

## Sex-related Differences in the Influence of Morphine on Ventilatory Control in Humans

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**Background:** Opiate agonists have different analgesic effects in male and female patients. The authors describe the influence of sex on the respiratory pharmacology of the  $\mu$ -receptor agonist morphine.

**Methods:** The study was placebo-controlled, double-blind, and randomized. Steady-state ventilatory responses to carbon dioxide and responses to a step into hypoxia (duration, 3 min; oxygen saturation,  $\sim 82\%$ ; end-tidal carbon dioxide tension, 45 mmHg) were obtained before and during intravenous morphine or placebo administration (bolus dose of 100  $\mu\text{g}/\text{kg}$ , followed by a continuous infusion of 30  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ) in 12 men and 12 women.

**Results:** In women, morphine reduced the slope of the ventilatory response to carbon dioxide from  $1.8 \pm 0.9$  to  $1.3 \pm 0.7$   $\text{l} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$  (mean  $\pm$  SD;  $P < 0.05$ ), whereas in men there was no significant effect (control =  $2.0 \pm 0.4$  vs. morphine =  $1.8 \pm 0.4$   $\text{l} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$ ). Morphine had no effect on the apneic threshold in women (control =  $33.8 \pm 3.8$  vs. morphine =  $35.3 \pm 5.3$  mmHg), but caused an increase in men from  $34.5 \pm 2.3$  to  $38.3 \pm 3$  mmHg,  $P < 0.05$ ). Morphine decreased hypoxic sensitivity in women from  $1.0 \pm 0.5$   $\text{l} \cdot \text{min}^{-1} \cdot \%\text{O}_2^{-1}$  to  $0.5 \pm 0.4$   $\text{l} \cdot \text{min}^{-1} \cdot \%\text{O}_2^{-1}$  ( $P < 0.05$ ) but did not cause a decrease in men (control =  $1.0 \pm 0.5$   $\text{l} \cdot \text{min}^{-1} \cdot \%\text{O}_2^{-1}$  vs. morphine =  $0.9 \pm 0.5$   $\text{l} \cdot \text{min}^{-1} \cdot \%\text{O}_2^{-1}$ ). Weight, lean body mass, body surface area, and calculated fat mass differed between the sexes, but their inclusion in the analysis as a covariate revealed no influence on the differences between men and women in morphine-induced changes.

**Conclusions:** In both sexes, morphine affects ventilatory control. However, we observed quantitative and qualitative differences between men and women in the way morphine affected the ventilatory responses to carbon dioxide and oxygen. Possible mechanisms for the observed sex differences in the respiratory pharmacology of morphine are discussed. (Key words: Carotid body; central chemoreceptors; dynamic end-tidal forcing; estradiol; female; hypercapnic ventilatory response; hypoxic ventilatory response; male;  $\mu$ -opiate receptor; opiates; peripheral chemoreceptors; progesterone; respiration; steady-state method; testosterone; ventilation.)

ANESTHESIOLOGISTS are often confronted with the large variable effects of opioids in the delivery of adequate analgesia in patients with acute and chronic pain. Animal studies, particularly those using inbred strains of mice and rats, indicate that this variability is related in part to genetic differences in pain sensitivity, in the analgesic potency of exogenously administered opioids, and in the response of the endogenous opioid system to pain and stress.<sup>1-5</sup> Many observations in humans have demonstrated sex-related differences in pain sensitivity.<sup>6,7</sup> Recent studies in rodents and humans revealed the existence of important sex-related differences in the antinociceptive responses to opiates.<sup>8-13</sup> For example, male rodents displayed significantly greater levels of  $\mu$ - and  $\kappa$ -opiate-induced analgesia compared with female animals.<sup>8-12</sup> Strain- and sex-related differences are not restricted to the analgesic properties of opioids but also involve other opioid-mediated behaviors such as locomotor activity, learning, memory, and addiction.<sup>4</sup>

Surprisingly, no studies have systematically investigated the interaction of genotype (sex and other genetic factors) and opioids on ventilatory control. Respiratory depression is a serious side effect, especially of  $\mu$ -opiate receptor (MOR) agonists. Therefore we designed this study to obtain information about the influence of sex on morphine-induced respiratory depression. The influence of intravenous morphine on the hypercapnic and hypoxic ventilatory responses was tested in healthy, young male and female volunteers in a placebo-controlled, double-blind, randomized study design.

This article is featured in "This Month in Anesthesiology." Please see this issue of ANESTHESIOLOGY, page 4A.

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## Materials and Methods

### *Participants and Apparatus*

Twenty-six healthy, nonsmoking volunteers (13 men, 13 women; aged 18–35 yr) were recruited after protocol approval by the Leiden University Committee on Medical Ethics. None of the volunteers was taking any medications. All women reported normal menstrual cycles. In this initial study, the phase of their menstrual cycle was not prospectively controlled. Sexually active women underwent a pregnancy test on the morning of the study. The volunteers were instructed not to eat or drink for at least 8 h before the study. They were not informed about respiratory physiology and the intentions of the study, but they did receive information on the nature and risks of the study as well as the possible side effects of morphine. All gave informed consent before their participation.

The volunteers were seated in a comfortable position and breathed through a face mask (Vital Signs, Totawa, NJ) positioned over the mouth and nose (a nose clip was not used). The inspired and expired gas flows were measured using a pneumotachograph connected to a differential pressure transducer (Hewlett Packard, Andover, MA) and electronically integrated to yield a volume signal. The volume signal was calibrated with a motor-driven piston pump. Corrections were made for changes in gas viscosity due to changes in oxygen concentration of the inhaled gas mixture. The subjects received a gas mixture with a flow of 45 l/min from a gas-mixing system consisting of three mass flow controllers (Bronkhorst High Tec, Veenendaal, The Netherlands) through which the flow of oxygen, carbon dioxide, and nitrogen could be set individually at a desired level. A PDP mini computer (Digital Equipment Co., Maynard, IA) provided control signals to the mass flow controllers so that the composition of the inspired gas mixture could be adjusted to obtain the desired end-tidal carbon dioxide concentration ( $P_{ET}CO_2$ ) and end-tidal oxygen concentration ( $P_{ET}O_2$ ). The oxygen and carbon dioxide concentrations of inspired and expired gas were measured using a gas monitor (Multicap; Datex, Helsinki, Finland). The gas monitor was calibrated with gas mixtures of known concentrations. A pulse oximeter (Satelite Plus; Datex) continuously measured the oxygen saturation ( $SpO_2$ ) of arterial hemoglobin with a finger probe.

