

# Simultaneous Measurement and Integrated Analysis of Analgesia and Respiration after an Intravenous Morphine Infusion

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**Background:** To study the influence of morphine on chemical control of breathing relative to the analgesic properties of morphine, the authors quantified morphine-induced analgesia and respiratory depression in a single group of healthy volunteers. Both respiratory and pain measurements were performed over single 24-h time spans.

**Methods:** Eight subjects (four men, four women) received a 90-s intravenous morphine infusion; eight others (four men, four women) received a 90-s placebo infusion. At regular time intervals, respiratory variables (breathing at a fixed end-tidal partial pressure of carbon dioxide of 50 mmHg and the isocapnic acute hypoxic response), pain tolerance (derived from a transcutaneous electrical acute pain model), and arterial blood samples were obtained. Data acquisition continued for 24 h. Population pharmacokinetic (sigmoid Emax)-pharmacodynamic models were applied to the respiratory and pain data. The models are characterized by potency parameters, shape parameters ( $\gamma$ ), and blood-effect site equilibration half-lives. All collected data were analyzed simultaneously using the statistical program NONMEM.

**Results:** Placebo had no systematic effect on analgesic or respiratory variables. Morphine potency parameter and blood-effect site equilibration half-life did not differ significantly among the three measured effect parameters ( $P > 0.01$ ). The integrated NONMEM analysis yielded a potency parameter of  $32 \pm 1.4$  nM (typical value  $\pm$  SE) and a blood-effect site equilibration half-life of  $4.4 \pm 0.3$  h. Parameter  $\gamma$  was 1 for hypercapnic and hypoxic breathing but  $2.4 \pm 0.7$  for analgesia ( $P < 0.01$ ).

**Conclusions:** Our data indicate that systems involved in morphine-induced analgesia and respiratory depression share important pharmacodynamic characteristics. This suggests similarities in central  $\mu$ -opioid analgesic and respiratory pathways (e.g., similarities in  $\mu$ -opioid receptors and G proteins). The clinical implication of this study is that after morphine administration, despite lack of good pain relief, moderate to severe respiratory depression remains possible.

MORPHINE is the most efficacious drug for the treatment of severe pain.<sup>1</sup> This remains true in contemporary medicine despite the alkaloid's many adverse effects. The most burdensome adverse effects for patients (and society) include nausea/vomiting, constipation, itching, hallucinations, sedation, respiratory depression, orthostatic hypotension, and addiction. Despite its many years

of prescription, various side effects of morphine remain understudied, especially when viewed in relation to its intended effect, analgesia. An example is the potentially life-threatening adverse effect of respiratory depression.<sup>2,3</sup> To the best of our knowledge, there are no studies that simultaneously assessed the influence of morphine on the chemical control of breathing and morphine analgesia. It may be argued that simultaneous assessment of both analgesia and chemical control of respiration is difficult or maybe even impossible because measurement of morphine-induced analgesia may affect respiratory testing and *vice versa*. Although, we believe that this is partially true, we made a special effort to measure respiration (*i.e.*, chemical control of breathing) and analgesia after a morphine infusion in a single group of volunteers and analyzed the data using an integrated pharmacokinetic-pharmacodynamic modeling approach. Results of this study will allow comparison of the morphine concentration range causing adequate analgesia and the range causing mild to severe respiratory depression. The study was placebo controlled and had a randomized, double-blind design.

## Materials and Methods

Sixteen healthy subjects (eight men, eight women; age range, 18–24 yr) participated in the study after approval of the protocol by the local Human Ethics Committee (Commissie Medische Ethiek, Leiden University Medical Center, Leiden, The Netherlands) and provided oral and written consent. All women were taking oral contraceptives. Subjects were asked not to eat or drink for at least 6 h before the study. They were comfortably seated in a hospital bed for the duration of the study.

Subjects were assigned randomly into four groups. Group 1 received 0.2 mg/kg intravenous morphine at 09:00 h ( $n = 4$ ), group 2 received 0.2 mg/kg intravenous morphine at 18:00 h ( $n = 4$ ), group 3 received intravenous placebo (0.9% NaCl) at 09:00 h ( $n = 4$ ), and group 4 received intravenous placebo (0.9% NaCl) at 18:00 h ( $n = 4$ ). In all groups, pharmacodynamic measurements continued for 24 h after the bolus drug infusion with the exception of the sleep period (from approximately 23:00 h to 07:00 h). Our design enabled us to obtain data points evenly spread out over the 24-h measurement period without the need to wake up subjects during their sleep period (fig. 1). The local pharmacy prepared the morphine solution (morphine hydrochloride in nor-

