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# Respiratory Effects of Morphine in Awake Unrestrained Rats[1]

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## ABSTRACT

This report describes a systematic analysis of opiate drug effects on ventilation and its components tidal volume and frequency in intact, awake and unrestrained rats. A whole-body plethysmographic method was used to measure these parameters of respiration while animals breathed air or various concentrations of $CO_2$ in air. Subcutaneous doses of morphine lower than 40 mg/kg exerted little or no apparent effect in rats breathing air; in rats breathing 4 to 8% of $CO_2$ these doses of morphine also failed to depress any of the ventilatory parameters below the level of saline controls breathing air. Doses (0.16 to 160 mg/kg) of morphine blunted the frequency response to $CO_2$ in a biphasic manner. The effects of morphine on tidal volume consisted of a slight increase at 0.16 and 0.63 mg/kg, a dose-dependent decrease at 2.5 to 40 mg/kg and a paradoxical rise at 160 mg/kg. These complex effects of morphine on tidal volume and frequency resulted in a simple sigmoid depression of minute volume. The slope of this sigmoid dose-response curve varied with the inspirate; it increased as the concentration of $CO_2$ was higher. Naloxone antagonized the frequency depression produced by 40 mg/kg of morphine in a dose-dependent manner at doses ranging from 0.01 to 0.16 mg/kg, but frequency decreased again at 0.63 mg/kg. The effects of naloxone on the tidal volume depression consisted of a paradoxical further decrease at 0.01 mg/kg, a dose-dependent antagonism of depression at 0.04 to 0.16 mg/kg and a stimulation above the normal control level at 0.63 mg/kg. These complex effects of naloxone on tidal volume and frequency resulted in a simple sigmoid antagonism of the minute volume depression produced by morphine. These and other experiments support the hypothesis that opiates depress the ventilatory response to $CO_2$, but several experimental conditions were identified in which the opiate action on minute volume was effected by intricate and perhaps paradoxical effects on tidal volume and frequency. The assumptions 1) that tidal volume is the sole directly controlled output variable of the $CO_2$ controller and 2) that opiates decrease the sensitivity of the $CO_2$ controller, do not seem to account in a parsimonious manner for all of the complex effects which opiates may exert on tidal volume and frequency of breathing in rats.

The respiratory effects that morphine-like drugs exert in humans and other mammals are thought (Borison, 1977b) to reflect an action of the opiates on the tidal volume and frequency control mechanisms of the respiratory center. Opiates presumably decrease the sensitivity of central chemoreceptors to changes in arterial pH which are due to changes in $PaCO_2$ (Mueller *et al.*, 1982). The opiate action is probably initiated in the medullary areas involved in the control of respiration in which opiate binding sites appear in high concentrations (Morin-Surun *et al.*, 1984a,b).

This report presents a systematic and somewhat detailed analysis of the effects of morphine on minute volume and its components: tidal volume and frequency. Ventilation was examined while the animals breathed air or various concentrations of $CO_2$ in air; this is because 1) opiates affect ventilation in subjects breathing air (Eckenhoff *et al.*, 1960; Jennett, 1968; Jordan, 1982; Kokka *et al.*, 1965; Ling *et al.*, 1983; Mueller *et al.*, 1982) whereas 2) ventilation may be more sensitive to opiates in subjects that are being exposed to a $CO_2$ challenge (*e.g.*, Lai *et al.*, 1978). Intact and unanesthetized subjects were used 1) to match the conditions in which the analgesic effects of opiates are typically examined and 2) to avoid the confounding influence that surgery, restraint, handling and anesthesia may have on ventilation (Borison, 1978; Brun-Pascaud *et al.*, 1982; Cushny, 1913; Eckenhoff and Oech, 1960; Isom *et al.*, 1969; Jordan, 1982; Lai *et al.*, 1978).

The purpose of the present study was to complement earlier, more fragmentary reports in which some aspects of the ventilatory effects of morphine were examined utilizing a limited range of inspired $CO_2$ and in diverse experimental conditions (*e.g.*, Arieli and Ar, 1979; Kokka *et al.*, 1965; McGilliard and Takemori, 1978; Wheeler and Farber, 1983). All experiments presented here were conducted in a constant set of experimental conditions and utilized a wholebody plethysmographic method; this is likely the only practical method for the investigation of patterns of ventilation in a manner which is wholly noninvasive and has no intrinsic effects on ventilation (Epstein and Epstein, 1978).

## Materials and Methods

**Animals.** The subjects were male Wistar rats weighing between 250 and 300 g; they arrived newly from the breeding quarters and, where

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necessary, were housed individually in standard rodent cages and had free access to food and water. Animals were used only once.

**Measurement of respiration.** Steady-state ventilation in rats breathing air or different concentrations of $CO_2$ in air was measured by means of a modification (Bartlett and Tenney, 1970) of the whole-body plethysmographic technique described by Drorbaugh and Fenn (1955). The animal was placed into an airtight, cylindrical 7-liter Plexiglas chamber and allowed to move about freely. It was left undisturbed for about 1 hr, during which time it could adapt to the new environment while the chamber was flushed with humidified air at a flow of about 5 liter/min. If injections were to be given, the animal was removed, injected and placed back into the chamber. The chamber was then flushed again with humidified air or the required gas mixture. The exposure to any gas mixture lasted at least 10 min before measurements were carried out. This exposure time served to reach ventilatory ($\dot{V}_E$, $V_T$, $f$) steady state; respiratory steady state ($Pa_{O_2}$, $Pa_{CO_2}$, pH) is also achieved under these conditions (Cragg and Drysdale, 1983; Polianski *et al.*, 1984). It may be of interest to note here that no evidence of fatigue was observed in preliminary experiments in which untreated rats were exposed continuously to 8% of $CO_2$ in air during a 2-hr period.