### *Study Design*

The study was placebo-controlled and had a double-blind, randomized design. Initially, all volunteers were

studied in two sessions separated by at least 2 weeks. In one of these sessions, control studies were followed by morphine studies (morphine session), and in the other, placebo (0.9% NaCl) studies followed the control studies (placebo session). The sequence of placebo and morphine sessions was randomized. Normal saline, morphine, or placebo were administered *via* an intravenous access line. To prevent any disturbance of the participants, the agents were delivered from an adjacent room by an infusion pump (Becton Dickinson, St. Etienne, France) connected to the participant *via* a 4-m line.<sup>14</sup> Saline was infused during control studies at a rate of 6 ml/h, and morphine and placebo (dissolved in saline; concentration = 0.5 mg/ml) were given as a bolus of 100  $\mu$ g/kg, followed by a continuous infusion of 30  $\mu$ g  $\cdot$  kg<sup>-1</sup>  $\cdot$  h<sup>-1</sup>. Morphine and placebo studies started 40 min after the bolus was given. After data analysis, we invited all the female participants for a third session to study the influence of the phase of the menstrual cycle on morphine-induced respiratory changes.

### *End-tidal Carbon Dioxide and Oxygen Tension Patterns*

The ventilatory responses to hypercapnia and acute hypoxia were obtained using the computer-driven "dynamic end-tidal forcing" technique. A detailed description of this technique has been published.<sup>15</sup> Briefly, by manipulating the inspired gas concentrations, independent of changes in ventilation or mixed venous return, this technique enabled us to force end-expiratory carbon dioxide and oxygen tensions ( $P_{ET}CO_2$  and  $P_{ET}O_2$ ) to follow a prescribed pattern in time. In this study, we obtained steady-state ventilatory carbon dioxide response and ventilatory responses to a step from normoxia into hypoxia.

Control and morphine/placebo studies started with the assessment of resting  $P_{ET}CO_2$  and  $\dot{V}_I$  during inhalation of a normoxic gas mixture without inspired carbon dioxide. Thereafter, hypoxic or hypercapnic studies were performed in a randomized order.

For the carbon dioxide study, 6 to 10 elevations in  $P_{ET}CO_2$  were applied to obtain data points for the steady-state ventilatory response. The elevations varied from 3–19 mmHg. The elevated  $P_{ET}CO_2$  readings lasted at least 8 min. When on-line analysis revealed that a ventilatory steady state had not been reached, the duration of hypercapnia was extended. The order of elevations was arbitrarily chosen. For the oxygen study,  $P_{ET}CO_2$  was maintained at a constant value of 45 mmHg



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to offset any increase in  $P_{ET}CO_2$  due to depression of  $\dot{V}_I$  by morphine during all protocols. The pattern of  $P_{ET}O_2$  was as follows: (1) 5–10 min at 110 mmHg, (2) a step decrease to 49 mmHg, (3) maintenance at 49 mmHg for 3 min, and (4) 7-min inhalation of a hyperoxic gas mixture (inspired oxygen fraction,  $\sim 0.70$ ).

### Nonrespiratory Data

Venous blood was drawn on each occasion before ventilatory measurements were made to determine serum progesterone and estradiol levels in women and testosterone and estradiol levels in men. To obtain information on nonrespiratory measures of morphine action, the following data were collected, at the end of control and placebo and morphine studies: the diameter of the pupil (obtained at constant indoor lighting), the central nervous system arousal state using a simple six-point observers' assessment of alertness and sedation scale (from 0 = normal alertness to 5 = closed eyes and unarousable; see Sarton *et al.*<sup>16</sup>), and the occurrence of nausea and itch.

Lean body mass (LBM) and body surface area (BSA) were calculated from weight (in kilograms) and height (in centimeters) using the following formulas<sup>17–19</sup>:

$$LBM \text{ (men)} = 1.1 \cdot \text{weight} - 128 \cdot (\text{weight/height})^2;$$

$$LBM \text{ (women)} = 1.07 \cdot \text{weight} - 148 \cdot (\text{weight/height})^2;$$

$$BSA = (\text{weight})^{0.425} \cdot (\text{height})^{0.725} \cdot 0.007184.$$

The body fat percentage was calculated as  $[(\text{weight} - LBM)/\text{weight} \cdot 100\%]$ .

### Data Analysis

**Resting Values.** Baseline  $P_{ET}CO_2$  and  $\dot{V}_I$  were assessed by taking the average of ten breaths after the volunteers had been breathing the normoxic gas mixtures for 10–15 min in the control studies and for about 50–55 min in the morphine and placebo studies (this period includes 40 min of morphine or placebo administration).

**Carbon Dioxide Studies.** The elevated  $P_{ET}CO_2$  and the corresponding  $\dot{V}_I$  breath-to-breath data were averaged for 10 breaths. Data points were obtained at the end of the  $P_{ET}CO_2$  elevation (that is, after at least 8 min). This procedure yielded 6 to 10 steady-state data points. We expressed  $\dot{V}_I$  as a linear function of  $P_{ET}CO_2$ :

$$\dot{V}_I = S(P_{ET}CO_2 - B)$$

where  $S$  is the ventilatory carbon dioxide sensitivity and

$B$  is the apneic threshold or extrapolated  $P_{ET}CO_2$  at zero  $\dot{V}_I$ . Parameters  $S$  and  $B$  were determined by linear regression of  $\dot{V}_I$  on  $P_{ET}CO_2$ .