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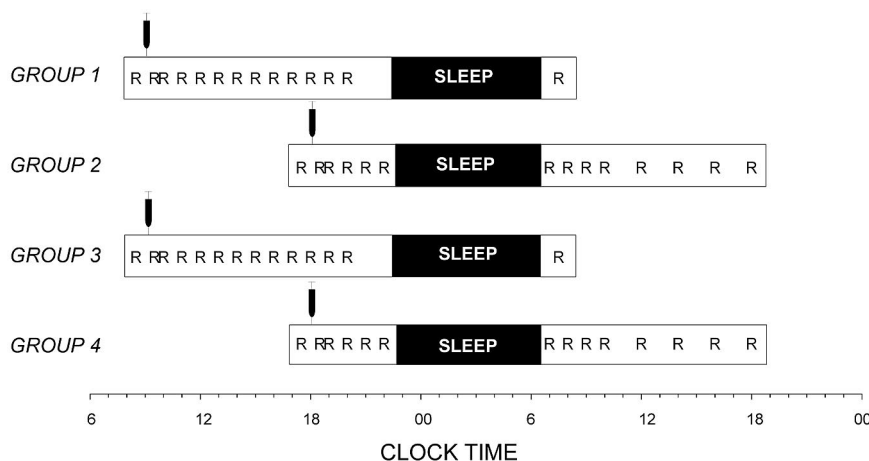


Fig. 1. Schematic diagram of the experimental protocol. Groups 1 and 2 received intravenous morphine at times 09:00 h (group 1) or 18:00 h (group 2). Groups 3 and 4 received placebo at times 09:00 h (group 3) or 18:00 h (group 4). The Rs indicate the times of respiratory testing. The dark period is the sleep period. In groups 2 and 4, blood sampling continued during sleep, but no other data were acquired.

mal saline; molecular weight of free base is 285 Da), performed the randomization, and prepared the syringes on the day before the experiment.

Because previous studies indicated that the A118G  $\mu$ -opioid receptor gene (*OPRM1*) single-nucleotide polymorphism (A118G SNP) has an important effect on opioid potency,<sup>4</sup> we tested all subjects for occurrence of this specific genetic polymorphism. Romberg *et al.*<sup>4</sup> provide a description of the DNA sequencing technique.

After arrival in the research unit, an arterial line for blood sampling was placed in the left or right radial artery during local anesthesia. In the contralateral arm an, intravenous line was inserted for drug infusion. After a 60-min pain assessment training session and resting period, baseline respiratory and pain measurements were performed. Baseline pain tolerance was assessed in triplicate. Next, the drug was infused over 90 s. Subsequently, pain assessment and respiratory measurements were performed at regular intervals for 24 h (with the exception of the sleep period).

#### Acute Pain Model

Acute pain was induced by an electrical current through two surface electrodes (Red Dot; 3M Health Care, Neuss, Germany) placed on the skin overlaying the tibial bone (shin bone) of the left leg. The electrodes were attached to a computer-interfaced current stimulator, which was locally designed and constructed. The stimulus was a 10-Hz tetanic pulse with a duration of 0.1 ms. The intensity of the noxious stimulation was increased from 0 mA in steps of 0.5 mA/1 s (cutoff = 128 mA). The subjects were instructed to press a button on a control box when no further increase in stimulus intensity was acceptable to them (pain tolerance). After the button was pressed, the stimulus train ended, and the current was collected and stored on the hard disk of a computer for further analysis. Before drug infusion, the subjects were trained on both sessions for approximately 1 h, during which several stimulus trains were applied. These data were discarded. The frequency of pain assessments is described in the Results.

To validate our pain model, we tested the model in a separate set of eight naive subjects who did not receive any drugs. We determined pain tolerance over a 6-h period with measurement frequencies identical to those of the current protocol. In seven subjects, after a 30-min training period (in which there was a small increase in pain tolerance currents), the current for pain tolerance was stable and, as judged by linear regression analysis, showed no systematic change over time. The mean coefficient of variation was 5%. In one subject, a slow, persistent linear increase in pain tolerance current of approximately 2.5 mA/h was observed over the 6-h period. These data indicate that the drug-related increases and decreases observed after morphine infusion are related to its pharmacologic properties rather than to habituation (causing an increase in time to response) or fear (causing a reduction in time to response), although we cannot exclude that these phenomena did occur. Especially the occurrence of a persistent increase in current in one subject (one of eight) suggests that habituation to the electrical stimulus in some subjects cannot be excluded.

#### Respiratory Measurements

The subjects 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 1,000 ml 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 45 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 mix-

tures could be adjusted to force end-tidal oxygen and carbon dioxide concentrations ( $P_{ETO_2}$  and  $P_{ETCO_2}$ ) to follow a specified pattern in time, independent of the ventilatory response (*i.e.*, dynamic end-tidal forcing).<sup>5</sup> The inspired and expired oxygen and carbon dioxide concentrations and the arterial hemoglobin-oxygen saturation ( $SpO_2$ ) were measured with a Datex Multicap gas monitor (near the mouth) and Datex Satellite Plus pulse oximeter (using a finger probe), respectively (Datex-Engstrom, Helsinki, Finland). The gas monitor was calibrated with gas mixtures of known concentration delivered by a gas-mixing pump (Wösthoff, Bochum, Germany).  $P_{ETCO_2}$ ,  $P_{ETO_2}$ , inspired minute ventilation ( $V_i$ ), and  $SpO_2$  were collected and stored on disk for further analysis.