After the inlet and outlet valves had been closed, changes in pressure between the animal chamber and an identical reference chamber were measured by means of a sensitive differential transducer (Validyne). The transducer's output was amplified and registered on graph paper. In this method, increases in pressure difference arise from the inhaled air's being heated and saturated further with water vapor; decreases arise from the cooling and condensation which occurs during expiration. In the course of each measurement, 0.25 ml of air was extracted from and later reinjected rapidly at end-expiration into the animal chamber for the purpose of calibration.

The two chambers were connected by a leak that had a half-life time of about 9.0 sec; the leak served to let leak away slow pressure differences such as those which occurred when the ambient temperature in the animal chamber increased.

Tidal volume ($V_T$, in milliliters) was computed by means of the equation:

$$V_T = \frac{P_T}{P_K} \times V_K \times \frac{T_B\,(P_B - P_C)}{T_B\,(P_B - P_C) - T_c\,(P_B - P_R)}$$

in which:

- $P_T$ = The pressure deflection associated with each tidal volume; the mean of at least six breaths was determined for this purpose.
- $P_K$ = The pressure deflection associated with the injection of the 0.25 ml calibrating volume ($V_K$).
- $T_B$ = Body temperature in °K. Oesophageal temperature was determined to the nearest 0.1°C before and after measurement; the temperature at the time that recordings were made was estimated by interpolation between these two values.
- $P_B$ = Barometric pressure, measured to the nearest 1 mm of Hg.
- $P_C$ = Vapor pressure of water at $T_C$, in millimeters of mercury.
- $T_C$ = Animal chamber temperature; it was measured to the nearest 0.1°C by means of four sensors that were suspended crosswise on the ceiling of the chamber. The means of these four readings was used and expressed in °K. Chamber temperature varied from about 22–25°C.
- $P_R$ = Vapor pressure of water at $T_B$ in millimeters of mercury.

Any given running record had a length of about 1 min and allowed $V_T$ to be computed at least 3 times from different samples each of which showed at least six breaths and a calibration. The sample yielding the median $V_T$ was used in data analysis. Ventilatory frequency ($f$; breaths per minute) was read directly from the pressure record. Minute volume ($\dot{V}_E$, in milliliters per minute per 100 grams) was adjusted for body weight and obtained from the equation:

$$\dot{V}_E = \frac{V_T \times f \times 100}{\text{B.W.}}$$

where body weight (B.W.) was expressed in grams.

All measurements were carried out while the animals appeared quiet but awake. Seemingly sleeping animals were aroused by a gentle knock on the chamber.

**$CO_2$ response curve.** Four groups of nine rats each were used. After the 1-hr adaptation period, the four groups were exposed, the first to 0, 1, 5 and 9, the second to 0, 2, 6 and 10, the third to 0, 3, 7 and 11 and the fourth to 0, 4, 8 and 12% of $CO_2$ in air. The sequence in which the animals were exposed to the four different inspirates was random.

The concentration of $O_2$ varied from 20.0% in the 0% $CO_2$ inspirate to 17.6% in the 12% $CO_2$ inspirate; an hypoxic drive is unlikely to occur in these conditions (Arieli and Ar, 1979; Holloway and Heath, 1984; Pepelko and Dixon, 1975).

**Morphine: time-effect relationship.** Rats were injected with either 10 mg/kg of morphine or saline ($n$ = 7 per group). Ventilation while animals breathed 4 and 8% $CO_2$ in air mixtures was measured at 1, 2, 3, 4, 6 and 8 hr after injection.

**Morphine: dose-effect relationship.** Rats were injected with doses of morphine ranging from 0.16 to 160 mg/kg or saline ($n$ = 7 per dose). The animals were exposed to air and 4, 6 and 8% of $CO_2$ in air mixtures, in random sequence. These exposures followed one after the other with intervals of about 10 min; the measurements were carried out between about 40 and 80 min after injection.

**Effects of naloxone.** Rats were injected with 0.63 mg/kg of naloxone or saline ($n$ = 7) and exposed to air and 4, 6 and 8% $CO_2$ in air mixtures, in random sequence. Measurements were carried out 30, 45, 50 and 75 min after injection. A second series of rats were injected with doses of naloxone ranging from 0.0025 to 0.63 mg/kg or saline ($n$ = 5 per dose) and, immediately thereafter, with 40 mg/kg of morphine. Animals were exposed to 4, 6 and 8% $CO_2$ in air mixtures, in random sequence. Measurements were carried out between 40 and 80 min after injections.

**Stereoisomeric compounds.** Rats ($n$ = 5 per dose) were injected with either 10 mg/kg of dextromoramide or 40 mg/kg of levomoramide. Animals were exposed to air and 4 and 8% $CO_2$ in air mixtures, in random sequence. Measurements were carried out at 45, 60 and 75 min after injection.

**Tolerance.** Rats were injected twice daily during 4 consecutive days with either 10 mg/kg of morphine or saline ($n$ = 5 per group). On the 5th day all rats were injected 10 mg/kg of morphine and exposed to air and 4, 6 and 8% $CO_2$ in air mixtures, in random sequence. Measurements were carried out 30, 45, 60 and 75 min after injection.

**Drugs.** All drugs were freshly prepared as aqueous solutions on the day of the experiment and injected s.c. in a volume of 1 ml/100 g b.wt.

The drugs being used were morphine HCl, naloxone HCl, levomoramide tartrate and dextromoramide tartrate. Doses were selected on the basis of preliminary experiments and are expressed in milligrams per kilogram of the salt (morphine and naloxone) or base (levomoramide and dextromoramide).

## Results

**$CO_2$ response curve.** The 36 animals breathing air in experiment 1 showed an average frequency of breathing of 100 breaths/min (S.E.M.: ±3.3). Tidal volume being 2.32 (±.06) ml, minute volume of ventilation averaged 76.9 (±2.61) ml/min/100 g. These base-line values correspond reasonably well with earlier measurements of tidal and minute volume by means of the wholebody plethysmographic method used here (Bartlett and Tenney, 1970; Hughson, 1980; Lai *et al.*, 1978, 1981; Maskrey *et al.*, 1981; Nattie, 1977; Olson and Dempsey, 1978; Pappenheimer, 1977).

