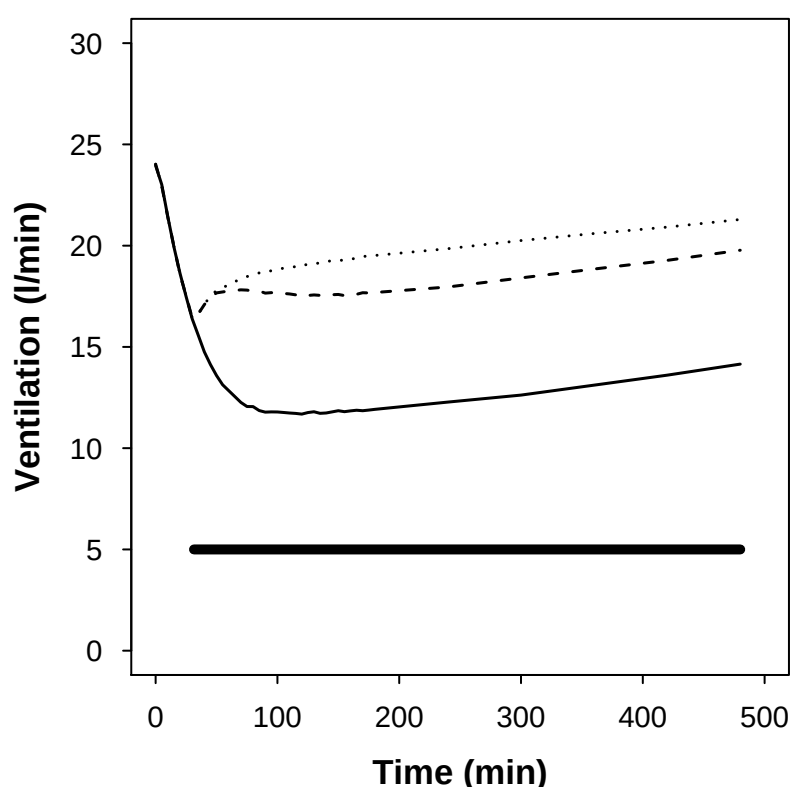


Figure 8. The influence of the mode of naloxone administration at the reversibility of buprenorphine-induced respiratory depression. Buprenorphine 0.2 mg/70 kg was administered, similar as in the original study design. After 30 min naloxone was infused continuously at a rate of 0 mg/hr (placebo: solid line), 2 mg/70 kg/hr (dashed line) or 4 mg/70 kg/hr (dotted line). The black box represent the duration of the naloxone infusion.

However, since there is no clear and conclusive explanation for the existence of such a bell-shaped curve, the final structure of a PK-PD model enabling the characterization of the bell-shaped curve remains unknown. Nevertheless, the current PK-PD model is valuable as it accurately predicts the reversal up to naloxone doses of 4 mg/70kg/hr. At a rate of 4 mg/70kg/hr almost complete reversal is accomplished and therefore the administration of higher doses of naloxone is not indicated.

The presently proposed competitive buprenorphine-naloxone interaction model constitutes a scientific basis for the characterization of the interactions between buprenorphine and other opioids such as the full agonists morphine and fentanyl. Clinically this is important since particularly with the use of partial agonists opioid rotation may compromise the analgesic effects of agonists with higher intrinsic efficacies (Thomsen *et al.*, 1999; Morgan *et al.*, 1999; Mizoguchi *et al.*, 2003; Kogel *et al.*, 2005). To date the utility of agonist-(partial) agonist interaction models to establish more rational opioid conversion tables on the basis of concentrations rather than dose models has not been fully explored. Nevertheless, it should be emphasized that the proposed competitive interaction PK-PD model may only be evaluated to describe the time course of respiratory depression after intravenous administration of buprenorphine and naloxone. Thus the utility of the agonist-antagonist interaction model to predict analgesia after opioid switching remains speculative. Evidently more data are needed to substantiate our hypothesis.

In conclusion, a competitive agonist-antagonist interaction model was derived that can describe and predict the reversibility of buprenorphine-induced respiratory depression after any mode and dose of buprenorphine and naloxone administration. The proposed pharmacodynamic interaction model is a first step towards predicting the degree and duration of reversal of buprenorphine-induced respiratory depression in human after any type of buprenorphine or naloxone administration. Continuous infusion of naloxone at a rate of 4 mg/70 kg/hr is recommended to maintain reversal.

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SECTION
V

**GENERAL DISCUSSION
AND PERSPECTIVES**

Chapter 12

MECHANISM-BASED PHARMACOKINETIC-PHARMACODYNAMIC MODELING OF THE EFFICACY AND SAFETY OF (SEMI-)SYNTHETIC OPIOIDS: SUMMARY, CONCLUSIONS AND PERSPECTIVES

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INTRODUCTION

This thesis describes a series of preclinical and clinical investigations on the pharmacokinetic-pharmacodynamic (PK-PD) correlation of the (semi-)synthetic opioids buprenorphine and fentanyl. In this chapter these investigations are briefly reviewed and discussed. In the last two decades important progress has been made in the understanding of the PK-PD correlation of (synthetic) opioids using quantitative EEG as a pharmacodynamic endpoint in both animal models and in man. Especially, the PK-PD correlations of the anilidopiperidine opioids, fentanyl, alfentanil, and sufentanil have been studied extensively (Scott *et al.*, 1985, 1991; Cox *et al.*, 1999). The results of these PK-PD investigations have contributed to a better understanding of the kinetics of onset of effect and the optimal dosing of these opioids in anesthesia. In addition to their use in anesthetic practice, potent opioids are also widely used as analgesics and are the first-choice for the treatment of acute post-operative and chronic (malignant) pain (WHO, 1996). Apart from their analgesic effect, opioids display various side-effects. Typical μ -opioid related side-effects are miosis, nausea/vomiting, sedation, constipation and respiratory depression (Vandermeulen, 2006). Most of these side-effects are mild in nature. However, acute respiratory depression remains a serious complication with a potentially fatal outcome (Baxter, 1994; Dahan *et al.*, 1998).

The development of opioids with an improved efficacy/safety balance remains an important goal. In theory, partial receptor agonists may have a greater selectivity of action, and could therefore be more easily titrated to the desired effect, with a reduced risk of serious side-effects (van Schaick *et al.*, 1998). This pharmacological concept has been illustrated in a series of preclinical investigations on adenosine A1 receptor agonists. Mechanism-based PK-PD modeling has been successfully applied to predict the selectivity of action of various N⁶-cyclopentyl-adenosine (CPA) analogues. This has enabled the design novel CPA analogues with significant antilipolytic activity, but reduced cardiovascular side effects, for the treatment of non-insulin dependent diabetes mellitus (Van der Graaf *et al.*, 1999).

In the late 1970s, buprenorphine has been introduced as a potent opioid analgesic with partial agonistic activity at the μ -opioid receptor (Cowan *et al.*, 1977a,b). It is anticipated that, due to its partial agonistic activity, buprenorphine may display a favorable efficacy/safety profile compared to full agonists like fentanyl and morphine. Another unique property relative to other opioids is that buprenorphine displays slow receptor association/dissociation kinetics in a variety of (*in vitro*) test systems (Boas and Villiger, 1985; Cassel *et al.*, 2005). This has important implications for the dosing of buprenorphine, in particular in relation to the reversal of buprenorphine-induced respiratory depression. In the mean time it has been demonstrated that the reversal of buprenorphine-induced respiratory depression by naloxone is indeed complicated by the slow receptor association/dissociation kinetics (Gal, 1989). However, this safety concern should be evaluated

against the background of the balance between the analgesic and the respiratory depressant effect. Specifically, buprenorphine would be of limited value if ceiling in analgesia would coincide with ceiling in respiratory depression in the therapeutic concentration range (Dahan *et al.*, 2006).

An important objective of the investigations described in this thesis was to characterize and predict the time course of buprenorphine's antinociceptive (efficacy outcome) and respiratory depressant effect (safety outcome) using integrated mechanism-based PK-PD models. In recent years pharmacokinetic-pharmacodynamic (PK-PD) modeling has developed as an independent scientific discipline, aiming at the prediction of the time course of drug effect *in vivo* (Danhof *et al.*, 2005). PK-PD modeling is already widely applied in drug development for optimization of the dosing and delivery of novel drugs in phase 2/3 clinical trials (Vozech *et al.*, 1996; Gieschke and Steimer, 2000). In the meantime however PK-PD modeling is increasingly also applied in pre-clinical investigations. Here the objective is the prediction of the PK-PD properties of novel drugs in man on the basis of information from *in vitro* bioassays and *in vivo* animal studies with the aim to optimize lead optimization and drug candidate selection (Visser *et al.*, 2003; Zuideveld *et al.*, 2004). The pertinent PK-PD information is subsequently used to optimize the design of early clinical trials. Mechanism-based PK-PD models contain specific expressions for processes on the causal path between drug administration and effect (Danhof *et al.*, 2005). This includes 1) the target site distribution, 2) the target binding and activation, 3) the transduction and 4) the effect of homeostatic feedback mechanisms. Thereby also effects on 5) disease processes are considered. Mechanism-based PK-PD modeling relies on the use of biomarkers and pharmacodynamic endpoints, which characterize in a strictly quantitative manner processes on the causal path between drug administration and pharmacological effect.

A specific objective of the investigations described in this thesis was the differentiation between the antinociceptive and respiratory depressant effects of buprenorphine and fentanyl by mechanism-based PK-PD modeling. The validation of the mechanism-based models is an integral and essential part of this research. The validation of mechanism-based PK-PD models requires challenging of the system by administering different doses and infusion rates. In this respect, preclinical investigations are very useful since in these investigations indeed wide dose ranges of new and existing drugs can be administered. Thus preclinical PK-PD studies offer unique possibilities for model discrimination enabling the identification and modeling of complex PK-PD relationships which can also occur in humans (Breimer and Danhof, 1997). Interestingly, in the present investigations we have also studied the PK-PD correlations in humans, using very similar pharmacodynamic endpoints, thus enabling us to characterize in detail the interspecies extrapolation. The objective of the investigations described in this thesis was to characterize the *in vivo* pharmacological and PK-PD properties of buprenorphine relative to fentanyl with respect to:

- kinetics of onset and offset of the pharmacological effects at the μ -opioid receptor
- selectivity of action (antinociception *versus* respiratory depression)
- the interspecies extrapolation of the PK-PD correlation of the antinociceptive and respiratory depressant effect
- the role of active metabolites
- kinetics of antagonism of the respiratory depressant effect

Fentanyl was selected as a comparator drug because of its distinctly different pharmacological properties. Specifically, fentanyl is a full agonist at the μ -opioid receptor and its binding to and dissociation from the μ -opioid receptor are fast (Boas and Villiger, 1985; Zuurmond *et al.*, 2002). Moreover, fentanyl is applied widely in clinical practice as an analgesic (Chelly *et al.*, 2004).

THE COMBINED BIOPHASE EQUILIBRATION-RECEPTOR ASSOCIATION/DISSOCIATION MODEL

The role of biophase distribution

For opioids, the time course of their pharmacological effects *in vivo* is dependent on a multitude of factors. This includes 1) an equilibrium delay between arterial and CNS biophase concentrations, 2) a delay between biophase opioid concentration and the occupancy of opioid receptor due to slow receptor association/dissociation kinetics and/or 3) a delay between receptor occupancy and the initiation of subsequent opioid effects (transduction). In principle these processes operate on different time scales and may each contribute to the time course of opioid action *in vivo* (Mager and Jusko, 2001).

