

# Comparative Ventilatory Effects of Intravenous Versus Fourth Cerebroventricular Infusions of Morphine Sulfate in the Unanesthetized Dog

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The ventilatory pharmacodynamics of morphine sulfate (MS) in the awake dog ( $n = 14$ ) were investigated. Two routes of MS administration were employed: 1) 4 h continuous intravenous (iv) infusion ( $1 \text{ mg} \cdot \text{kg}^{-1}$  loading dose,  $10 \text{ } \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  thereafter); and 2) fourth ventricle to cisterna magna perfusion (VCP) at increasing infusate morphine concentrations ( $0.1$ – $100 \text{ } \mu\text{g} \cdot \text{ml}^{-1}$ ). The former was associated with a constant plasma and cisternal CSF (and presumably tissue) free morphine concentration. The latter produced, over 1 h at a constant infusate morphine delivery, a cisternal CSF free morphine concentration that leveled off by 30 min, little or no distribution of drug beyond superficial dorsal and superficial ventral brainstem tissue, and no detectable levels of morphine in plasma. When comparing the two routes of administration, ventilatory depression for a given cisternal free morphine level in the iv infusion studies was of a much greater magnitude than that seen in VCP experiments. Differences in the ventilatory patterns were also noted. Thus, iv delivery produced a decrease in tidal volume ( $V_T$ ) and no change or reduced respiratory frequency ( $f$ ) with prolonged exposure. VCP delivery was also associated with reduction in  $V_T$  but produced significant increases in  $f$ . An apparent maximal ventilatory depression with 1 h VCP administration was observed at morphine infusate levels of  $>10 \text{ } \mu\text{g} \cdot \text{ml}^{-1}$ , with higher infusate concentrations and extension of the perfusion period to 3 h producing no significant additional changes. Finally, VCP delivery of the  $\mu$ -antagonist nalbuphine could only partially reverse the ventilatory depression accompanying iv morphine administration. These findings suggest that the ventilatory depression associated with iv morphine is a result of interactions with brain  $\mu$ -opioid receptors in superficial brainstem tissue and in deep brainstem and/or suprapontine tissue as well. (Key words: Anesthetics, intravenous: morphine. Brain: brainstem; respiratory center; ventriculocisternal perfusion. Carbon dioxide. Cerebrospinal fluid. Ventilation: carbon dioxide response.)

THE VENTILATORY DEPRESSANT actions of  $\mu$ -agonist opioids like morphine are well documented.<sup>1</sup> Previous work has suggested that opioid-induced ventilatory depression is a function of an opioid-receptor association with respiratory-related neurons in the brainstem.<sup>1-7</sup> In fact, Taveira da Silva *et al.*<sup>7</sup> concluded from their work

in anesthetized cats, that the ventilatory depression resulting from intravenous diacetylmorphine was, to a substantial degree, mediated at the chemosensitive areas on the ventrolateral medullary surface. On the other hand, the time course of ventilatory depression following intravenous,<sup>8</sup> intrathecal,<sup>9</sup> or epidural<sup>10</sup> morphine in dogs was found to correspond poorly with morphine concentration changes in cerebrospinal fluid (CSF) taken from the cisterna magna. If morphine-induced ventilatory changes were solely mediated at sites on the ventral medullary surface, then one would expect a close correlation between cisternal morphine levels and ventilation. It might be speculated that this lack of correlation is a function of slow morphine equilibration within brainstem tissue<sup>8</sup> or that suprapontine sites are involved.

The present study addresses primarily the question of the relative roles of superficial brainstem and suprapontine or deep brainstem sites in mediating the ventilatory depressant action of morphine. We employed a continuous morphine infusion protocol to minimize problems associated with slow equilibration and nonsteady state conditions. To this end, two routes of morphine sulfate administration in awake dogs were used. The continuous intravenous infusion protocol allowed a constant plasma and cisterna magna CSF (and presumably tissue) free morphine concentration and provided generally for morphine delivery to the entire brain. The fourth cerebral ventricle to cisterna magna perfusion (VCP) procedure produced a constant cisternal CSF morphine concentration with no measurable passage of morphine into blood and essentially involved pontomedullary surface tissue only. By comparing the ventilatory responses as a function of cisternal CSF morphine levels, when employing the above two methods for morphine delivery, we were able to assess the relative influence of pontomedullary surface tissue, in relation to whole brain involvement, in the mediation of the ventilatory depression associated with intravenous morphine administration.

## Materials and Methods

The present study was approved by the Institutional Animal Care and Use Committee. Adult male mongrel dogs were used (25–30 kg). Only dogs that were infection- and parasite-free, displayed a quiet temperament, and readily tolerated handling and prolonged restraint were

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selected for use. The animals were surgically prepared during 1.0–1.5% halothane anesthesia, with indwelling femoral arterial and venous catheters, guide cannulae for insertion of spinal needles into the fourth ventricle and cisterna magna, and a tracheostomy. The guide cannulae were approximately 2 cm in length and consisted of a hollow plastic cylinder (ID 3 mm) covered at one end with a rubber membrane. For insertion of the guide cannulae, the skin and musculature overlying the occipital bone were retracted. For the fourth ventricular cannula, a depression was made into the occipital crest, using a grinding device, and a small portion of the dura overlying the cerebellum was exposed. The fourth ventricular guide was positioned over the midline of the caudal portion of the cerebellum at a rostrally directed angle approximately 120° relative to the sagittal crest and held in place with bone wax. Next, a groove was ground into the occipital bone to accommodate the placement of the cisternal guide cannula. When in place, the cannula was directed toward the atlanto-occipital membrane. Eight to 12 stainless steel screws were randomly inserted into the bone to facilitate adhesion of dental acrylic. Both cannulae were then fixed into position with the acrylic. Spinal needle (22 g) insertion depths for subsequent experiments were then determined. Penetration into the cisterna magna was confirmed by aspiration of CSF. The spinal needle insertion depth for the fourth ventricle was determined by infusing sterile saline ( $0.3 \text{ ml} \cdot \text{min}^{-1}$ ) into the needle while at the same time monitoring the needle tip pressure during advancement of the needle through the cerebellum. A sudden drop in pressure confirmed entry into the ventricle. The musculature and skin were then sutured around the cannulae. The dogs were allowed 10–14 days to recover prior to study.