The effects of different percentages of inspired $CO_2$ are shown in figure 1. Frequency of breathing started to rise from 2% $CO_2$ on and reached an apparent peak at 9% $CO_2$; $CO_2$ concentrations beyond 9% failed to cause a further increase in frequency. One to 3% of $CO_2$ exerted no apparent effect on

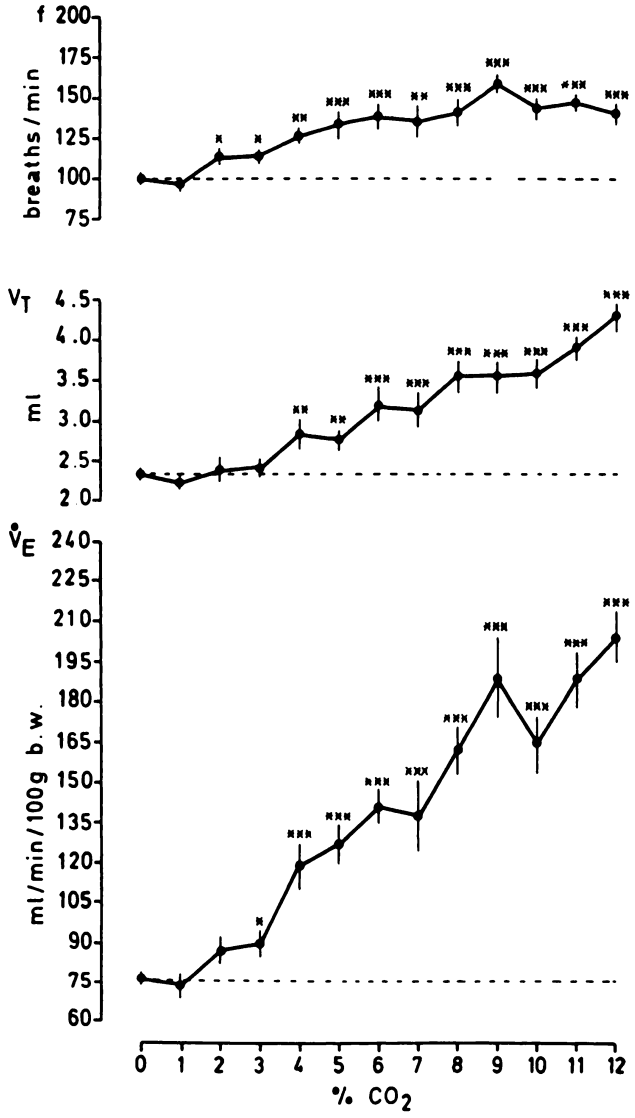


**Fig. 1.** Effects of different concentrations of inhaled carbondioxide on frequency ($f$), tidal volume ($V_T$) and minute volume ($\dot{V}_E$) of respiration in rat. Each data point represents the mean (±1 S.E.M.) of nine animals. The asterisks refer to the difference from animals breathing air and indicate one-tailed probability to be P ≦ .05 (*), P ≦ .01 (**) or p ≦ .005 (***) (Mann-Whitney $U$ test).

tidal volume. From 4% on, tidal volume increased as a function of the percentage of inspiratory $CO_2$; the stimulatory effect continued to augment at concentrations up to 12%. As a result of these increases in frequency and tidal volume, minute volume of ventilation increased at 3% $CO_2$ and continued to do so up to 9%; the average values of the 9 to 12% $CO_2$ data points did not appear to differ greatly.

Regression lines were computed through those data points along the $CO_2$ response curves that yielded statistical significance (P < .05). For the purpose of these computations, experimental values were converted into percentages relative to the 0% $CO_2$ data point; the conversion served to account for the differences in the absolute values in which the three parameters of ventilation are expressed. The parameter $b$ of the regression equation $y = a + bx$ was 3.27, 7.45 and 14.39 for the curves of frequency, tidal volume and minute volume, respectively. It thus appears that minute volume was the most responsive to $CO_2$ challenge among the three parameters of ventilation being examined.

To characterize the $CO_2$ response curve, further experiments utilized 4, 6 and 8% $CO_2$ for the following reasons: 1) 4% was the lowest percentage which stimulated both frequency and tidal volume significantly and 2) 8% $CO_2$ was the highest percentage which, for both frequency and tidal volume, was still in the rising segment of the $CO_2$ response curve at a point at which the curve had not yet reached its ceiling.

**Morphine: time-effect relationship.** One hour after injection, morphine reduced minute volume (not shown) by 36% relative to saline controls on exposure to 4% $CO_2$ (P < .01), and by 45% on exposure to 8% $CO_2$ (P < .005). At none of the other intervals (*i.e.*, 2–8 hr) were there any significant differences with saline controls in this experiment. Morphine thus appeared to exert its peak effect on respiration 1 hr after s.c. injection (see also: de Vry *et al.*, 1981; Isom *et al.*, 1969; Kokka *et al.*, 1965), and observations in further experiments involving morphine were made at or about 60 min after injection of the drug.

**Morphine: dose-effect relationship.** The effects of different doses of morphine on frequency, tidal volume and minute volume in rats breathing air and 4, 6, and 8% $CO_2$ are summarized in figure 2.

Doses of morphine up to 40 mg/kg had no reliable effect on frequency while the rats breathed air, and only at 160 mg/kg was frequency slightly decreased (P = .062). Tidal volume, on air, was not reliably affected by doses of morphine up to 10 mg/kg. Both the 40- and the 160-mg/kg dose produced a reliable decrease, but the apparent dose-response curve assumed a biphasic shape. Minute volume, on air, was increased slightly at 0.16 mg/kg (P > .05); it was lower than control at 40 (P = .062) and 160 mg/kg (P < .05), but the magnitude of the depression was almost equal at these doses.