**Oxygen Studies.** Mean values of the breath-to-breath  $\dot{V}_I$  and  $SpO_2$  were calculated for the last 10 breaths of normoxia and the last 10 breaths of hypoxia (*i.e.*, during 3 min of hypoxia). The difference between these two data points, expressed as  $l \cdot \text{min}^{-1}$  [change in  $SpO_2$  (%) ]<sup>-1</sup> is called hypoxic ventilatory sensitivity ( $H$ ). The normoxic data point (obtained at  $P_{ET}CO_2 = 45$  mmHg) is abbreviated  $N_{45}$ .

**Blood Samples.** Serum progesterone, estradiol, and testosterone levels were determined by radioimmunoassay (Diagnostic Products Co., Los Angeles, CA; Orion Diagnostics, Espoo, Finland; Byk-Sangtec Diagnostica, Dietzenbach, Germany, respectively). The intra- and interassay coefficients of variation for progesterone were 3.9% and 5.6%, for estradiol 7.3% and 5.1%, and for testosterone 6.3% and 7.4%, respectively. Reference values (95% range) obtained from women without fertility problems are for progesterone: follicular phase = 0.5 – 4.6 nM (median = 1.3 nM) and luteal phase = 5.1 – 67 nM (median = 22 nM); and for estradiol: follicular phase = 37 – 285 pM and luteal phase 51 – 872 pM.

### Statistical Analysis

**Treatment versus Control.** To detect an effect of placebo *versus* control or morphine *versus* control, paired  $t$  tests were performed on the individual parameter values in both sexes. In men, linear regression analysis of testosterone and estradiol concentrations (obtained in the morphine sessions) on morphine-induced changes in the individual values were performed. Probability values  $< 0.05$  were considered significant.

**Men versus Women.** Because each morphine or placebo study was preceded by a control measurement,  $t$  tests were performed on the parameter differences between control and treatment (*i.e.*,  $\Delta S$ ). The differences of the means were considered significant at  $P < 0.05$ . The *a posteriori* statistical power for the test on the morphine-induced changes in  $S$ ,  $B$ , and  $H$  with sex as treatment was 69%, 53% and 78%, respectively.<sup>20</sup>

To determine if factors such as body weight, height, LBM, BSA, and calculated fat percentage significantly influenced morphine-induced changes in  $S$ ,  $B$ , and  $H$ , an analysis of covariance was performed for sex and these factors as covariates. An effect of covariates was considered significant at  $P < 0.05$ .

**Follicular versus Luteal Phase.** For the effect of the follicular or luteal phase of the menstrual cycle



Table 1. Anthropometric Data of the Subjects

	Men	Women
No. of subjects	12	12
Age (yr)	25.6 ± 1.7	24.8 ± 4.5
Weight (kg)	76.6 ± 6.7	64.9 ± 9.9*
Height (cm)	182.0 ± 6.9	172.0 ± 4.5*
Body surface area (m <sup>2</sup> )	1.97 ± 0.1	1.76 ± 0.14*
Lean body mass (kg)	61.6 ± 3.4	47.8 ± 4.5*
Calculated body fat mass (%)	19.7 ± 3.3	24.5 ± 4.1*

Values are mean ± SD.

\*  $P < 0.05$ , Student's  $t$  test.

paired  $t$  tests were performed on individual values. Finally, linear regression analysis of progesterone and estradiol concentrations on morphine-induced changes in individual values were performed.

## Results

### Sex Differences

One man and one woman did not complete the morphine session because of nausea and vomiting. Their data were discarded. As expected, analysis of the anthropometric data showed that the men, compared with the women in our group, were heavier, taller, and had higher LBM and BSA values and lesser calculated fat mass (table 1). Table 2 shows the nonrespiratory variables. They indicate that the occurrence or magnitude of the nonrespiratory effects of morphine was similar in men and women. With respect to the observers' assessment of alertness and sedation scale, all participants were either not sedated (score = 0) or drowsy and light-headed with open eyes and responsive to command (score = 1).

Table 3. Influence of Morphine on Ventilatory Control in Men and Women

	Control	Morphine
Men		
P <sub>ET</sub> CO <sub>2</sub> (mmHg)*	39.0 ± 1.5	42.8 ± 2.3‡
$\dot{V}_I$ (L · min <sup>-1</sup> )*	10.8 ± 1.6	9.5 ± 1.8‡
S (L · min <sup>-1</sup> · mmHg <sup>-1</sup> )	2.0 ± 0.4	1.8 ± 0.4
B (mmHg)	34.5 ± 2.3	38.3 ± 3.0‡
N <sub>45</sub> (L · min <sup>-1</sup> )	20.4 ± 4.5	12.1 ± 7.0‡
H (L · min <sup>-1</sup> · % <sup>-1</sup> )	1.0 ± 0.5	0.9 ± 0.5
Women†		
P <sub>ET</sub> CO <sub>2</sub> (mmHg)*	37.5 ± 2.3§	41.3 ± 3.0‡
$\dot{V}_I$ (L · min <sup>-1</sup> )*	9.4 ± 1.6	7.9 ± 1.3‡§
S (L · min <sup>-1</sup> · mmHg <sup>-1</sup> )	1.8 ± 0.9	1.3 ± 0.7‡§
B (mmHg)	33.8 ± 3.8	35.3 ± 3.0
N <sub>45</sub> (L · min <sup>-1</sup> )	19.1 ± 9.4	10.6 ± 6.3‡
H (L · min <sup>-1</sup> · % <sup>-1</sup> )	1.0 ± 0.5	0.5 ± 0.4‡§

Values are mean ± SD.

\* Data obtained without inspired carbon dioxide.

† Follicular phase,  $n = 5$ ; luteal phase,  $n = 7$ .‡  $P < 0.05$ , morphine versus control.§  $P < 0.05$ , women versus men.

