

# Sex Differences in Morphine Analgesia

## An Experimental Study in Healthy Volunteers

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**Background:** Animal and human studies indicate the existence of important sex-related differences in opioid-mediated behavior. In this study the authors examined the influence of morphine on experimentally induced pain in healthy male and female volunteers.

**Methods:** Young healthy men and women (10 of each sex) received intravenous morphine (bolus 0.1-mg/kg dose followed by an infusion of 0.030 mg · kg<sup>-1</sup> · h<sup>-1</sup> for 1 h). Pain threshold and pain tolerance in response to a gradual increase in transcutaneous electrical stimulation, as well as plasma concentrations of morphine and its major metabolites (morphine-6-glucuronide and morphine-3-glucuronide) were determined at regular intervals up to 7 h after the start of morphine infusion. A population pharmacodynamic model was used to analyze the morphine-induced changes in stimulus intensity. The improvement of the model fits by inclusion of covariates (sex, age, weight, lean body mass) was tested for significance. The model is characterized by baseline current, a rate constant for equilibrium between plasma and effect-site morphine concentrations ( $k_{e0}$ ), and analgesic potency ( $AC_{50}$ , or the morphine concentration causing a 100% increase in stimulus intensity for response).

**Results:** The inclusion of the covariates age, weight, and lean body mass did not improve the model fits for any of the model parameters. For both pain threshold and tolerance, a significant dependency on sex was observed for the parameters  $k_{e0}$  (pain threshold: 0.0070 ± 0.0013 (± SE) min<sup>-1</sup> in men vs. 0.0030 ± 0.0005 min<sup>-1</sup> in women; pain tolerance: 0.0073 ±

0.0012 min<sup>-1</sup> in men vs. 0.0024 ± 0.0005 min<sup>-1</sup> in women) and  $AC_{50}$  (pain threshold: 71.2 ± 10.5 nM in men vs. 41.7 ± 8.4 nM in women; pain tolerance: 76.5 ± 7.4 nM in men vs. 32.9 ± 7.9 nM in women). Baseline currents were similar for both sexes: 21.4 ± 1.6 mA for pain threshold and 39.1 ± 2.3 mA for pain tolerance. Concentrations of morphine, morphine-3-glucuronide, and morphine-6-glucuronide did not differ between men and women.

**Conclusions:** These data show sex differences in morphine analgesia, with greater morphine potency but slower speed of onset and offset in women. The data are in agreement with observations of sex differences in morphine-induced respiratory depression and may explain higher postoperative opioid consumption in men relative to women. (Key words: Gender; gender differences; modeling; opioid.)

ANIMAL studies indicate the existence of important sex-related differences in opioid-induced analgesia.<sup>1-4</sup> Male rats and mice almost uniformly display greater opioid analgesia across several nociceptive assays.<sup>1</sup> For example, male rats are more sensitive than females to the antinociceptive properties of the  $\mu$ -opioid receptor agonists morphine and alfentanil.<sup>3,4</sup> The contention that these sex differences are likely caused by differences in pharmacodynamics is supported by the greater analgesia observed in male relative to female animals after supraspinal administration of morphine.<sup>2,5</sup>

Prospective human studies on the interaction of sex and the analgesic effects of opioids are scarce. Gear *et al.*<sup>6,7</sup> showed that post-dental surgery pain relief by opioids acting at the  $\kappa$ -receptor was greater in women than men. However, in most studies that compare the analgesic effects of opioids in men and women, sex comparisons were not the primary focus of investigation, resulting in inadequate controls for confounding variables such as underlying disease, age, and plasma concentrations of morphine and its metabolites. *Post hoc* comparisons have focused on patient-controlled analgesia and thus measured opioid consumption rather than analgesia.<sup>8,9</sup>

We designed a prospective study to compare the analgesic effects of a bolus and short (1-h) infusion of morphine in healthy male and female volunteers by measuring pain threshold and pain tolerance using an experimental pain model. Furthermore, the arterial plasma concentrations of morphine and its major metabolites, morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G), were determined. Analgesic effect and plasma concentration measurements continued up to 6 h after termination of the morphine infusion. To

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relate the analgesic effects to morphine concentrations, we analyzed the data using a pharmacokinetic-pharmacodynamic model consisting of a part that describes the pharmacokinetics, a part that describes the time lag between morphine plasma concentrations and effect-site concentrations, and a part that translates the effect-site concentration into analgesia.<sup>10</sup> This approach enabled us to determine if putative sex-related differences in morphine analgesia have a pharmacokinetic (sex differences in morphine, M6G, or M3G concentrations) or a pharmacodynamic (a potency difference or a difference in equilibration speed between plasma and effect-site morphine concentration) origin, or both.

## Methods

Twenty volunteers (10 men, 10 women; age, 21–36 yr) were recruited to participate in this protocol, which was approved by the local Human Ethics Committee. The subjects were healthy, did not take medication, and did not have a history of illicit substance abuse. All women reported normal menstrual cycles (they did not use oral contraceptives). Because we did not study the influence of the menstrual cycle *per se*, we did not prospectively control for the phase of the menstrual cycle. All subjects gave oral and written informed consent. The subjects were asked not to eat or drink for at least 6 h before the study.

After arrival in the laboratory, a catheter was inserted in the radial artery during local anesthesia. An intravenous catheter was inserted in the contralateral arm. Over the next 30 min, the subjects were familiarized with the transcutaneous electrical stimulation (6–8 training trials). After a subsequent resting period, baseline pain threshold and pain tolerance were assessed in replicate trials (see below). The morphine infusion started at 8:30 AM. Morphine was given as an intravenous bolus dose of 100  $\mu\text{g}/\text{kg}$  followed by a 1-h infusion of 30  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ .

