Mechanism-Based Pharmacokinetic-Pharmacodynamic Modeling of the Respiratory-Depressant Effect of Buprenorphine and Fentanyl in Rats

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ABSTRACT

The purpose of this investigation was to develop a mechanism-based pharmacokinetic/pharmacodynamic (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 (\( k_{eo} \)), receptor association (\( k_{on} \)), and dissociation (\( k_{off} \)) were 0.0348 min\(^{-1}\) (95% confidence interval (CI), 0.0193–0.0503 min\(^{-1}\)), 0.57 ml/ng/min (95% CI, 0.38–0.76 ml/ng/min), and 0.0903 min\(^{-1}\) (95% CI, 0.035–0.196 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.

Natural, semisynthetic, 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, \( \mu \)-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 toward 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 relation-
ship 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).

Therefore, the objective of the present investigation was 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. 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 ($P_{a,CO_2}$) as a surrogate biomarker of minute ventilation ($V_e$) (Ohtani et al., 1997; Megarbane et al., 2005). 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 were determined by a novel method in which minute ventilation is measured by whole-body plethysmography at a fixed inspired CO2 concentration of 6.5% (Romberg et al., 2003). Apart from an increased sensitivity, the application of 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 biophase distribution model in combination with a fractional sigmoid $E_{max}$ pharmacodynamic model, a receptor/association dissociation model with a linear transduction function, and a combined biophase distribution receptor association/dissociation model with a linear transduction function.

**Materials and Methods**

**Animals.** Male Wistar rats, weighing 225 to 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 10 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 i.m. injection of 0.1 mg/kg medetomidine hydrochloride (1 mg/ml Domitor; Pfizer, Capelle a/d IJssel, The Netherlands) and 1 mg/kg ketamine base (50 mg/ml Ketalar; Parke-Davis, Hoofddorp, The Netherlands). Two days before the experiment, in-dwelling 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, whereas the cannula in the left femoral artery was used for serial collection of arterial blood samples. The cannulae were made from pyrogen-free, nonsterile polyethylene tubing. One day before surgery, cannulae were disinfected in a 1% benzalkoniumchlorid solution. The venous cannula consisted of 3-cm polyethylene tubing (0.28-mm i.d.; Portex Limited, King of Kingdom) heat-sealed to 9-cm polyethylene tubing (0.58-mm i.d.; Portex Limited). 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 TA10TFA-F40 system; Data Sciences International, St. Paul, MN) was implanted under the skin in the neck for the measurement of body temperature. The cannulae were tunneled s.c. 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. To prevent clotting and cannula obstruction, the cannulae were filled with a 25% (w/v) polyvinylpyrrolidone solution (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 2 drops of polysorbate 80 (Hospital Pharmacy, Leiden University Medical Center). To accelerate solubility, the solution was placed in an ultrasonification 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 Electronics Inc., Winchester, UK). In brief, 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.). 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, were controlled by mass flow controllers (5850S/BC Mass Flow Controller; Brooks Instruments, Venendaal, The Netherlands) connected to a microprocessor control and read-out unit (model 0152; Brooks Instruments). O2 and CO2 levels in the chambers were monitored continuously using a Datex-Atos 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 back-
ground 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 ($V_e$, where $V_e = V_T \times RR$) were obtained from changes in chamber pressure using a low-pressure differential transducer connected to preamplifier modules (MAX2270; Buxco Electronics Inc.). The signals were digitized at a rate of 200 Hz using a CED 1401plus 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) 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-breadth basis. Minute ventilation was averaged over the total number of breaths obtained in 1 min 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).

**Measurement of Buprenorphine and Fentanyl Plasma Concentrations.** Two different bioassays 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 of plasma, 25 μl of internal standard (4 μg/100 μl [^3]H,buprenorphine) was added. Subsequently, 25 μl of concentrated ammonia was added, and the samples were extracted by liquid/liquid extraction with 600 μl 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, 3μ, C18A, 2 mm; Varian) and guard column (Atlantis C18 column, 3μ, 100 × 2.1 mm (Waters, Eschborn, Germany). The lower limit of quantification 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 vehicle, buprenorphine, fentanyl, or vehicle via a zero order i.v. infusion using an infusion pump (BAS Bioanalytical Systems Inc., West Lafayette, IN), minute ventilation was measured at the following predefined timepoints: 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; and 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 nonlinear 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 interanimal variability, was undertaken using the NONMEM software version V, level 1.1. The population analysis approach, which takes into consideration both intra-animal and interanimal variability, was undertaken using the first-order conditional estimation method with $\eta$ fixed. The pharmacokinetic and pharmacodynamic parameters of buprenorphine and fentanyl were estimated using nonlinear 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 interanimal variability, was undertaken using the first-order conditional estimation method with $\eta$ fixed. The pharmacokinetic and pharmacodynamic parameters of buprenorphine and fentanyl were estimated using nonlinear 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 interanimal variability, was undertaken using the first-order conditional estimation method with $\eta$ fixed. The pharmacokinetic and pharmacodynamic parameters of buprenorphine and fentanyl were estimated using nonlinear 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 interanimal variability, was undertaken using the first-order conditional estimation method with $\eta$ fixed.