In contrast to placebo, which left all determined variables and parameters unaffected, morphine affected ventilatory control in both sexes (table 3). However, carbon dioxide and oxygen responses were affected by morphine in a different way in the male and female participant groups. Figure 1 shows the steady state ventilatory responses to carbon dioxide of a male and female volunteer. It exemplifies the general observation in men that, after morphine is given, the ventilatory carbon dioxide response curve is shifted to higher P<sub>ET</sub>CO<sub>2</sub> levels with no change in S, whereas in women, S is reduced, with no change in B (see table 3 for means). Morphine shifted normoxic ventilation at P<sub>ET</sub>CO<sub>2</sub> of 45

Table 2. Nonrespiratory Data: Sedation Score, Occurrence of Itching, Nausea/Vomiting, and Change in Pupil Diameter from Control ( $\Delta$ )

	Placebo		Morphine	
	Men	Women	Men	Women
LOAA/S score 0 (n)	10	9	8	7
LOAA/S score 1 (n)	2	3	4	5
Itch (n)	0	0	3	3
Nausea/vomiting (n)	0	0	2	4
$\Delta$ pupil diameter (mm)	0.1 ± 0.2	0.0 ± 0.2	-2.2 ± 0.9	-2.2 ± 0.6

LOAA/S score = Leiden observers' assessment of alertness/sedation score (score 0 = normal alertness; score 1 = drowsy or light headed with open eyes); n is number of subjects, total number of subjects is 24 (12 men and 12 women).



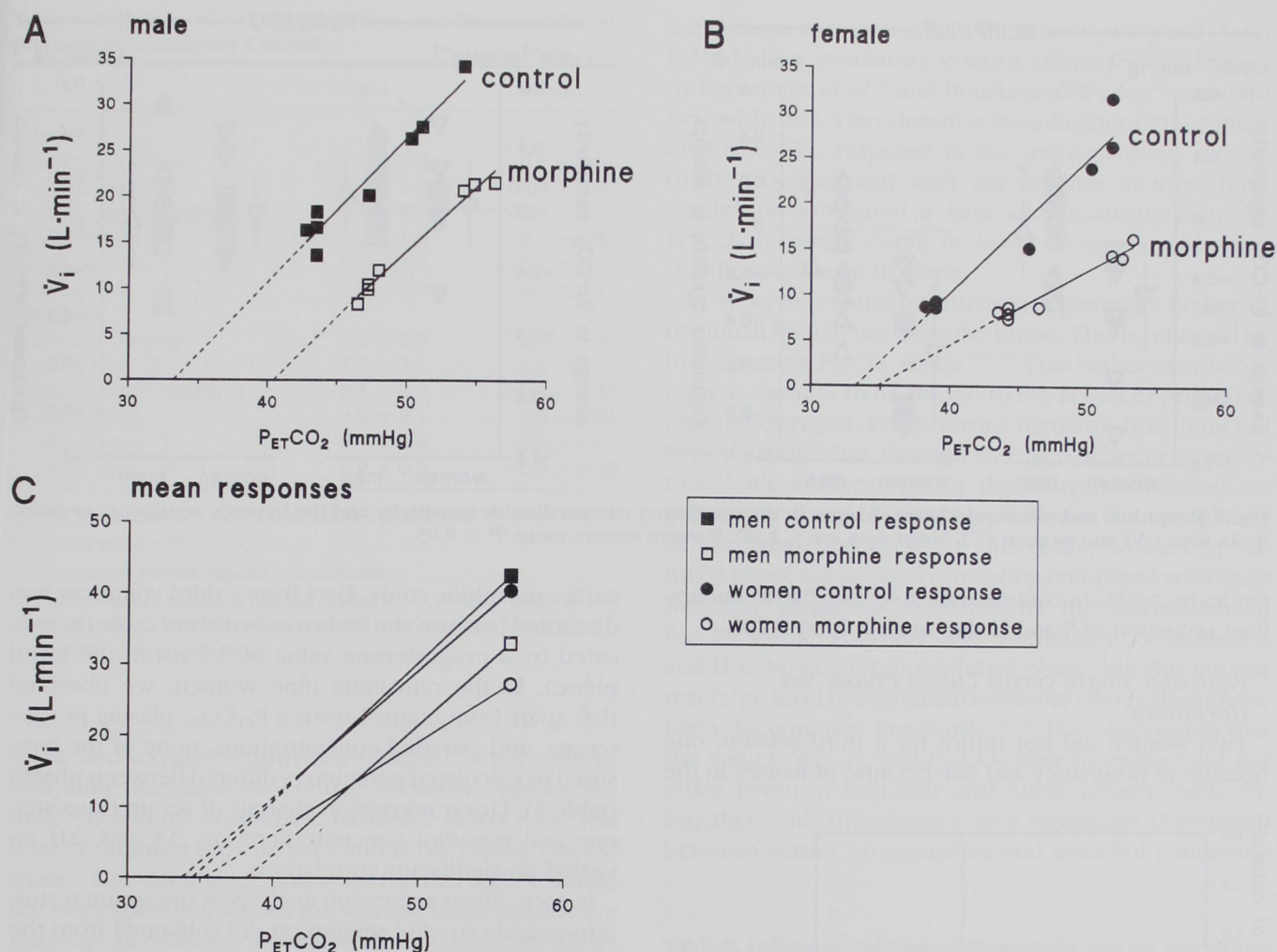


Fig. 1. The ventilatory response to carbon dioxide obtained in a man (A) and a woman (B). The squares and circles are steady state  $\dot{V}_i$ - $P_{ET}CO_2$  data points. The continuous lines through the data points are the linear regression lines. The slope of the regression line is the ventilatory carbon dioxide sensitivity or S. Broken lines are drawn to illustrate the values of the apneic threshold (that is, the extrapolated intercept with the x axis or B). (A) Control:  $S = 1.6 \text{ l} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$ ,  $B = 33 \text{ mmHg}$ ; morphine:  $S = 1.4 \text{ l} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$ ,  $B = 40.8 \text{ mmHg}$ . (B) Responses obtained from a woman in the follicular phase of her menstrual cycle. Control:  $S = 1.5 \text{ l} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$ ,  $B = 33 \text{ mmHg}$ ; morphine:  $S = 0.8 \text{ l} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$ ,  $B = 34.5 \text{ mmHg}$ . (C) Morphine-induced respiratory depression in men and women (mean responses, both groups  $n = 12$ ). Morphine increased the magnitude of B in men without affecting S; In women, S was reduced but B remained unchanged.

mmHg to the same extent in men as in women. This is not surprising, because the ventilatory response curves after morphine administration in men and women pivoted at a  $P_{ET}CO_2$  of about 45 mmHg (fig. 1). However, in contrast to the response in men, the hypoxic sensitivity was depressed in women by about 50%. Table 3 lists the mean values of the parameters.