### *Induction of Acute Pain*

Experimental pain was induced by an electrical current through two electrodes (Red Dot; 3M, London, Ontario, Canada) placed on the skin overlaying the tibia of the left leg (transcutaneous electrical stimulation).<sup>11</sup> The electrodes were attached to an electrostimulator (Innervator NS 242; Fisher & Paykel, Auckland, New Zealand). The intensity of the noxious stimulus (pulse duration 0.2 ms at 2 Hz) was increased at 6-s intervals in steps of 10 mA. Initial current was 10 mA, and maximal current possible was 80 mA. During exposure to the stimulus train, the subjects were instructed to state “pain” when the stimulus became painful (pain threshold) and “stop” when no further increase in stimulus intensity was acceptable (pain tolerance). Stimulation was ended at the pain tolerance level. The stimulus

intensities at pain threshold and tolerance were collected. This procedure was performed twice at fixed times before, during, and after morphine administration ( $t = 5, 10, 20, 30, 40, 50, 60, 65, 70, 80, 90, 100, 110, 120, 130, 140, 150, 180, 210, 240, 300, 330, 360, 390,$  and 420 min after the morphine bolus dose). The two values were averaged for further analysis. To avoid possible confounding influences of social sex roles in pain reporting, male subjects were tested by a male researcher and female subjects by a female researcher.<sup>12</sup>

Blindly including a 80-mA cutoff in the analysis would result in the loss of important information. Subjects that reached 80 mA either had no response (*i.e.*, pain tolerance is not yet reached) or stated “stop” (*i.e.*, pain tolerance). In those circumstances in which pain tolerance was not reached at 80 mA, we therefore included a value of 80+ mA in the analysis (see below). For plotting purposes only, the 80+ mA value was fixed, somewhat arbitrarily, to 90 mA if two values of 80+ mA were detected and to 85 mA if the combination of 80+ mA and 80 mA was observed.

### *Plasma Concentrations of Morphine and Its Metabolites*

At fixed times ( $t = 5, 10, 20, 30, 40, 50, 60, 65, 70, 80, 100, 130, 180, 300,$  and 420 min after the morphine bolus dose), 5 ml arterial blood was drawn for determination of plasma concentrations ( $C_p$ ) of morphine, M3G, and M6G (and arterial carbon dioxide partial pressure; see Web site supplement for a description of the opioid analysis technique). Comparisons of the male and female morphine, M6G, and M3G concentrations were made by two-tailed *t* tests. In addition, the male and female area under the concentration–time curves, determined from the log-linear trapezoidal rule, were compared using *t* tests. *P* values less than 0.05 were considered significant.

### *Analysis of the Pharmacokinetic and Pharmacodynamic Data*

The pharmacokinetics (see web site supplement) and pharmacodynamics of morphine were determined separately with NONMEM, version V, level 1.1 (a data analysis program for nonlinear mixed-effects modeling)<sup>13</sup> using a population approach. A two-stage approach was chosen to determine the pharmacodynamics of morphine. First, to obtain optimal estimates of the plasma morphine concentration when the analgesic effect was assessed but no blood samples were taken, a three-compartment pharmacokinetic model was fitted to the measured morphine concentrations. This was performed for each subject separately. In the second stage, population pharmacodynamic model parameters were determined with fixed individual pharmacokinetic model parameters. To eliminate the hysteresis between the estimated morphine concentrations and analgesic effect, an effect compartment was postulated. This effect com-

partment equilibrates with the plasma compartment (given by a three-compartment pharmacokinetic analysis) with a rate constant,  $k_{e0}$ .<sup>10</sup>

The verbal response to the noxious stimuli (identifying pain threshold and tolerance) during the study was modeled by assuming that morphine caused the attenuation of the signal propagation or central signal processing. As a consequence, stronger stimuli are needed for the same response. Assuming complete analgesia is possible and morphine is solely responsible for the analgesic effect, the attenuation ( $A$ ) was modeled by an inhibitory sigmoid  $E_{\max}$  model given by:

$$A = 1/[1 + (C_e(t)/AC_{50})^\gamma] \quad (1)$$

where  $AC_{50}$  is the effect-site concentration causing 50% attenuation (or the concentration causing a 100% increase in stimulus intensity for response),  $\gamma$  is a shape parameter, and  $C_e(t)$  is the effect-site morphine concentration at time  $t$ .

For both pain threshold and tolerance, a verbal response occurs when the pain sensation exceeds the corresponding response thresholds (see figure in Web Enhancement):

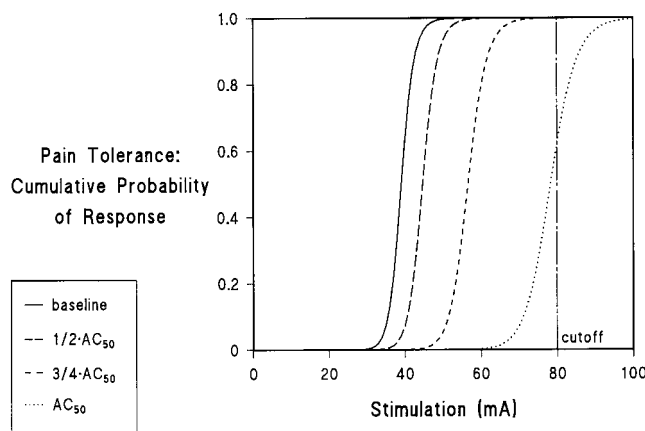
$$\text{current}(t) \cdot A \geq \text{Response Threshold} \quad (2)$$

Currents exceeding the response threshold are given by:

$$\begin{aligned} \text{current}(t) &\geq \text{Response Threshold}/A = \\ &\geq \text{baseline current} \cdot [1 + (C_e(t)/AC_{50})^\gamma] \quad (3) \end{aligned}$$

Before morphine infusion, the response threshold equals baseline current ( $A = 1$ ;  $C_e = 0$ ).