On the day of study, the dogs were restrained without anesthesia in an apparatus that immobilized the head and provided support for the torso,<sup>11</sup> an endotracheal tube was inserted into the tracheostomy and 22 g spinal needles were inserted to the depths established during surgery. The endotracheal tube was connected to a one-way valve that permitted separation of inspired from expired air. The expiratory side was used to measure minute ventilation employing a Bourne respiratory monitor. Respiratory rate could be taken from the expired CO<sub>2</sub> record (Gould capnograph). Tidal volume was then calculated. Arterial blood, rectal temperature, and fourth ventricular CSF pressure were continuously monitored throughout the study. Body temperature did not vary by more than 1° C from the initial reading (38–40° C) during the course of any experiment. A low O<sub>2</sub> flow was added to inspired air to ensure that ventilation throughout the study would not be affected by a decline in PaO<sub>2</sub>.

Two modes of morphine administration were employed, intravenous (iv) infusion and VCP. All of the an-

imals in the iv series ( $n = 6$ ) were also employed in the VCP experiments. To avoid the effects of morphine tolerance, studies in the same dog were performed with a minimum interval of 2 weeks. In three of the dogs given iv morphine, the order of study was iv morphine, then (in  $\geq 2$  weeks) VCP morphine. In the other three animals, the reverse order was employed. In the iv infusion experiments, following a 1-h control period, the dogs were given a loading dose of  $1 \text{ mg} \cdot \text{kg}^{-1}$  morphine sulfate (MS) followed by a constant infusion at  $10 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  over 4 h. Included in the infusate was [<sup>14</sup>C] morphine (Amersham). Arterial blood was taken at 15 min intervals for analysis of PaO<sub>2</sub>, PaCO<sub>2</sub>, pH, plasma <sup>14</sup>C activity and plasma morphine base. Cisternal CSF samples (0.5 ml) were obtained every 30 min and analyzed for <sup>14</sup>C activity and morphine base concentrations. Ventilatory drive (CO<sub>2</sub> response) assessments were performed every 30–60 min using a CO<sub>2</sub> rebreathing system and were based upon the 1 s change in inspiratory occlusion pressure (dp/dt) versus PaCO<sub>2</sub>. The pressure changes were actually measured over the first 200–300 msec following occlusion and extrapolated to 1 s. A minimum of 9 PaCO<sub>2</sub> versus dp/dt data points were obtained during each rebreathing episode. To facilitate comparisons among experimental groups, we used the dp/dt at a PaCO<sub>2</sub> of 70 mmHg (dp/dt<sub>70</sub>) during rebreathing, based on linear regression analysis, as our measure of ventilatory drive. Without exception, this mode of analysis was associated with highly significant correlations between dp/dt and PaCO<sub>2</sub> ( $r > 0.9$ ).

For VCP experiments ( $n = 11$ ) the dogs were prepared for study as described above. Morphine (including [<sup>3</sup>H] morphine [Amersham]) was administered in an artificial CSF solution.<sup>11</sup> The labeled morphine was employed to trace the time-related changes in morphine concentration in cisternal fluid and, *via* arterial plasma analysis, to assess whether the present VCP morphine delivery protocol produces increases in blood morphine levels. The CSF solution was equilibrated with CO<sub>2</sub> at 39° C and had the composition P<sub>CO<sub>2</sub></sub> = 45 mmHg, pH = 7.30, and [HCO<sub>3</sub>] =  $22 \text{ mmol} \cdot \text{l}^{-1}$ . Solution sterility was attained by twice filtering the CSF, first through 0.45 μm, then 0.2 μm Millipore filters. The CSF was delivered into the fourth ventricle at a rate of  $0.3 \text{ ml} \cdot \text{min}^{-1}$ . The inflow catheter was surrounded by a "heating jacket" maintained at 39° C to ensure that the fluid entering the ventricle did not vary in temperature. The distal end of the cisternal outflow catheter was held at ear level and the CSF outflow was collected into tubes changed every 5 min. To monitor system patency, VCP pressure was continuously recorded and in nearly all cases remained at 5–15 mmHg. All studies were preceded by 1 h of VCP with drug-free CSF alone (control). In a preliminary investigation in five dogs, 4 h of continuous VCP with drug-free CSF produced no significant differences among PaCO<sub>2</sub> and dp/dt<sub>70</sub> values

measured at 15 and 30 min intervals, demonstrating the viability of our experimental preparation and the adequacy of the animal selection process. The infusate MS concentrations evaluated were 0.1, 0.5, 1.0, 10.0, and  $100 \mu\text{g} \cdot \text{ml}^{-1}$  artificial CSF. Each concentration was delivered *via* VCP for 1 h. Based on a protocol of increasing concentrations, no more than three MS concentrations were studied on a given day, with the exception that in five animals the perfusion at  $100 \mu\text{g} \cdot \text{ml}^{-1}$  was extended by 2 h. During the control period and during MS infusions arterial samples were taken every 10 min and two ventilatory drive assessments were performed. In two dogs, a 60 min VCP with [ $^{14}\text{C}$ ] morphine-containing CSF was carried out. The animals were then killed with an intravenous injection of sodium pentobarbital followed by KCl. The whole brain was removed and frozen in freon 22. Parallel sections of frozen brainstem tissue (0.5–0.8 mm thick) were made starting at the floor of the fourth ventricle and proceeding to the ventral medullary surface. Additional 0.5 mm thick slices were prepared from other regions in direct contact with intracerebral CSF: ventral pons (pontomedullary subarachnoid space), periaqueductal gray (aqueduct), thalamus (third ventricle), periventricular white matter and caudate nucleus (lateral ventricles) and cerebral cortex (cortical subarachnoid space). The tissue was then processed for scintillation counting according to procedures described in an earlier report.<sup>12</sup>

