Pharmacokinetic-Pharmacodynamic Modeling of the Respiratory Depressant Effect of Norbuprenorphine in Rats

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Received October 20, 2006; accepted February 2, 2007

ABSTRACT

The objective of this investigation was to characterize the pharmacokinetic-pharmacodynamic (PK-PD) correlation of buprenorphine’s active metabolite norbuprenorphine for the effect on respiration in rats. Following i.v. 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 PK of norbuprenorphine was best described by a three-compartment PK model with nonlinear elimination. A saturable biophase distribution model with a power PD 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 based on 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 based on a six-compartment PK model with nonlinear elimination of norbuprenorphine. This analysis showed that following i.v. administration, 10% of the administered dose of buprenorphine is converted into norbuprenorphine. By simulation it is shown that following i.v. administration of buprenorphine, the concentrations of norbuprenorphine reach values that are well below the values causing an effect on respiration.

Recently, the pharmacokinetic-pharmacodynamic (PK-PD) relationship of buprenorphine for the effect on the respiratory response has been studied in rats (Yassen et al., 2006) and humans (Yassen et al., 2007). In these investigations, buprenorphine has been shown to display ceiling of the 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 on 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 opiates 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 opiate (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; and 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 because in many instances such metabolites cannot be directly administered to humans (Breimer and Danhof, 1997). Previously, the pharmacological properties of the major metabolites of remifentanil and midazolam in humans have been successfully predicted based on 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 objectives were 1) to determine the rate-limiting steps in the time course of norbuprenorphine-induced respiratory depression and 2) to characterize the in vivo concentration-respiratory depressant effect relationship. Finally, an integrated drug-metabolite population PK model was developed to explore the contribution of the effect of norbuprenorphine to the observed respiratory depressant effect following i.v. administration of the parent drug.

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 before 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 (Domitor 1 mg/ml; Pfizer, Capelle a/dIJssel, The Netherlands) and 1 mg/kg ketamine base (Ketalar 50 mg/ml; Parke-Davis, Hoofddorp, The Netherlands). Two days before the experiment, indwelling cannulas 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, whereas the cannula in the left femoral artery was used for serial collection of arterial blood samples. The cannulas were made from pyrogen-free, nonsterile polyethylene tubing. One day before surgery, cannulas were disinfected in a benzalkonium chloride 1% solution. The venous cannula consisted of polyethylene tubing (0.58 mm i.d.). Furthermore, a telemetric transmitter (Physiotel Instruments, Rijswijk, The Netherlands) was implanted under the skin in the neck for the measurement of respiratory depression; and the back of the neck with a rubber ring. The skin in the neck and of body temperature. The cannulas were tunneled s.c. and fixed at the skin-muscle junction. To prevent clotting and cannula obstruction, 0.5 cm of polyethylene tubing (0.28 mm i.d.) heat-sealed to 21 cm of polyethylene tubing (0.58 mm i.d.; Portex, Winchester, UK) for the quantification of ventilation. 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.).

**Measurement of Respiratory Depression.** Respiratory depression was determined in unrestrained, conscious animals using whole-body plethysmography (model PLYT3223; Buxco Electronics Inc., Woonsocket, UK) for the quantification of ventilation. In brief, the chambers 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, a 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 (5850SB/C Mass Flow Controller; Brooks Instruments, Rijswijk, 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 Multicap gas monitor (Datex-Engstrom, Helsinki, Finland). In each animal, the effects of norbuprenorphine 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 (VT), breathing frequency (RR), and minute ventilation (V̇e) were obtained from changes in chamber pressure using a low-pressure differential transducer connected to a preamplifier module (MAX2270; Buxco Electronics Inc.). The signals were digitized at a rate of 200 Hz using a CED 1410plus, 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-breath 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).

**Drug Analysis.** Norbuprenorphine plasma concentrations were determined by high-performance liquid chromatography coupled to tandem mass spectrometry (Yassen et al., 2005). In brief, 25 μl of internal standard (4 g/100 ml) was added to 50 μl of plasma. Subsequently, 25 μl of concentrated ammonia was added, and the samples were extracted by liquid/liquid extraction with 600 μl of methyl tertiary-buty1 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/tandem mass spectrometry system (Applied Biosystems, Darmstadt, Germany). Chromatography was performed on a precolumn (Metaguard Polaris, 3 μm, C18-A, 2 mm; Varian, Darmstadt, Germany) guarded Synergi 4-μm Hydro-RP 80A column, 75 × 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.