Although the model parameters can be estimated using least squares regression, this is suboptimal because this procedure cannot take into account censored data. Censoring occurs because the stimulus is applied in multiples of 10 mA and because of the presence of a cutoff in the stimulus. The maximum likelihood method can handle censored data when the likelihood of the occurrence of a set of recorded stimuli can be computed. This is accomplished by assuming that the intraindividual variability of the response threshold is governed by a log-logistic (cumulative) probability distribution function. The distribution resembles the log-normal distribution but has the advantage that it can be evaluated analytically by the fitting procedure. The likelihood of a response at, for example, 60 mA, is proportional to the probability that the response lies between 50 and 60 mA. Likewise, the likelihood of the event that the response threshold is not reached at the cutoff of 80 mA (*i.e.*, 80+ mA data), is proportional to the probability that the stimulus lies between 80 mA and infinity. We assume that the median of the log-logistic distribution depends on the effect-site concentration as given by equation 3.



**Fig. 1.** The cumulative probability of the occurrence of pain tolerance for four effect-site morphine concentrations ( $C_e$ ). At a  $C_e = 0$  nM, the probability of pain tolerance at 39.1 mA is 0.5. At a  $C_e$  equal to  $AC_{50}$ , the probability that pain tolerance occurs at a current greater than 80 mA (the cutoff) is 0.37.

Figure 1 shows the cumulative probabilities of attaining pain tolerance for four possible effect-site morphine concentrations. At baseline ( $C_e = 0$  nM) the probability of pain tolerance at 39.1 mA is 0.5. At a  $C_e$  equal to  $AC_{50}$ , the probability that pain tolerance occurs at a current above the 80 mA cutoff is 0.37. Figure 1 is based on observations made in this study for pain tolerance and applies to men and women.

The interindividual variability of each of the model parameters ( $AC_{50}$ , baseline, and  $\gamma$ ) was assumed to be log-normally distributed and was characterized by percent coefficient of variation.

Using the maximum likelihood method, the pharmacodynamic model parameters were estimated by maximizing the product of the probabilities of the occurrence of the measurements (*i.e.*, two data points at times  $t = 0, 5, 10, \dots, 420$  min). The improvement of the model fit by inclusion of covariates sex, age, weight, and lean body mass was tested using the likelihood ratio criterion.<sup>14</sup> Lean body mass was calculated as the difference between measured weight and body fat. Body fat was determined from skinfold measurements.<sup>15</sup>

Separate analyses were performed on pain threshold and pain tolerance data.  $P$  values less than 0.01 were considered significant. Further details of the analysis of the pharmacodynamic data are given on the web site supplement.

## Results

All subjects completed the study without major side effects. Compared with the women in our group, the men were taller and heavier and had a higher lean body mass (table 1). Table 2 shows the side effects that occurred during the study.

Morphine caused significant increases in electrical currents needed for pain threshold and tolerance in both

**Table 1. Anthropometric Data of the Subjects**

Variable	Men	Women
No. of subjects	10	10
Age (yr)	26.4 ± 1.6	24.5 ± 0.9
Weight (kg)	75.9 ± 2.4	64.8 ± 1.9*
Height (cm)	183.0 ± 1.7	169.8 ± 1.9*
LBM (kg)	62.8 ± 1.2	45.7 ± 1.4*
Phase of menstrual cycle (F/L)†	—	6/4

Values are mean ± SEM.

\*  $P < 0.05$  ( $t$  test). † No. of subjects in follicular phase (F) or luteal phase (L).

LBM = lean body mass.

sexes. In figure 2, the plasma morphine concentration *versus* pain tolerance relations of a male and a female subject are plotted, showing counterclockwise hysteresis loops (the arrows indicate the time course). When the time lags were taken into account (fig. 2, right), the loops collapsed. Examples of data fits of a male and female subject whose pain tolerance data did not exceed cutoff values are given in figure 3. When testing for tolerance, 80+ mA values were reached in two men and two women. Despite the occurrence of these censored pharmacodynamic data, we were able to fit the model to the data (fig. 4). The normalized deviance (for an explanation, see web site supplement),<sup>16</sup> a measure of goodness of fit, ranged from 0.12 to 0.88 bits (10–90% percentile = 0.23–0.77 bits; mean value = 0.52 bits). The normalized deviance of the curves shown in figures 2–4 are given in the legends.

The population pharmacodynamic model parameters are given in table 3. The inclusion of the covariates age, weight, and lean body mass did not improve the model fits for any of the model parameters. A significant sex dependency was observed for model parameters  $AC_{50}$  and  $k_{e0}$  for both pain threshold and tolerance, with higher  $k_{e0}$  and  $AC_{50}$  values in men. With respect to  $k_{e0}$ , the equivalent blood—effect-site concentration equilibration half-lives ( $t_{1/2}k_{e0}$ ) for pain threshold are 1.6 h and

3.8 h, and for pain tolerance are 1.6 h and 4.8 h, in men and women, respectively. The absence of a significant effect of sex on the model parameter baseline indicates similar premorphine pain thresholds and tolerances in both sexes (table 3). The individual and mean Bayesian estimates of the analgesic responses are given in figure 5. In figure 6, the steady state morphine concentration *versus* transcutaneous electrical stimulation is plotted for pain threshold and pain tolerance. Note that when effect-site morphine concentration equals  $AC_{50}$ , the current needed for threshold and tolerance detection is twice the baseline current.

The observed sex differences in morphine analgesia were unrelated to sex differences in the pharmacokinetics of morphine (see web site supplement). Plasma concentrations and area under the concentration–time curves of morphine, M3G, and M6G did not differ between men and women (see fig. 7).