We measured two respiratory variables: (1) respiration at a fixed end-tidal partial pressure of carbon dioxide ( $P_{CO_2}$ ) of 50 mmHg ( $V_i$ (normoxia)), and (2) the acute hypoxic ventilatory response (AHR). To obtain these variables, we performed steps from normoxia ( $P_{ETO_2}$ , 110 mmHg for 8 min;  $P_{ETCO_2}$ , 50 mmHg) into hypoxia ( $P_{ETO_2}$ , 45 mmHg—values were reached within 4–6 breaths;  $P_{ETCO_2}$ , 50 mmHg; duration of hypoxia, 3 min) were applied. The  $P_{ETCO_2}$  was clamped at 50 mmHg to offset any depressant effect of the opioids on  $P_{ETCO_2}$ . The breath-to-breath data of the last 10 breaths of normoxia,  $V_i$ (normoxia), and the last 10 breaths of hypoxia,  $V_i$ (hypoxia), were averaged. Because the relation between ventilation and arterial oxygen saturation is found to be linear, we calculated the difference between the hypoxic and normoxic  $V_i$  and the  $SpO_2$  data points and expressed the AHR or sensitivity as follows<sup>6,7</sup>:

$$AHR = \frac{V_i(\text{hypoxia}) - V_i(\text{normoxia})}{SpO_2(\text{normoxia}) - SpO_2(\text{hypoxia})} \quad (1)$$

(units:  $l \cdot \text{min}^{-1} \cdot \% \text{ desaturation}^{-1}$ ). Figure 1 and the Results section describe the frequency of respiratory measurements. Pain measurements always followed respiratory measurements with at least a 10-min resting period in between.

#### Plasma Morphine and Glucuronide Concentrations

At fixed times ( $t = 5, 10, 15, 20, 25, 30, 40, 50, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360, 390, 420, 460, 510, \text{ and } 540$  after the morphine bolus), 5 ml blood was drawn for determination of morphine and its two major metabolites, morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). In groups 2 and 4, the drawing of blood samples continued while the subjects were asleep. Plasma was separated within 15 min of blood collection and was stored at  $-20^\circ\text{C}$ . Morphine and its glucuronides were assayed with liquid chromatography-mass spectrometry. The lower limits of quantification were set at 2.0 ng/ml for morphine and its glucuronides. The coefficient of variation varied from 4% to 8% over the calibration range of 2–10,000 ng/ml.

#### Pharmacokinetic and Pharmacodynamic Data Analysis

The pharmacokinetics and pharmacodynamics of morphine were determined separately with NONMEM, version V, level 1.1 (San Francisco, CA), using a population approach.<sup>8</sup> First, two- and three-compartment pharmacokinetic models were fitted to the pharmacokinetic data. Next, the respiratory effect (by both the power and the Leiden pharmacodynamic model) and analgesia data were analyzed using fixed individual pharmacokinetic model parameters (*i.e.*, individual Bayesian estimates). To eliminate a possible hysteresis between opioid plasma concentrations, as described by the pharmacokinetics model, and pharmacodynamic effects, an effect compartment was postulated. This effect compartment equilibrates with the plasma compartment with a half-life,  $t_{1/2}k_{e0}$  (blood-effect site equilibration half-life).

#### Respiratory Pharmacodynamic Models

We described the relation between effect site opioid concentration and respiratory effect by the power or Leiden pharmacodynamic model and the sigmoid Emax model. The Leiden model is of the form<sup>7,9</sup>:

$$E(t) = E_0 \cdot \left[ 1 - \left( \frac{C_E(t)}{C_{50}} \right)^\gamma \cdot 0.5 \right], \quad (2)$$

where  $E(t)$  is the effect at time  $t$ ,  $E_0$  is the baseline (predrug) effect,  $C_E(t)$  is the effect site concentration at time  $t$ ,  $C_{50}$  is the effect site or steady state concentration causing a 50% depression in effect, and  $\gamma$  is a dimensionless shape parameter. The sigmoid Emax model is of the form:

$$E(t) = E_0 \cdot \left[ 1 + \left( \frac{C_E(t)}{C_{50}} \right)^\gamma \right]^{-1}. \quad (3)$$

#### Analgesia Model

We assume that morphine attenuates the response to the applied noxious stimuli by inhibition of signal propagation and/or central signal processing. As a consequence, stronger stimuli are needed before a subject presses the pain tolerance button. The attenuation ( $A$ ) was described by an inhibitory sigmoid Emax model<sup>10</sup>:

$$A = \left[ 1 + \left( \frac{C_E(t)}{AC_{50}} \right)^\gamma \right]^{-1}, \quad (4)$$

where  $AC_{50}$  is the effect site concentration causing 50% attenuation. Because a response of the subject occurs when his or her pain sensation exceeds the response threshold (for pain tolerance), we may rewrite equation 4 into the following<sup>10</sup>:

$$E(t) = E_0 \cdot \left[ 1 + \left( \frac{C_E(t)}{AC_{50}} \right)^\gamma \right], \quad (5)$$

where  $E(t)$  is the current at time  $t$ ,  $E_0$  is the baseline (predrug) current,  $C_E(t)$  is the morphine effect site con-



**Table 1. Nonrespiratory Adverse Effects of Morphine**

Subject ID	Sex	Nausea	Rash	Urticaria	Itching
4	Female	—	—	—	—
10	Male	—	—	—	—
34	Female	—	—	—	—
36	Male	—	+	—	—
38	Male	+	+	—	+
45	Female	+	—	—	—
51	Female	+	+	+	—
55	Male	—	—	—	—

centration at time  $t$ ,  $AC_{50}$  is the morphine effect site or steady state concentration causing a 50% increase in attenuation (or a doubling of tolerable current), and  $\gamma$  is a dimensionless shape parameter.