Morphine-induced changes in ventilatory carbon dioxide sensitivity ( $\Delta S$ ), apneic threshold ( $\Delta B$ ), and hypoxic sensitivities ( $\Delta H$ ) differed significantly between men and women (table 4 and fig. 2). Inclusion of the

anthropometric factors weight, height, LBM, BSA, and calculated fat mass as covariates in the analysis revealed that none had a significant influence on the difference in  $\Delta S$ ,  $\Delta B$ , or  $\Delta H$  between men and women.

In figure 3 we plotted the hypoxic sensitivities against the carbon dioxide sensitivities (obtained from the morphine sessions) of individual participants. Correlation between carbon dioxide and oxygen studies was good ( $r = 0.84$ ;  $n = 24$ ;  $P < 0.0001$ ). Morphine caused about 60% greater changes in H than in S. Note that 11 women showed a reduction of the hypoxic and hypercapnic sensi-



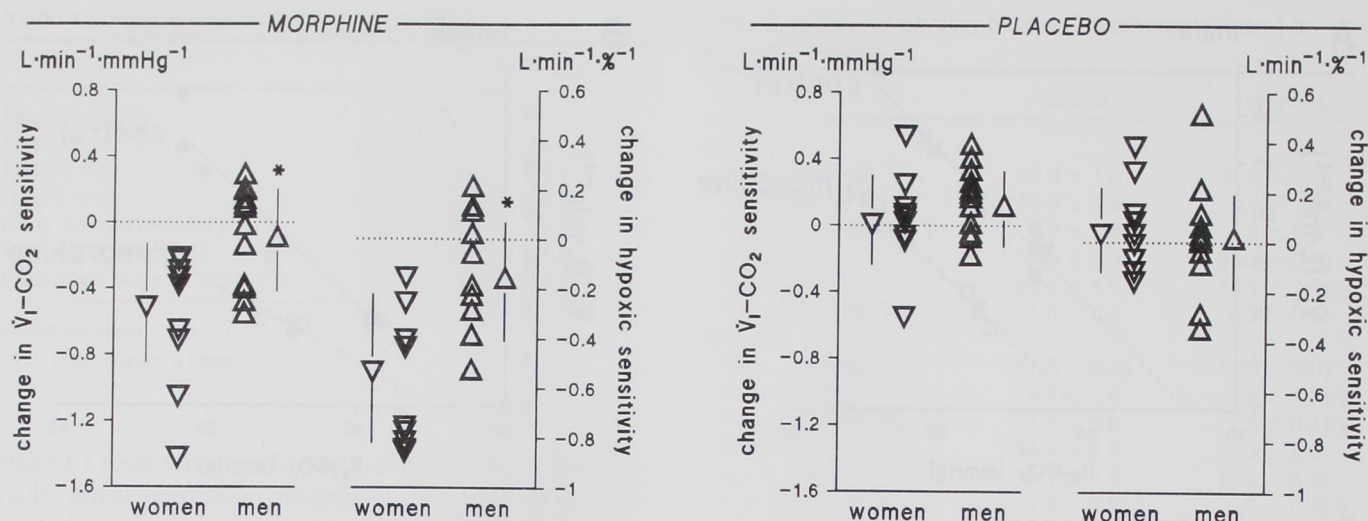


Fig. 2. Morphine- and placebo-induced changes in the ventilatory carbon dioxide sensitivity and the hypoxic ventilatory sensitivity in men ( $\Delta$ ) and women ( $\nabla$ ). Mean data are  $\pm$  1 SD. Women versus men: \* $P$  < 0.05.

tivities by >20%. In contrast, only four men had a concomitant reduction of S and H due to morphine >20%.

#### Follicular Phase versus Luteal Phase, Sex Hormones

Two women did not return for a third session, one because of pregnancy and one because of nausea in the

earlier morphine study. Data from a third volunteer was discarded because she had an anovulatory cycle (as indicated by a progesterone value of 5.7 nm in the luteal phase). In the remaining nine women, we observed that apart from control resting  $P_{ET}CO_2$ , plasma progesterone, and estradiol concentrations, none of the measured or calculated parameters differed between phases (table 5). Linear regression analysis of serum progesterone and estradiol concentrations on  $\Delta S$  and  $\Delta H$  revealed no significant correlation.

In men, linear regression analysis of the serum testosterone and estradiol session on  $\Delta B$  (obtained from the morphine session) showed no significant correlation.