## Discussion

Using an experimental pain model to test for sex differences in opioid analgesia in healthy volunteers, we observed the following after intravenous morphine administration: (1) morphine has a greater potency in women; (2) morphine shows a slower speed of onset and offset of analgesic effect in women; (3) similar results were obtained for pain threshold and pain tolerance; and (4) the absence of sex differences in plasma concentrations of morphine and its metabolites over the time range studied.

To assess pain threshold and tolerance, we applied a gradually increasing electrical current (from 0 to 80 mA) to the skin and assessed the response of the subjects. The electrical pain test is sensitive to opioid and nonopioid analgesics, and stimulation to the level of pain tolerance can produce a pain experience that resembles clinical pain.<sup>17</sup> To quantify the potency of morphine and the time lag between morphine  $C_p$  and the analgesic effect, we analyzed the data using a pharmacokinetic–pharmacodynamic model consisting of a hypothetical effect compartment and an inhibitory sigmoid  $E_{max}$  model. The sigmoid  $E_{max}$  model relates morphine  $C_e$  to analgesic effect by assuming that morphine dampens the central perception of afferent noxious stimuli. As a consequence, stronger stimuli are needed for pain threshold and tolerance. Although the model permits complete analgesia ( $C_e \rightarrow \infty$ ,  $A = 0$ ; equation 1), conclusions drawn from our analysis should be restricted to the range of morphine concentrations measured. To handle the fact that we obtained censored data (because of the application of stimuli in multiples of 10 mA, and the 80 mA cutoff), we assumed that the intraindividual variability of the response thresholds (fig. 1) is log-logistically distributed. This approach enables us to predict

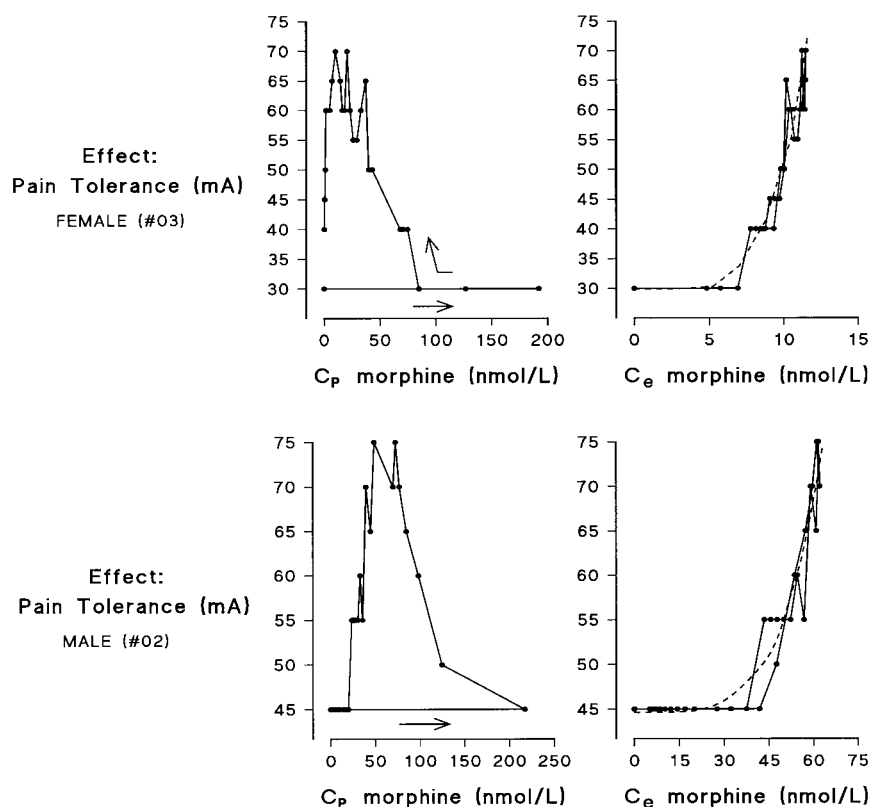
**Table 2. Side Effects of Morphine**

Side Effect	Men	Women
Nausea/Vomiting	4	5
Headache	0	1
Vertigo/Lightheadedness	2	6
Heavy feeling	6	4
Warm feeling	4	6
Tiredness	5	8
Sedation	4	6
Orthostatic hypotension	1	2
Itching	0	2
Flushing	2	1
Rash	2	3
Urticaria	0	2
Respiratory depression*	9	10
Total	39	56

Values are the number of subjects.

\* Defined by an increase in arterial  $P_{CO_2}$  of 2 mmHg or more.

**Fig. 2.** (Left) Examples of pain tolerance *versus* plasma morphine concentration ( $C_p$ ) in a representative female (top) and male (bottom) subject. Counterclockwise hysteresis loops are observed in both subjects. The arrows indicate the time course. (Right) Corresponding pain tolerance *versus* estimated effect-site morphine concentration ( $C_e$ ) and model fits (broken line). Note the collapse of the hysteresis loop and the potency difference between the male and female subjects. The normalized deviance, a measure of goodness of fit, was 0.71 bits for the female data fit and 0.42 bits for male data fit.