### Integrated Analysis

For the respiratory data, we initially assessed which model (Leiden model or sigmoid Emax model) performed best by performing a separate analysis on  $V_i$  (normoxia) and AHR. Model selection was done on the basis of the Akaike's Information-theoretic Criterion, parameter estimation errors, and visual inspection of the data fits. Subsequently, the best model for that specific endpoint was used in the integrated data analysis in NONMEM (*i.e.*, combining all respiratory and analgesia data in one analysis). Likelihood ratio tests were performed to determine whether parameters (baseline, potency parameters  $AC_{50}$  and  $C_{50}$ , half-lives, and the shape parameters) differed among the three endpoints and whether  $\gamma$  equaled 1. The presence of first-level random effects ( $\eta$ s) was tested on each of the model parameters and quantified by percent coefficient of variation (a measure of between subject variability). The improvement of the model fits by inclusion of covariates sex, age, weight, and time of infusion was tested using the likelihood ratio criterion.  $P$  values of less than 0.01 were considered significant. Values are reported as population value  $\pm$  SE.

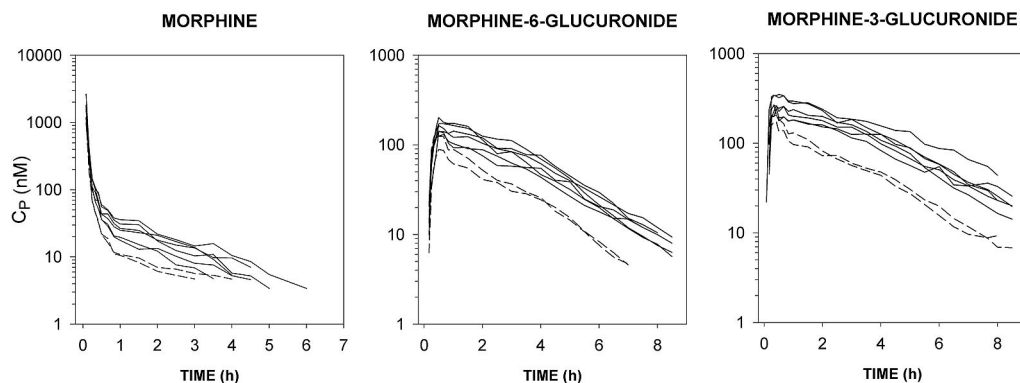
### Simulations

Using the parameters obtained from 1,000 bootstrap replicate data sets, population responses were simulated, and median values and 95% confidence intervals were estimated. We simulated a single 90-s infusion of 0.2 mg/kg morphine, a single 90-s infusion of 0.2 mg/kg combined with a continuous infusion of  $1 \text{ mg} \cdot 70 \text{ kg}^{-1} \cdot \text{h}^{-1}$ , and four 90-s infusions of 0.1 mg/kg at 6-h intervals.

### Results

All subjects completed the study without major adverse effects. Adverse effects that did occur are given in table 1. All adverse effects occurred in only four subjects. The symptoms were mild and did not necessitate treatment. Two subjects (both in group 1, Nos. 4 and 34) inadvertently received 0.13 mg/kg morphine rather than 0.2 mg/kg. These data were included in the analysis. Three of the 16 subjects were carriers of the A118 mutation; all of these subjects were in the placebo arm of the study, making the morphine group (groups 1 and 2) homogenous with respect to the  $\mu$ -opioid receptor gene. The individual morphine, M3G, and M6G plasma concentrations are shown in figure 2. A three- rather than a two-compartment pharmacokinetic model best described the morphine pharmacokinetic data (table 2). Placebo had no systematic effect on either analgesia or respiration, suggesting the absence of large interactive influences of the simultaneous measurements of analgesia and respiration on the study outcome.

The respiratory model selection showed that for AHR, the sigmoid Emax model was the best model (improvement in NONMEM objective function by 10 points); for  $V_i$  (normoxia), significant differences between the fits analyzed with the Leiden or sigmoid Emax models were not indicated by the Akaike's Information-theoretic Criterion, the estimation errors, or visual inspection of the data fits. However, the combined data analysis of all data did indi-



**Fig. 2.** Individual plasma concentrations ( $C_p$ ) of morphine (*left*), morphine-6-glucuronide (*middle*), and morphine-3-glucuronide (*right*) after an intravenous 90-s morphine infusion. All but two subjects received 0.2 mg/kg morphine; two subjects inadvertently received 0.13 mg/kg (represented by dashed lines).

**Table 2. Population Pharmacokinetic Model Parameters**

	$\Theta$	SE	%CV
$V_1$ , l/kg	0.106	0.013	19
$V_2$ , l/kg	0.276	0.044	*
$V_3$ , l/kg	1.820	0.121	15
$Cl_1$ , l · min <sup>-1</sup> · kg <sup>-1</sup>	0.028	0.002	19
$Cl_2$ , l · min <sup>-1</sup> · kg <sup>-1</sup>	0.019	0.002	*
$Cl_3$ , l · min <sup>-1</sup> · kg <sup>-1</sup>	0.020	0.002	*

\* Parameter not included in the statistical model.