An additional group of dogs ( $n = 3$ ) was studied by combining the iv and VCP delivery protocols. Thus, at 3–4 h of iv morphine administration, while continuing the iv morphine infusion, a 1 h VCP of the  $\mu$ -receptor antagonist nalbuphine (at  $10 \mu\text{g} \cdot \text{ml}^{-1}$ ) was initiated. The nalbuphine was delivered in a mock CSF solution containing morphine ( $0.1 \mu\text{g} \cdot \text{ml}^{-1}$ ). This morphine concentration was chosen to approximate the cisternal CSF morphine concentration already present (see below). Forty-five minutes VCP of the mock CSF solution alone (*i.e.*, without nalbuphine) produced no changes in the iv morphine-induced ventilatory depression. Nalbuphine was employed instead of the more conventional antagonist drug naloxone for the following reasons. First, in the dog, evidence has been presented suggesting that the  $\mu$ -receptor action of nalbuphine, including ventilatory effects, is as a pure antagonist with *no* agonist potency.<sup>13,14</sup> Second, preliminary VCP experiments with  $^3\text{H}$ -labeled nalbuphine (a gift from Dr. W. K. Schmidt, DuPont) or naloxone (Amersham) demonstrated that the cisternal outflow/infusate concentration ratio at 1 h VCP of nalbuphine (0.50–0.55) was similar to that of morphine (0.60–0.65, see Results and fig. 4), while the ratio obtained with [ $^3\text{H}$ ] naloxone (0.20–0.25) fell far below that of morphine. This latter result is very likely a reflection of the greater lipophilicity of naloxone<sup>15</sup> compared to the other two drugs. Thus, according to Herz and Teschemacher,<sup>16</sup> one

can predict a greater loss of naloxone to the blood and a more limited penetration of this drug into the tissue surrounding the fourth ventricle and cisterna magna. With the possibility that naloxone would not remain confined to this tissue and be distributed in the blood to other intracerebral loci, or perhaps even be unable to reach subsurface receptors (as in the case of intracerebroventricular delivery of the highly lipophilic drug, fentanyl—see ref. 16, 17), nalbuphine was judged the better choice in the above model. Based upon the outflow/inflow ratio for nalbuphine given above and a cisternal CSF morphine base concentration of 0.05–0.10  $\mu\text{g} \cdot \text{ml}^{-1}$  it was estimated that the protocol employed would produce a 50–100-fold excess of nalbuphine over morphine in the CSF. A nalbuphine/morphine infusate concentration ratio of one in VCP experiments has been shown to completely reverse the ventilatory depression resulting from morphine delivery *via* VCP alone.<sup>13</sup> The nalbuphine concentration used in the present study should, therefore, considerably exceed the levels required to completely reverse morphine-induced ventilatory depression mediated at the brainstem level.

Total morphine concentrations in the iv series were calculated from the specific activity of the injectate and the measured  $^{14}\text{C}$  activity in plasma or CSF. Morphine base concentrations were determined using an HPLC system (Waters), essentially according to procedures described by Todd *et al.*<sup>18</sup> Nalorphine was employed as an internal standard. The modest but consistent difference in the extraction efficiencies of morphine and nalorphine, as determined in a separate series of evaluations, was taken into consideration in the final calculation of morphine concentrations. Radioactivity was measured employing a liquid scintillation counter (Searle Analytic 81) with quench corrections based on an external standard. Arterial gases and *pH* were determined using an IL 1303 blood gas/*pH* analyzer. For the iv series, a repeated measures analysis of variance, with Bonferroni corrections for multiple comparisons, was used for statistical analyses. Otherwise, a nonparametric Mann-Whitney U-test was employed.

## Results

### CONTROL CONDITIONS

Values for mean arterial pressure, arterial  $\text{P}_{\text{O}_2}$ ,  $\text{P}_{\text{CO}_2}$ , and *pH*, ventilatory drive ( $dp/dt_{70}$ ), minute ventilation ( $V_{\text{min}}$ ), respiratory rate ( $f$ ), and tidal volume ( $V_{\text{T}}$ ) obtained in dogs from both experimental series prior to drug administration are given in table 1. The dogs used in the present study did not exhibit any clear signs of stress as indicated by a relatively constant mean arterial pressure and  $\text{Pa}_{\text{CO}_2}$  during the control period (complete data not