## Discussion

We observed differences in the influence of an analgesic dose of morphine on the steady state ventilatory response to carbon dioxide and the isocapnic ventilatory response to acute hypoxia in men and women. Both sexes showed respiratory depression after morphine, as demonstrated by the increase in resting  $P_{ET}CO_2$  and decrease in resting  $\dot{V}_I$  (table 3). However, women showed a decrease of the ventilatory carbon dioxide and hypoxic sensitivities by 30% and 50%, respectively. This contrasts sharply with our findings in men that showed no significant effect of morphine on the ventilatory oxygen and carbon dioxide sensitivities. Their oxygen and carbon dioxide responses were shifted in a parallel manner to lower  $\dot{V}_I$  levels. The 95% confidence intervals for the male morphine carbon di-

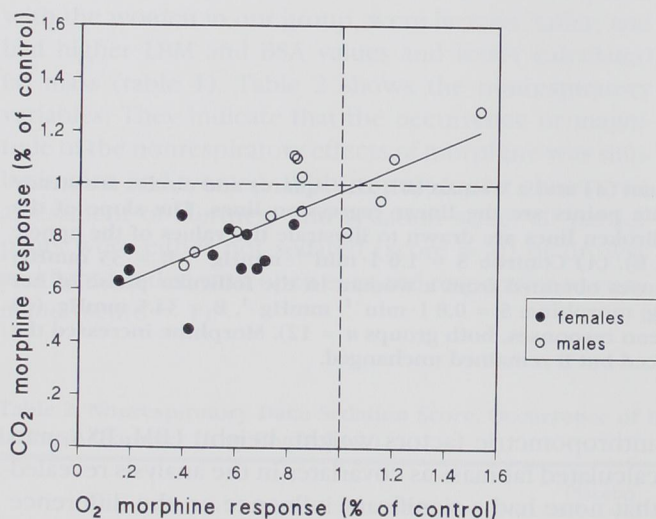


Fig. 3. The ventilatory carbon dioxide sensitivity after morphine versus the hypoxic ventilatory sensitivity after morphine in men ( $\circ$ ) and women ( $\bullet$ ). The values are percentages of control. The correlation between hypoxic and hypercapnic data is good:  $r = 0.84$ ,  $P < 0.0001$ . The line through the data is obtained from linear regression analysis on all data points ( $n = 24$ , slope = 0.43). Note that all 12 women and 5 of the men have a concomitant depression of hypoxic and hypercapnic responses after morphine administration (data points are shown in the lower left quadrant of the figure).



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**Table 4. Influence of Sex on Morphine- and Placebo-induced Changes in Ventilatory Control**

	Men	Women†
Placebo		
$\Delta P_{ETCO_2}$ (mmHg)*	$-0.12 \pm 0.9$	$-0.1 \pm 0.8$
$\Delta \dot{V}_I$ (L · min <sup>-1</sup> )*	$0.6 \pm 1.8$	$-0.04 \pm 1.0$
$\Delta S$ (L · min <sup>-1</sup> · mmHg <sup>-1</sup> )	$0.1 \pm 0.2$	$0.04 \pm 0.3$
$\Delta B$ (mmHg)	$0.3 \pm 1.5$	$0.7 \pm 1.9$
$\Delta N_{45}$ (L · min <sup>-1</sup> )	$0.3 \pm 2.4$	$-0.2 \pm 3.3$
$\Delta H$ (L · min <sup>-1</sup> · % <sup>-1</sup> )	$0.0 \pm 0.2$	$0.1 \pm 0.2$
Morphine		
$\Delta P_{ETCO_2}$ (mmHg)*	$3.5 \pm 0.9$	$4.0 \pm 1.3$
$\Delta \dot{V}_I$ (L · min <sup>-1</sup> )*	$-1.3 \pm 1.1$	$-1.6 \pm 1.5$
$\Delta S$ (L · min <sup>-1</sup> · mmHg <sup>-1</sup> )	$-0.1 \pm 0.3$	$-0.5 \pm 0.4\ddagger$
$\Delta B$ (mmHg)	$4.2 \pm 2.1$	$1.5 \pm 3.0\ddagger$
$\Delta N_{45}$ (L · min <sup>-1</sup> )	$-8.4 \pm 3.9$	$-8.5 \pm 5.5$
$\Delta H$ (L · min <sup>-1</sup> · % <sup>-1</sup> )	$-0.2 \pm 0.2$	$-0.5 \pm 0.3\ddagger$

$\Delta$  = difference between morphine or placebo and control

Values are mean  $\pm$  SD.

\* Data obtained without inspired carbon dioxide.

† Placebo session: women in follicular phase,  $n = 7$ ; in luteal phase,  $n = 5$ ; morphine session: women in follicular phase,  $n = 5$ ; in luteal phase,  $n = 7$ .

‡  $P < 0.05$ , women versus men.

oxide and oxygen sensitivities (defined as the ratio of morphine response to control response) places the male morphine responses between 0.86–1.04 and 0.69–1.00 times the control S and H responses, respectively. The confidence intervals for the men exclude the corresponding values for women (95% confidence intervals of women: morphine S = 0.65–0.80 times control S; morphine H = 0.35–0.63 times control H). Thus, although we cannot exclude a small effect on H and S in men, our data indicate quantitative differences in the morphine responses between men and women. Further, the absence of a shift of the  $\dot{V}_I$ - $P_{ETCO_2}$  response in women compared with a shift in men may reflect a qualitative rather than a quantitative difference. It suggests that the mechanism of ventilatory depression by morphine differs between the sexes.

Although it is of interest to compare our findings with studies from the literature (see references 21 through 29), direct comparisons are difficult for various reasons: (1) none of the studies was designed to test the influence of sex on opioid-induced respiratory depression; (2) the sample sizes were small; (3) there were differences in agents,<sup>21,22</sup> doses,<sup>23,24</sup> species,<sup>25,26</sup> and central nervous system arousal states<sup>25,27</sup>; and (4) the methods to assess carbon dioxide- and oxygen-mediated control of breathing differed from our steady state method (*i.e.*,

a technique that generates a constant  $P_{ETCO_2}$  and  $P_{ETO_2}$  input to the ventilatory control system).<sup>23,24,28</sup> Studies by Loeschcke *et al.*<sup>29</sup> and Bourke and Warley<sup>28</sup> compare best with ours. They describe the influence of morphine on the  $\dot{V}_I$ - $CO_2$  response in six and four men, respectively. In agreement with our findings in men, both studies demonstrated a shift of the steady state  $\dot{V}_I$ - $P_{ETCO_2}$  response curve to lower  $\dot{V}_I$  levels without a significant change in slope.