**PK-PD Experiments.** To minimize the influence of circadian rhythms, all the 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 h. On
administration of norbuprenorphine or vehicle via a constant i.v.
rate infusion using an infusion pump (BAS Bioanalytical Systems
Inc., West Lafayette, IN), minute ventilation was measured during 1
min at the following predefined time-points: dose 1, 0 (baseline), 5,
20, 35, 50, 65, 90, 120, 150, and 180 min; and dose 2, 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, and 300 (doses 2 and 3) min.
In cases when blood sampling coincided with the ventilation mea-
surement, ventilation measurement preceded blood sampling to min-
imize stress for the animals. Serial arterial blood samples (100
µl) were collected in heparinized microtubes. Plasma (50
µl) was sepa-
rated from the blood by centrifugation at 5000 rpm for 15 min and
frozen at $-20^\circ$C until analysis.

**PK-PD Modeling Software.** Nonlinear mixed effects modeling
using the NONMEM software package (version V, level 1.1) (Beal
and Sheiner, 1999) was used to characterize the PK-PD relationship
of norbuprenorphine in rats. The Fortran compiler Compaq Visual
Fortran version 6.1 was used for compilation. The PK-PD param-
ters were estimated using the first-order conditional estimation
method with $\eta - e$ interaction. Evaluation of NONMEM outputs and
graphical analysis were performed using S-PLUS 6.0 (Insightful
Corp., Seattle, WA).

**PK Analysis of Norbuprenorphine.** To determine the basic
structural PK model for norbuprenorphine, one-, two-, and three-
compartment models with linear and nonlinear elimination were
tested. Model selection and identification were based on the likeli-
hood ratio test, PK 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. Initially, linear PK models were tested.
Thereafter, the individual PK parameter estimates were examined
for norbuprenorphine dose dependence. Based on the model selection
criteria, a three-compartment model with concentration-dependent
elimination was selected for norbuprenorphine according to:

$$k_{40} = \frac{V_m k_m}{C_p + K_m}$$

where $V_m k_m$ is the maximum elimination rate, which is $k_{40}$ at norbu-
prenorphine plasma concentration $C_p = 0$, and $K_m$ is the concentra-
tion at which the elimination rate constant is half-minimal. The model algorithm was programmed in ADVAN6 subroutine in NON-
MEM and was parameterized in terms of rate constants and volume of
central distribution (Fig. 1).

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

**PD Analysis.** To characterize the observed hysteresis in the time
course of respiratory depression relative to the plasma concentra-
tion, various structurally different PK-PD models, incorporating dif-
f erent link models, were evaluated for their appropriateness to char-
acterize the time course of norbuprenorphine’s respiratory depressant effect: 1) receptor association-dissociation model in com-
bination 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 $E_{max}$ PD model (Sheiner
et al., 1979); 4) a linear biophase distribution model with a power PD
model (Sarton et al., 2000); and 5) a saturable biophase distribution
model with a power PD model.

In the final model, the concentration-effect relationship of norbu-
prenorphine was described using the power PD model. Time depen-

![Fig. 1. A schematic representation of the integrated parent-metabolite six-
compartment PK-PD model to charac-
terize the conversion of bupe
norphine into norbuprenorphine and
predict the contribution of norbu-
prenorphine to the overall respiratory
depressant effect following the admin-
istration of buprenorphine.](https://example.com/figure1.png)
dependencies in norbuprenorphine’s PD were explained based on nonlinear biophase distribution kinetics:

\[ k_{eq} = \frac{V_{m}^{b} \times K_{m}^{b}}{C_{e} + K_{m}^{b}} \]  

where \( C_{e} \) is the norbuprenorphine biophase concentration, \( V_{m}^{b} \) is the rate constant of biophase distribution \( k_{eq} \) at \( C_{e} = 0 \), and \( K_{m}^{b} \) is the concentration at which the rate constant of biophase equilibration \( k_{eq} \) is half-minimal. A schematic representation of the population PK-PD model is shown in Fig. 1. The power PD model is of the form:

\[ E = E_{0} \times \left[ 1 - 0.5 \times \left( \frac{C_{e}}{EC_{50}} \right)^n \right] \]  

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.

**Statistical Analysis.** A one-way analysis of variance was performed to assess the effect of norbuprenorphine dose at baseline and at time reaching maximum respiratory depression. Post hoc comparisons were performed by using the Tukey’s test for multiple comparisons. Statistical tests were performed using SigmaStat for Windows version 3.5 (Systat Software, San Diego, CA). All the data are expressed as mean \( \pm \) S.D., and a level of 5% was taken as significant.