More marked effects of morphine on respiration became apparent after exposing the animals to $CO_2$.

Doses of morphine lower than 160 mg/kg reduced significantly frequency at 4% (2.5 and 10 mg/kg), 6% (0.16–40 mg/kg) and 8% $CO_2$ (2.5–40 mg/kg). The morphine dose-response curve was biphasic in that the slope of the 0.63 to 40 mg/kg segment of the curve was shallower than that of the 40 to 160 mg/kg segment. The curve's shape changed, however, as exposure to $CO_2$ increased; the difference in slope between the two segments appeared to diminish as the percentage of $CO_2$ challenge was larger (fig 2).

Exposure to 4, 6 and 8% $CO_2$ also revealed biphasic effects of morphine on tidal volume. Lower doses (*i.e.*, 0.16 and 0.63 mg/kg) slightly increased tidal volume at all $CO_2$ challenges, but at no point was this increase statistically significant. Ten and 40 mg/kg of morphine decreased tidal volume in all gas conditions in a dose-related manner whereas, in all gas conditions, tidal volume at 160 was larger than at 40 mg/kg (P < .05; <.01; <.01; and <.01 at 0, 4, 6 and 8% $CO_2$, respectively).

As a result of these complex effects on frequency and tidal volume, morphine's effects on minute volume also gave rise to a biphasic dose-response curve. That is, the slope of the 0.63- to 40-mg/kg segment was steeper than that of the 40- to 160 mg/kg segment, and the difference seemed to become more marked as the percentage of $CO_2$ challenge was larger (fig. 2).

Using the method of least of sum of squares, regression lines

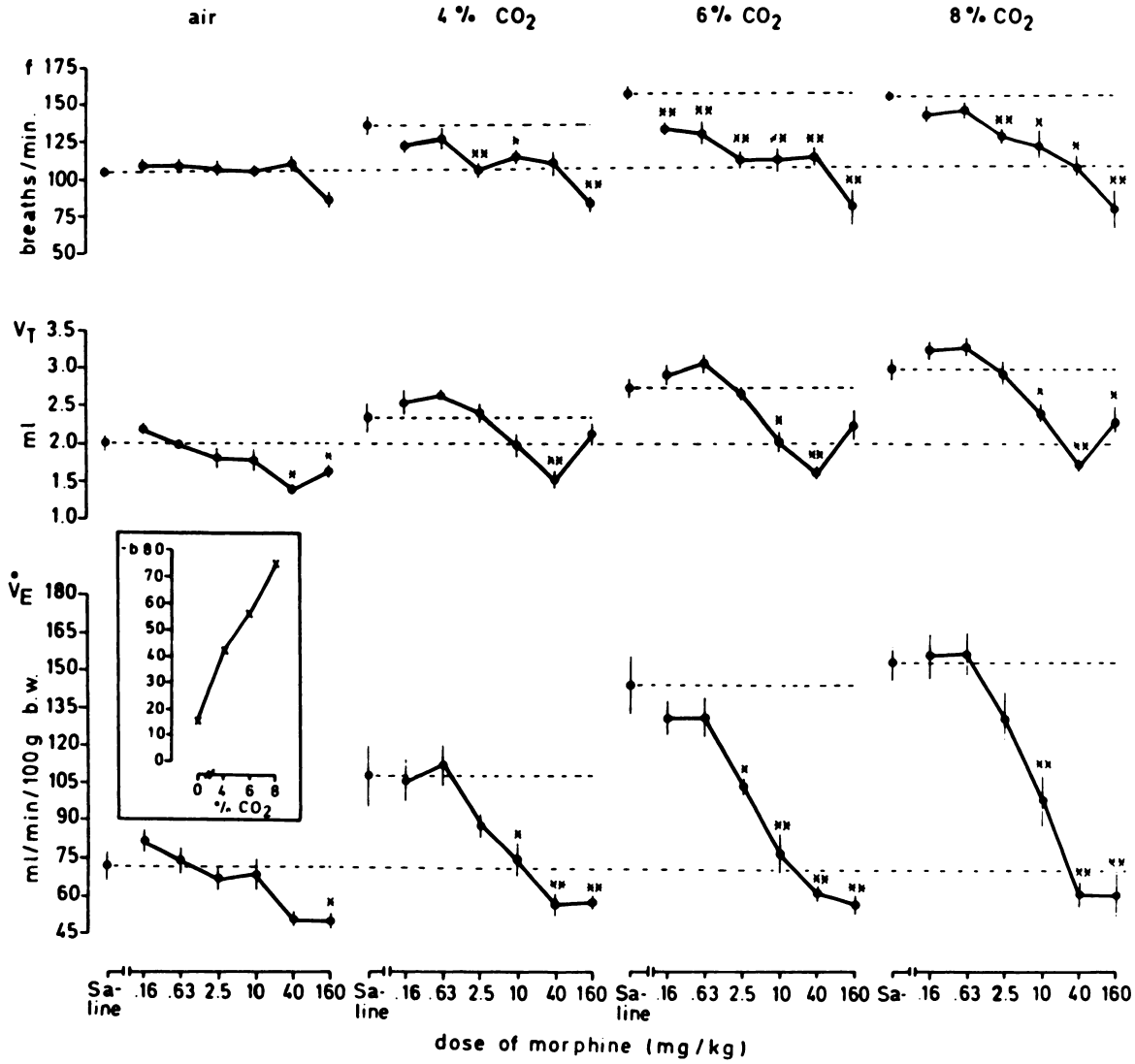


**Fig. 2.** Dose-response curves of s.c. morphine on frequency ($f$), tidal volume ($V_T$) and minute volume ($\dot{V}_E$) of breathing while rats were breathing air or 4, 6 and 8% $CO_2$ in air mixtures. Each data point is the mean ±1 S.E.M. of seven animals per dose of morphine or saline. Saline values are represented by horizontal broken lines. Asterisks refer to the difference with saline controls at the same gas mixture, and indicate two-tailed probability $P \leq .05$ (*) or $P \leq .01$ (**) (Mann-Whitney $U$ test). Insert: slope of the morphine dose-response curve for minute volume as a function of the concentration of $CO_2$ in the inspirate. $b$ represents the parameter $b$ in the regression equation $y = a + bx$; the equation was resolved (in log-linear coordinates) for doses 0.63, 2.5, 10 and 40 mg/kg.