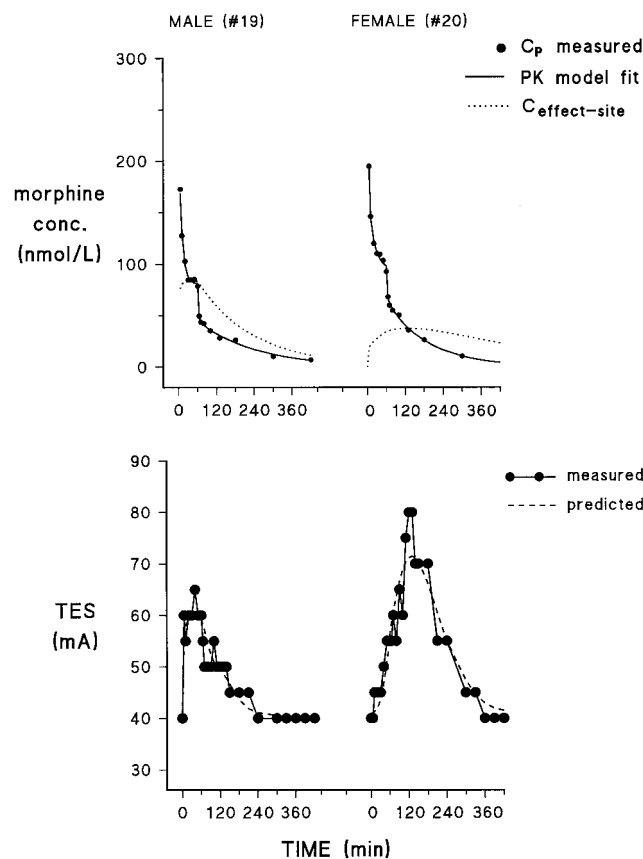
analgesic behavior above the cutoff current (figs. 1 and 4). Luks *et al.*<sup>18</sup> used a similar approach to account for censored time-to-response data, examining opioid-induced analgesia in neonatal dogs.

On the administration of morphine, all men and women showed an increase in pain threshold and tolerance. Part of these increases may be related to an expectation or placebo effect.<sup>19</sup> We refrained from the inclusion of placebo controls because our study relates to possible sex differences rather than to the absolute size of morphine effect. Furthermore, because we may assume that expectation effects occurred in both sexes, we do not feel that the lack of a placebo arm influenced the outcome and conclusions of our study significantly.

Our analysis revealed that parameters  $AC_{50}$  and  $k_{e0}$  differed significantly between men and women. Previous clinical studies reported conflicting results concerning the influence of sex on the pharmacodynamics of  $\mu$ -opioids. Dahan *et al.* studied the influence of morphine on ventilatory control and observed greater depression of the hypoxic drive (*i.e.*, the increase in ventilation caused by a short-term isocapnic hypoxic challenge) and the slope of the ventilatory response to inspired carbon dioxide in women compared with men.<sup>20,21</sup> Minto *et al.*<sup>22</sup> studied the influence of remifentanyl on the spectral edge frequency of the electroencephalogram in patients and found no effect of sex on  $EC_{50}$  (the remifentanyl concentration that causes 50% of the effect). Drover and Lemmens<sup>23</sup> compared plasma remifentanyl concentrations for adequate anesthesia with 66% nitrous oxide

during elective abdominal surgery in men and women and observed the need for greater opioid concentrations in women. However, the conclusions of that study were potentially confounded by the fact that the pain stimuli, *i.e.*, the type of operations, were different between men and women (urologic *vs.* gynecologic). Miaskowski and Levine<sup>8</sup> reviewed all available studies ( $n = 18$ ) on  $\mu$ -opioid patient-controlled analgesia for postoperative pain from 1966 to 1998 in which data for men and women were compared. In 10 studies, opioid consumption was higher in men, whereas in the remainder no differences were found between the sexes. We consider the differences in study end points as the main reason for these conflicting results: ventilatory depression *versus* electroencephalogram effect *versus* suppression of somatic stimuli *versus* opioid consumption *versus* analgesia (present study). These examples illustrate that the occurrence and direction of sex differences depend on the specific end point examined. Our present observations of greater analgesic potency of morphine in women is in agreement with our previous studies on respiration<sup>20,21</sup> and suggests a common biologic basis for sex differences in the analgesic and ventilatory effects of morphine. Interestingly, sex differences are not restricted to  $\mu$ -opioids. Gear *et al.*<sup>6,7</sup> showed that opioids acting at the  $\kappa$ -receptor are more effective analgesics in women in a post-dental surgery pain model.

The time lag between morphine plasma concentration and analgesic effect depends on several factors, including (1) cardiac output-dependent delivery of morphine

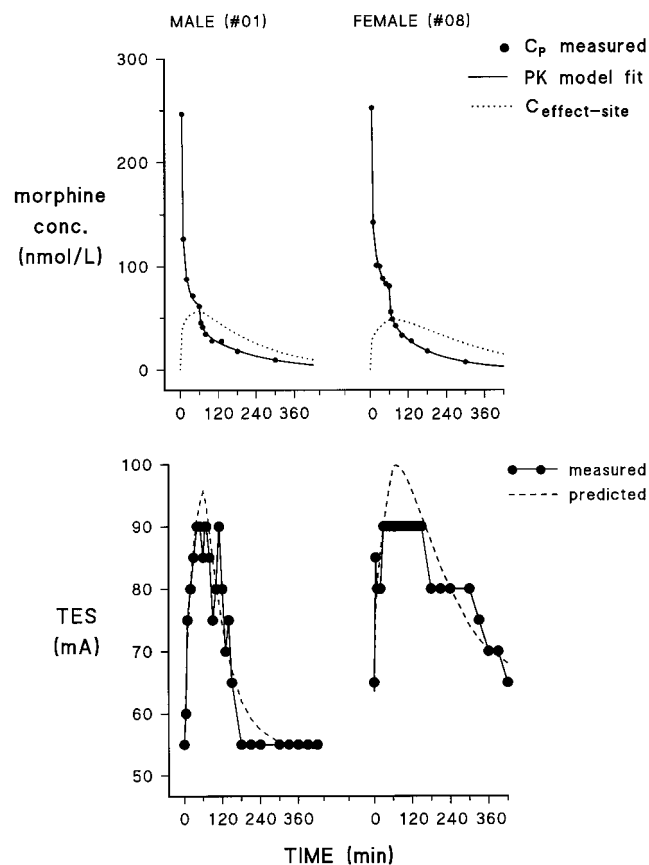


**Fig. 3.** Influence of intravenous morphine infusion (bolus dose of  $100 \mu\text{g}/\text{kg}$  at time  $t = 0$ , followed by an infusion of  $30 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  for 1 h) on pain tolerance in representative male (*left*) and female (*right*) volunteers. (*Top*) Measured morphine plasma concentrations (filled circles), pharmacokinetic (PK) model fit (continuous line), and morphine effect-site concentration (dashed line). (*Bottom*) Measured (filled circles) and predicted (broken line) currents at which pain tolerance is reached. Both subjects reached pain tolerance at or below the 80-mA cutoff during the study. The normalized deviance, a measure of goodness of fit, was 0.45 bits for the male data fit and 0.68 bits for female data fit. Conc. = concentration; TES = transcutaneous electrical stimulation.