%CV = percent coefficient of variation;  $\Theta$  = typical value.

cate a significant improvement in data fit when the sigmoid Emax model over the Leiden model was chosen for both respiratory variables. Hence, the sigmoid Emax model was chosen for both AHR and  $V_1$ (normoxia).

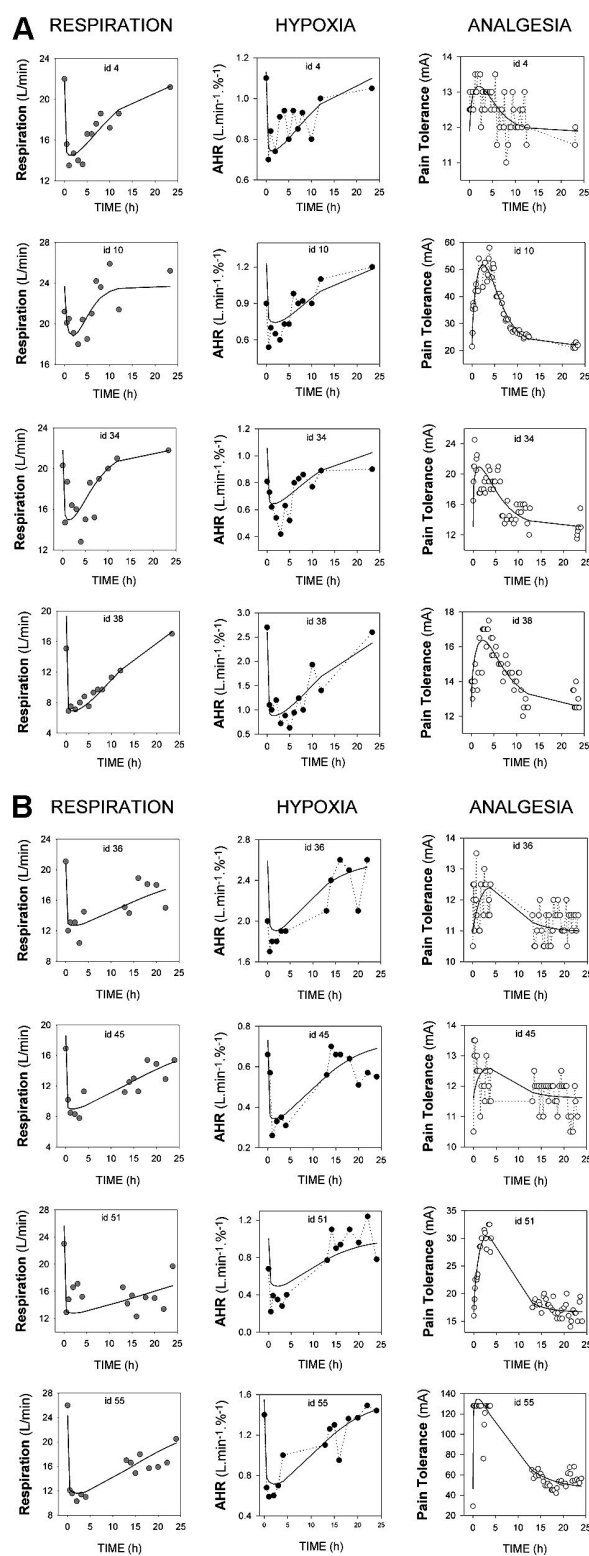
The effect of morphine on  $V_1$ (normoxia), AHR, and pain tolerance for all subjects together with the individual predicted responses is given in figure 3. All data fits seemed to be adequate. For none of the model parameters did inclusion of the covariates sex, age, weight, and time of infusion improve the model fits. The potency parameters and blood-effect site equilibration half-lives did not differ significantly among the three tested endpoints (table 3):  $AC_{50}$  and  $C_{50}$  values were  $32 \pm 1.4$  nM ( $9.0 \pm 0.4$  ng/ml);  $t_{1/2k_{e0}}$  was  $4.4 \pm 0.3$  h. The shape parameter  $\gamma$  did differ between respiration and analgesia (for both respiratory endpoints,  $\gamma = 1$ ; for analgesia,  $\gamma = 2.4 \pm 0.7$ ), giving the distinct difference in shape of the steady state morphine effect responses (fig. 4). Figure 4 shows further that (mild to moderate) respiratory depression already tends to occur at morphine concentrations not causing any analgesic effect ( $< 10$  nM). At greater morphine concentrations (10–100 nM), the gain in analgesic effect (*i.e.*, slope) is greater than the gain in respiratory depression.

The results of the simulations are given in figure 5. It shows the median analgesic and respiratory responses  $\pm 95\%$  prediction intervals. The large intervals for analgesia are related to the large value and SE of parameter  $\gamma$  (table 3). The simulations show that the median respiratory effect is not likely to exceed 40% of baseline (at least not for these simulations based on clinical dosing regimens).