For lipid soluble intravenous anesthetics, the rate of equilibration between blood and brain has been shown to be an important determinant of the kinetics of action *in vivo*. This is therefore an important factor in the PK-PD analysis of, not only the EEG effect but also the antinociceptive and respiratory depressant effect of opioids (Dutta *et al.*, 1998). Apart from plasma pharmacokinetics, mechanisms that govern the biophase distribution include 1) blood-brain barrier transport and 2) subsequent kinetic processes within the brain that affect the target-site distribution and elimination. The blood-brain barrier forms the largest dynamic interface between blood and brain and its major function is to protect the CNS from neuroactive and potentially toxic compounds circulating in the plasma (Ohtsuki, 2004; Pardridge, 2007). This is accomplished by regulating the exchange of endogenous and exogenous substances between blood and CNS (de Lange, 2004; Pardridge, 2005). Blood-brain barrier transport is a major determinant of the effect of CNS active drugs (Xie *et al.*, 1999). In mechanistic terms this transport is determined by:

1) the functionality of the brain-brain barrier and 2) the physicochemical characteristics of the drug (Oldendorf, 1970, 1974; Rubin and Staddon, 1999). Moreover, in theory transport across the blood-brain barrier can be affected by changes in the perfusion of the central nervous system (cerebral blood flow-limited) and by changes in the functionality of active transport mechanisms which are operative at the blood-brain barrier (Ludbrook and Upton, 2002; Upton *et al.*, 2004). For instance, for *d*-tubocurarine, it has been demonstrated that the value of the biophase equilibration rate constant k_{e0} is lower under halothane anesthesia relative to nitrous oxide anesthesia, which could be explained by the reduction in skeletal blood flow (Stanski *et al.*, 1979). Likewise it has been demonstrated that for various drugs the blood-brain equilibration is influenced by the functionality of active efflux transporters (de Lange *et al.*, 1998).

It is generally believed that for lipid-soluble intravenous anesthetics, the rate of their uptake in the brain is determined by the cerebral blood flow (cerebral blood flow-limited transport) (Dutta *et al.*, 1998). Drug-induced hemodynamic changes during anesthesia or changes in arterial CO₂ concentration may significantly alter the cerebral blood flow auto regulation, thereby directly impacting drug uptake and delivery in the brain (Ludbrook and Upton, 1997; Upton *et al.*, 1999). In addition, it has been convincingly demonstrated that the delivery of opioids in the brain can be modulated by active transport mechanisms (Silverman, 1999; Dagenais *et al.*, 2004). Particularly for relatively hydrophilic opioids which are substrates for the active transporters target site distribution is restricted (Loo and Clarke, 2005). Morphine is an opioid with a low lipid solubility and a substrate for P-glycoprotein (Thompson *et al.*, 2000). This may explain the slow onset of morphine's pharmacological effects *in vivo*. For fentanyl, sufentanil and alfentanil the situation appears to be different as these drugs have adequate lipid solubility to allow rapid, passive blood-brain barrier transport (Roy and Flynn, 1989). It has been proposed that time delays in the time course of the pharmacological effects of these synthetic opioids is caused, at least in part, by partitioning within the brain (Scott *et al.*, 1985, 1991). This is consistent with the observation that their respective biophase equilibration half-times are correlated with their lipophilicity (Dutta *et al.*, 1998). Given buprenorphine's high lipophilicity it is anticipated that biophase equilibration is also an important determinant of the kinetics of buprenorphine's analgesic and respiratory depressant effect *in vivo*. The role of active transport mechanisms in the kinetics of onset of buprenorphine's effect is less pronounced, since to the best of our knowledge it is not known whether buprenorphine is a substrate for P-glycoprotein or other saturable outward transport mechanisms. Most likely, taking into account buprenorphine's high lipophilicity, its delivery to the brain is mainly driven by passive diffusion.

The PK-PD correlations of fentanyl and buprenorphine as model drugs were determined both in pre-clinical studies in rats and in clinical studies in healthy volunteers (chapters 4-7). Mechanism-based PK-PD models were based on receptor theory and contained specific expressions for the rate constant of biophase distribution as well as for the rate constants of receptor association and dissociation at the μ -opioid receptor. The

investigations showed the biophase distribution kinetics are nearly identical for buprenorphine's antinociceptive and respiratory depressant effect, indicating that the differences in the kinetics of onset and offset of buprenorphine's pharmacological effects are mainly attributed to differences in receptor association and dissociation kinetics. The relatively long half-time of buprenorphine's biophase distribution compared to fentanyl is in agreement with buprenorphine's higher lipophilic nature. Figure 1 shows the relationship between the half-time of biophase distribution and the brain-plasma partitioning ratio. The relationship is linear and the regression line appears to predict buprenorphine's half-time of biophase distribution accurately. This confirms that the rate constant of biophase distribution is indeed a mechanistic parameter characterizing the biophase distribution kinetics of buprenorphine in a correct and accurate manner.

The combined biophase distribution-receptor association/dissociation model was also fitted to the time course of respiratory depressant effect of the active metabolite norbuprenorphine (chapter 8). Pharmacokinetic-pharmacodynamic analysis showed that no sensible estimates of the rate constants of receptor association/dissociation could be obtained, a situation which was previously also encountered with fentanyl. This indicates that hysteresis in norbuprenorphine's time course of respiratory depression can be fully explained by biophase distribution. In addition, the kinetics of onset and offset of norbuprenorphine's respiratory depressant effect were found to be non-linear, as the rate constant of biophase distribution was found to be concentration-dependent (chapter 8). In line with norbuprenorphine's low lipophilicity, a characteristic also shared by morphine, transport across the blood-brain barrier appears to be a rate-limiting factor in the onset of the respiratory depressant effect. This suggests that active efflux transport vectors operating at the blood brain barrier might be responsible for the brain extrusion of norbuprenorphine at low (therapeutic) concentrations. When the active efflux transport mechanisms become readily saturated at relatively high norbuprenorphine concentrations, its transport to the brain is enhanced, yielding ultimately a pharmacological effect. An interesting question is whether norbuprenorphine behaves in the same manner as the peripherally acting opioid loperamide. Experimental animal studies show that when given along with P-glycoprotein inhibitors, loperamide turns into a centrally acting opioid (Schinkel *et al.*, 1996), displaying potent opioid activity at the μ -opioid receptor (analgesia, respiratory depression and EEG) (Groenendaal *et al.*, 2007a,b). Although we postulate that norbuprenorphine is a substrate for active transport vectors, no direct evidence has been provided for our observations. Clearly, more *in vivo* mechanistic studies need to be performed to substantiate our hypothesis. *In vivo* intracerebral microdialysis offers a novel technique to study the brain disposition characteristics of norbuprenorphine and changes in norbuprenorphine transport across the blood brain barrier by inhibition of P-glycoprotein (Bickel, 2005). Previously, this technique has been successfully applied to investigate the role of blood-brain transport on the central effects of morphine and the impact of P-glycoprotein inhibition on the kinetics of morphine's action in the brain (Letrent *et al.*, 1999; Bouw *et al.*, 2000). This underlines the importance of the appli-

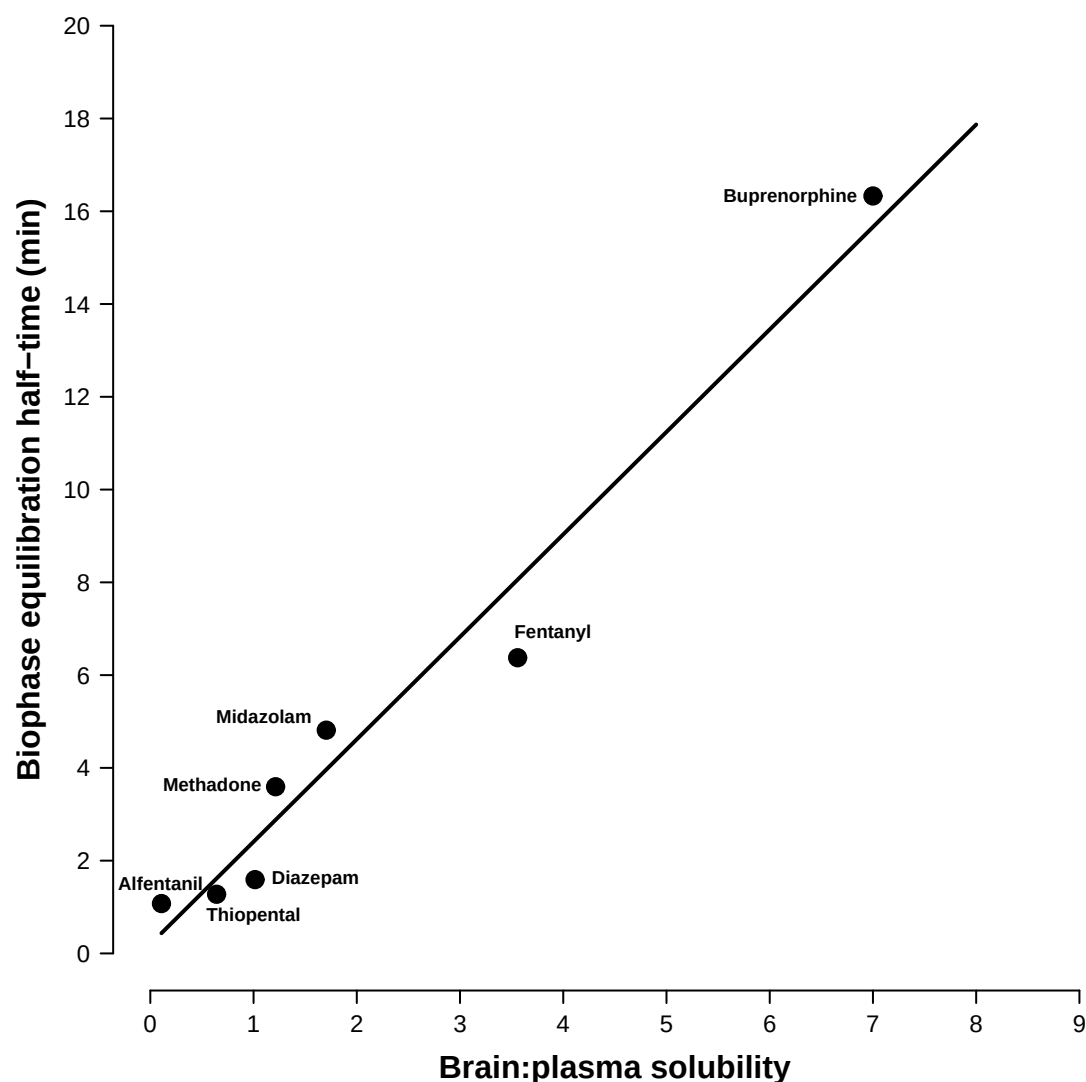


Figure 1. The relationship between half-time of biophase equilibration and brain:plasma solubility for various lipid soluble intravenous anesthetics. This relationship was originally adapted from Dutta *et al.* (1998). For buprenorphine the brain:plasma partitioning ratio was obtained from Ohtani *et al.* (1995). The corresponding *in vivo* half-time of biophase distribution for a rat weighting 300 g was calculated from equation 2 chapter 10.

cation of *in vivo* microdialysis to study the pharmacokinetic-pharmacodynamic properties of drugs and is therefore a powerful tool for PK-PD analysis (Brunner *et al.*, 2005; de Lange *et al.*, 2005).

The role of receptor association/dissociation kinetics

Results from early dedicated *in vitro* binding studies indicate that buprenorphine possesses relatively slow receptor association/dissociation kinetics at the μ -opioid recep-

tor. In this thesis it is unequivocally demonstrated that this property contributes to the slow onset and long duration of buprenorphine's analgesic and respiratory depressant effect *in vivo* (chapters 4-7). Although buprenorphine is not the only long-acting opioid analgesic, it is the slow receptor association/dissociation kinetics which distinguishes buprenorphine from the other long-acting opioid analgesics, like methadone. Therefore, the pharmacokinetic-pharmacodynamic modeling of the *in vivo* slow receptor association/dissociation kinetics is considered very valuable as it constitutes a scientific basis for the understanding of the *in vivo* time course of buprenorphine's pharmacological effects. Furthermore, on the basis of this analysis an estimate of the dissociation equilibration constant K_D is obtained (calculated by the ratio of k_{off} to k_{on}), which serves as a direct measure of the *in vivo* potency. These *in vivo* potency estimates are valuable to study buprenorphine's *in vivo* selectivity of action with respect to antinociception and respiratory depression. Also, estimated *in vivo* K_D values can be compared with *in vitro* binding affinity constants, enabling the establishment of *in vitro-in vivo* K_D correlations.