TABLE 1. Physiologic Variables during Control Conditions

|                        | MABP<br>(mmHg) | PaO <sub>2</sub><br>(mmHg) | PaCO <sub>2</sub><br>(mmHg) | pHa           | dp/dt <sub>70</sub><br>(mmHg · sec <sup>-1</sup> ) | V <sub>min</sub><br>(l · min <sup>-1</sup> ) | f<br>(breaths/min) | V <sub>T</sub> (ml) |
|------------------------|----------------|----------------------------|-----------------------------|---------------|--|--|--------------------|---------------------|
| IV infusion series (6) | 117 ± 4        | 229 ± 14                   | 38.5 ± 0.9                  | 7.327 ± 0.015 | 71.1 ± 7.7   | 2.74 ± 0.05                                  | 16.7 ± 2.4         | 194 ± 33            |
| VCP series (11)        | 122 ± 5        | 178 ± 19                   | 40.7 ± 0.6                  | 7.313 ± 0.020 | 55.9 ± 6.9   | 2.10 ± 0.18                                  | 11.3 ± 0.6         | 195 ± 19            |

All values are means ± SE. Number of animals in parenthesis. V<sub>min</sub> = minute ventilation; f = respiratory rate; V<sub>T</sub> = tidal volume.

shown). The data for PaCO<sub>2</sub>, PaO<sub>2</sub>, and pH presented in table 1 represents a mean of six to seven arterial samples taken in each animal over a 1 h span preceding onset of morphine administration. The control dp/dt<sub>70</sub> value for each dog was derived from the pooled PaCO<sub>2</sub> versus dp/dt data derived during two separate episodes of CO<sub>2</sub> re-breathing done 30 min apart.

### INTRACEREBRAL MORPHINE DISTRIBUTION DURING VCP

Figure 1 describes the relative distribution of labeled morphine within the brain following 60 min of VCP in two dogs. Figure 1 (left) shows that morphine penetration into the brain stem is rather limited, involving only the outer 1.5–2 mm of tissue on both the dorsal and ventral surfaces. The right portion of figure 1 represents the relative morphine distribution among different brain regions in contact with ventricular or subarachnoid CSF. These results are taken from the average values obtained from only two experiments. Thus, the standard error bars are not included. Very little distribution of morphine into the lateral and third ventricles and the cortical subarachnoid space appears to occur during VCP. This is demonstrated by the finding of minimal radioactivity in tissue in contact with the above CSF compartments, such as the caudate nucleus and periventricular white matter (lateral ventricle), sensorimotor and parietal cortices (cortical subarachnoid space), and thalamus (third ventricle). However, we observed a significant spread of morphine into the cerebral aqueduct (at least the caudal portion) and the pontobulbar subarachnoid space, as indicated by the involvement of ventral medullary and pontine tissue. No measurable radioactivity could be detected in the blood at any time during those experiments or in any of the other experiments (see below) where labeled morphine was given *via* VCP.

### VENTILATORY CHANGES DURING VCP MORPHINE ADMINISTRATION

In figure 2 are shown the ventilatory changes as a function of infusate morphine concentrations in VCP experiments. Ventilatory changes are also expressed both as change in PaCO<sub>2</sub> from control (ΔPaCO<sub>2</sub>, fig. 2, left) and as a percent control dp/dt<sub>70</sub> (fig. 2, right). The results dem-

onstrate a dose dependent increase in ventilatory depression starting at an infusate morphine concentration of 0.5 μg · ml<sup>-1</sup>, with a maximum depression occurring somewhere between 10 and 100 μg · ml<sup>-1</sup>. This latter finding was confirmed by results in several animals (data not presented) where infusate morphine levels >100 μg · ml<sup>-1</sup> were given following 60 min at 100 μg · ml<sup>-1</sup> and no further ventilatory depression was observed. Minute ventilation (V<sub>min</sub>), respiratory rate (f), and tidal volume (V<sub>T</sub>) changes at increasing infusate morphine concentrations are presented in figure 3. Significant reductions (P < 0.05) from control in V<sub>min</sub> and V<sub>T</sub> were seen at infusate concentrations ≥1.0 μg · ml<sup>-1</sup> with the magnitude of the reductions being virtually identical at 1.0, 10, and 100 μg · ml<sup>-1</sup>. In addition, VCP-delivered morphine resulted in significant 30% increases (P < 0.05) in respiratory rates at 0.1, 10, and 100 μg · ml<sup>-1</sup>.

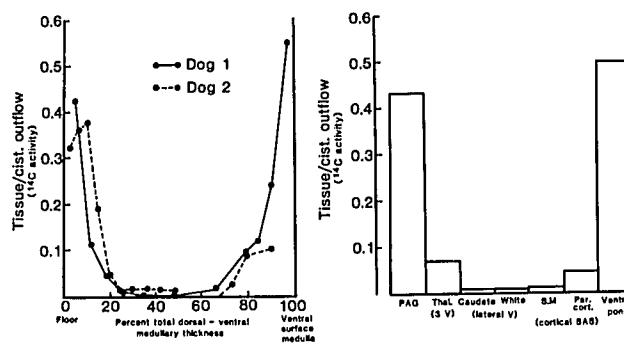


FIG. 1. The distribution of [<sup>14</sup>C] morphine within medullary tissue (left) and in tissue from additional brain regions in direct contact with cerebrospinal fluid (right) in two dogs following 1 h of fourth ventricle to cisterna magna perfusion with [<sup>14</sup>C] morphine-containing mock CSF. The tissue <sup>14</sup>C activity is expressed as a fraction of the <sup>14</sup>C activity in cisternal outflow CSF. The profile of morphine penetration into medullary tissue is derived from measurements of relative tissue <sup>14</sup>C activities in a series of parallel slices of varying thickness taken from a cylindrical plug of tissue extending from the dorsal to the ventral surface of the medulla. Penetration depth is expressed as a percentage of the distance from the dorsal (floor of the fourth ventricle) to ventral surface (10 mm). Relative <sup>14</sup>C activities in the remaining regions are taken from 0.5 mm-thick slices of tissue. The CSF regions are in parentheses. The terms and abbreviations used are identified as follows: PAG = periaqueductal gray; thal. = thalamus; white = periventricular white matter; 3V = third ventricle; S.M. cort. = sensorimotor cortex; Par. cort. = parietal cortex; SAS = subarachnoid space. The vertical bars represent the averages of measurements made in the two dogs.