**Integrated Buprenorphine-Norbuprenorphine PK 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 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 PK model with a rate constant \( k_{conv} \), characterizing the conversion of buprenorphine to norbuprenorphine (Fig. 1). This rate constant \( k_{conv} \) was subsequently used to calculate the corresponding conversion fraction into norbuprenorphine:

\[ F_{conv} = \frac{k_{conv}}{k_{conv} + k_{10}} \times 100\% \]  

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

\[ P_{i} = P_{tot} \times \exp(\eta_{i}) \]  

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 \( \eta_{i} \) is the normally distributed interanimal random variable with mean zero and variance \( \sigma^{2} \). The coefficient of variation of the structural 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:

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

where \( C_{obs,ij} \) is the \( j \)-th observed concentration in the \( i \)-th individual, \( C_{pred,ij} \) is the predicted concentration, and \( \epsilon_{ij} \) 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 misspecification. To refine the stochastic model, correlation between PK parameter estimates was tested by conducting covariance matrix analysis (OMEGA BLOCK option). A significant correlation between two parameters was assumed when the decrease in OFV was more than 6.6 points (\( p < 0.01 \)).

**Results**

**Norbuprenorphine PK.** The PK of norbuprenorphine was nonlinear as indicated by the nonlinear relation between dose and area under the curve (AUC) (Fig. 2). A three-compartment model with nonlinear elimination best described the PK of norbuprenorphine. The rate of elimination decreased with increasing plasma concentrations of norbuprenorphine (Fig. 2). The observed and mean population predicted concentration time courses of norbuprenorphine stratified to treatment are shown in Fig. 3. All the PK 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. Interanimal variability of the PK parameters \( V_{m}^{b} \) and \( k_{64} \) was 13.4 and 28.1%. The parameter estimates obtained with the PK model are presented in Table 1.

**Norbuprenorphine PD.** The baseline values of ventilation (± S.D.) were 59.1 ± 10.2, 58.8 ± 5.03, and 59.8 ± 4.78 ml/min and were not significantly different among the doses 1 through 3, respectively (\( p > 0.05 \), Tukey’s test). 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 (± S.D.) of the minimum ventilation of 30.6 ± 6.22, 28.5 ± 3.68, and 17.5 ± 2.94 ml/min for doses 1 through 3, respectively. The minimum ventilatory response was not significantly different between animals that received 0.32 (dose 1) or 0.84 mg (dose 2) of norbuprenorphine (p < 0.05, Tukey’s test). The minimum ventilatory response was not significantly different between 0.32 and 0.84 mg of norbuprenorphine (p > 0.05, Tukey’s test).

To show 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_m) 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 PD model was equal to the E_{max} PD model, indicating that E_{max} had not been reached yet at 1.848 mg of norbuprenorphine as judged by the OFV (Table 2).

The power model with linear biophase equilibration showed a dependence of k_m on norbuprenorphine concentration (Fig. 4). The power model with nonlinear 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 nonlinear elimination from the brain. Although the OFV 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 PD model was able to successfully describe the individual respiratory depressant effect-time profiles, yielding estimates of V_{m}, K_{m}, and EC_{50} for norbuprenorphine. Figure 5 shows the observed and mean population predicted effect-time profiles of norbuprenorphine stratified to treatment. The typical values of V_{m}, K_{m}, and K_{m} were estimated at 0.113 min^{-1} [95% confidence interval (CI), 0.0895–0.137 min^{-1}] and 35.9 ng/ml (95% CI, 31.4–40.9 ng/ml). The PD 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 (Fig. 4). The in vivo potency (EC_{50}) was estimated at 72.8 ng/ml (95% CI, 40.5–105.1 ng/ml).

### 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 PK of buprenorphine, norbuprenorphine, and the formation of norbuprenorphine. A schematic representation of the population PK model is shown in Fig. 1. Unique and precise PK 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. Interanimal variability was estimated for k_{m}, k_{31}, V_{m}, k_{conv}, V_{m}, 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%.

### Table 1

Parameter estimates of the final population PK model for norbuprenorphine

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>CV of Parameter Estimate</th>
<th>Interanimal Variability</th>
<th>CV of Variability Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>V_{m}, min^{-1}</td>
<td>0.246</td>
<td>20.1</td>
<td>13.4</td>
<td>43.0</td>
</tr>
<tr>
<td>K_{m}, ng/ml</td>
<td>331</td>
<td>29.7</td>
<td>N.E.</td>
<td></td>
</tr>
<tr>
<td>K_{m}, min^{-1}</td>
<td>0.050</td>
<td>41.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>k_{12}, min^{-1}</td>
<td>0.002</td>
<td>44.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K_{m}, min^{-1}</td>
<td>0.219</td>
<td>15.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>k_{45}, min^{-1}</td>
<td>0.036</td>
<td>10.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V_{m}, ml</td>
<td>218</td>
<td>15.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportional error, %</td>
<td>22.8</td>
<td>11.9</td>
<td></td>
<td></td>
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</tbody>
</table>