were computed for mean frequency, tidal volume and minute volume values with each of the different gas mixtures; these values were converted into percentages relative to the 0% $CO_2$ data points for each dose of morphine. A decrease in the slope of the $CO_2$ response curve of frequency, tidal volume and minute volume occurred at doses of morphine in excess of 2.5 mg/kg (fig. 3). A steep and linear decrease in slope occurred at doses from 2.5 and 40 mg/kg; a ceiling then appeared to occur at 40 and 160 mg/kg.

Morphine's dose-response curve on frequency slopes was similar to its curve on tidal volume slope. Morphine's response curve on minute volume slope, however, was considerably steeper, thus indicating that minute volume of ventilation constituted the most sensitive measure of the drug's ventilatory effects.

**Effects of naloxone.** In rats breathing 8% of $CO_2$ 0.0025 mg/kg of naloxone exerted no apparent effect on the depression of all parameters of ventilation produced by 40 mg/kg of morphine (fig. 4). However, at 0.01 mg/kg, frequency was somewhat increased ($P = .075$) whereas tidal volume was reliably decreased ($P < .05$) relative to the saline *plus* morphine control group (fig. 4). From this dose on, tidal volume increased as a function of naloxone dose, and the antagonism was complete at 0.16 mg/kg. At 0.63 mg/kg, however, tidal volume increased further to reach a value greater ($P < .05$) than saline *plus* saline-treated animals. Doses of naloxone (0.01–0.16 mg/kg) also increased frequency relative to that in saline *plus* morphine-treated rats, and frequency in rats pretreated with 0.16 mg/kg of naloxone no longer differed ($P > .05$) from saline *plus* saline control. At 0.63 mg/kg, however, frequency again

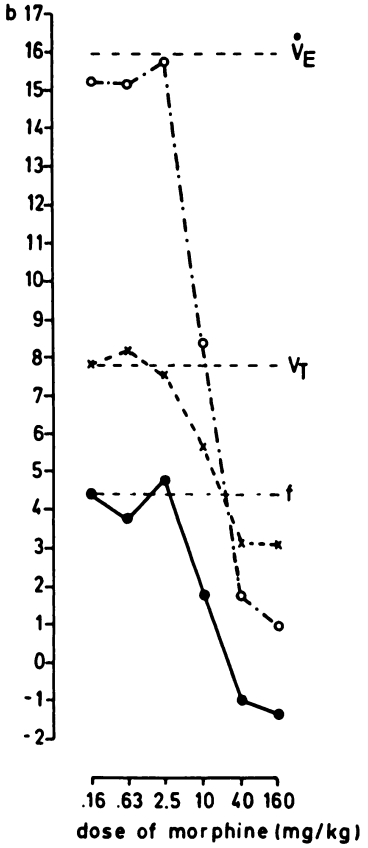


**Fig. 3.** Dose-response curves of s.c. morphine on the slope $b$ ($y = a + bx$) of the carbondioxide response curve (4, 6, and 8% $CO_2$) for frequency ($f$) (●), tidal volume ($V_T$) (×) and minute volume ($\dot{V}_E$) of respiration (○). For each dose of morphine and saline all parameters on 4, 6, and 8% $CO_2$ ($f$, $V_T$ and $\dot{V}_E$) are expressed in values relative to their 0% $CO_2$ values. Regression lines were computed with the method of least sum of squares.

decreased; at this point, the antagonist effect failed to reach statistical significance, whereas frequency again differed significantly from the saline *plus* saline control value.

The result of these complex effects of naloxone on frequency and tidal volume was that the drug antagonized the morphine-induced depression of minute volume in a surprisingly orderly manner; minute volume in naloxone *plus* morphine-treated animals no longer differed from that in the saline *plus* saline control group at 0.16 and 0.63 mg/kg of naloxone.

Results obtained at 4 and 6% $CO_2$ (not shown) were generally consistent with those obtained at 8%; the dose-response curve of naloxone in affecting the ventilatory effects of morphine showed a similar intricacy in the 0.01 to 0.63 mg/kg range of naloxone dose.

At 0.63 mg/kg, naloxone had no apparent intrinsic effect on either parameter of ventilation in rats breathing air or 4, 6 or 8% of $CO_2$ (data not shown).

**Stereoisomeric compounds.** Data obtained with 10 mg/kg of dextromoramide and 40 mg/kg of levomoramide are summarized in figure 5. While breathing air, rats injected with dextromoramide showed a significant decrease in frequency compared with saline controls ($P < .05$). There was no effect on tidal volume but the effect on minute volume was again reliable ($P < .05$). After exposure to $CO_2$, rats injected with dextromoramide were completely unresponsive to $CO_2$ (slope of minute volume response curve: $b = -0.3$).

Levomoramide (40 mg/kg) induced a significant increase in frequency while the animals breathed air ($P < .01$). This increase in frequency also occurred during $CO_2$ exposure. Levomoramide had no significant effect on tidal volume either during air breathing or after $CO_2$ exposure. The net result was that levomoramide produced an increase in minute volume of breathing which proved to be significant after exposure to 8% $CO_2$ ($P < .05$).

**Tolerance.** This experiment examined the effects of 10 mg/kg of morphine after rats had been treated twice a day during 4 days with either 10 mg/kg of morphine or saline.