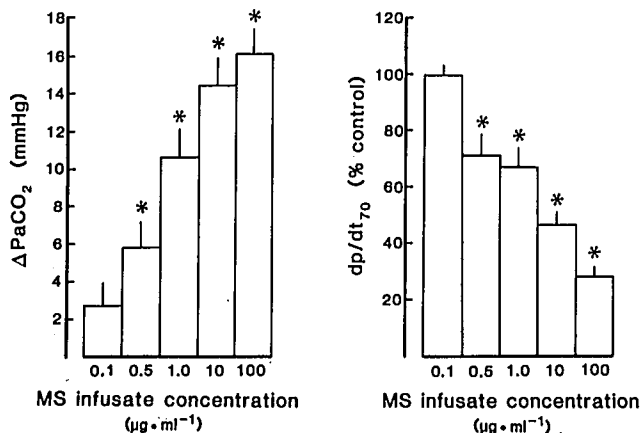


FIG. 2. The dose-response relationship of increasing infusate morphine sulfate (MS) concentrations on ventilation in VCP experiments. Each concentration was maintained for 1 h. The ventilatory variables represented are the  $\text{PaCO}_2$  change from control ( $\Delta\text{PaCO}_2$ , left panel) and the  $\text{CO}_2$  response relative to control (% control  $\text{dp}/\text{dt}_{70}$ , right panel). Five dogs were studied at 0.1, 0.5, and 1.0  $\mu\text{g}\cdot\text{ml}^{-1}$ ; 11 were evaluated at 10  $\mu\text{g}\cdot\text{ml}^{-1}$  and eight evaluated at 100  $\mu\text{g}\cdot\text{ml}^{-1}$ . Asterisk denotes statistical significance ( $P < 0.05$ ) compared to control. Vertical bars are means  $\pm$  SE. See text for further explanation.

Further evaluation of the data indicated that the ventilatory changes occurring over 60 min of VCP closely followed the time-related changes in morphine levels in

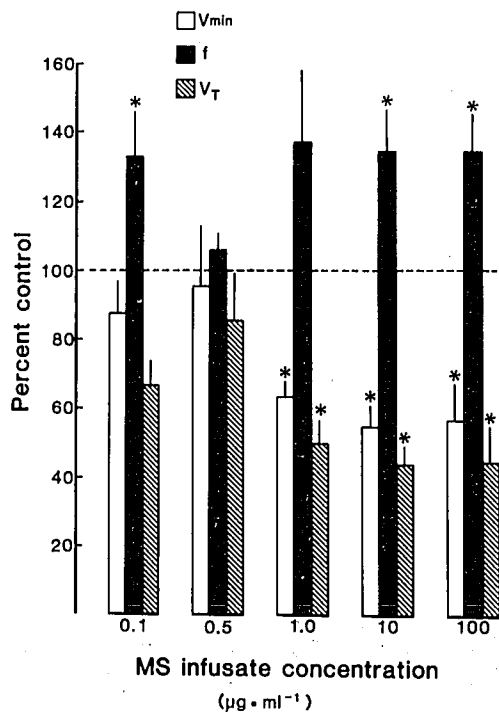


FIG. 3. The relationship between infusate morphine sulfate (MS) concentration and minute ventilation ( $V_{\text{min}}$ ), respiratory frequency ( $f$ ) and tidal volume ( $V_T$ ) in VCP experiments. The ventilatory variables, expressed as a percent of the control values, are presented as means  $\pm$  SE. Asterisk denotes statistical significance ( $P < 0.05$ ) compared to control. For additional information, see figure 2 legend and text.

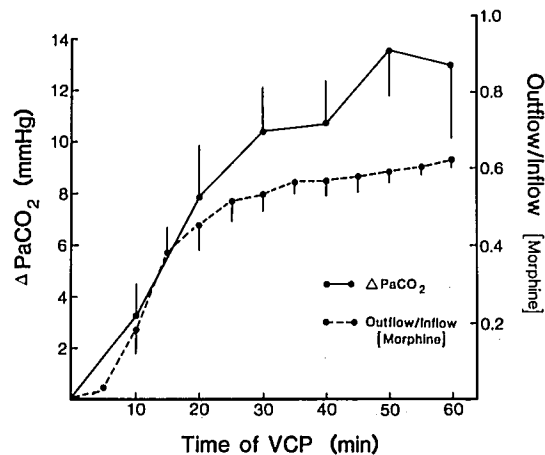


FIG. 4. The relationship over time between arterial  $\text{PCO}_2$  changes from control ( $\Delta\text{PaCO}_2$ ) and changes in relative cisternal morphine levels (expressed as a fraction of the infusate concentration of 10  $\mu\text{g}\cdot\text{ml}^{-1}$  in VCP experiments). These results were taken from six dogs where 10  $\mu\text{g}\cdot\text{ml}^{-1}$  was the initial level evaluated on the day of study and are expressed as means  $\pm$  SE. All values, starting at 10 min, were significantly different from control ( $P < 0.05$ ).

the cisternal outflow. Thus, as shown in figure 4, we found the  $\text{PaCO}_2$  and relative cisternal morphine concentration to increase gradually over the initial 20–30 min with no significant further increases beyond 30 min. The observation that the cisternal morphine concentration achieved a level about 60% of that of the inflow concentration (fig. 4) is probably a reflection of the dilution of the perfusate by native CSF, distribution of morphine into the surrounding tissue, and perhaps some loss to blood,<sup>16</sup> although no morphine could ever be detected in the blood in the VCP experiments. When the perfusion period was extended to 3 h in five dogs (at 100  $\mu\text{g}\cdot\text{ml}^{-1}$ ), no further changes from the 1 h value occurred in  $\text{dp}/\text{dt}_{70}$  or  $\text{PaCO}_2$ .