Previously, a slow receptor association/dissociation model has been successfully applied to predict the time course of the antihypertensive effect of eight calcium channel blockers (Shimada *et al.*, 1996). In that investigation, unique and independent estimates of *in vivo* equilibrium dissociation constants for the calcium channel blockers were obtained which nicely correlated with the respective affinity binding constants obtained from *in vitro* binding studies. This suggests that the time course of the *in vivo* antihypertensive effect of a calcium channel blocker like nifedipine can be predicted on the basis of information obtained from *in vitro* binding assays. The unique application of the slow receptor binding model to the data obtained from Japanese hypertensive patients contributed to a better understanding of the *in vivo* pharmacodynamics of long-acting calcium antagonists. In a similar fashion, a mechanism-based PK-PD model incorporating a specific expression for receptor association/dissociation kinetics can enhance our understanding of the kinetics of buprenorphine's action at the μ -opioid receptor.

An important difference between the calcium channel receptor binding model and the PK-PD model developed to characterize buprenorphine's time courses of pharmacological effects is that the latter model also incorporates a specific expression for biophase distribution. In the (pre)-clinical investigations discussed in this thesis, accurate and precise estimates of the rate constants of biophase distribution and receptor association/dissociation have been obtained, indicating that the combined biophase distribution-receptor association/dissociation model is indeed identifiable by using the experimental *in vivo* data. Model identification is critically important in the development of mechanism-based PK-PD models. The more information is available from the data the more likely the data will support the development of a more complex PK-PD model. The development of such models requires tests by different doses and infusion rates and the application of complex study designs. In this respect, pre-clinical investigations involving the use of instrumented animal models offer a unique opportunity for the development and validation of mechanism-based PK-PD models.

Pharmacogenetics

Considerable inter-individual variability was observed in the time course of buprenorphine's antinociceptive effect in volunteers. This indicates that the kinetics of onset and offset of buprenorphine's analgesic effect, as well as the effective concentration ranges can differ significantly between patients. In situations where optimization and individualization of drug therapy becomes a need, population PK-PD modeling can be applied to identify the factors which explain the observed inter-individual variability [53]. Meanwhile this approach has been widely applied to analyze inter-individual variability in pharmacokinetics (Viberg *et al.*, 2006; Li *et al.*, 2006). Although variability in pharmacokinetics is an important factor, inter-individual (genetic) variability in pharmacodynamics should also be considered (Romberg *et al.*, 2005; Lack *et al.*, 2005). This is particularly important for drugs where the variability in pharmacodynamics is more pronounced than the variability in pharmacokinetics and for drugs with a small therapeutic window (Levy, 1998).

Given the relatively small inter-individual variability in buprenorphine pharmacokinetics, it appears that the observed inter-individual variability in analgesic effect is largely caused by variability in biophase distribution kinetics and/or pharmacodynamics. With regard to the biophase distribution, the transport of opioids across the blood-brain barrier might be modulated by drug efflux transport vectors. P-glycoprotein is a product of the Multi Drug Resistance (MDR1) gene and is expressed at the luminal or apical membrane of endothelial cells at the blood-brain barrier (Schinkel and Jonker, 2003). Morphine, a commonly used opioid, has been shown to interact with *MDR1a* P-glycoprotein (Callaghan and Riordan, 1993). For morphine the interaction with P-glycoprotein has been shown to significantly alter the onset and intensity of its pharmacological effects. Specifically, Thompson *et al.* (2000) reported an increased morphine-induced antinociceptive response in mice lacking P-glycoprotein. Also, the *MDR1* gene has been shown to be highly polymorphic (Marzolini *et al.*, 2004), implying that the expression of P-glycoprotein at the blood-brain barrier is a source of variability (Loo and Clarke, 2005). At present it is unknown whether buprenorphine interacts with specific drug efflux transport mechanisms. Taking into account buprenorphine's high lipophilicity, the transport of buprenorphine across the blood-brain barrier is most likely mediated by passive diffusion rather than by active transport processes. The evidence is however not conclusive.

For fentanyl, another highly lipophilic opioid, it has recently been shown that analysis of polymorphism in the *ABC1* gene, encoding P-glycoprotein, is clinically relevant for the prediction of the degree of respiratory depression following intravenous fentanyl administration (Park *et al.*, 2006). These results underline the importance of the pharmacogenetic profiling of the clinical effects of opioids and the use of such information in PK-PD models to facilitate understanding of the origin of inter-individual variability in the *in vivo* pharmacological effect of opioids. In the meantime, the implications of variation in transporter expression at the blood-brain barrier on the clinical effects of buprenorphine remain unclear. Evidently, more studies are needed to substantiate the generality of the

findings by Park *et al.* (2006) for other opioids like buprenorphine.

Another important determinant of buprenorphine's effect is the interaction at the μ -opioid receptor. In theory, in the case of a high homology in receptors between species, the receptor association/dissociation kinetics are drug-specific parameters which are independent of the species. For the μ -opioid receptor a high degree of structural homology exists between the rat and human μ -opioid receptor suggesting that the receptors functions in a nearly identical manner in these species (Rothman *et al.*, 1995). Although the estimates of the rate constants of receptor association and dissociation are in the same range for rat and human (chapter 10), considerable inter-subject variability was observed. The discovery of a single nucleotide polymorphism (SNP) of the G118 allele for the receptor, has yielded a (genetic) basis for the observed inter-individual variability in analgesic response (Befort *et al.*, 2001). The variant 118G allele results from substitution of a single nucleotide A by G at amino acid position 40 (position 118 of the receptor gene). Due to this nucleotide exchange the amino acid asparagine is replaced by aspartate in the mutated μ -opioid receptor gene (Compton *et al.*, 2003). Recently, it has been demonstrated that patients carrying the G118 allele require higher doses of alfentanil to reach even less pronounced analgesia compared to wild-type patients. In addition, a correlation between the μ -opioid genotype and morphine- and morphine-6-glucuronide-induced pupil diameter constriction has been observed (Skarke *et al.*, 2003). Specifically, it was shown that presence of the G118 allele decreases potency of morphine-6-glucuronide by a factor of 2.4 (heterozygous carriers) and 2.6 (homozygous carriers). For morphine, a decrease in potency by a factor of 2.1 and 3.6 for heterozygous and homozygous carriers was shown. The importance of G118 allele mutation as a key determinant of analgesia had also been addressed by Romberg *et al.* (2004). They found that mutation in the G118 allele is a significant predictor for the analgesic potency after intravenous administration of morphine-6-glucuronide. It can not be excluded that also other, recently discovered single point mutations in the μ -opioid receptor gene contribute to the observed variability in analgesic action of opioids (Befort *et al.*, 2001). For morphine, morphine-6-glucuronide and alfentanil there is substantial evidence that indicates that μ -opioid receptor mutations cause inter-individual variability of the clinical effects of opioids. For buprenorphine, the relevance of μ -opioid receptor mutations for its clinical effects needs yet to be established. The observed inter-individual variability in estimates of the rate constants of receptor association/dissociation kinetics and the pharmacogenetic evidence gathered for morphine provides a solid basis for continuing research in this field.

Recent advancements in pharmacogenetics and system biology are adding a new dimension to PK-PD analysis (Housman and Ledley, 1998; Weinshilboum and Wang, 2004). The development of mechanism-based PK-PD models incorporating pharmacogenetic information enhances understanding of inter- and intra-variability in drug response. Especially, mechanism-based PK-PD models which contain specific expressions on the causal path between drug administration and pharmacological effect are of particular interest for the identification of genetic variability (Danhof *et al.*, 2007). This applies also for the

kinetics of receptor association and dissociation. For example, one variant form of the μ -opioid receptor had been shown to have a three times higher affinity for β -endorphin than the wild-type μ -opioid receptor (Bond *et al.*, 1998). For opioids, differences in *in vivo* receptor binding to variant forms of the receptor (provided that genetic information is available) can be analyzed using a dynamical receptor model. In the present investigation, the slow receptor dissociation kinetics is reflected by the rate constant k_{off} . The relatively large inter-individual variability on this parameter provides considerable margin to explore the importance of genetic polymorphism in the μ -opioid receptor gene in part of the observed variability in buprenorphine-induced antinociception. Mechanism-based PK-PD models in conjunction with the analysis of pharmacogenetics information offer a unique opportunity for the optimization and individualization of pharmacotherapy. It is anticipated that the use of pharmacogenetic information to support mechanism-based PK-PD model development and application will increase significantly the coming years as the interdisciplinary area of PK-PD and pharmacogenetics is rapidly evolving and these resources become more widely available to the scientific community.

THE SELECTION OF PHARMACODYNAMIC ENDPOINTS

Characterization of the time course of the antinociceptive effect

In preclinical investigations the analgesic effect of opioids is typically quantified as an antinociceptive effect. This involves 1) production of a pain-like state by presentation of a painful stimulus and 2) measurement of behavioral responses, which are indicative of the perceived stimulus-induced pain (Negus *et al.*, 2006). In such investigations only stimuli that are of modest intensity and produce no irreversible tissue damage can be used. In the investigations described in this thesis, the tail flick latency test was used to assess antinociception. In this model the rat's tail is exposed to a radiant heat source causing a tail withdrawal response. Following the administration of an opioid, the prolonged tail withdrawal latency is used as a measure of antinociception. For the characterization of the antinociceptive effect of buprenorphine in healthy volunteers a nearly identical experimental model of acute pain was used, with the understanding that instead of the application of radiant heat to produce a pain-like state, acute pain was induced by an electrical pain stimulus. The subjects were asked to press a button to indicate when the stimulus became painful. The time between onset of the electrical stimulus with the start of the timer and stop of the painful stimulus when the button is pressed is a measure of the antinociceptive effect of opioids. Previous studies have shown that acute pain models can be successfully applied for the prediction of opioids' analgesic potency in acute and chronic pain patients (Sarton *et al.*, 2000). A limitation of antinociceptive tests is that the pharmacodynamic endpoint (e.g. time to tail withdrawal) is restricted to a preset cut-off time, to avoid tissue damage. As a result, the observed maximum effect is restricted to the

selected cut-off time. This complicates the characterization of opioid analgesics in terms of intrinsic efficacy and in particular the differentiation between partial and full agonistic activity (Yassen *et al.*, 2005). Moreover if not taken into account appropriately, the use of restrictive pharmacodynamic endpoints in PK-PD modeling may result in inaccurate and biased pharmacodynamic parameter estimates (Beal, 2005). One approach to obtain relevant pharmacodynamic information from censored data is by incorporating statistical distribution models in the PK-PD models, allowing for predictions beyond the cut-off time (Cox and Oakes, 1984). The Weibull and log-logistic distribution are two of the most widely used distribution for the analysis of time-to-event data. Previously, the maximum likelihood parameter estimation approach in NONMEM had been successfully used for the estimation of the shape and scale parameters of statistical distribution models to censored pharmacodynamic data together with pharmacodynamic parameters characterizing the time course of drug effect (Luks *et al.*, 1998; Sarton *et al.*, 2000; Yassen *et al.*, 2005).

Characterization of the time course of the respiratory depressant effect

The side-effects of opioids have been known for centuries and it is now recognized that respiratory depression is a serious adverse effect, contributing to opioid related deaths (Bailey *et al.*, 2000; Lotsch *et al.*, 2006). Respiratory depression is defined as the increase in arterial CO₂ concentration ($P_a\text{CO}_2$) or decrease in pH and arterial O₂ ($P_a\text{O}_2$) concentration without a compensatory increase in ventilatory response (Yeadon and Kitchen, 1989; Ko *et al.*, 2003). The respiratory system is a rather complex system and opioids may affect all elements of respiration resulting in a decrease in respiratory rate or minute volume and irregular breathing patterns. Not surprisingly, since opioids impact on distinct aspects of the respiratory system, various biomarkers have been proposed to monitor opioid-induced modulation of breathing. In theory, respiratory depression could be relatively easily quantified by measuring the respiratory rate, oxygen saturation or arterial carbon dioxide concentration. In our investigations, initially, $P_a\text{CO}_2$ was selected as a surrogate endpoint for respiratory depression. In a series of experiments, different doses of buprenorphine and fentanyl were administered to rats and the time course of changes in $P_a\text{CO}_2$ was characterized. The effect of buprenorphine on $P_a\text{CO}_2$ is shown in figure 2.