**Pharmacokinetic Analysis.** 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 \( \chi^2 \) distributed. The following goodness-of-fit plots were subjected to visual inspection to detect systematic 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 ADVAN5 TRANS4 and ADVAN11 TRANS4 subroutines in NONMEM. For example, for fentanyl, the pharmacokinetic parameters, clearance (\( CL \)), the intercompartmental 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 interanimal variability in pharmacokinetic parameters and assumed a lognormal distribution of all model parameters over the population. Therefore, an exponential distribution model was used to account for interanimal variability:

\[
P_i = P_{\text{tot}} \times \exp(\eta_i)
\]

in which \( P_i \) is the individual value of model parameter \( P \), \( P_{\text{tot}} \) is the typical value (mean population value) of parameter \( P \) in the population, and \( \eta_i \) is the normally distributed interanimal random variable with mean zero and variance \( \sigma^2 \). The coefficient of variation of the appropriate residual error model parameters is expressed as percentage of the root mean square of the interanimal 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:

\[
C_{\text{obs},ij} = C_{\text{pred},ij} \times (1 + \eta_i)
\]

in which \( C_{\text{obs},ij} \) is the observed concentration in the \( ij \)th individual, \( C_{\text{pred},ij} \) is the predicted concentration, and \( \eta_i \) is the normally distributed residual random variable with mean zero and variance \( \sigma^2 \). The residual error term contains all the error terms that cannot 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 analyses of the pharmacokinetic and pharmacodynamic data are computer-intensive. Therefore, the sequential (two-stage) approach was preferred above simultaneous PK/PD analysis. Unless the pharmacokinetic model is mis-specified, 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 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). The validation procedure consisted of a bootstrap validation procedure. For the bootstrap validation procedure, 1000 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, S.E., coefficient of variation, and 95% confidence intervals of all model parameters were calculated and compared with 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: biophase equilibrium model with a fractional sigmoid \( E_{\text{max}} \) pharmacodynamic model, receptor association/dissociation model with a linear transduction function, and combined biophase equilibrium-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 equilibrium-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:

\[
d\left[C_{\text{biophase}}\right] \over dt = k_{\text{in}} \times \left[C_{\text{pl}}\right] - \left[C_{\text{biophase}}\right]
\]

where \( k_{\text{in}} \) is the first order rate constant for distribution in the effect compartment, and \( C_{\text{pl}} \) represents the plasma concentration and \( C_{\text{biophase}} \) the effect site concentration. At the site of action, the drug can bind to the \( \mu \)-opioid receptor. Following the law of mass action, the rate of drug receptor binding reaction is proportional to the concentration of drug \( C_{\text{pl}} \) and free receptor \( [R] \):

\[
d\left[C_{\text{R}}\right] \over dt = k_{\text{on}} \times \left[C_{\text{pl}}\right] \times [R] - k_{\text{off}} \times \left[C_{\text{R}}\right] / [R]_{\text{tot}}
\]

Under the assumption that the concentration of drug is in excess compared with the free receptor concentration and that the total number of receptors ([R]_{\text{tot}}) is equal to the sum of drug-bound ([C_{\text{R}}]) and unbound receptors ([R]), eq. 4 can be rearranged:

\[
dp_{\text{app}} \over dt = k_{\text{on}} \times \left[C_{\text{pl}}\right] \times (1 - \rho_{\text{app}}) - k_{\text{off}} \times \rho_{\text{app}}
\]

in which \( \rho_{\text{app}} \) is the apparent fractional receptor occupancy ([C_{\text{R}}] / [R]_{\text{tot}}). In the present analysis, a linear transduction function was used to characterize the fractional receptor occupancy-respiratory-depressant effect relationship:

\[
E = E_0 \times (1 - \alpha \times \rho_{\text{app}})
\]

where \( E \) is the ventilatory response, \( E_0 \) is the baseline ventilation, and \( \alpha \) 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 (eq. 3 in combination with the fractional sigmoid \( E_{\text{max}} \) pharmacodynamic model, which is of the form:

\[
E = E_0 \times \left(1 - \alpha \times \frac{C_{\text{pl}}}{C_{\text{pl}} + EC_{50}}\right)
\]

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

**Results**

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. Interanimal variability could be characterized for the pharmacokinetic parameters of buprenorphine and fentanyl indicated in Tables 2 and 3. Figures 1 and 2 show the time course of buprenorphine and fentanyl concentration, respectively. The mean population-predicted time courses 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 Tables 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 i.v. administration.