In table 4, we give the time to peak effect values for the measured and predicted data. These values may be useful to link pharmacokinetic and pharmacodynamic data from separate studies.<sup>11</sup>

## Discussion

In this study, we quantified two important measures of morphine in a single group of volunteers over an identical time span: respiratory depression and analgesia. Somewhat arbitrarily, the term *analgesia* is chosen above *antinociception*, although it was antinociception



**Fig. 3. Individual data fits of all eight subjects receiving morphine: respiration at a fixed end-tidal partial pressure of carbon dioxide (left column), the acute hypoxic response (AHR, middle column), and pain tolerance (right column). Dotted lines are the measured data, and the continuous lines are the model (Bayesian) prediction. (A) Subjects 4, 10, 34, and 38 received morphine at 9:00 AM; (B) subjects 36, 45, 51, and 55 received morphine at 6:00 PM. Subjects 4 and 34 received 0.13 mg/kg, and the others received 0.2 mg/kg.**

**Table 3. Model Parameters for Analgesia,  $V_i$  (Normoxia), and AHR (Integrated NONMEM Analysis)**

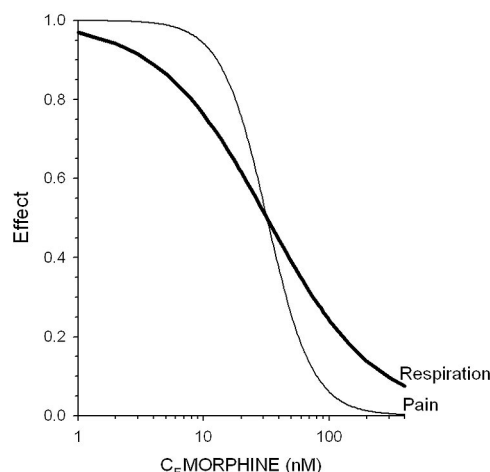
	$\Theta$	SE	%CV	95% CI*
Baseline analgesia, mA	16.0	2.5	12	12.1–25.5
Baseline $V_i$ (normoxia), l/min	21.8	1.2	46	19.4–24.3
Baseline AHR, l · min <sup>-1</sup> · % <sup>-1</sup>	1.3	0.2	44	1.0–1.8
$t_{1/2}k_{e0}$ analgesia, h			79	
$t_{1/2}k_{e0}$ $V_i$ (normoxia), h	4.4	0.3	†	3.4–5.1
$t_{1/2}k_{e0}$ AHR, h			†	
AC <sub>50</sub> analgesia, nM			†	
C <sub>50</sub> $V_i$ (normoxia), nM	32.0	1.4	63	27.0–37.5
C <sub>50</sub> AHR, nM				
$\gamma$ Analgesia	2.4	0.7	72	0.7–3.3
$\gamma$ $V_i$ (normoxia)	1		89	
$\gamma$ AHR	1		†	
WSV analgesia	0.19			
WSV $V_i$ (normoxia)	0.09			
WSV AHR	0.17			

Vertical bar (|) indicates parameter has a similar value as that of the middle entry in its group of three parameters.

\* Derived from the bootstrap analysis. † Parameter not included in the statistical model.

AC<sub>50</sub> = effect site concentration causing 50% attenuation; AHR = acute hypoxic ventilatory response; C<sub>50</sub> = effect site or steady state concentration causing a 50% depression in effect; CI = confidence interval; %CV = percent coefficient of variation;  $\gamma$  = shape parameter;  $t_{1/2}k_{e0}$  = blood–effect site equilibration half-life;  $\Theta$  = typical value;  $V_i$  (normoxia) = normoxic, hypercapnic minute ventilation; WSV = within-subject variability =  $[\sqrt{\sigma^2}]/\text{Baseline}$ , where  $\sigma^2$  is the variance of the residual error.

that was tested in the healthy, young humans without pain, underlying disease, and inflammation. The influence of morphine on breathing and pain relief has been tested previously using a pharmacokinetic–pharmacodynamic approach.<sup>7,10</sup> Our study is the first to assess morphine's analgesic and respiratory effects simultaneously. The outcome of the study is in close agreement with these previous studies (see Romberg *et al.*<sup>7</sup> and Sarton *et*



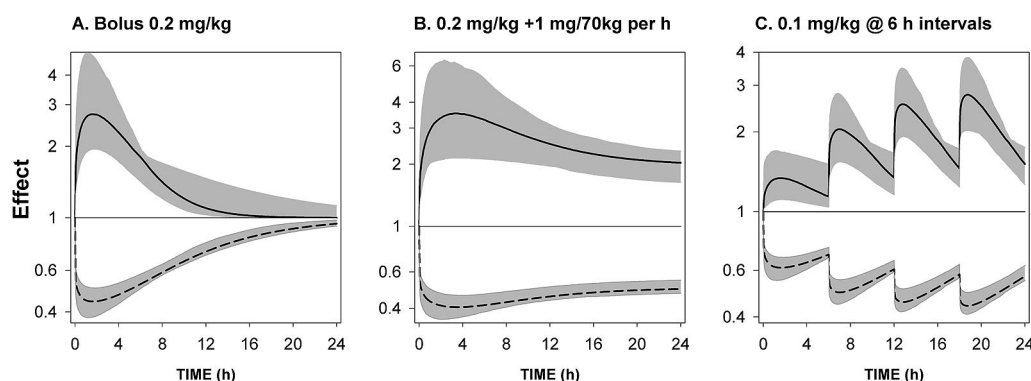
**Fig. 4.** Steady state relations between morphine and pain (1 = no pain relief, 0 = maximum pain relief; cf. parameter A in equation 4) and morphine and respiration (acute hypoxic response and respiration at a fixed end-tidal partial pressure of carbon dioxide; 1 = no respiratory depression, 0 = apnea).  $C_E$  = effect site concentration.