CV, coefficient of variation; N.E., not estimated.
TABLE 2
Results of the model discrimination to characterize the time course of respiratory depression following i.v. administration of norbuprenorphine

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
<th>Model 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>OFV</td>
<td>1300</td>
<td>1099</td>
<td>1030</td>
<td>1029</td>
<td>987</td>
</tr>
<tr>
<td>(k_{so}) min(^{-1})</td>
<td>0.192</td>
<td>0.024</td>
<td>0.023</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>(k_{on}), ml/ng/min</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(k_{off}), min(^{-1})</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Model 1, receptor association-dissociation model with linear transduction function; Model 2, combined biophase equilibration-receptor association-dissociation model with a linear transduction function; Model 3, linear biophase distribution model with a sigmoid \(E_{\text{max}}\) PD model; Model 4, linear biophase distribution model with a power PD model; Model 5, saturable biophase distribution model with a power PD model.

Fig. 4. Nonlinear PD of norbuprenorphine illustrated by nonlinear dose-dependent biophase equilibration kinetics. The population 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.

Representative simultaneous PK fits of buprenorphine and norbuprenorphine of six animals are shown in Fig. 6.

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 because metabolites often cannot 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 \(\mu\)-opioid receptor association-dissociation kinetics. This is consistent with results from in vitro binding studies, which show that norbuprenorphine binding to the \(\mu\)-opioid receptor is more rapid and reversible compared with 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 based on the biophase distribution model with the sigmoid \(E_{\text{max}}\) PD 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 \(\mu\)-opioid receptor, displaying full respiratory depression. This is confirmed by the observation that the concentration-effect relationship was equally well described with a power PD model and a sigmoid \(E_{\text{max}}\) PD model, with a value of the intrinsic activity \(\alpha\) of 1 (Table 2). Norbuprenorphine's in vivo full agonistic activity at the \(\mu\)-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 cells, expressing the \(\mu\)-opioid receptor, using a \([^{35}]\)Sguanosine-5’-O-(\(\gamma\)-thio)triphosphate functional binding assay (Huang et al., 2001). The results of these in vitro studies show that norbuprenorphine is a full agonist at the \(\mu\)-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 based on 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 based on the biophase distribution model with the power PD model revealed a dose-dependent decrease in \(k_{so}\), 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 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 (P-gp) in brain disposition has been well established (Xie et al., 1999). For example, it has been shown that inhibition of P-gp 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-gp 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

![Fig. 5. Changes in ventilation in time following administration of norbuprenorphine. For each treatment group (1–3) the observed (symbols) and mean population predicted (solid line) time course of respiratory depression effect is shown.](image)

<table>
<thead>
<tr>
<th>TABLE 3</th>
<th>Population PD estimates and their respective variability of norbuprenorphine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td>Population Estimate</td>
</tr>
<tr>
<td>$V_{m, n}$, min$^{-1}$</td>
<td>0.113</td>
</tr>
<tr>
<td>$K_{m, n}$, ng/ml</td>
<td>35.9</td>
</tr>
<tr>
<td>EC$_{50}$, ng/ml</td>
<td>72.8</td>
</tr>
<tr>
<td>$E_{o, n}$, ml/min</td>
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<tr>
<td>$n$</td>
<td>0.14</td>
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<tr>
<td>Additive error</td>
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</table>

N.E., not estimated.

<table>
<thead>
<tr>
<th>TABLE 4</th>
<th>Parameter estimates of the final integrated buprenorphine-norbuprenorphine population PK model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td>Population Estimate</td>
</tr>
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<td>$h_{10, n}$, min$^{-1}$</td>
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</tr>
<tr>
<td>$h_{21, n}$, min$^{-1}$</td>
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</tr>
<tr>
<td>$h_{22, n}$, min$^{-1}$</td>
<td>0.082</td>
</tr>
<tr>
<td>$h_{31, n}$, min$^{-1}$</td>
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</tr>
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<td>$h_{41, n}$, min$^{-1}$</td>
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</tr>
<tr>
<td>$V_{m, n}$, ml</td>
<td>184</td>
</tr>
<tr>
<td>Proportional error, %</td>
<td>23.7</td>
</tr>
</tbody>
</table>