**Mechanism-Based PK/PD Model.** Vehicle treatment had no systematic effect on ventilation over the measurement period. Predrug ventilation (S.E.M.) 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. The biophase equilibration model in combination with the sigmoid $E_{\text{max}}$ pharmacodynamic model, the receptor association/dissociation model, and the combined biophase equilibration-receptor model with the fractional sigmoid transduction function were 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 (Fig. 3).

The changes in observed ventilation following the administration of 0.05 to 0.3 mg/kg buprenorphine are shown in Fig. 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, 0.035–0.146 min$^{-1}$), which corresponds to $t_{1/2,\text{off}} = 19.9$ min. The typical values for the rate constants of receptor association ($k_{on}$) and dissociation ($k_{off}$) were 0.57 ml/ng/min (95% CI, 0.38–0.76 ml/ng/min) and 0.0903 min$^{-1}$ (95% CI, 0.035–0.046 min$^{-1}$), respectively. The $t_{1/2}$ 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 $E_{\text{max}}$ 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

**TABLE 2**

Parameter estimates of the final population pharmacokinetic model for buprenorphine and the stability of the parameters using the bootstrap resampling procedure

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Original Data Set</th>
<th>1000 Bootstrap Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Cl$ (ml/min)</td>
<td>26.0 ± 4.2</td>
<td>26.0 ± 4.2</td>
</tr>
<tr>
<td>$V_1$ (ml)</td>
<td>225 ± 14.3</td>
<td>214 ± 21.5</td>
</tr>
<tr>
<td>$V_2$ (ml)</td>
<td>559 ± 12.9</td>
<td>558 ± 16.3</td>
</tr>
<tr>
<td>$V_3$ (ml)</td>
<td>1960 ± 7.5</td>
<td>1966 ± 8.2</td>
</tr>
<tr>
<td>$Q_2$ (ml)</td>
<td>32.9 ± 10.4</td>
<td>34.1 ± 17.8</td>
</tr>
<tr>
<td>$Q_3$ (ml)</td>
<td>16.4 ± 6.1</td>
<td>16.6 ± 9.2</td>
</tr>
<tr>
<td>Interanimal variability</td>
<td>22.6 ± 29.3</td>
<td>21.9 ± 30.6</td>
</tr>
<tr>
<td>Proportional error (%)</td>
<td>21.7 ± 8.0</td>
<td>17.4 ± 21.8</td>
</tr>
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CV, coefficient of variation.
Therefore, the combined biophase equilibration-receptor association/dissociation model can be simplified to the parsimonious biophase equilibration model with the fractional sigmoid $E_{\text{max}}$ pharmacodynamic model. The latter model was able to successfully describe the time courses of respiratory depression for all animals, yielding estimates for $k_{\text{eo}}$ and $\text{EC}_{50}$. Figure 5 shows the changes in observed ventilation for all individual animals stratified to dose. The respiratory-depressant effects in typical animals, predicted on the basis of the population mean pharmacodynamic parameter estimates, are depicted as solid lines. In Fig. 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 min$^{-1}$), which corresponds to $t_{1/2}k_{\text{eo}} = 1.6$ min. The value of $k_{\text{eo}}$, obtained with the combined biophase equilibration/receptor association/dissociation model, was 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 $\text{EC}_{50}$ of fentanyl was 4.83 ng/ml (95% CI, 3.19–6.47 ng/ml). The intrinsic activity of fentanyl was 0.85 (95% CI, 0.71–1.00). The par-
macodynamic parameter estimates, their respective estimates of interanimal variability, and the corresponding relative S.E. 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 Fig. 7.

**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. In addition, the goodness-of-fit plots for both PK/PD models are nearly identical (Fig. 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 (A. Yassen, J. Kan, E. Olofsen, E. Suidgeest, A. Dahan, and M. Danhof, unpublished data).