#### INTRAVENOUS MORPHINE

With the 4 h intravenous morphine delivery protocol employed in this study, plasma total morphine (*i.e.*, morphine base plus metabolites) and plasma morphine base levels remained relatively constant from 15–30 min out to the end of the 4 h infusion period (fig. 5, upper portion). The average plasma morphine base concentration over this period (0.23  $\mu\text{g}\cdot\text{ml}^{-1}$ ) represented approximately 15% of the average total plasma morphine. Over the same time period, cisternal CSF morphine base levels (mean value over 30–240 min = 0.08  $\mu\text{g}\cdot\text{ml}^{-1}$  or 35% of the plasma morphine base value) were found to be unchanging. However, total cisternal CSF morphine increased gradually during the experimental period, rising from a concentration of 0.08  $\mu\text{g}\cdot\text{ml}^{-1}$  at 30 min to a concentration at 240 min of 0.27  $\mu\text{g}\cdot\text{ml}^{-1}$ .

In the lower portion of figure 5 are represented the ventilatory changes, % control  $dp/dt_{70}$  and  $\Delta Pa_{CO_2}$ , over the 4 h intravenous infusion period. Briefly, most of the ventilatory changes occurred over the first hour (both  $Pa_{CO_2}$  and  $dp/dt_{70}$  were significantly changed from control at 30 min) with a modest trend toward increasing depression over time. In all instances comparisons with control achieved statistical significance ( $P < 0.05$ ). However, no statistically significant differences were found when comparisons were made among measurements obtained during the infusion of morphine. Changes from control in  $V_{min}$ ,  $f$ , and  $V_T$  during intravenous morphine administration are presented in figure 6. Significant reductions in  $V_{min}$  and  $V_T$  were observed starting at 1 h following the initiation of morphine delivery with the changes being essentially maintained throughout. No significant changes in respiratory rate were observed until 4 h when a reduction in  $f$  to 70% of control ( $P < 0.05$ ) was found.

#### VENTILATORY DEPRESSION: RELATIONSHIP TO CSF MORPHINE LEVELS AND ROUTE OF ADMINISTRATION

In figure 7, ventilatory changes, related to cisternal CSF morphine base levels, are directly compared in animals receiving morphine intravenously *versus via* VCP. The differences are striking. Thus, for a given cisternal CSF morphine base concentration, the ventilatory depression in the intravenously infused animals far exceeds that in the VCP animals. For example, following 4 h of iv morphine administration, a cisternal CSF morphine base concentration of  $0.08 \mu\text{g} \cdot \text{ml}^{-1}$  was associated with a  $\Delta Pa_{CO_2} > 20$  mmHg and a  $dp/dt_{70}$  reduced to  $<30\%$  of control. A similar cisternal CSF concentration in VCP experiments was associated with little or no ventilatory depression. Even if one relates the depression in the intravenous series to free morphine concentrations in plasma, which should represent the highest possible intracerebral morphine concentration, this difference is not appreciably altered. In fact, one could never achieve the same degree of ventilatory depression as in intravenously infused animals with any concentration of VCP delivered morphine, including CSF levels 10–20 times higher than represented here (see above).

#### VCP NALBUPHINE DURING IV MORPHINE ADMINISTRATION

As summarized in figure 8, 1 h of nalbuphine delivery *via* VCP during iv morphine infusion only partially reversed the ventilatory depression associated with iv morphine. Thus, the  $dp/dt_{70}$  returned to a value still signifi-

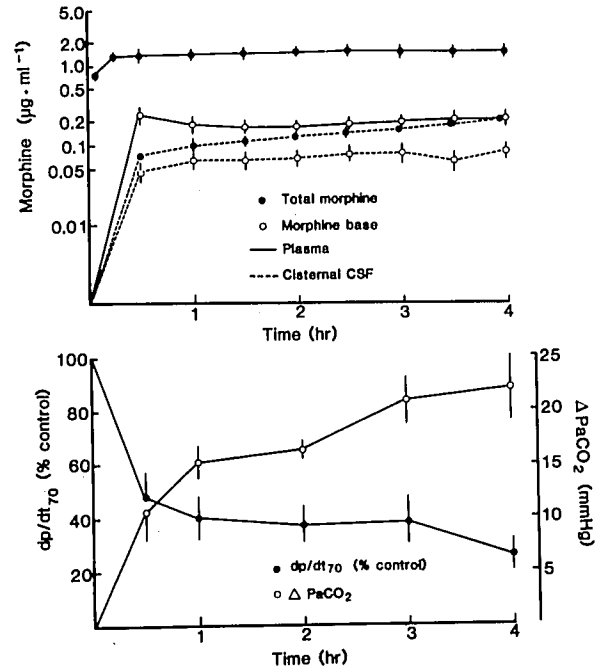


FIG. 5. Time-related changes in the log-concentrations of total morphine (*i.e.*, morphine base plus metabolites) and morphine base in arterial plasma and CSF (upper panel) along with the arterial  $P_{CO_2}$  and  $CO_2$  response changes over the same time period (lower panel) during continuous intravenous morphine infusions at  $10 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , following a loading dose at  $1 \text{mg} \cdot \text{kg}^{-1}$  ( $n = 6$ ). Each point is expressed as mean  $\pm$  SE. All ventilatory changes achieved statistical significance when compared to control ( $P < 0.05$ ). Control ventilatory variables are given in table 1.

icantly below control ( $P < 0.05$ ) but significantly greater ( $P < 0.05$ ) than the  $dp/dt_{70}$  measured immediately prior to nalbuphine administration.