*al.*<sup>10</sup>) showing (1) similar morphine sensitivities for those systems involved in opioid-induced analgesia and respiratory effect, (2) similar onset/offset times for morphine-induced analgesia and respiratory depression, and (3) a difference in shape of the morphine dose–effect relation for analgesia and respiration. Items 1 and 2 indicate that the two systems assessed by us (respiration and analgesia) share important pharmacodynamic characteristics when exposed to morphine. This suggests similarities in central  $\mu$ -opioid analgesic and respiratory pathways. Crucial components of these pathways are the  $\mu$ -opioid receptors themselves and the  $\mu$  receptor-linked G proteins. Our data then suggest that similar receptor/G protein complexes are expressed on neurons involved in respiration and those involved in pain processing and analgesia. However, there are some, albeit weak, indications in animals that  $\mu$ -opioid-related analgesic and respiratory effects are mediated *via* distinct receptor subtypes,  $\mu_1$  and  $\mu_2$ , with different receptor binding kinetics.<sup>12</sup> The  $\mu_1$ -opioid receptor is held responsible for the analgesic effect of opioids, and the  $\mu_2$  receptor is held responsible for their respiratory effects. Our current data do not support the existence of these specific receptor subtypes in humans. In case of their existence, we would have expected more significant differences in pharmacodynamics than just the difference in parameter  $\gamma$ , such as differences in morphine potency and onset/offset times (see next paragraph). The difference in parameter  $\gamma$  among endpoints remains unexplained, but a value of  $\gamma > 1$  (and large SE) for the analgesia data may be related to the conscious processing of the pain sensation. Our findings corroborate studies in exon 2  $\mu$ -opioid receptor gene knockout mice, showing that the  $\mu$ -opioid receptor mediates the analgesic and respiratory effects of morphine and the  $\mu$ -opioid receptor (*OPRM*) gene is the molecular site of action of these effects.<sup>13–15</sup> Whether our results are specific to morphine or relate to other  $\mu$  opioids (such as morphine's active metabolite M6G) should be studied further.

The finding of similar half-lives for the analgesic and respiratory effects of morphine may be expected if one takes into account that the central neuronal network involved in the control of pain transmission is in close proximity to and overlaps with neurons involved in ventilatory control, especially in the rostral ventromedial and lateral medulla and pons.<sup>16</sup> However, the magnitude of the parameter  $t_{1/2}k_{e0}$  is not caused only by transport delays to the effector sites within the central nervous system, but also to neuronal dynamics. For example, animal studies indicate that more than 50% of the antinociceptive delay of morphine is related to drug distribution within the brain compartment, receptor–agonist binding kinetics, and neuronal dynamics.<sup>17</sup>

We previously assessed the respiratory effects of morphine without obtaining morphine plasma concentra-





**Fig. 5.** Simulations using 1,000 bootstrap replicates on a log-linear scale. Data are presented as median  $\pm$  95% prediction intervals for analgesia (continuous line) and respiration (dashed line) relative to baseline (= 1). A value of 0.4 indicates 60% depression of respiration; a value of 2 indicates doubling of current for pain threshold. (A) A single 90-s infusion of 0.2 mg/kg morphine at  $t = 0$ . (B) Morphine 90-s infusion at  $t = 0$ , followed by a continuous infusion of  $1 \text{ mg} \cdot 70 \text{ kg}^{-1} \cdot \text{h}^{-1}$ . (C) Four 90-s infusions of 0.1 mg/kg morphine at 6-h intervals.

tions.<sup>7</sup> We did perform a population pharmacokinetic-pharmacodynamic analysis on that specific data set using previously obtained pharmacokinetic data in a different but comparable group of volunteers (in terms of age, weight, health, sex, and other items).<sup>10</sup> It is of interest to compare the results of our current study, in which we did obtain morphine pharmacokinetic data, and those of previous study, from which it may be concluded that the morphine potency ratio analgesia: $V_I$ (normoxia):AHR = 1:1:1.<sup>7</sup> The results of these two studies are in accord. For  $V_I$ (normoxia) and AHR, the blood-effect site equilibration half-lives were 3.8 and 4.3 h in our previous studies versus 4.4 h in the current study. Similar observations were made for  $C_{50}$  (previous values were 56 and 33 nM for  $V_I$ (normoxia) and AHR, respectively, vs. 32 nM in this study) and parameter  $\gamma$  (1 in both studies). Conclusions on the respiratory pharmacodynamics of morphine are in fact identical for both studies. These observations indicate that reliable pharmacodynamic parameter estimates may be obtained using a separate pharmacokinetic data set (provided the pharmacokinetic data are obtained from a comparable subject group, volunteers or patients). This statement is important because the influence of blood sampling (and other disturbing activities such as pain testing and subject coaching) on the measurement of respiration is seldom acknowledged by investigators. Blood sampling and pain testing have a significant effect, often causing hypoventilatory or hyperventilatory responses due to activation of behav-

ioral respiratory drives.<sup>18–20</sup> In the current study, we tried to minimize the complex interactive effects of pain testing, respiration measurement, and blood sampling by keeping ample time between respiratory measurements, pain testing, and blood sampling and by abstaining from subject coaching. However, despite our efforts and the observation that population parameter values were comparable, within-subject data variability (table 3) was greater in the current respiratory studies (in which blood sampling and pain testing occurred) compared with our previous study, in which only respiration was measured (compare fig. 3 in both studies).<sup>7,10</sup> We relate this to activation of behavioral control of respiration.