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 \( \alpha \) 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 cells, expressing the human \( \mu \)-opioid receptor, using a \(^{[\text{35}S]}\text{GTP}_Y\text{S-functional binding assay} \) (Huang et al., 2001). The results of that in vitro study show that buprenorphine is a potent partial agonist at the \( \mu \)-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 \(^{[\text{35}S]}\text{GTP}_Y\text{S binding to the human neuroblastoma SK-N-SH cells expressing the human \( \mu \)-opioid receptor was estimated at 0.14 nM (Selley et al., 1997). The in vivo \( K_d \) of buprenorphine is in the same range as 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 \( t_{1/2} \) of receptor dissociation kinetics is also consistent with point estimates obtained by other techniques. The \( t_{1/2} \) of receptor dissociation kinetics was 7.7 min. The dissociation kinetics of buprenorphine from the \( \mu \)-opioid receptor have been determined in an in vivo opioid receptor imaging study using \(^{11}\text{C-buprenorphine as a tracer} \) (Shiue et al., 1991). The dissociation rate constant was estimated to be 0.069 min\(^{-1}\), which corresponds to \( t_{1/2} \), \( k_{off} \) of 0.693/\( k_{on} \) of 10 min. In separate in vivo investigations, the receptor dissociation kinetics of buprenorphine from the \( \mu \)-opioid receptor had been also determined for the antinociceptive effect in rat. The \( t_{1/2} \) of receptor dissociation was 9.5 min in rat (Yassen et al., 2005). The in vivo \( t_{1/2} \), \( k_{off} \) value of 7.7 min obtained in the present investigation is remarkably similar to those in vivo \( t_{1/2} \), \( k_{off} \) values.

In addition to the slow receptor association/dissociation kinetics, biophase equilibration kinetics also contribute to the observed delay in the respiratory-depressant effect following the i.v. administration of buprenorphine. Data from animal studies indicate that buprenorphine readily penetrates the blood-brain barrier following i.v. administration (Ohtani et al., 1995). In baboon brain, peak concentration of buprenorphine was achieved after 15 to 20 min following bolus i.v. administration of radiolabeled buprenorphine (Galynker et al., 1996). In the present study, after i.v. administration of 0.1 mg/kg buprenorphine, the maximum predicted biophase concentration was reached after 20 to 35 min (Fig. 8) and is in good agreement with the previously 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 \( E_{\text{max}} \) 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 the common belief that delay in the central nervous system effects of fentanyl are attributed to an equilibrium delay between the arterial and biophase concentration of fentanyl (Scott et al., 1991). The \( t_{1/2} \) of biophase equilibration for fentanyl was 1.6 min. The \( t_{1/2} \) 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 effects are mediated through activation of \( \mu \)-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 \( E_{\text{max}} \) pharmacodynamic model.

### Table 5

<table>
<thead>
<tr>
<th>Population Parameter Estimate</th>
<th>CV of Parameter Estimate</th>
<th>Interanimal Variability</th>
<th>CV of Variability Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_{on} ) (ml/ng/min)</td>
<td>0.572</td>
<td>17.1</td>
<td>N.E.</td>
</tr>
<tr>
<td>( k_{off} ) (min(^{-1}))</td>
<td>0.0903</td>
<td>31.2</td>
<td>75.4</td>
</tr>
<tr>
<td>( K_d ) (ng/ml)</td>
<td>0.16</td>
<td>31.2</td>
<td>N.E.</td>
</tr>
<tr>
<td>( h_{on} ) (ml/ng/min)</td>
<td>0.0348</td>
<td>22.7</td>
<td>79.1</td>
</tr>
<tr>
<td>( h_{off} ) (ml/ng/min)</td>
<td>59.8</td>
<td>4.0</td>
<td>20.9</td>
</tr>
<tr>
<td>( E_{\text{c}} ) (ml/min)</td>
<td>0.48</td>
<td>3.4</td>
<td>13.6</td>
</tr>
<tr>
<td>( \alpha )</td>
<td>11.8</td>
<td>25.1</td>
<td>N.E.</td>
</tr>
</tbody>
</table>

N.E., not estimated.

Secondary parameter (\( K_d = k_{off} h_{on} \)).
An important feature of the combined biophase distribution-receptor association/dissociation 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. An important feature of the combined biophase equilibration-receptor association/dissociation kinetics 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 respira-
phase 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-species similarity. There is evidence that the \( \mu \)-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 a first step toward the prediction of the contribution of buprenorphine’s active metabolite norbuprenorphine to the overall respiratory-depressant effect in human. Since norbuprenorphine cannot 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 remifentanil’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 are subjects 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 that 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.

References


Cox EH, Kerbusch T, Van der Graaf PH, and Danhof M (1999). Currently, extrapolation of the respiratory-depressant effect of fentanyl is subject of ongoing investigations at our laboratory.

Another intriguing question is whether the combined biophase concentration (left y-axis); dashed line, apparent fractional receptor occupancy in time (right y-axis).

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

**Fig. 8.** The predicted changes in buprenorphine biophase concentration and apparent fractional receptor occupancy. Solid line, time course of biophase concentration (left y-axis); dashed line, apparent fractional receptor occupancy in time (right y-axis).