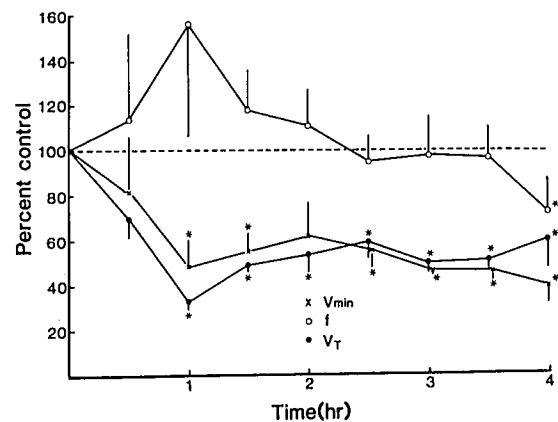


FIG. 6. Time-related changes in minute ventilation ( $V_{min}$ ) respiratory frequency ( $f$ ) and tidal volume ( $V_T$ ) during continuous intravenous morphine infusion. Each value is expressed as a percent of the control value given in table 1 and is given as the mean  $\pm$  SE. Asterisk denotes statistical significance ( $P < 0.05$ ) when compared to control.

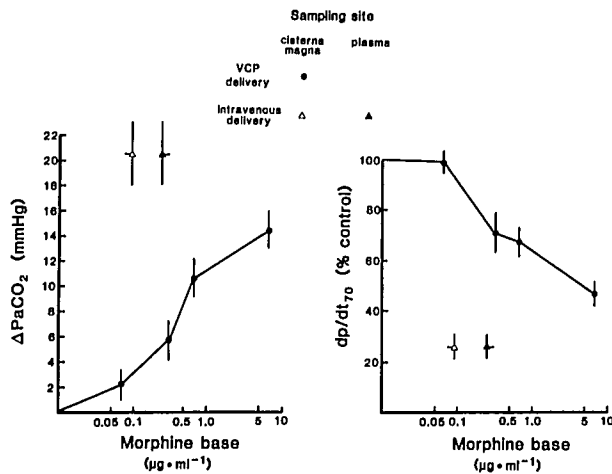


FIG. 7. Direct comparison of ventilatory changes ( $\Delta PaCO_2$ , [left panel] and percent control  $dp/dt_{70}$  [right panel]) related to log-morphine base concentrations in cisternal CSF (and plasma) in fourth ventricular *versus* continuous intravenous morphine infusion experiments. The concentration values measured in both plasma (closed triangles) and cisternal CSF (open triangles) at 4 h of intravenous morphine delivery are presented. Measurements during VCP experiments (closed circles) were made following 1 h at each concentration and are related solely to the cisternal CSF morphine base level. All values are means  $\pm$  SE. See text.

### Discussion

The ventilatory depression observed in the intravenous infusion experiments was associated with a substantially lower cisternal CSF free morphine concentration when compared with that present during VCP studies. However, a significant limitation in comparing dose-dependent ventilatory responses resulting from the two modes of morphine delivery is that the relevant brainstem tissue morphine concentration associated with a specific level of ventilatory depression in both iv and VCP experiments cannot be accurately determined from cisternal CSF measurements alone. In iv studies it is reasonable to assume, in spite of the possible, albeit disputable,<sup>16</sup> existence of an intracerebral transport mechanism for morphine,<sup>19,20</sup> that the brainstem tissue-free morphine concentration has a value bounded by the cisternal CSF level and the plasma level. In VCP studies, as demonstrated in figure 1, 1 h of perfusion produced a rather steep gradient of diminishing morphine concentration in the brainstem from both dorsal and ventral tissue surfaces inward, with only the outer 1.5–2 mm of tissue being involved. However, previous studies involving direct application of an agonist drug to the ventral medullary surface in anesthetized cats have demonstrated a fairly rapid ventilatory depressant effect that levels off within minutes and remains constant as long as drug exposure is maintained,<sup>5,6</sup> suggesting an effect mediated in tissue lying at or near the surface. In fact, the chemosensitive cells of the ventrolateral medulla have been implicated in this effect. In

cats, the bulk of these cells lie within 1 mm of the tissue surface.<sup>21</sup> In the rat, a concentration of opiate receptors has been found near the dorsal medullary surface in association with the respiratory-related neurons of the nucleus tractus solitarius.<sup>22</sup> Our finding of apparently parallel changes in  $PaCO_2$  and cisternal morphine levels (fig. 4) and the absence of additional ventilatory changes, when extending the period of  $100 \mu g \cdot ml^{-1}$  morphine VCP out to 3 h, suggests a superficially mediated effect but in tissue in direct contact with (or close proximity to) cisternal fluid. This would include the regions mentioned above. These findings and the results represented in figure 1 suggest that the tissue morphine concentration responsible for a given ventilatory effect in our VCP experiments has a value between 10% and 100% of the cisternal CSF morphine level. Thus, even if one shifts the VCP dose/response curve of figure 7 to the left by a factor of 10, the differences in the magnitude of the ventilatory depression when comparing iv and fourth ventricular morphine administration are still quite pronounced.