Parameter estimates norbuprenorphine

| Parameter | Population Estimate | CV of Parameter Estimate | Interanimal Variability | CV of Variability Estimate |
| $h_{42, n}$, min$^{-1}$ | 0.017 | 17.0 | 35.5 | 37.5 |
| $h_{53, n}$, min$^{-1}$ | 0.254 | 12.4 | 46.9 | 33.1 |
| $K_{m, n}$, ng/ml | 169 | 21.1 |  |  |
| $h_{61, n}$, min$^{-1}$ | 0.049 | 27.8 |  |  |
| $h_{62, n}$, min$^{-1}$ | 0.003 | 21.3 |  |  |
| $h_{63, n}$, min$^{-1}$ | 0.263 | 11.8 |  |  |
| $h_{64, n}$, min$^{-1}$ | 0.036 | 9.1 | 29.2 | 45.0 |
| $V_{m, n}$, ml | 205 | 15.2 |  |  |
| Proportional error, % | 24.3 | 8.2 |  |  |
present study, an integrated population PK model was developed for the conversion of buprenorphine into norbuprenorphine. PK analysis revealed that the elimination of norbuprenorphine is nonlinear. 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 PK of buprenorphine and norbuprenorphine were simultaneously analyzed after buprenorphine administration at a wide dose range of 0.1 to 30 mg/kg. Interestingly, it was shown, in contrast to the present data, the PK of norbuprenorphine was not dose-dependent. It should be noted, however, that the measured norbuprenorphine concentrations in their study following the i.v. administration of 30 mg/kg buprenorphine or 1.0 mg/kg norbuprenorphine were lower than the measured norbuprenorphine concentrations in the present study on i.v. administration of 0.32, 0.84, or 1.848 mg of norbuprenorphine, indicating that high doses of norbuprenorphine (>0.84 mg) must be administered to identify nonlinear PK behavior. On the other hand, in the same study by Gopal et
al. (2002), it was shown that buprenorphine displays dose-dependent PK, whereas in the present study, linear PK is assumed for buprenorphine. Again, this is because the present dose range in which the disposition of buprenorphine was studied did not allow identification of dose-dependent PK. In view of the present results and those obtained by Gopal et al. (2002), it seems 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 with or even exceeds the concentration of buprenorphine following chronic sublingual administration in humans (Kuhlman et al., 1998).

The PK parameters of buprenorphine 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 i.v. infusion over 20 min). This is based on the assumption of a zero-interaction between buprenorphine and norbuprenorphine. The predicted time courses of norbuprenorphine concentration and respiratory depression are shown in Fig. 7. Only following the administration of high-dose 30 mg/kg buprenorphine does the peak concentration exceed that of the estimated EC50 value for norbuprenorphine of 72.8 ng/ml. The lower buprenorphine doses yield maximum norbuprenorphine concentrations that are much lower than the EC50 value. The PK interaction between buprenorphine and norbuprenorphine, which has been described previously by Gopal et al. (2002), is not taken into account. In the present drug-metabolite PK 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 with 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 nonlinear PK on the predicted contribution of norbuprenorphine’s respiratory depressant effect is minimal, also taking into account that 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 based on the relative target affinities (Jonker et al., 2005). However, it cannot be excluded that the PD interaction is indeed more complex, also in the light that in addition to the µ-opioid receptor, the δ-opioid receptor subtype also may be involved in the respiratory depressant effect (Su et al., 1998; Gengo et al., 2003).

The implications for dosing of buprenorphine in humans are not known, especially on chronic use. An important utility of the integrated parent-metabolite population PK-PD model for further investigations is the prediction of the concentration-effect relationship of norbuprenorphine in humans. There is evidence that allometric models are accurate predictors for animal to human PK extrapolation of renally excreted drugs or high hepatic extraction drugs (i.e., buprenorphine) (Holford, 1996; West et al., 1997). With respect to the PD, 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 electroencephalogram 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 the subject of further investigation in our laboratory.

In conclusion, the PD of the metabolite norbuprenorphine is distinctly different from the PD of the parent compound buprenorphine with regard to the receptor association-dissociation kinetics, the in vivo potency, and the intrinsic efficacy for the respiratory depressant effect. Following i.v. 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.

Acknowledgments
We thank Dr. Rolf Ter Linden and Nicole Kohl for skillful support with the bioanalytical measurements performed in the frame of this work.

References
Breimer DD and Danhof M (1997) Relevance of the application of pharmacokinetic work.

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References
Cowan A, Duxey JC, and Harry EJ (1977) The animal pharmacology of buprenorphine. This is in line with the experience from clinical use of buprenorphine in patients.

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