In women, ventilatory drives are generally higher in the luteal than in the follicular phase. This is reflected by lower resting  $P_{ETCO_2}$  levels.<sup>30–32</sup> This higher ventilatory drive is thought to be related to the action of progesterone and estrogen. Progesterone increases breathing *via* hypothalamic sites through estrogen-dependent receptors.<sup>33</sup> We anticipated that the morphine-induced depression of S and H could vary between the two phases of the menstrual cycle. In our study, women in the luteal phase were hyperventilating compared with their follicular phase, as indicated by the lower control  $P_{ETCO_2}$  (table 5). There was a tendency for control S and H to be greater in the luteal phase, but this did not reach the level of significance. Similar observations have been documented previously.<sup>30,34</sup> The observation that the values of S, H, and  $P_{ETCO_2}$  after morphine did not differ between follicular and luteal phases (table 5), together with the absence of a significant correlation between serum progesterone and estradiol concentra-

**Table 5. Influence of Phase of Menstrual Cycle on Morphine-induced Respiratory Depression**

	Follicular Phase	Luteal Phase
$P_{ETCO_2}$ control (mmHg)*	$38.3 \pm 2.3$	$36.5 \pm 2.3\ddagger$
$P_{ETCO_2}$ morphine (mmHg)*	$41.3 \pm 3.1$	$39.8 \pm 3.2$
$\dot{V}_I$ control (L · min <sup>-1</sup> )*	$10.2 \pm 1.7$	$10.2 \pm 1.2$
$\dot{V}_I$ morphine (L · min <sup>-1</sup> )*	$8.4 \pm 1.0$	$8.3 \pm 1.4$
S control (L · min <sup>-1</sup> · mmHg <sup>-1</sup> )	$1.7 \pm 0.8$	$2.0 \pm 1.1$
S morphine (L · min <sup>-1</sup> · mmHg <sup>-1</sup> )	$1.3 \pm 0.6$	$1.5 \pm 0.8$
B control (mmHg)	$33.0 \pm 4.5$	$33.0 \pm 4.5$
B morphine (mmHg)	$34.5 \pm 6.8$	$33.0 \pm 5.8$
$N_{45}$ control (L · min <sup>-1</sup> )	$19.4 \pm 7.3$	$22.5 \pm 12.0$
$N_{45}$ morphine (L · min <sup>-1</sup> )	$12.1 \pm 6.5$	$13.7 \pm 7.5$
H control (L · min <sup>-1</sup> · % <sup>-1</sup> )	$1.1 \pm 0.6$	$1.2 \pm 0.6$
H morphine (L · min <sup>-1</sup> · % <sup>-1</sup> )	$0.6 \pm 0.4$	$0.5 \pm 0.4$
Day of menstrual cycle	$6 \pm 2$	$23 \pm 3$
Progesterone (nM)	$2.1 \pm 0.8$	$21.0 \pm 6.6\ddagger$
Estradiol (pM)	$170.3 \pm 164.6$	$348.7 \pm 133.3\ddagger$

Values are mean  $\pm$  SD;  $n = 9$ .

\* Data obtained without inspired carbon dioxide.

‡  $P < 0.05$ .



tions and  $\Delta S$  and  $\Delta H$  leads us to conclude that temporal changes in female gonadal hormones did not influence opioid-induced respiratory depression in nonpregnant women. Whether these findings would also hold true at higher progesterone concentrations, as during pregnancy,<sup>32,35</sup> deserves further study. The disappearance of the difference in  $P_{ET}CO_2$ s between the two phases of the menstrual cycle during morphine administration suggests that the effect of morphine on metabolic rate overrides the progesterone effect.

### *Mechanisms of Sex-related Differences*

We did not measure morphine or morphine metabolite concentrations in plasma or cerebrospinal fluid. It is therefore possible that at least part of the observed differences in the effects of morphine on the ventilatory control between men and women is due to sex differences in morphine's pharmacokinetics. For example, lower morphine concentrations or lower levels of morphine-6-glucuronide, one of morphine's active metabolites, in the brain stem or at other target sites (e.g., MOR occur in the carotid bodies)<sup>36</sup> in men compared with women may explain, to some extent, the absence of a significant depression of the hypoxic and hypercapnic ventilatory sensitivities in men compared with a significant depression in women. We reviewed animal and human data from the literature and took into account the indirect data from our study to obtain information on the existence of sex differences in the kinetics of morphine that could explain our results.

**Animal Studies.** Cicero *et al.*<sup>8,9</sup> showed identical peak concentrations of morphine in blood and brain and elimination half-lives in male and female rats after subcutaneous administration (dose range, 2.5–20 mg/kg). Furthermore, they report no sex differences in free and protein-bound morphine concentrations in blood.<sup>9</sup> Candido *et al.*,<sup>37</sup> on the other hand, showed a 20% higher brain morphine concentration in male compared with female mice after a 4 mg/kg injection. Craft *et al.*<sup>38</sup> observed no sex differences in blood and brain morphine concentrations 20 min after subcutaneous morphine, but they did find higher brain levels in male rats 60 min after injection. It is of interest to note that sex-related differences in the analgesic properties of morphine are observed after intracerebroventricular injection of morphine in rats (dose range, 1–40  $\mu$ g).<sup>39</sup> It is clear that the results of these studies do not correspond with the hypothesis of differential pharmacokinetics as an explanation of our findings because none

showed lower blood or brain morphine concentrations in male animals.