The ventilation of the two groups was similar upon exposure to air. Upon exposure to $CO_2$, frequency of breathing in the experimental group was at no point higher ($P > .05$) than in the control group (fig. 6). In contrast, tidal volume in the experimental group exceeded the control tidal volume at all concentrations of inhaled $CO_2$. The difference was significant at 6 and 8% $CO_2$. As a consequence, minute volume of the

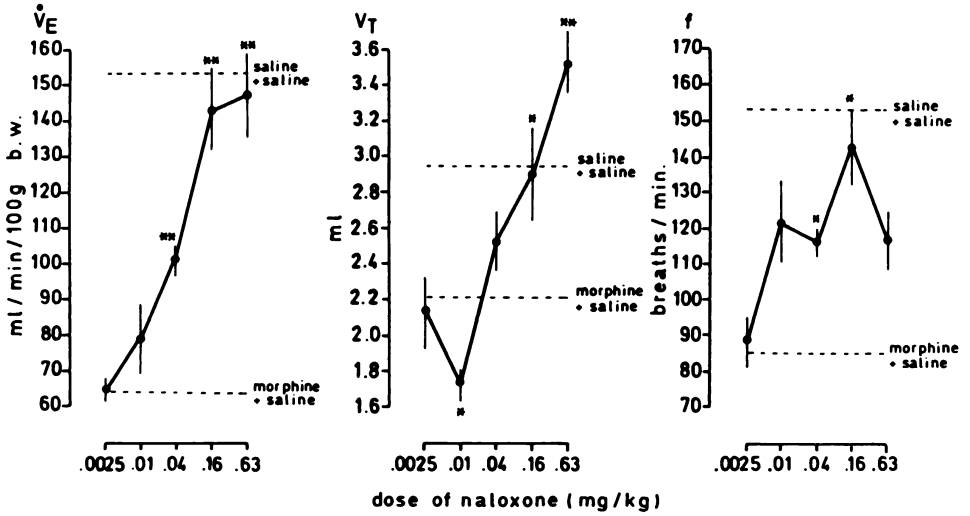


**Fig. 4.** Effects of increasing doses of s.c. naloxone (0.0025–0.63 mg/kg) on the respiratory effects of 40 mg/kg of s.c. morphine. Each data point represents the mean ±1 S.E.M. of five animals per dose of s.c. naloxone on minute volume ($\dot{V}_E$), tidal volume ($V_T$) and frequency ($f$) after exposure to 8% $CO_2$ in air. Saline-morphine and saline-saline controls ($n = 5$ per group) are given in horizontal lines. Asterisks refer to the difference with the saline-morphine control group and indicate one-tailed probability to be $P \leq .05$ (*) and $P \leq .01$ (**) (Mann-Whitney $U$ test).

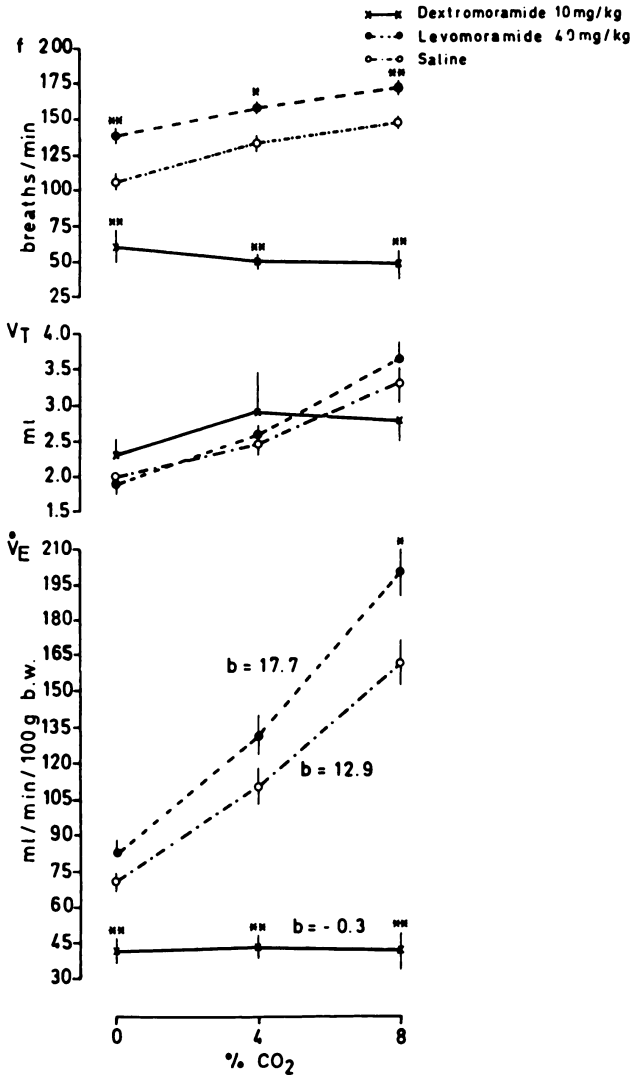


Fig. 5. Respiratory effects [frequency ($f$), tidal volume ($V_T$) and minute volume ($\dot{V}_E$) of breathing] of 10 mg/kg s.c. dextromoramide (×) or 40 mg/kg s.c. levomoramide (●) and saline controls (○). Each data point represents the mean ±1 S.E.M. of five animals per group after exposure to 0, 4, and 8% $CO_2$ in air. Asterisks refer to the differences from the saline control group and indicate two-tailed probability to be $P \leq .05$ (*) and $P \leq .01$ (**) (Mann-Whitney $U$ test). The corresponding slopes of the carbondioxide response curve (4–8% $CO_2$) for minute volume of breathing are indicated.