Irrespective of the dose, buprenorphine showed a relatively small increase in $P_a\text{CO}_2$ of 1-1.5 kPa, suggesting that buprenorphine has only minimal *in vivo* respiratory depressant activity. Moreover an irregular time course of changes in $P_a\text{CO}_2$ was observed. These observations can be explained by the complex homeostatic feedback mechanisms that are operative *in vivo*. These physiological regulatory feedback mechanisms operate at faster time scales than the time to onset of buprenorphine's respiratory depressant effect, thereby enabling immediate compensation for the increase in $P_a\text{CO}_2$ by increasing the pulmonary ventilation. This may also explain the observed oscillatory type behavior of $P_a\text{CO}_2$ in time. Evidently, $P_a\text{CO}_2$ is not a sufficiently sensitive biomarker for monitoring the respiratory depressant effect of opioids (Dahan *et al.*, 2005). Different biomarkers

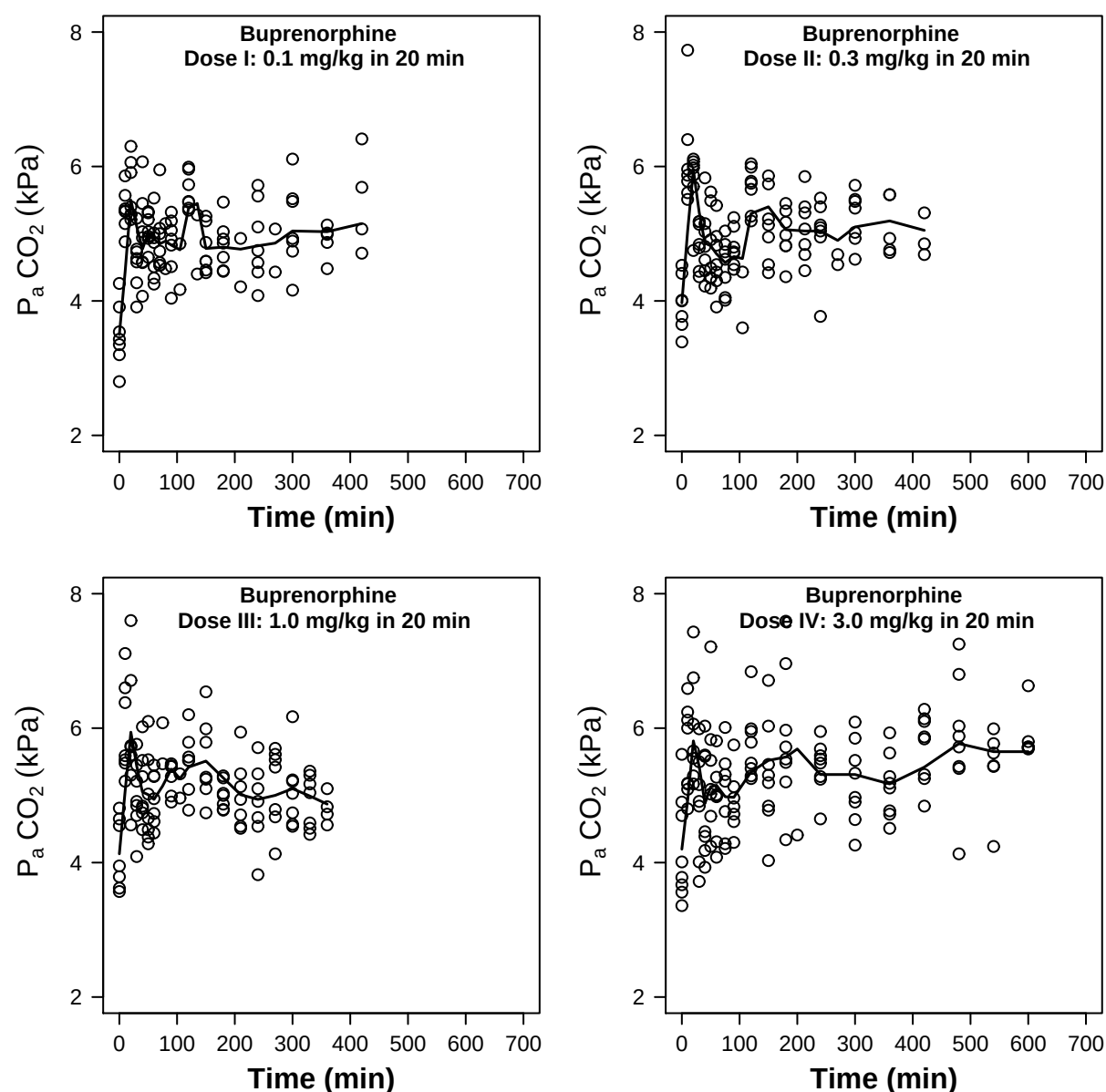


Figure 2. Time course of arterial CO₂ ($P_a\text{CO}_2$) concentration following the administration of different doses of buprenorphine.

which offer a dynamic and potentially more sensitive measure of respiratory depression are required for the characterization opioid-induced respiratory depression (Berkenbosch *et al.*, 1992). To overcome the problem of the lack of sensitivity of arterial CO₂ measurements, a new rat model for respiratory depression was developed. In this novel experimental setup, whole-body plethysmography was employed to characterize the impact of opioids on the respiratory system in freely moving rats. Minute ventilation was measured at a fixed inspiratory CO₂ concentration, thereby minimizing the influence of compensatory feedback mechanisms at the ventilatory response. Besides biomarker sensitivity, the use of whole-body plethysmography for the measurement of minute ventilation, allows for a direct comparison with results obtained in humans, as the read-out parameter

(minute ventilation) is obtained in a nearly identical manner in both rats and humans (dynamical end-tidal forcing).

ANIMAL-TO-HUMAN EXTRAPOLATION OF BUPRENORPHINE'S PHARMACOLOGICAL EFFECTS

An important issue in drug development is the use of mechanism-based PK-PD modeling for the prediction of pharmacodynamics in humans on the basis of information from pre-clinical investigations. As such mechanism-based PK-PD modeling constitutes a scientific basis for drug candidate selection and lead optimization (Danhof *et al.*, 2005). Moreover it also enables optimization of early proof-of-mechanism and/or proof-of-concept trials in man.

Prediction of pharmacodynamics in humans involves prediction of 1) dose-exposure relationship and 2) the exposure-response relationship. For the prediction of the dose-exposure relationship, two distinct approaches can be employed: 1) pharmacokinetic allometric scaling and 2) physiologically-based pharmacokinetic (PBPK) modeling. The allometric scaling of the time course of drug concentration frequently relies on the use of empirical compartmental models. In this type of scaling models, the body is described in terms of a number of well-stirred compartments mimicking the drug's absorption, distribution and elimination. Typically these compartmental models have no physiological or anatomical significance and therefore their application in animal to human pharmacokinetic scaling is limited. In some cases, however, when the elimination route of the drug is simple (e.g. single route of elimination) and well-defined, compartmental models have proven valuable for the prediction of the pharmacokinetics in humans by allometric scaling. This especially concerns high-extraction ratio drugs which are eliminated via the hepatic route (flow-limited clearance) (Knibbe *et al.*, 2005). Physiologically-based PK models are considered more mechanistic as the time course of the drug concentration is described in physiologically meaningful terms. Specifically in physiologically-based PK modeling compartments often represent a body organ or tissue and the flow of the drug to each specific organ/tissue is described by their volumes, blood flows and blood-tissue partitioning coefficients (Blakey *et al.*, 1997; Rowland *et al.*, 2004). However, this approach is mathematically more complex and time consuming than the conventional compartmental approach (Nestorov, 2003).

Buprenorphine is a high extraction ratio drug and its clearance is largely determined by the hepatic blood flow (Bullingham *et al.*, 1980). Generally, for high-extraction-ratio drugs, compartmental allometric scaling appears to provide satisfactory prediction of drug disposition in man (Knibbe *et al.*, 2005). The high cross-species similarities in buprenorphine's pharmacokinetics, with the strong dependency of clearance of hepatic blood flow, may explain the accurate and precise pharmacokinetic estimates and their respective allometric exponents obtained with the population pharmacokinetic model on the basis of

two species (chapters 4-7).

With respect to the scaling of the pharmacodynamics, mechanism-based PD models differ from empirical PD models in that they contain specific expressions to characterize the processes on the causal path between drug administration and effect. An important feature of mechanism-based PK-PD models is the distinction between drug- and system-specific parameters. In theory, the rate constants of receptor association and dissociation kinetics are drug-specific and, given the high homology of the μ -opioid receptor in rats and human, display cross-species similarity. In contrast the rate constant of biophase equilibration is a system-dependent parameter and should be scaled from animal to human. Ultimately, an allometric power function was used for animal to human extrapolation of biophase distribution kinetics (chapter 10). This demonstrates that mechanism-based PK-PD models constitute a realistic approach for animal to human extrapolation of pharmacodynamics. An important observation was that the value of the rate constant of biophase distribution was identical for the antinociceptive and respiratory depressant effect. In conceptual terms, biophase distribution reflects the partitioning of buprenorphine between blood and brain. Theoretically, the blood-brain partitioning is determined by the physico-chemical properties and plasma protein binding (Dutta *et al.*, 1998). Since the antinociceptive and respiratory depressant effects occur in the same concentration range, it is not surprising that biophase distribution is identical for both pharmacological effects. Differences in the onset and offset of the pharmacological effects appear to be related to differences in receptor association and dissociation kinetics. In this respect, an important observation was that for the antinociceptive and respiratory depressant effect different K_D values were estimated, indicating that buprenorphine's pharmacological effects are mediated through activation of distinct μ -opioid receptor subtypes.

PHARMACODYNAMIC INTERACTION BETWEEN BUPRENORPHINE AND NALOXONE

Chapter 11 focuses on the *in vivo* interaction between buprenorphine and naloxone at the μ -opioid receptor. In chapters 6 and 7 it was shown that time-dependency in the pharmacodynamics of buprenorphine can be partly explained by slow receptor association/dissociation. Evidently this pharmacological characteristic complicates the *in vivo* reversal of respiratory depression by naloxone, which is consistent with results from *in vitro* displacement studies, showing a complex competitive interaction between buprenorphine and naloxone. A few studies have addressed the ability to reverse the respiratory depressant effect of buprenorphine. Just two studies from the literature published in 1980's as well as some anecdotal data indicate that the respiratory depression from buprenorphine is resistant to antagonism by naloxone (Gal, 1989; Bowdle, 2003; Metha *et al.*, 2005). While relatively low bolus doses of intravenous naloxone had no effect, high doses (2.5 to 10 mg) caused only partial reversal of buprenorphine's respiratory effects. These results may be

explained by the short duration of action of a bolus dose of naloxone resulting from rapid elimination, combined with the slow receptor association/dissociation kinetics of buprenorphine. Consequently, a bolus dose of naloxone may be unable to completely displace buprenorphine from the μ -opioid receptor. Interestingly, simulations on the basis of the combined biophase distribution-receptor association/dissociation model predicted that a different mode of naloxone administration, including a continuous naloxone infusion, is required to accomplish complete reversal of respiratory depression. On the basis of these simulations preclinical experiments were performed to prospectively test the predictive power of the combined biophase distribution-receptor association/dissociation model in accurately predicting the time course of reversal of buprenorphine-induced respiratory depression in rats. The experiments indeed confirmed that continuous infusion of naloxone is indicated to fully reverse respiratory depression (figure 3).

A previously proposed mechanism-based PK-PD model (chapter 10) for animal to human extrapolation of the time course of buprenorphine's pharmacological effects was used to simulate new naloxone dosing regimens for the reversal of respiratory depression in healthy volunteers. The clinical data from healthy volunteers confirmed the prediction of the reversal on the basis of the data obtained in rats. Figure 4 shows the relationship between the time of naloxone administration and the time course of reversal of buprenorphine-induced respiratory depression. The model predicts that continuous infusion of naloxone is needed for complete reversal of respiratory depression.