to the brain, (2) diffusion of morphine to brain sites containing  $\mu$ -opioid receptors (*i.e.*, crossing of the blood-brain barrier), (3) receptor-binding, and (4) neuronal dynamics. Because the rate constant  $k_{e0}$  combines the dynamics of all of these factors, differences in  $k_{e0}$  reflect differences in any of these factors. For example, the  $k_{e0}$  of morphine in neonatal dogs ( $0.086 \text{ min}^{-1}$ ) is 10–30 times greater than that observed in our study.<sup>18</sup> We relate this to the evident species difference and possibly to a difference in maturity of the blood-brain barrier between dogs up to 1 month of age and adult humans (factor 2). The causes for sex differences in  $k_{e0}$  observed in our study remain elusive. The values of  $k_{e0}$  of morphine observed in this study are small compared with those of other clinically used opioids (fentanyl,  $0.10 \text{ min}^{-1}$ ; sufentanil,  $0.11 \text{ min}^{-1}$ ; alfentanil,  $0.63 \text{ min}^{-1}$ ; remifentanyl,  $0.52 \text{ min}^{-1}$ ).<sup>22,24,25</sup> Although these differences may be related to factors 1–4, it is important to

note that in these studies an electroencephalogram-derived parameter (95% spectral edge frequency)—not analgesia—was chosen as the opioid effect.<sup>22,24,25</sup> It is possible that distinct end points (*i.e.*, effects originating at distinct sites in the central nervous system) may behave differently over time. Interestingly, the value of  $k_{e0}$  observed in women in our study is in close agreement with the  $k_{e0}$  estimate for morphine-induced pupil constriction.<sup>26</sup> Finally, our findings are in agreement with our clinical observation of a slow onset of the analgesic effect of morphine.

In our pharmacokinetic–pharmacodynamic analysis, we assumed that the measured analgesia was caused by the action of morphine at the  $\mu$ -receptor. We did not incorporate any effect of the most important metabolites of morphine, M6G and M3G. The metabolites appear in



**Fig. 4.** Influence of intravenous morphine infusion (bolus dose of  $100 \mu\text{g}/\text{kg}$  at time  $t = 0$  followed by an infusion of  $30 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  for 1 h) on pain tolerance in male (*left*) and female (*right*) volunteers. (*Top*) Measured morphine plasma concentrations (filled circles), pharmacokinetic (PK) model fit (continuous line), and morphine effect-site concentration (dashed line). (*Bottom*) Measured (filled circles) and predicted (broken line) currents at which pain tolerance is reached. Both subjects did not reach pain tolerance at the cutoff of 80 mA (*i.e.*, 80+ mA in the analysis and 85 and 90 mA in these plots; see Methods for details). Note that at times when the cutoff is reached, the pharmacodynamic analysis predicts higher currents for pain tolerance as if no cutoff was present. The normalized deviance, a measure of goodness of fit, was 0.57 bits for the male data fit and 0.33 bits for female data fit. Conc. = concentration; TES = transcutaneous electrical stimulation.

**Table 3. Population Pharmacodynamic Parameters**

Model Parameter	Pain Threshold		Pain Tolerance	
	Men	Women	Men	Women
Baseline (mA)				
Value		21.4		39.1
SE of estimate		1.6		2.3
%CV		28		25
AC <sub>50</sub> (nM)				
Value	71.2	41.7	76.5	32.9
SE of estimate	10.5	8.4	7.4	7.9
%CV	32	49	23	70
k <sub>e0</sub> (min <sup>-1</sup> )				
Value	0.0070	0.0030	0.0073	0.0024
SE of estimate	0.0013	0.0005	0.0012	0.0005
%CV	45	45	55	55
γ				
Value		2.6		2.8
SE of estimate		0.6		0.4
%CV		48		50
σ (log mA)				
Value		0.095		0.079
SE of estimate		0.009		0.007
%CV		36		28

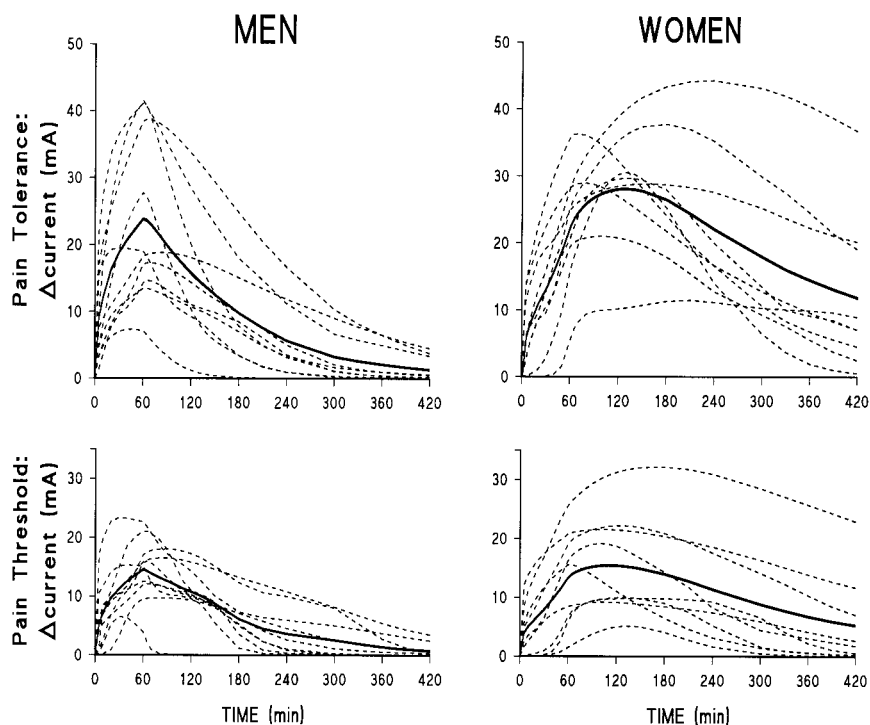
%CV (coefficient of variation) is a measure of the interindividual variability.