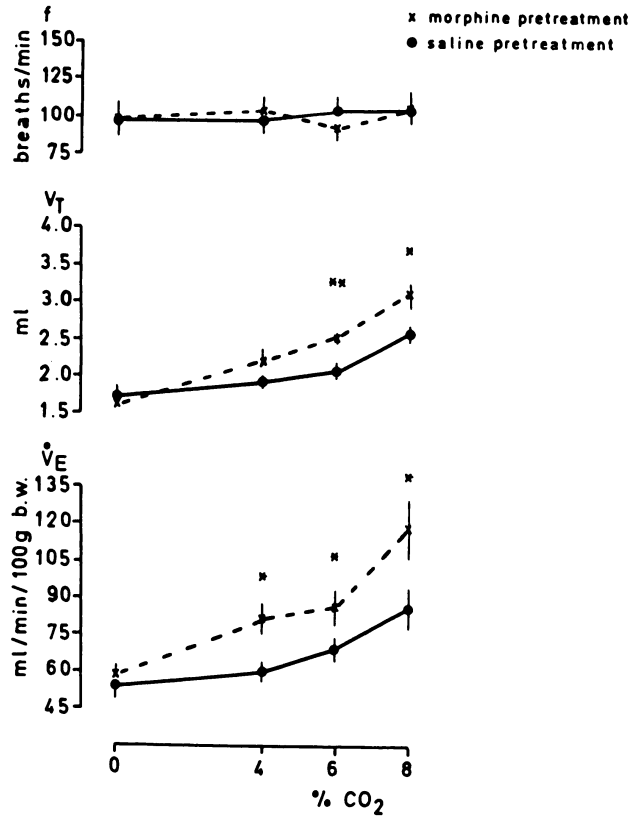


Fig. 6. Effects of pretreatment during 4 days with 10 mg/kg s.c. morphine twice daily on minute volume ($\dot{V}_E$) of breathing 1 hr after 10 mg/kg s.c. morphine. Each data point represents the mean ±1 S.E.M. of seven animals after exposure to 0, 4, 6 and 8% $CO_2$ in air. The asterisks refer to the difference between the morphine- and saline-pretreated group and indicate one-tailed probability to be $P \leq .05$ (*) (Mann-Whitney $U$ test). $f$, frequency; $V_T$, tidal volume.

experimental animals reliably exceeded that of the controls at all three concentrations of $CO_2$ (fig. 6).

**Drug effects on body temperature.** The measurement of ventilation required that body temperature be determined. These data revealed the following effects. Relative to saline control (mean: 37.3 ± 0.3°C), morphine lowered ($P < .05$) temperature at 40 (36.1 ± 0.4°C) and 160 mg/kg (36.5 ± 0.6°C), but not at lower doses. Naloxone (0.63 mg/kg) exerted no intrinsic effect (37.5 ± 0.1°C), but antagonized the hypothermia produced by 40 mg/kg of morphine (saline *plus* morphine control group: 36.7 ± 0.4°C) at 0.16 (38.1 ± 0.2°C; $P < .05$) and 0.63 mg/kg (37.8 ± 0.2°C; $P < .05$). Dextromoramide (10 mg/kg) (34.8 ± 0.4°C), but not 40 mg/kg of levomoramide (37.5 ± 0.2°C) also decreased ($P < .05$) temperature. Other manipulations or drug administrations had no significant effect.

## Discussion

The first of the experiments reported here characterized the effects on frequency, tidal volume and minute volume of exposing untreated rats to concentrations of $CO_2$ in the inspirate that varied from 0 to 12%. Similar to results obtained by Lai *et al.* (1978), exposure to $CO_2$ increased minute volume; the effect was reliable from 3% $CO_2$ on, and then increased further in the 3 to 9% $CO_2$ range (fig. 1). The minute volume increase in response to $CO_2$ resulted from an increase in both frequency and tidal volume which occurred from 2 and 4% $CO_2$ on, respectively. Stimulation by inspired $CO_2$ of frequency without change in tidal volume has been observed earlier in rodents (Holloway and Heath, 1984; Maskrey *et al.*, 1981) and dogs (Bartoli *et al.*, 1974), and may reflect the direct effects that airway $CO_2$ has on stretch receptor activity (Bartlett and Sant'Ambrogio, 1976; Bartoli *et al.*, 1974). Frequency reached an apparent ceiling averaging about 150 breaths/min at 9 to 12% $CO_2$; tidal volume continued to increase at $CO_2$ concentrations of up to 12%.

Rats breathing air were essentially unresponsive to doses of morphine lower than 40 mg/kg (see also: Isom *et al.*, 1969; Kokka *et al.*, 1965), but marked effects of morphine on ventilation did become apparent when the animals were exposed to 4 to 8% of $CO_2$. Morphine diminished in a dose-dependent

manner the stimulating effects of $CO_2$ on frequency; this effect was at all times reliable at 2.5 mg/kg, but doses as low as 0.16 mg/kg were also effective with some inspirates (fig. 2). Across all three concentrations of $CO_2$, 0.16 and 0.63 mg/kg of morphine increased tidal volume, be it that this increase failed to reach statistical significance. Morphine reliably blunted the tidal volume response to $CO_2$ at 10 and 40 mg/kg, but 160 mg/kg exerted less effect (fig. 2). The latter is difficult to interpret, but two considerations may perhaps be relevant. One is that the paradoxical increase in tidal volume which occurred at 160 mg/kg compensates the abrupt decline in frequency which appeared at this dose. A second consideration is that the tidal volume effects of morphine may perhaps be proportional to the effects of the drug on metabolic rate; morphine depresses metabolic rate, and its dose-response curve in exerting this action is also biphasic (Kokka *et al.*, 1965; Nelson and Elliot, 1967).