It may be hypothesized that the competitive buprenorphine-naloxone interaction model can also be used to study the interaction between the partial agonist buprenorphine and full agonists like morphine and fentanyl. The interaction model is derived from classical receptor theory and provides a theoretical framework to describe competitive interactions between full agonists and buprenorphine at the μ -opioid receptor. Clinically this is relevant since opioid rotation may compromise the analgesic effects of agonists of higher intrinsic efficacies (Morgan *et al.*, 1999; Thomsen *et al.*, 1999; Mizoguchi *et al.*, 2003; Kogel *et al.*, 2005). To date the utility of agonist-(partial) agonist interaction models to establish more rational opioid conversion tables on the basis of concentrations rather than dose models has not been fully explored. Nevertheless, it should be emphasized that the proposed competitive interaction PK-PD model may only be applied to describe the time course of respiratory depression after intravenous administration of buprenorphine and naloxone. Practically, the utility of the agonist-antagonist interaction model to predict analgesia after opioid switching remains speculative. Evidently more data are needed to substantiate our hypothesis.

It is foreseen that the animal model for respiratory depression can be employed to study the interaction between buprenorphine and benzodiazepines. Although this interaction is not directly important in contemporary practice in anesthesia, it is relevant to the clinical practice in addiction medicine (Drummer, 2005). Heroin addiction remains a major worldwide concern (Darke and Zador, 1996). Nowadays, buprenorphine is considered first-choice option in the treatment of addiction (Ling *et al.*, 1994). Due to its slow

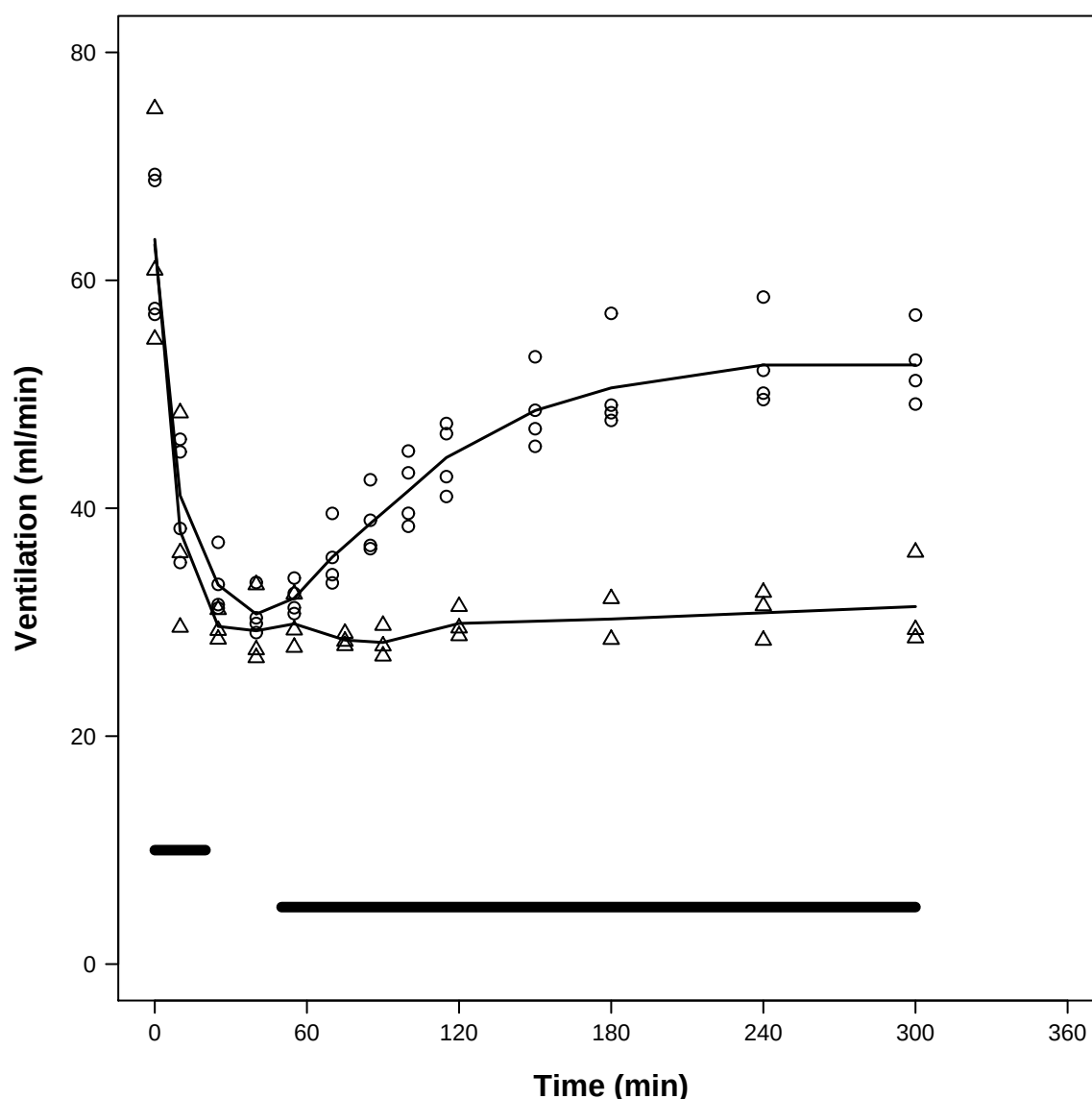


Figure 3. Time course of reversal of buprenorphine-induced respiratory depression following the administration of 0.1 mg/kg buprenorphine to rats during 20 minutes. Continuous infusion of naloxone or saline was started after 50 minutes upon start of buprenorphine infusion. In each group (naloxone/saline) 4 animals were tested. The triangles and circles represent the mean time course of reversal following saline or naloxone administration, respectively.

receptor association/dissociation kinetics, resulting in a prolonged duration of action, and its partial agonistic activity at the μ -opioid receptor, it is believed that buprenorphine has low abuse potential with less withdrawal symptoms (Jasinski *et al.*, 1978; Reisinger, 1985). Although buprenorphine is a relatively safe opioid, fatalities have been reported in relation to buprenorphine use in humans, frequently associated with the concomitant intake of psychotropic drugs, including benzodiazepines (Reynaud *et al.*, 1998; Pirnay *et al.*, 2004). Since both buprenorphine and benzodiazepines are not known to display potentially life-threatening respiratory depression when given separately, it is the com-

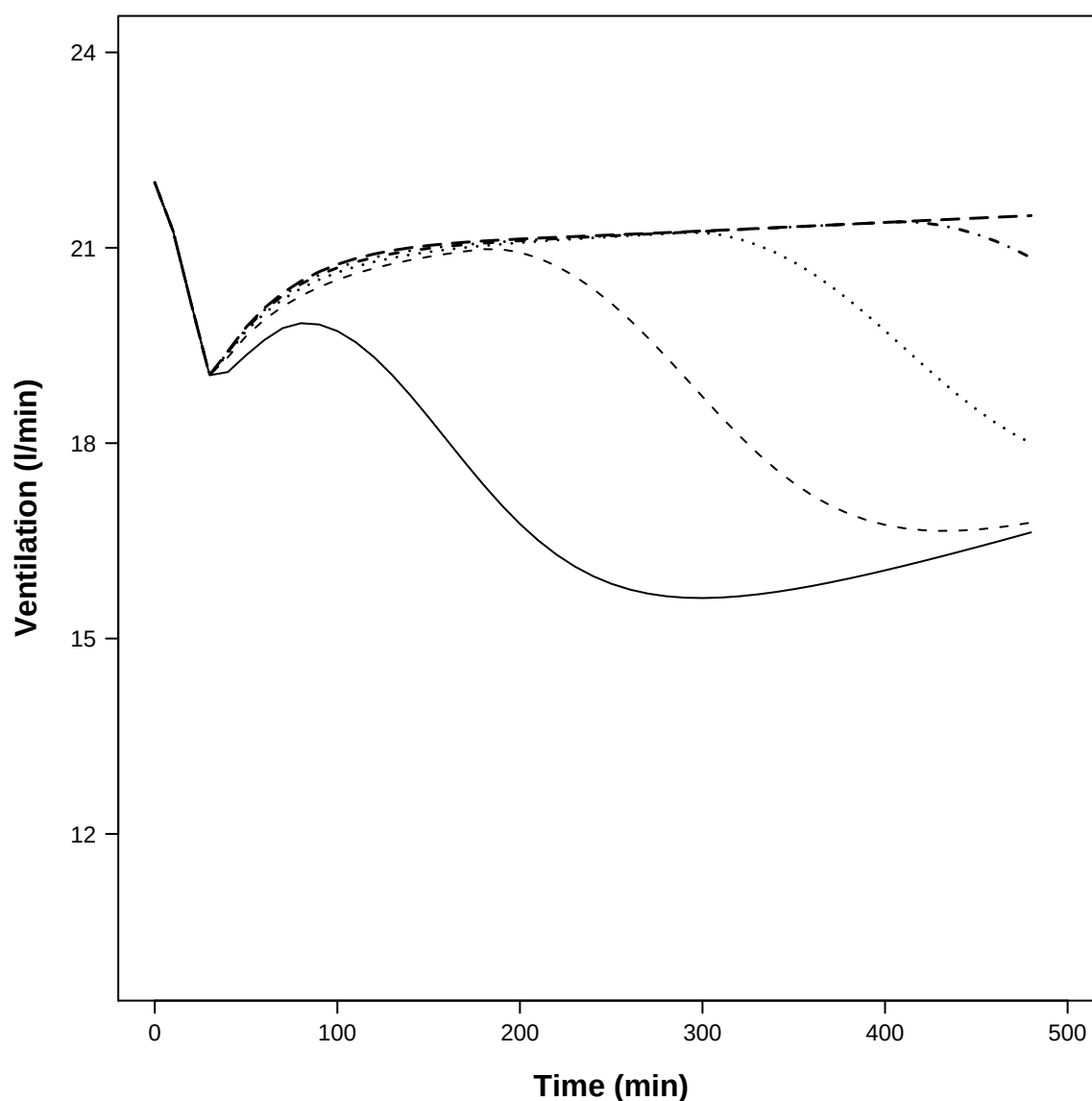


Figure 4. The time course of reversal of respiratory depression following the administration of 0.2 mg/70 kg buprenorphine (bolus i.v.). At $t = 30$ min naloxone infusion is started at a rate of 4 mg/70 kg/hr during 30 (solid line), 144 (dotted line), 258 (dashed line), 372 (dashed-dotted-dotted line) and 486 (long dashed line) min.

bination of buprenorphine and benzodiazepines which appears to cause fatalities. In this respect it is important that buprenorphine and benzodiazepines do not exert their respiratory depressant action through a single receptor alone but rather due to activation of two different receptors. As a consequence the interaction between buprenorphine and a benzodiazepine can be most likely described by a so-called two-receptor interaction model. In contrast to competitive interactions, the two-receptor interaction between buprenorphine and a benzodiazepine can lead to synergy (Jonker *et al.*, 2005). Synergism between buprenorphine and a benzodiazepine may result in exaggerated respiratory depression, exceeding the intensity of the ceiling respiratory depressant effect observed

when buprenorphine is administered alone. In this particular case, a consequence of drug synergism is potentially life-threatening respiratory depression. Although several studies have studied the respiratory depressant effect following the combination of buprenorphine and benzodiazepines, PK-PD modeling of the non-competitive interaction between opioids and benzodiazepines has yet not been achieved. Recently, a theoretical concept has been presented for the modeling of two-receptor interactions (Jonker *et al.*, 2005). A future challenge is the modeling of the slow receptor association/dissociation kinetics within the proposed theoretical framework of non-competitive drug-drug interactions.