None of the subjects participating in the morphine arm of the study had an A118G point mutation of the  $\mu$ -opioid receptor gene. Consequently, because the A118G mutation is the single most significant single nucleotide polymorphism of the *OPRM1* gene (G118 allelic frequencies in the general population range from 10% to 20%),<sup>4,21</sup> we may consider our group of eight volunteers to be genetically homogeneous with respect to the *OPRM1* gene. We previously observed a difference in M6G analgesic potency between wild-type subjects and A118G mutants (threefold lesser potency in mutants) causing large between-subject variability ( $> 150\%$ ).<sup>4</sup> Whether the A118G mutation reduces opioid potency in general or the effect is restricted to M6G remains unknown. Our current protocol was not designed to detect sex differences or study chronopharmacology. Not surprisingly, we did not find a significant effect of covariates sex and the time of infusion on our model parameters. We relate this to the relatively small number of subjects in the morphine arm of the study (four men, four women; four infusions at 8:00 AM, four at 6:00 PM).

We analyzed our pharmacodynamic data using both the Leiden model (equation 2) and the classic sigmoid Emax model (equation 3). We have a slight preference for the Leiden (or power) model above the sigmoid

**Table 4.** Time to Peak Effect

	Measured, min	Predicted, min
Analgesia	80 $\pm$ 23	119 $\pm$ 16
$V_I$ (normoxia)	111 $\pm$ 23	97 $\pm$ 11
AHR	98 $\pm$ 31	90 $\pm$ 11

Values are presented as mean  $\pm$  SEM.

AHR = acute hypoxic response;  $V_I$  (normoxia) = normoxic, hypercapnic minute ventilation.

model for various reasons. In contrast to the sigmoid model, the Leiden model is able to describe linear responses, negative responses, and loss of respiratory activity at realistic opioid concentrations without the need for additional parameterization (e.g., using probit or logistic analysis).<sup>22,23</sup> Evidently, the correct predictive nature of a model is of clinical and experimental importance. We observed little difference in parameter estimates between models (data not given), and objective criteria indicated that although the sigmoid Emax model best described the hypoxic responses, no difference was observed between models for  $V_i$ (normoxia). Taking into account the results of the integrated analysis (showing significant better fits using the sigmoid Emax model on both respiratory variables), we here give the results of the analysis with the sigmoid Emax model. We do not believe that our current study is a true test for respiratory descriptive models for the reasons that the observed plasma concentration range was limited and no apnea was observed in our subjects. A true test for the two models will be the analysis of data that include periods of opioid-induced apnea. We are currently performing studies to resolve this matter.

In this study, we measured both morphine and M6G concentrations. Using an approach similar to that of Lötsch *et al.*<sup>24</sup> and M6G data from Romberg *et al.*,<sup>4,7</sup> we were able to calculate the contribution of M6G to morphine's analgesic and respiratory effects. The M6G contribution was small; only 5–10% for both analgesia and respiration could be attributed to M6G. We relate this to the low potency of M6G relative to morphine and slow equilibration half-life. With Lötsch *et al.*,<sup>25</sup> we therefore conclude that "M6G barely contributes to the central nervous opioid effects after infusion of analgesic doses of morphine."

Our  $AC_{50}$  and  $C_{50}$  values have pharmacologic importance and allow comparison of distinct endpoints but lack direct clinical relevance. Still, our study contains implications for the treatment of acute pain. Morphine concentrations needed to treat severe pain in postoperative and cancer patients range from 30 to 90 nM (9–25 ng/mL).<sup>26,27</sup> Our results and simulations indicate that respiration in healthy volunteers is severely depressed at these opioid concentrations (60% depression of breathing, i.e., 40% of control values; figs. 4 and 5). In acute pain or postoperative patients, respiration is related to the fragile balance between depression from opioids, sleep/sedation, and residual anesthetics on one hand and stimulation from pain, arousal, stress, and inflammation on the other. Although pain and stress stimulate breathing, they do so *via* activation of behavioral control, without affecting (i.e., restoring) chemical control.<sup>18,19</sup> Assuming a worst case scenario (analgesia and respiratory depression at the lower confidence interval bounds, i.e., minimum analgesia and maximum respiratory depression), the current data together with the simulations

do indicate that (severe) respiratory depression is possible despite the occurrence of (severe) pain. Furthermore, when postoperative patients cycle between awake and sleep states, they may be in pain and breathing while awake but severely respiratory depressed when asleep. During these sleep/sedated periods, respiratory depression may even increase to values much less than 40% of control (40% of control is equivalent to an increase of 10–15 mmHg end-tidal  $P_{CO_2}$  together with a reduction of minute ventilation by 40–50% in spontaneously breathing patients not stimulated by carbon dioxide),<sup>28,29</sup> or patients may even stop breathing completely. This is due to the synergistic interaction of sleep/sedation and opioids on breathing.<sup>30,31</sup> Further studies are needed to study the pharmacokinetics-pharmacodynamics of morphine in (postoperative) patients in pain.

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