As a result of these complex effects on frequency and tidal volume, the effects of morphine on minute volume had the following characteristics: 1) morphine exerted little effect in rats breathing air, and only a dose as high as 160 mg/kg reliably depressed minute volume. 2) Morphine blunted the minute volume response to $CO_2$ exposure; its dose-response curve in exerting this effect was biphasic, and the animals became entirely unresponsive to $CO_2$ at doses of 40 to 160 mg/kg. The blunting of the minute volume response to $CO_2$ 3) was dynamic and linear in the 0.63 to 40 mg/kg range of doses, irrespective of the $CO_2$ concentration to which the animals were exposed. However, 4) the slope of the morphine dose-response curve was steeper as the $CO_2$ concentration was higher (fig. 2). According to pharmacological theory (Ariëns, 1964), the slope of a dose-response curve is determined by the characteristics of the drug-receptor interaction that is involved, and it is not immediately apparent by what mechanism the $CO_2$ concentration of the inspirate may determine the slope of the dose-response curve. One possible explanation may be that exposure to various concentrations of $CO_2$ results in changes in the pH of cerebral blood and cerebrospinal fluid; pH changes may profoundly influence the receptor binding (Pert and Snyder, 1973) and, presumably therefore, the pharmacological activity of opiates.

Naloxone antagonized the effects of morphine on minute volume in a dose-dependent manner (fig. 4). This finding is consistent with abundant evidence (*e.g.* Flórez and Pazos, 1982; Holaday, 1982; McGilliard and Takemori, 1978; Pazos and Flórez, 1983) that naloxone blocks the respiratory effects of opiates in laboratory animals. However, further analysis of the data reveals that this particularly orderly antagonism of minute volume depression was effected through complex interactions of naloxone with frequency and tidal volume. That is, the slight antagonism of minute volume depression which occurred with 0.01 mg/kg of naloxone resulted from an increase by about 50% in frequency which was counteracted in part by a paradoxical further decrease in tidal volume (fig. 4). In contrast, the near complete antagonism of minute volume depression at 0.63 mg/kg of naloxone resulted from a stimulation of tidal volume above the normal control level, whereas frequency was again depressed relative to this level. These complexities did not seem to result from a possible intrinsic action of naloxone as naloxone alone exerted no reliable effects in the present experiments. The latter is consistent with findings in awake cats (Wheeler and Farber, 1983). However, naloxone increased the minute volume response to $CO_2$ in anesthetized rabbits (Buebler, 1980) and stimulated frequency and tidal volume in stressed rats (Isom and Elshowihy, 1982). These apparent differences in intrinsic ventilatory effects of naloxone highlight the necessity of studying ventilation in conditions in which such variables as stress and level of anesthesia are strictly controlled (Hayashi *et al.*, 1983).

Prior treatment with 10 mg/kg twice daily during 4 days induced significant tolerance to the minute volume depression produced by 10 mg/kg of morphine. Interestingly, this tolerance resulted from tolerance to the depression of tidal volume, whereas frequency in tolerant rats was similar to that in nontolerant animals (fig. 6). It is, again, conceivable that these changes in tidal volume, unlike frequency, relate to changes in metabolic rate; Kokka *et al.* (1965) reported that tolerance developed to the depression of metabolic rate produced by 10 mg/kg after rats had been exposed to a similar schedule of drug administration.

Current concepts of $CO_2$ regulation (Borison, 1977a) specify that tidal volume is the sole directly controlled output variable of the $CO_2$ controller. Frequency is adjusted through proprioceptive feedback in the vagus nerve that is elicited at pulmonary stretch receptors and acts upon the brain stem respiratory pacemaker. Opiates are thought (Mueller *et al.*, 1982) to decrease the sensitivity of medullary chemoreceptors to changes in arterial pH that are due to changes in $PaCO_2$. Opiates must be expected, therefore, to alter the tidal volume response to $CO_2$, while changes in frequency presumably reflect secondary adjustments to an altered tidal volume. Some of the present findings are not readily explained by this proposed mechanism: 1) relative to 40 mg/kg, 160 mg/kg of morphine increased tidal volume with each of the four inspirates used, while frequency continued to decrease. 2) When given as a pretreatment before 40 mg/kg of morphine, 0.01 mg/kg of naloxone reliably decreased tidal volume while frequency was, if anything, increased. 3) In the same experiment, 0.63 mg/kg of naloxone increased further tidal volume relative to the 0.16-mg/kg dose, while frequency again decreased. 4) Dextromoramide depressed markedly and levomoramide increased frequency in the absence of any detectable effect on tidal volume. 5) Tolerance developed to the tidal volume depression produced by 10 mg/kg of morphine while frequency remained depressed. These findings suggest that opiates may not only decrease chemoreceptor sensitivity to $CO_2$ (Mueller *et al.*, 1982), but interact in a complex manner with the control of both frequency and tidal volume (Borison, 1977b). The mechanisms of these interactions are unclear, but it is not inconceivable that opiate effects on, for example, metabolic rate (Kokka *et al.*, 1965; Nelson and Elliot, 1967) and tone of intercostal muscle and diaphragm (Jaffe and Martin, 1980) may contribute indirectly to the observed changes in tidal volume and frequency. While further work is required to resolve the issue, the findings mentioned above make it apparent that the widespread use of frequency in animals breathing air as the sole measure of opiate effects on ventilation (*e.g.* Flórez and Pazos, 1982; Freye *et al.*, 1983; Holaday, 1982; McGilliard and Takemori, 1978; Petts and Pleuvry, 1983; Sitsen *et al.*, 1982; Ward and Takemori, 1983) may obscure several of the complexities of these opiate effects.

In summary, the present report describes a systematic analysis of the effects of morphine on minute volume and its components tidal volume and frequency in awake unrestrained rats that breathed air or 4 to 8% of $CO_2$. Other experiments characterized the $CO_2$ response curves and analyzed the effects of

naloxone on the ventilatory action of morphine. Some aspects of stereospecificity and tolerance were also examined. The data are generally consistent with the hypothesis that opiates depress the minute volume response to $CO_2$, but several conditions were identified in which the opiate interaction with minute volume appeared to be effected by intricate effects on tidal volume and frequency. These complexities perhaps result from multiple respiratory as well as nonrespiratory actions of opiates.

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