EFFICACY AND SAFETY OF OPIOIDS

Drug safety is an important issue in drug development and regulation. The recent concerns regarding the cardiovascular safety of drugs have raised the question to what extent drug safety could be predicted from pre-clinical investigations (Rolan *et al.*, 2007). In recent years, mechanism-based PK-PD models have been successfully applied for the prediction of drug efficacy (Danhof *et al.*, 2007). Evidently these types of PK-PD models, with their much improved properties for extrapolation and prediction also constitute a scientific basis for the prediction of drug safety (Jonker *et al.*, 2005). For anesthetic drugs and in particular opioids prescribed for post-operative pain relief or chronic (malignant) pain syndromes, safety issues associated with opioid administration are of major concern. In chapter 9, a therapeutic utility function is proposed to assess the therapeutic utility of buprenorphine and fentanyl in rats. It was shown that buprenorphine has a higher therapeutic index relative to fentanyl. Since buprenorphine and fentanyl function in a nearly identical manner in humans with regard to receptor affinity and intrinsic activity, it is likely that the therapeutic indices estimated in rats also apply to the human situation. Although these findings suggest that buprenorphine is relatively safe, one needs to be aware that data were obtained in healthy volunteers and animals free of underlying disease, pain and co-medication. How the therapeutic index changes when buprenorphine is administered to elderly patients with pain and underlying disease is unknown. Highly likely the pharmacological response in this group of patients with multiple underlying patho-physiological conditions is more variable than in healthy volunteers. Aging results in profound changes in the anatomical and physiological functioning of virtually all vital organs, including the cardiovascular and renal system (Muhlberg and Platt, 1999). Much attention had been devoted to studying the effect of age on the kidney. In this respect, age-related changes in renal function may significantly alter drug disposition characteristics and thus the drug exposure-response relationships (Crooks *et al.*, 1976). The lack of understanding of variability in drug response in elderly patients makes drug administration to geriatric patients a difficult challenge. To this end, PK-PD models may facilitate the search for mechanisms that govern changes in the (patho)-physiology providing a better understanding of variability in drug response in the elderly. There is ample evidence

that pain has a significant effect on breathing, often stimulating respiration (Borgbjerg *et al.*, 1996; Dahan and Teppema, 2003). Again, this may alter the therapeutic index of buprenorphine significantly. The exact relationship between changes in pain state and the stimulatory effect on respiration and its clinical implications for the safety issues of opioids is still poorly understood. Another important issue is how the therapeutic utility of opioids is changed when given in combination with naloxone. Naloxone is a μ -opioid receptor antagonist and is expected to reverse μ -opioid receptor mediated pharmacological effects like respiratory depression and analgesia. Since there is evidence that buprenorphine exert its analgesic and respiratory depression via activation of distinct μ -opioid receptor subtypes, the degree and intensity of reversal of respiratory depression and analgesia can be different (Dahan *et al.*, 2006). This depends on the dose of naloxone administered and the affinity of buprenorphine for the two receptor subtypes. Preferably, one would like to completely antagonize respiratory depression without reversing analgesia. To accomplish selective antagonism, naloxone dosing regimens need to be established, minimally compromising the analgesic effectiveness of opioids. In theory, for each combination of buprenorphine and naloxone concentrations a therapeutic utility can be calculated, hereby realizing a response surface. The response surface methodology enables us to select the optimum doses of naloxone which effectively reverses respiratory depression and concurrently minimally compromising the analgesic effectiveness of the opioid (response surface therapeutic utility models). Also, another important aspect in opioid therapy is how changes in the biological pathway alter the therapeutic indices of opioid analgesics. For instance, it is known that upon chronic exposure of opioids tolerance may occur, resulting in less sensitivity to the analgesic effect of opioids. Therapeutic utility functions may prove valuable in the selection of new doses of the opioids in order to optimize analgesic opioid therapy.

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Chapter 13

Nederlandse samenvatting
(Synopsis in Dutch)

INLEIDING

Opiaten nemen een belangrijke plaats in de behandeling van postoperatieve, acute en/of chronische pijn. Tot op heden is morfine de meest gebruikte pijnstiller voor de bestrijding van ernstige pijn. Toch hebben morfine en andere opiaten vele ongewenste neveneffecten. De belangrijkste bijwerkingen van opiaten die aangrijpen op de μ -opiaat receptor zijn misselijkheid, braken, jeuk, constipatie, duizeligheid en ademhalingsdepressie. Hoewel patiënten veel van deze bijwerkingen als hinderlijk kunnen ervaren zijn deze niet levensbedreigend en kunnen waar gewenst symptomatisch worden behandeld. Een ernstige bijwerking die wel de nodige aandacht behoeft is ademhalingsdepressie. Na toediening van een opiaat kan het ademhalingsdepressieve effect zich binnen enkele minuten maar ook pas na enkele uren manifesteren met een soms dodelijke afloop. De ontwikkeling van nieuwe opiaten die een gunstige pijnstillende werking laten zien, maar niet of minder de bijwerking op het ademhalingsstelsel vertonen is om de bovengenoemde reden van groot belang. Vanuit theoretisch oogpunt kunnen partiële agonisten, geneesmiddelen met een lager maximaal effect, een verbeterde selectiviteit van werking hebben en daarmee in principe een verbeterde balans tussen werkzaamheid -en veiligheid. In de praktijk betekent deze verbeterde balans dat de dosering op relatief veilige manier opgetitreerd kan worden totdat de gewenste werking is bereikt zonder een verhoogd risico op het ontstaan van (schadelijke) bijwerkingen.

Buprenorfine is een semi-synthetisch opiaat en al in de beginjaren '70 ontwikkeld als een krachtige pijnstiller. Toentertijd hebben verscheidene studies aangetoond dat buprenorfine zich gedraagt als een partiële agonist voor de μ -opiaat receptor in buiten het lichaam gekweekte cellen. In principe heeft buprenorfine door zijn partiële intrinsieke activiteit op de μ -opiaat receptor een verbeterde selectiviteit van werking in vergelijking tot volle μ -opiaat receptor agonisten zoals morfine, fentanyl en methadon. Toch is de toegevoegde klinische waarde van buprenorfine gering indien de (sub-)maximale pijnstillende werking in hetzelfde therapeutische concentratiebereik valt als het submaximale ademhalingsdepressieve effect. Naast de partieel agonistische activiteit op de μ -opiaat receptor onderscheidt buprenorfine zich van de andere opiaten door de trage μ -opiaat receptor associatie en dissociatie kinetiek. Dit heeft tot gevolg dat het pijnstillende effect van buprenorfine langzaam intreedt. De trage inwerking van het effect bemoeilijkt het snel optitreren van de buprenorfine dosering tot het niveau waarbij adequate pijnstilling wordt bereikt. Aan de andere kant zorgt de hechte en langdurige binding van buprenorfine aan de receptor voor een verlengde duur van pijnstillende werking. Ter vergelijking, de pijnstillende werking na intraveneuze toediening van een eenmalige dosering van morfine houdt ongeveer 3 tot 4 uur aan, terwijl voor buprenorfine het pijnstillende effect 6 tot 8 uur aan kan houden. Buprenorfine hoeft hierdoor minder frequent te worden toegediend gedurende de dag. De trage receptor dissociatie kinetiek bemoeilijkt echter ook het tegengaan van optredende ademhalingsdepressies door toediening van de μ -opiaat re-

ceptor antagonist naloxon. De intraveneuze bolus toediening van gebruikelijke klinische dosering van 0.2 tot 0.4 mg naloxon is in de meeste gevallen niet afdoende om volledige en onomkeerbare blokkering van de ademhalingsdepressieve werking van buprenorfine te bewerkstelligen.

De duur en de intensiteit van de effecten van opiaten worden bepaald door de farmacokinetische (PK) en farmacodynamische (PD) eigenschappen van het toegediende opiaat. De farmacokinetiek beschrijft de wijze waarop geneesmiddelen door het lichaam worden geabsorbeerd, verdeeld, door enzymen worden afgebroken en uitgescheiden. De farmacodynamiek beschrijft de wijze waarop het geneesmiddel op het lichaam werkt en heeft o.a. betrekking op de volgende aspecten: de binding van een geneesmiddel aan de receptor, de activatie van de receptor en de signaal-transductie cascade resulterend in het effect. De resultante van de farmacokinetische en farmacodynamische processen bepaalt het farmacologische profiel van het geneesmiddel. Op basis van wiskundige modellen kan het tijdsverloop van het effect van het geneesmiddel als de resultante van de farmacokinetische en farmacodynamische processen worden beschreven en voorspeld d.m.v. geïntegreerde farmacokinetische-farmacodynamische (PK-PD) modellering. Het kunnen voorspellen van de effect-tijd relatie heeft bewezen nut bij de ontwikkeling en de toepassing (o.a. optimalisatie en individualisering van farmacotherapie) van (nieuwe) geneesmiddelen.

De afgelopen jaren zijn er belangrijke ontwikkelingen geweest op het gebied van de PK-PD modellering. In de eerste plaats heeft er een verschuiving plaatsgevonden van het gebruik van de traditionele beschrijvende PK-PD modellen naar de toepassing van meer voorspellende mechanistische PK-PD modellen. Deze laatste modellen beschrijven het tijdsverloop van het effect gebaseerd op beschikbare fysiologische en farmacologische informatie over het achterliggende werkingsmechanisme van een geneesmiddel of de geneesmiddelengroep waartoe het geneesmiddel behoort. Recent zijn verscheidene mechanistische PK-PD modellen ontwikkeld waarmee complexere effect-tijd profielen kunnen worden beschreven.

Het onderzoek beschreven in dit proefschrift heeft betrekking op de ontwikkeling van mechanistische PK-PD modellen waarmee het verloop van het pijnstillende (werkzaamheid) en ademhalingsdepressieve (veiligheid) werking van opiaten in de tijd kan worden beschreven en voorspeld. Op basis van de farmacologische eigenschappen van buprenorfine en bestaande principes van de receptor theorie is te verwachten dat een PK-PD model dat in staat is om receptor associatie en dissociatie kinetiek te karakteriseren en kwantificeren, de werkzaamheid en veiligheid van buprenorfine kan voorspellen. Een waardevolle eigenschap van het beschreven onderzoek in dit proefschrift is dat zowel data uit proefdierstudies als data verkregen op basis van studies met gezonde vrijwilligers beschikbaar zijn. Het voordeel van studies in proefdieren is dat vaak veel hogere doseringen van een geneesmiddel kunnen worden toegediend dan in studies met gezonde vrijwilligers en/of patiënten. Hierdoor kan vaak de gehele concentratie-werkings relatie worden bestudeerd en beschreven. Op basis van de concentratie-werkings relatie kan vervolgens o.a. de intrinsieke effectiviteit (volle of partiële agonist) en de potentie wor-

den geschat. Tevens kunnen in proefdieren experimentele en/of niet-geregistreerde geneesmiddelen worden uitgetest. Een bekend voorbeeld is het toedienen van metabolieten van geneesmiddelen om vervolgens hun farmacologische activiteit te meten.

In dit proefschrift zijn mechanistische PK-PD modellen ontwikkeld en gevalideerd op basis van gegevens verkregen uit proefdierstudies. Deze PK-PD modellen zijn vervolgens toegepast in fase I studies bij gezonde vrijwilligers. Het ontwikkelde mechanistische PK-PD model stelt ons in staat om zowel de verdeling van buprenorfine naar de hersenen als receptorbezetting (door kwantificering van de receptor associatie en dissociatie kinetiek) te voorspellen. De relatie tussen receptorbezetting en het effect wordt beschreven met een lineair transductie model beschreven. De specifieke doelstellingen van het onderzoek dat in dit proefschrift beschreven zijn het karakteriseren van *in vivo* farmacologische en PK-PD eigenschappen van buprenorfine in vergelijking tot fentanyl met betrekking tot:

- de snelheid waarmee de pijnstillende en ademhalingsdepressieve effecten intreden en de duur van de effecten
- de selectiviteit van werking (balans tussen effectiviteit en veiligheid)
- het extrapoleren, in strikt kwantitatieve zin van de PK-PD eigenschappen van buprenorfine van proefdieren naar mensen
- de bijdrage van actieve metabolieten aan het ademhalingsdepressieve effect van buprenorfine
- het opheffen van ademhalingsdepressies door het toedienen van naloxon

In het onderzoek in dit proefschrift worden de pijnstillende en ademhalingsdepressieve effecten van buprenorfine vergeleken met de effecten van fentanyl. Fentanyl is een volledig synthetisch opiaat, in tegenstelling tot buprenorfine wat semi-synthetisch is, met volle agonistische activiteit op de μ -opiat receptor. Dit houdt onder meer in dat de pijnstillende maar ook de ademhalingsdepressieve werking in volle omvang kan worden gekwantificeerd. Dit laatste kan tot levensbedreigende situaties leiden waarbij, zelfs bij toediening van therapeutische doseringen de ademhaling volledig kan worden onderdrukt. Naast het feit dat fentanyl een volle agonist is voor de μ -opiat receptor is, bindt fentanyl heel snel aan de μ -opiat receptor en is de dissociatie van de binding aan de receptor eveneens snel (snelle receptor associatie-dissociatie kinetiek). Fentanyl is daarom een opiaat met volledig andere farmacologische eigenschappen dan buprenorfine en dient in dit onderzoek als een "comparator" geneesmiddel.