**Human Studies.** There is a paucity of data with respect to the influence of sex on the pharmacokinetics of morphine. We retrieved two studies from the literature. Both indicate the absence of sex influences on morphine's kinetics. The first studied morphine and morphine-3-glucuronide levels in blood and cerebrospinal fluid after intradural administration of morphine in adults,<sup>40</sup> and the second determined kinetic parameters during continuous intravenous administration in a pediatric population with sickle cell disease.<sup>41</sup>

A sex difference in the metabolism of morphine may be responsible for our results. Metabolites appear in the blood within 10 min after morphine administration.<sup>42,43</sup> The major metabolite morphine-3-glucuronide (55%) is inactive, but morphine-6-glucuronide (10–15%) is a respiratory depressant when given intracerebroventricularly.<sup>44</sup> In humans with normal renal function, intravenous administration of morphine-6-glucuronide caused no respiratory depression (as expressed by an increase in  $P_{ET}CO_2$ ) or other signs of toxicity (e.g., sedation or vomiting).<sup>45,46</sup> This is probably due to the poor permeability of morphine-6-glucuronide through the blood–brain barrier.<sup>47</sup>

Another possible explanation for our findings is that blood effect-site equilibration times for morphine were greater in men than in women. As a consequence, morphine levels in the brain in men may not have reached female (steady state) values at the time of the hypoxic and hypercapnic studies. To understand the dynamics of  $P_{ET}CO_2$  and  $\dot{V}_I$  during morphine infusion, a one-component exponential was fitted through the  $\dot{V}_I$  data. A similar procedure was performed on the  $P_{ET}CO_2$  data. The estimated time constants did not differ between men and women and ranged from 2–7 min for each of the data sets. This indicates that the time between the start of morphine and the execution of the respiratory responses (40 min) was at least six times the blood (respiratory) effect-site time constant, and a steady state in  $\dot{V}_I$  and  $P_{ET}CO_2$  was attained before experiments were begun in all participants.

The most compelling evidence that morphine's effect on the slope of the carbon dioxide response curve is independent of dose comes from a study by Bourke and Warley.<sup>28</sup> They determined steady state ventilatory carbon dioxide responses, in a group of men only, after 0.07 and 0.21 mg/kg doses of intravenous morphine. They showed a parallel right shift of the carbon dioxide response curve at 0.07 mg/kg and a further rightward



shift at 0.21 mg/kg. At both doses (their highest dose is twice the loading dose used in our study), the slope of the response curve (*i.e.*, the carbon dioxide sensitivity) remained identical to the control. They concluded that the parallel shift of the steady-state carbon dioxide response curve is specific to drugs that act on opioid receptors (*i.e.*, a qualitative effect). Because they did not study women, it seems logical at this point to add the qualification "in male participants."

In our study, analysis of covariance showed that none of the anthropometric factors that could influence the plasma morphine concentrations (weight, height, LBM, BSA, and fat content) had a significant influence on the differences between men and women in the effect of a dose of morphine given based on weight on  $\Delta S$ ,  $\Delta H$ , and  $\Delta B$ . Further, we did not observe any differences in the measured nonrespiratory effects of morphine (table 2). We are aware that the power of these tests may have been inadequate to observe significant effects. However, when viewed in light of the data from the literature, we find no indications that our tests were flawed (*i.e.*, a type II error). It is evident that further studies on the kinetics of morphine and its metabolites in men and women are needed before we can conclude definitely that morphine concentrations at target receptor sites do not differ between men and women. For now, all available information indicates that such differences were not sufficiently large to explain the sex differences in the respiratory pharmacology of morphine in our study.

We suggest that the sex differences in morphine-induced ventilatory depression are genetic in origin and that the underlying biological mechanisms are related to the effects of sex steroids. Endogenous gonadal hormones could mediate sex-related differences in neurobehavioral responses through at least two possible mechanisms. (1) They could be due to the mere presence or absence of sex steroids (*i.e.*, acute effects). An example, relevant to our study, is the observation that testosterone administration in hypogonadal adult men causes an increase in  $\dot{V}_I$  and hypoxic response largely related to an increase in metabolic rate.<sup>48</sup> Although an acute effect of testosterone could explain our findings, control and placebo studies did not show sex-related differences. An acute effect of female sex hormones seems unlikely, because we observed no difference in the respiratory effects of morphine in the follicular and luteal phases of the menstrual cycle. (2) The differences could also be due to long-term developmental and organizational effects of sex steroids that occur in prenatal and early postnatal life.<sup>49</sup> As a result,

there are differences in brain neurobiology and structure (sexual dimorphism) between men and women. It is generally accepted that this mechanism is responsible for the observation that the analgesic potency of morphine and endogenous opioids is higher in men than in women<sup>4,5,8-12</sup> and the finding that the sexes modulate pain using neurochemically and genetically distinct mechanisms.<sup>1,2</sup> This mechanism implies intrinsic sex-specific differences in the central processing of information from activated MOR in brain areas directly or indirectly involved in respiratory control. Candidate areas are the locus ceruleus and the median preoptic area because both may be specially involved in ventilatory control, possess MOR, and show sexual dimorphism.<sup>50-55</sup>

We cannot exclude that, apart from sex-dependent genetic determinants, sex-independent factors also may have played a role in the outcome of our study. Some indication for this is shown in figure 3. Morphine caused a decrease of both the hypoxic and hypercapnic responses of >20% in 15 participants (4 men, 11 women) compared with 9 volunteers who had relatively unaffected hypoxic or hypercapnic responses.

In conclusion, we observed qualitative differences in morphine-induced depression of respiration as determined by ventilatory carbon dioxide and oxygen responses between men and women. Although we cannot exclude sex differences in the bioavailability of morphine to the brain or the involvement of non-sex-related determinants, we hypothesize that the observed differences are caused by sex differences in the central nervous system response to activated MOR involved in the induction of respiratory depression. Our study raises many questions and issues for further study. For example: How do our results relate to the influence of endogenous opioids on respiratory control? Is it possible to extrapolate our findings to all drugs acting at MOR and agents acting at  $\kappa$ - and  $\delta$ -opiate receptors? Is there a sex difference in the magnitude of the secondary depression of  $\dot{V}_I$  due to (long-term) hypoxia after morphine?<sup>56</sup> Our findings warrant further study of the sex-dependent and sex-independent genetic factors on opioid- and anesthetic-induced respiratory depression. Inbred strains of mice and rats may serve as the best model for this purpose.

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