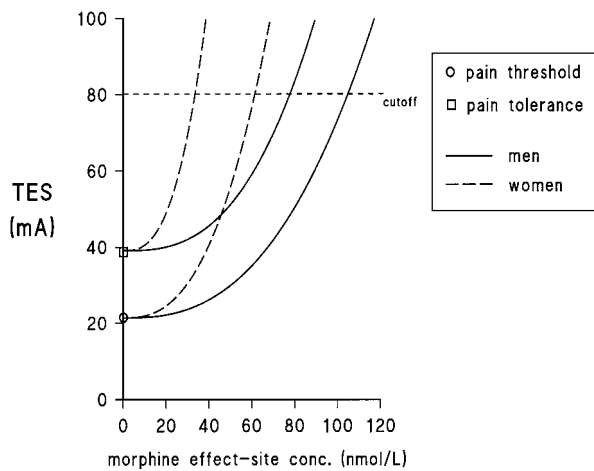
AC<sub>50</sub> = morphine concentration causing a 100% increase in stimulus intensity for response; k<sub>e0</sub> = rate constant; see the Web Enhancement for an explanation of σ.

the blood within 10 min after morphine administration (fig. 7). The major metabolite, M3G (55% of morphine is metabolized to M3G), has long been regarded as inactive.<sup>27</sup> However, there are indications in rats that M3G may functionally antagonize opioid-induced analgesia and ventilatory depression.<sup>28</sup> This may be especially relevant during prolonged administration of morphine.<sup>29</sup> Approximately 10% of morphine is metabolized to

M6G.<sup>27</sup> Rat studies show that central administration of M6G causes potent analgesia (potency 90–600 times greater than morphine after intracerebrovascular and intrathecal administration, respectively).<sup>27,30</sup> Intravenous infusion of M6G in rats produces analgesia five times more potent than morphine, but with a considerably longer time delay (*i.e.*, smaller k<sub>e0</sub>) than morphine (half-lives of 1.4 h and 0.4 h for M6G and morphine,

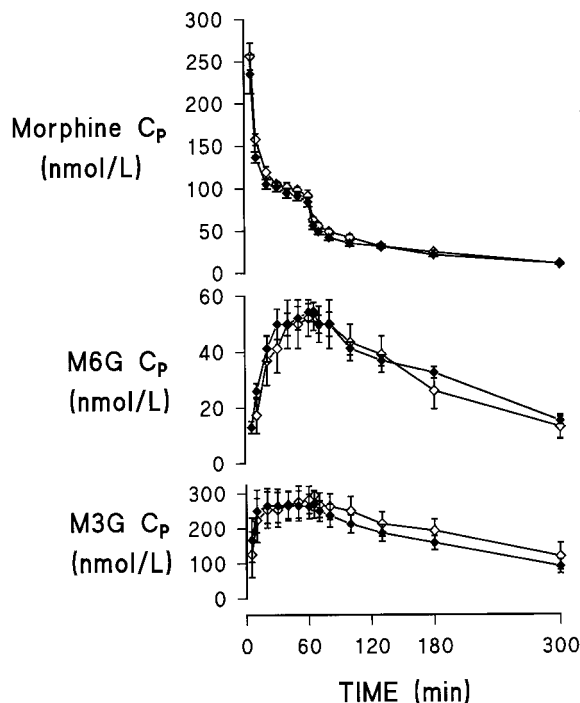
**Fig. 5. Individual (dashed lines) and mean (continuous lines) Bayesian estimates of the analgesic responses. (Left) Male responses: pain tolerance (top) and threshold (bottom). (Right) Female responses: pain tolerance (top) and threshold (bottom).**