PK-PD MODELLERING VAN HET PIJNSTILLENDE EFFECT

Het onderzoek beschreven in **hoofdstuk 4** heeft betrekking op het beschrijven en het voorspellen van het beloop van het pijnstillende effect van buprenorfine en fentanyl in

ratten. Voor het karakteriseren van het pijnstillende effect is gebruik gemaakt van een acuut pijnmodel. In dit model wordt de tijd tot het wegtrekken van de staart na het continu aanbieden van een externe prikkel (in dit geval hitte) als maat genomen voor de pijnstillende werking. Na intraveneuze toediening van buprenorfine of fentanyl is er een vertraging in het pijnstillende effect waarneembaar t.o.v. de concentratie in het bloed. Met andere woorden het maximale pijnstillende effect is nog niet bereikt op het moment dat de concentratie in het bloed maximaal is. Dit wordt hysteresis genoemd. De vertraging in het effect was aanzienlijk langer voor buprenorfine dan voor fentanyl. In **hoofdstuk 4** is een mechanistisch PK-PD model ontwikkeld voor het karakteriseren van de hysteresis tussen het pijnstillende effect en de concentratie in het bloed. Een unieke eigenschap van dit (gecombineerde) model is dat daarmee zowel de snelheid van distributie naar de hersenen als de snelheden van associatie en dissociatie aan de μ -opiaat receptor onafhankelijk kunnen worden gekwantificeerd. Op basis van dit PK-PD model kon worden vastgesteld dat de hysteresis in het pijnstillende effect van buprenorfine wordt veroorzaakt door zowel de verdeling naar de hersenen (biofase) als trage receptor associatie-dissociatie kinetiek (receptor binding). Echter in tegenstelling tot wat algemeen werd aangenomen is niet de trage receptor associatie-dissociatie kinetiek maar vooral de verdeling naar de hersenen de belangrijkste snelheidsbepalende stap. Voor fentanyl leverde de PK-PD analyse hoge schattingen op voor de snelheidsconstanten van receptor associatie-dissociatie kinetiek op. Dit betekent dat voor fentanyl de bijdrage van receptor binding aan de vertraging in het pijnstillende effect minimaal is. De waarneming van snelle receptor associatie-dissociatie kinetiek is in overeenstemming met *in vitro* receptor binding gegevens uit de literatuur. Als gevolg hiervan kan het PK-PD model voor fentanyl worden vereenvoudigd tot het biofase distributie model waarin de hysteresis volledig kon worden verklaard door verdeling van fentanyl naar de hersenen.

Een nadeel van het toegepaste diermodel voor acute pijn is dat de pijnprikkel gedurende maximaal 10 seconden aangeboden mag worden om weefselschade aan de staart te voorkomen. Om die reden wordt, indien in het experiment een blootstelling langer dan 10 seconden nodig zou zijn, de prikkeltoevoer handmatig onderbroken. In dat geval is 10 seconden dus het maximale pijnstillende effect. Dit neemt niet weg dat het pijnstillende effect langer dan 10 seconden kan zijn, maar dat dit niet experimenteel kan worden vastgesteld. Er is dan sprake van gecensureerde data boven de 10 seconden. Indien in de PK-PD analyse geen rekening wordt gehouden met de aanwezigheid van gecensureerde data kan dit leiden tot bias in de schattingen van de farmacodynamische parameters. In dit onderzoek is dit ondervangen door gebruik te maken van een statistische verdeling die voorspellingen van het pijnstillende effect boven de 10 seconden mogelijk maakt. Door gebruik te maken van deze statistische verdeling in de PK-PD analyse is gebleken dat zowel buprenorfine als fentanyl een volledig pijnstillende werking laten zien.

In **hoofdstuk 5** is het pijnstillende effect van buprenorfine in gezonde vrijwilligers onderzocht. Het principe van het meten van het pijnstillende effect is hetzelfde als bij proefdieren met uitzondering dat bij gezonde vrijwilligers gebruik is gemaakt van elek-

trische pijnprikkels die werden toegediend op het scheenbeen. Een belangrijke vraagstelling is of hetzelfde PK-PD model dat is gebruikt bij de analyse van de data beschreven in **hoofdstuk 4** ook hier van toepassing kan zijn. Na intraveneuze toediening van buprenorfine (0.05 - 0.6 mg/70kg) is ook in de humane vrijwilligers hysteresis waargenomen. Op basis van het ontwikkelde model kon ook in de humane vrijwilligers een onderscheid worden gemaakt tussen de bijdrage van de verdeling van buprenorfine naar de hersenen en de receptor associatie en dissociatie kinetiek aan de geobserveerde hysteresis. De analyse leverde schattingen op voor de snelheidsconstanten voor receptor associatie en dissociatie kinetiek als die vergelijkbaar waren met de in **hoofdstuk 4** gevonden waarden. De snelheidsconstante die de verdeling naar de hersenen karakteriseert was verschillend tussen proefdieren en mensen. Tenslotte is d.m.v. PK-PD analyse aangetoond dat het pijnstillende effect van buprenorfine in het onderzochte concentratiebereik niet wordt beperkt door een voor partiële agonisten karakteristiek plafondeffect.

PK-PD MODELLERING VAN HET EFFECT OP DE ADEMHALING

In **hoofdstuk 6** is onderzocht of het ontwikkelde PK-PD model kan worden toegepast om de ademhalingsdepressieve werking van buprenorfine in proefdieren te kunnen beschrijven en voorspellen. Belangrijke vragen in dit verband zijn ten eerste of de hysteresis in concentratie-werkings relatie kan worden verklaard door zowel de verdeling van buprenorfine naar de hersenen en langzame receptor associatie/dissociatie kinetiek. Ten tweede, of er sprake is van een plafondeffect in de ademhalingsdepressieve werking van buprenorfine. Op basis van gecombineerde PK-PD model kon voor buprenorfine een kwantitatief onderscheid worden gemaakt in de bijdrage van de verdeling naar de hersenen en de receptor associatie/dissociatie kinetiek aan de waargenomen hysteresis. Een uitermate interessante bevinding is dat er voor buprenorfine, in tegenstelling tot fentanyl, wel sprake is van een plafondeffect in de ademhalingsdepressieve werking. Dit plafondeffect is niet gevonden voor de pijnstillende werking van buprenorfine. Dit kan worden verklaard door het feit dat de pijnstillende en ademhalingsdepressieve effecten zich afspelen in verschillende gedeelten van de hersenen, als gevolg van o.m. verschillen in receptor dichtheid. Buprenorfine kan zich daardoor als een partiële agonist gedragen in een effect en als een volle agonist voor een ander effect. Het verschil in de geschatte evenwichtsdissoctatie constante voor het pijnstillende en ademhalingsdepressieve effect van buprenorfine suggereert dat er twee verschillende μ -opiat receptor subtypen betrokken zijn bij het tot stand komen van de betreffende effecten.

Het onderzoek in **hoofdstuk 7** heeft betrekking op de ademhalingsdepressieve werking van buprenorfine en fentanyl in humane vrijwilligers. De ademhalingsdepressieve werking van buprenorfine (0.05-0.6 mg/70 kg) en fentanyl (0.05-0.6 mg/70 kg) is onderzocht bij verschillende doseringen. Op basis van het PK-PD model kon voor buprenorfine een onderscheid worden gemaakt in de bijdrage van de verdeling naar de hersenen en

de trage receptor associatie/dissociatie kinetiek als oorzaak van het relatief traag intreden van het ademhalingsdepressieve effect. Voor fentanyl kon deze vertraging volledig worden toegeschreven aan de trage verdeling naar de hersenen. Daarnaast vertoonden de concentratie-werkings relaties van buprenorfine en fentanyl grote onderlinge verschillen, waarbij net als bij proefdieren er sprake is van een partieel agonistisch effect op de ademhaling van buprenorfine en een volledige ademhalingsdepressieve werking van fentanyl (volle agonist).

In **hoofdstuk 8** is het ademhalingsdepressieve effect van buprenorfine's primaire metabooliet norbuprenorfine in proefdieren onderzocht. Drie verschillende doseringen norbuprenorfine zijn intraveneus toegediend aan proefdieren. De effect-tijd profielen laten een duidelijke vertraging zien in het intreden van het ademhalingsdepressieve effect van norbuprenorfine. Het gecombineerde PK-PD model is vervolgens toegepast om de hysteresis te kunnen verklaren. Op basis van de PK-PD analyse is gebleken dat, in tegenstelling tot buprenorfine, kan worden verklaard door verdeling van norbuprenorfine naar de hersenen. De bijdrage van receptor associatie/dissociatie kinetiek is minimaal. Tevens is gebleken dat de duur van het ademhalingsdepressieve effect meer dan evenredig toeneemt bij hogere dosering (niet-lineaire toename in effect). Een mogelijke verklaring voor deze waarneming is dat transportmechanismen op de bloed-hersen barrière verhinderen dat norbuprenorfine van het bloed naar de hersenen wordt getransporteerd. Bij hogere doseringen raken deze transportmechanismen verzadigd waardoor norbuprenorfine moeilijker vanuit de hersenen naar de bloedbaan wordt getransporteerd. Norbuprenorfine stapelt zich daardoor als het ware op in de hersenen met als gevolg dat het ademhalingsdepressieve effect langer aanhoudt. Het PK-PD model is aangepast om deze niet-lineariteit in de verdeling van het bloed naar de hersenen te kunnen beschrijven. Daarnaast is op basis van PK-PD analyse aangetoond dat norbuprenorfine een volledig ademhalingsdepressieve werking heeft. Dit kan er toe leiden dat na toediening van buprenorfine er zoveel norbuprenorfine wordt gevormd dat er een volledige ademhalingsdepressie kan optreden. Echter de potentie van norbuprenorfine is aanzienlijk lager dan die van buprenorfine waardoor niet duidelijk is in hoeverre norbuprenorfine de ademhalingsdepressieve werking van buprenorfine kan beïnvloeden. Om de bijdrage van norbuprenorfine aan de totale ademhalingsdepressieve werking na toediening van buprenorfine te bestuderen is een PK-PD model ontwikkeld waarin in de eerste plaats de omzetting van buprenorfine naar norbuprenorfine wordt beschreven en in de tweede plaats de bijdrage van norbuprenorfine aan het totale effect kan worden voorspeld. Deze analyse toont aan dat, indien buprenorfine in therapeutische doseringen wordt toegediend, de bijdrage van norbuprenorfine aan de ademhalingsdepressieve werking minimaal is.

MODELLERING VAN DE BELANS TUSSEN WERKZAAMHEID EN VEILIGHEID

Op basis van de resultaten van **hoofdstuk 4 t/m 7** kan worden geconcludeerd dat het ontwikkelde mechanistische PK-PD model gebruikt kan worden voor het voorspellen van het tijdsverloop van de pijnstillende en ademhalingsdepressieve werking van buprenorfine. De uitgevoerde PK-PD analyses laten zien dat er verschillen bestaan in de snelheid van het intreden van de ademhalingsdepressieve en pijnstillende werking en dat de duur van de effecten (bij gelijke dosering) verschillend is. Daarnaast is de potentie van buprenorfine voor de ademhalingsdepressieve werking lager dan voor de pijnstillende werking en bestaan er verschillen in de intrinsieke effectiviteit. Op basis van deze afzonderlijke analyses is het bijna onmogelijk om een uitspraak te doen over de balans tussen de werkzaamheid en veiligheid van buprenorfine. **Hoofdstuk 9** beschrijft het gebruik van een zgn. 'utility functie' in de PK-PD analyse om een uitspraak te kunnen doen over de balans tussen werkzaamheid en veiligheid van buprenorfine en fentanyl. Daartoe wordt op basis van kansberekening de kans op pijnstilling en ademhalingsdepressie bij een bepaalde concentratie in bloed bepaald. De resultante van deze twee kansen (utility) bepaalt de balans tussen de pijnstillende en ademhalingsdepressieve werking. De PK-PD analyse laat zien dat de balans tussen werkzaamheid en veiligheid positief is voor buprenorfine terwijl voor fentanyl deze balans negatief is in het therapeutische concentratiebereik. Dit heeft belangrijke gevolgen voor het doseren van buprenorfine en fentanyl. Buprenorfine blijkt een relatief veilig geneesmiddel waarbij de dosering relatief veilig opgetitreerd kan worden. Voor fentanyl is de therapeutische index klein en dient met enige voorzichtig te worden gedoseerd om levensbedreigende ademhalingsdepressies te voorkomen.

EXTRAPOLATIE VAN PROEFDIEREN NAAR DE MENS

In **hoofdstuk 10** wordt de bruikbaarheid van het mechanistische PK-PD model onderzocht voor extrapolatie van de gegevens uit proefdieren naar de mens. Hierbij is de hypothese dat de parameters die de receptor associatie-dissociatie kinetiek beschrijven identiek zijn voor proefdieren en mensen, maar dat de waarden daarvan wel verschillend kunnen zijn voor het pijnstillende en ademhalingsdepressieve effect. De waarde van de parameter die de snelheid van verdeling van buprenorphine naar de hersenen beschrijft verschilt bij proefdieren en de mens, omdat die onder meer mede bepaald wordt door het volume waarover buprenorfine wordt verdeeld. Dit volume is weer afhankelijk van hersengewicht. Voor het schalen van deze parameter van proefdieren naar mensen wordt daarom gebruik gemaakt van een allometrische wiskundige vergelijking. Op basis van een simultane PK-PD analyse van de verkregen data (pijnstilling en ademhaling) uit proefdieren en mensen is gebleken dat de snelheidsconstante die de verdeling van buprenorfine naar de hersenen in mensen karakteriseert goed voorspeld kan worden op basis van de gegevens verkregen van proefdieren. Tevens zijn de waarden van de parameters die de receptor associ-

atie/dissociatie kinetiek karakteriseren identiek in proefdieren en mensen. Deze bevinding wordt ondersteund door gegevens uit de literatuur. De μ -opiaat receptor van ratten en mensen vertoont namelijk een hoge mate van homologie waardoor aangenomen mag worden dat de bindingseigenschappen van buprenorfine voor de μ -opiaat receptor gelijk zijn voor ratten en mensen. Uit de snelheidsconstanten die de receptor associatie en dissociatie kinetiek karakteriseren kan de evenwichtsdissociatie constante (K_D), een maat voor de affiniteit van buprenorfine voor de μ -opiaat receptor, worden geschat. De waarde van de K_D is verschillend voor het pijnstillende en ademhalingsdepressieve effect van buprenorfine. Deze bevinding suggereert dat de pijnstillende en ademhalingsdepressieve werking van buprenorfine tot stand komen na activatie van twee onafhankelijke subtypen van de μ -opiaat receptor. De aanwezigheid van verschillende subtypen van μ -opiaat receptoren wordt ondersteund door gegevens uit de literatuur, waarin is aangetoond dat buprenorfine met zowel hoge als lage affiniteit kan binden aan μ -opiaat receptoren.

MODELLERING VAN DE INTERACTIE MET NALOXON

In **hoofdstukken 4 t/m 10** is de werkzaamheid en veiligheid van buprenorfine besproken. De resultaten van PK-PD analyses uitgevoerd op basis van data verkregen middels uitgevoerde experimenten in zowel proefdieren als humane vrijwilligers tonen aan dat receptor associatie/dissociatie kinetiek een belangrijke rol speelt in totstandkoming en de totale duur van het pijnstillende en ademhalingsdepressieve effect van buprenorfine. De receptor associatie/dissociatie kinetiek bepaalt in grote mate de selectiviteit van werking van buprenorfine (balans tussen werkzaamheid en veiligheid). Een ander aspect dat betrekking heeft op de balans tussen werkzaamheid en veiligheid is de mate waarin de ademhalingsdepressieve werking van buprenorfine tegengegaan kan worden door toediening van naloxon. In **hoofdstuk 11** is de farmacodynamische interactie tussen buprenorfine en de opiaat antagonist naloxon in gezonde vrijwilligers bestudeerd. Aan de hand van klinische trial simulaties is aangetoond dat, indien naloxon als een intraveneuze bolus injectie wordt toegediend, de ademhalingsdepressieve werking van buprenorfine zich moeilijk laat opheffen. Dit kan verklaard worden door de langzame receptor dissociatie kinetiek van buprenorfine en de snelle uitscheiding van naloxon uit de hersenen. Uit de simulaties is gebleken dat naloxon volgens een intraveneuze continue infusieschema dient te worden gegeven om volledige omkering van ademhalingsdepressie te kunnen bewerkstelligen. Experimentele data verkregen van mensen bevestigen de uitkomsten van de simulaties en tonen aan dat continue infusie van naloxon noodzakelijk is om langdurige en volledige omkering van de ademhalingsdepressie te bewerkstelligen.

SAMENVATTING EN CONCLUSIES

Samenvattend kan worden gesteld dat er PK-PD modellen zijn ontwikkeld voor het voorspellen van het tijdsverloop van de pijnstillende en ademhalingsdepressieve werking van (semi-)synthetische opiaten. Het PK-PD model is gebaseerd op zowel het karakteriseren van de verdeling van het opiaat van het bloed naar de hersenen als de μ -opiaat receptor associatie/dissociatie kinetiek. Het mechanistische PK-PD model heeft goede eigenschappen voor inter- en extrapolatie van de pijnstillende en ademhalingsdepressieve werking van buprenorfine. Door gebruik te maken van een utility functie is een eerste aanzet gegeven tot het modelleren van de balans tussen werkzaamheid en veiligheid. Hieruit is gebleken dat buprenorfine een verbeterde selectiviteit van werking heeft met betrekking tot ademhalingsdepressie. In **hoofdstuk 12** zijn alle resultaten bediscussieerd en wordt verder ingegaan op factoren (o.a. actieve transportsystemen op de bloed-hersen barrière) die de verdeling van opiaten naar de hersenen kunnen beïnvloeden. Tevens wordt ingegaan op de rol die farmacogenetica speelt bij het voorspellen van het tijdsverloop van de pijnstillende en ademhalingsdepressieve effecten van opiaten en de daarmee samenhangende variatie tussen de proefpersonen. Tenslotte wordt ingegaan op mogelijke andere toepassingen van het ontwikkelde PK-PD model voor het modelleren van de farmacodynamische interacties tussen buprenorphine en een ander opiaat of tussen buprenorfine en andere centraalwerkende geneesmiddelen zoals de benzodiazepinen. Deze laatste interactie is in het bijzonder van belang omdat gelijktijdig gebruik van buprenorfine en een benzodiazepine wel kan leiden tot levensbedreigende ademhalingsdepressies.

LIST OF ABBREVIATIONS AND SYMBOLS

ABC	ATP-binding cassette
ARE	Additive residual error
ATP	Adenosine triphosphate
AUC	Area under the curve
BBB	Blood-brain barrier
C_{100}	Drug concentration producing 100 % response relative to baseline
C_e	Drug biophase concentration
C_eR	Drug-receptor complex
CHO cells	Chinese Hamster Ovary cells
Cl	Clearance of the drug from the body
C_{max}	Maximum concentration of the drug in blood after dosing
CNS	Central nervous system
CO ₂	Carbon dioxide
C_p	Drug plasma concentration
E_0	Baseline drug effect
EC ₅₀	Drug concentration producing half maximal effect
ECD	Electron capture detector
EEG	Electro-encephalogram
E_{max}	Maximal effect
GC	Gas chromatography
GTP	Guanosine triphosphate
HPLC	High performance liquid chromatography
IAV	Inter-animal variability
IIV	Inter-individual variability
IU	International units
iv	Intravenous
K_D	Equilibrium dissociation constant
k_{e0}	Rate constant of biophase distribution
k_{off}	Rate constant of receptor dissociation
k_{on}	Rate constant of receptor association
MDR	Multidrug resistance
MS	Mass spectrometry
N ₂	Nitrogen
n	Slope parameter of the sigmoid E_{max} pharmacodynamic model
NE	Not estimated
NaCl	Saline
NaOH	Sodium hydroxide

O_2	Oxygen
OFV	Objective function value
OPRM1	Human μ -opioid receptor gene
OR	Odds ratio
ORL1	Opioid receptor-like 1
P_aCO_2	Arterial carbon dioxide pressure
PET	Positron Emission Tomography
$P_{ET}CO_2$	End-tidal pressure of carbon dioxide
$P_{ET}O_2$	End-tidal pressure of oxygen
PK-PD modeling	Pharmacokinetic-pharmacodynamic modeling
pK_a	Negative logarithm of the acid dissociation constant
P-gp	P-glycoprotein
PRE	Proportional residual error
Q_2, Q_3	Inter-compartmental clearance
R	Receptor
rpm	Revolutions per minute
RR	Respiratory rate
RSE	Relative standard error
R_{tot}	Total receptor number
SK-N-SH	Human neuroblastoma cell line
SpO_2	Oxygen saturation
$t_{1/2,ke0}$	Half-life of biophase distribution
$t_{1/2,koff}$	Half-life of receptor dissociation
T_{max}	Time to maximum drug concentration in blood
V_1, V_2, V_3	Volumes of distribution of the compartments 1, 2 and 3
V_{i50}	Inspired minute ventilation at $P_{ET}CO_2$ of 50 mmHg
V_i	Inspired minute ventilation
V_T	Tidal volume
α	Maximal response by the drug, intrinsic activity
ρ_{app}	Apparent receptor occupancy
χ^2	Chi-square distribution
η	Inter-individual variability
ϵ	Residual error

NAWOORD

Promoveren doe je nooit alleen! Mijn naam mag dan wel op de cover van het proefschrift staan, het beschreven onderzoek in dit proefschrift is direct en indirect het werk van een hoop mensen.

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CURRICULUM VITAE

Ashraf Yassen werd op 21 mei 1977 geboren te Vlaardingen. In 1996 behaalde hij het VWO diploma aan de S.G. Groen van Prinsterer te Vlaardingen, waarna hij begon met de studie Farmacie aan de Universiteit Utrecht. Tijdens zijn studie heeft hij, in het kader van een farmacologische onderzoeksstage, onderzoek gedaan naar het ontstaan van tandvleeshyperplasie bij niertransplantatiepatiënten. Dit onderzoek werd uitgevoerd onder begeleiding van Prof. Dr. A. Vulto, Dr. P.J Smak Gregoor, Dr. H. R. de Jonge binnen de afdelingen Klinische farmacie en Nefrologie van het toenmalige Academisch Ziekenhuis Rotterdam (tegenwoordig Erasmus MC) en de afdeling Biochemie van de Erasmus Universiteit. Het doctoraaldiploma Farmacie behaalde hij in augustus 2000, waarna hij begon aan de apothekersopleiding aan de Universiteit Utrecht. Het apothekersexamen werd behaald in mei 2006.

Tijdens zijn opleiding tot apotheker werkte hij tevens als onderzoeker in opleiding bij de sectie Farmacologie van het Leiden/Amsterdam Center for Drug Research (maart 2002-september 2006). In samenwerking met de afdeling Anesthesiologie van het Leids Universitair Medisch Centrum werden de in dit proefschrift beschreven studies uitgevoerd onder begeleiding van Prof. Dr. M. Danhof (promotor) en Prof. Dr. A. Dahan (promotor). Voor het onderzoek ontving hij in 2007 de Graduate Student Award in Pharmacokinetics, Pharmacodynamics & Drug Metabolism and Clinical Pharmacology & Translational Research van de American Association of Pharmaceutical Scientists (AAPS).

Sinds oktober 2006 is de auteur werkzaam als wetenschappelijk onderzoeker binnen de afdeling Anesthesiologie van het Leids Universitair Medisch Centrum.

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