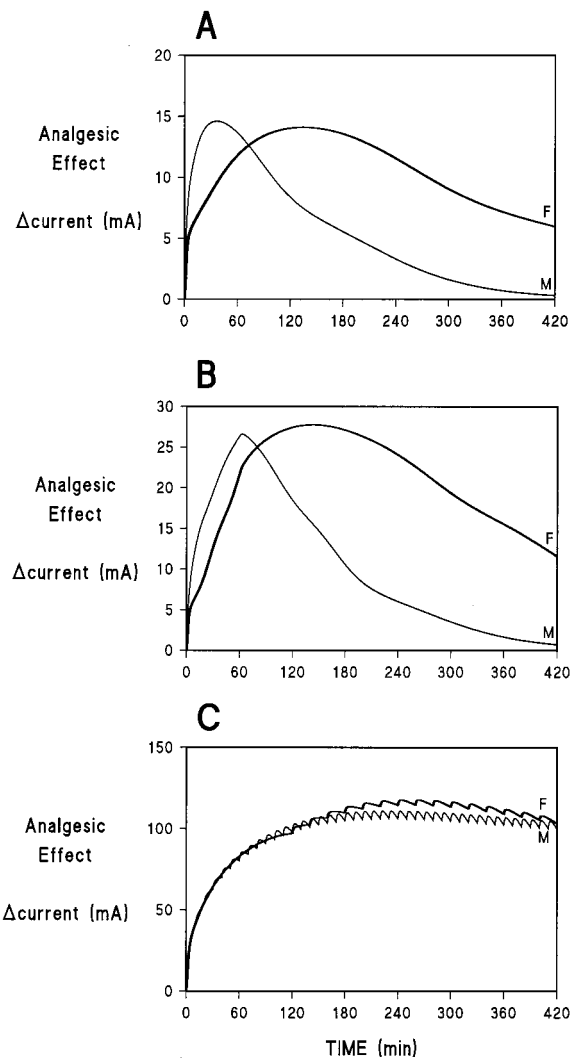


**Fig. 6.** Model prediction of the effect-site morphine concentration *versus* electrical current at which pain threshold and tolerance are observed in men and women. For both pain threshold and tolerance, morphine potency is higher in women than men. Conc. = concentration; TES = transcutaneous electrical stimulation.

respectively).<sup>31</sup> This observation is explained by the poor permeability of M6G through the blood-brain barrier.<sup>32</sup> In humans, the degree of contribution of M6G to morphine analgesia is still unresolved. In a placebo controlled study, Lötsch *et al.*<sup>33</sup> showed that, in contrast to morphine, 4-h intravenous infusions of low-dose M6G (maximum bolus dose, 2 mg/70 kg) produced no analgesia (or signs of toxicity) using an experimental pain model based on pain-related cortical potentials after stimulation of nasal nociceptors with short pulses of



**Fig. 7.** Averaged morphine, morphine-6-glucuronide (M6G), and morphine-3-glucuronide (M3G) plasma concentrations ( $C_p$ )  $\pm$  SEM in men (closed symbols) and women (open symbols).



**Fig. 8.** Simulations using the pharmacokinetic-pharmacodynamic model of the analgesic effect (pain tolerance) in men (M, thin lines) and women (F, thick lines). (A) Bolus dose of 0.1 mg/kg morphine at  $t = 0$ . Despite the lesser potency of morphine in men, the analgesic effect in men is initially greater because of the faster equilibration of morphine concentration between plasma and effect site. (B) Bolus dose of 0.1 mg/kg morphine at  $t = 0$  followed by  $30 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  for 1 h. The area under the curve is greater for women than men by a factor of 2. (C) Simulations intended to obtain similar analgesic profiles (equal peak effects and area under the curves) in men and women using a regimen of a bolus dose plus patient-controlled analgesia. A 12.0-mg bolus dose was administered in men (weight = 75 kg) at  $t = 0$ , followed by 0.5-mg infusions at 10-min intervals throughout the 7-h simulation. A 15.4-mg bolus dose was administered in women (weight = 75 kg), followed by no morphine for 120 min and then subsequent 0.34-mg infusions at 20-min intervals for the remainder of the simulation. The total amount of morphine used during the 7-h simulation was 33.0 mg in men and 20.4 mg in women.

high concentrations of carbon dioxide. On the other hand, Buetler *et al.*<sup>34</sup> observed potent analgesia within 60 min after a single bolus dose of M6G (5 mg administered intravenously) using an electrical pain test similar to the one used in our study.<sup>34</sup> Both Lötsch *et al.* and Buetler *et al.* exclusively included healthy male volun-



teers in their study. Clinical studies (using patient-controlled analgesia, repeated oral dosing strategies, and sustained-release tablets) indicate the importance of the contribution of M6G in the analgesia produced by morphine, with a potency of M6G to morphine of 2:1, in men and women.<sup>35-37</sup> A recent study in exon-1  $\mu$ -opioid receptor knockout mice provided genetic evidence for a unique receptor site for M6G analgesia.<sup>38</sup> Further studies are needed to determine the importance of M6G and the M6G receptor in the observation of greater morphine potency in women.

Our results are in disagreement with the majority of animal studies on the interaction of sex and  $\mu$ -opioid-induced antinociception. In general,  $\mu$ -opioid analgesia is greater in male compared with female rodents. As recently reviewed by us,<sup>1</sup> discrepant findings in opioid-related sex differences may be related to species differences, differences in nociceptive assay, and quantification of analgesia. With respect to the quantification of analgesia, the absence of assessing the time lag between plasma opioid concentration and effect in most animal studies should be considered as cause for differences in study outcomes. For example, if we had restricted our analgesic testing to a single measurement at  $t = 30$  min after a 0.1-mg/kg bolus dose of morphine, we would have concluded that morphine analgesia was greater in men (see simulation A in fig. 8). However, this is not a result of greater morphine potency (as defined by parameter  $AC_{50}$ ), but of a faster equilibration between plasma and effect-site morphine concentrations (as defined by parameter  $k_{e0}$ ) in males compared with females. Furthermore, there is an absence of significant metabolism of morphine into M6G in mice and some rat strains.<sup>29,39</sup> If further studies indicate that M6G plays a role in the observed sex differences in morphine analgesia in humans, this would lead to tenuous comparisons between rodent and human populations. Animals that do not produce M6G after morphine infusion are an optimal model for the assessment of sex differences in the potency of morphine *per se*.

We observed similar plasma concentrations of morphine, M6G, and M3G in men and women. Extrapolation of the present findings to our previous studies on sex-related differences in the influence of morphine on ventilatory control (in which no blood samples were collected)<sup>20,21</sup> suggests that the greater depression by morphine of ventilatory responses in women was unrelated to sex differences in the pharmacokinetics of morphine.

In conclusion, our data show that sex differences in morphine-induced analgesia have a pharmacodynamic origin. Compared with men, women showed greater morphine potency but slower onset and offset of analgesic effect. The data are in agreement with observations of sex differences in morphine-induced respiratory de-

pression and may explain higher postoperative opioid consumption in men relative to women (see simulation C of fig. 8).

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