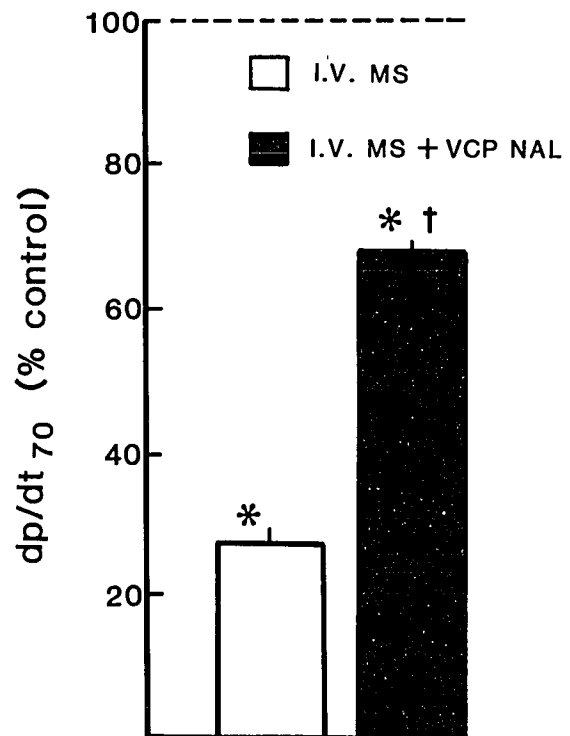


FIG. 8. Effect on  $dp/dt_{70}$  (expressed as percent control) of a 45 min to 1 h VCP with a mock CSF solution containing  $10 \mu g \cdot ml^{-1}$  nalbuphine hydrochloride (NAL) in combination with a continuous iv morphine sulfate (MS) infusion. The nalbuphine was introduced at 3.5–4 h following the start of iv morphine delivery and the iv morphine infusion was maintained during the period of nalbuphine exposure. The iv MS bar represents measurements made immediately prior to the start of the VCP with nalbuphine. The bars are means  $\pm$  SE. Definition of symbols: \* $P < 0.05$ , compared to control; † $P < 0.05$ , compared to iv MS.

Previous studies in anesthetized cats suggested, as we have demonstrated, that after accounting for drug dilution, considerably less drug is required for a given level of ventilatory depression with iv administration than with delivery intracerebroventricularly or directly to the ventral medullary surface.<sup>2,3,6,7</sup> Taveira da Silva *et al.*<sup>7</sup> speculated that the substantially lower ventilatory effect of intracerebral administration was entirely a result of slow penetration of drug to subsurface sites, or removal *via* the blood, reducing the actual drug concentration at the site(s) of action. In fact, these authors found that application of naloxone directly to Mitchell's or Schlaefke's area on the ventral medullary surface could completely reverse the ventilatory depression resulting from iv diacetylmorphine administration. This suggested that virtually all of the ventilatory depression associated with iv diacetylmorphine was mediated at these sites. However, this finding should be viewed with some caution considering the extensive surgical preparation involved, the use of anesthetics and questions regarding the appropriateness of employing the cat for this type of study (see below). In contrast, we found that VCP administration of the  $\mu$ -antagonist nalbuphine (see Materials and Methods) was only able to partially reverse the ventilatory depression accompanying iv morphine. This occurred in spite of the fact that nalbuphine was presented at a relative concentration far in excess of what we previously found to completely reverse the ventilatory depressant effects of VCP-delivered morphine.<sup>13</sup> This finding supports the concept that the ventilatory depressant actions of intravenous morphine are mediated at intracerebral loci in addition to pontomedullary surface tissue sites.

One might question the adequacy of using the cat as a model for studying the ventilatory depressant actions of  $\mu$ -agonists. Unlike humans and dogs, awake cats demonstrate excitation and ventilatory stimulation during systemic morphine administration.<sup>23</sup> In the presence of anesthesia, ventilatory depression results.<sup>1</sup> However, the character of the ventilatory changes differs from that in humans or dogs. Thus, iv or intramuscular morphine administration in the absence of anesthesia, in healthy, normal humans<sup>1,24-27</sup> and dogs<sup>28</sup> (see also above), leads to reductions in tidal volume, with little or no reduction in respiratory rate, except at high concentrations<sup>1</sup> or after prolonged exposure (fig. 6). In anesthetized cats, on the other hand, the response is one of reduced rate with no change in tidal volume.<sup>2,7</sup> This could be related to the possibility that, in the cat, as opposed to dog or human, different intracerebral sites are involved in mediating the ventilatory actions of  $\mu$ -agonists (see below) and/or that opiate/anesthetic interactions had some influence. The use of anesthetics in studies on the ventilatory actions of opiates complicates interpretation of results. In addition to the well-known ability of most anesthetics (particularly

volatile anesthetics and barbiturates) to depress ventilation, there are strong indications that interactions between opiates and anesthetics given together can provide an exaggerated or, at a minimum, an altered effect. For example, in dogs, Freye and Hartung<sup>17</sup> reported little or no ventilatory effects of fourth ventricular infusions of fentanyl or exposure to 0.75% halothane presented separately. However, when given together, a substantial dose-dependent ventilatory depression resulted.

One must also consider the possible additional influences associated with the substantial presence of drug peripherally in iv delivery experiments. VCP delivery of morphine does not produce any detectable drug levels in blood. Previous studies in cats have indicated that peripheral sites are not generally involved in mediating the ventilatory effects of iv  $\mu$ -agonist drugs. Thus, the ventilatory depressant effects of diacetylmorphine were not affected by peripheral chemodenervation.<sup>7</sup> Furthermore, the intracarotid morphine levels needed to produce any suppression at carotid body activity<sup>29</sup> are far greater than the intraarterial morphine concentrations achieved in the present or other studies involving iv morphine administration. In the dog, the effects of morphine on carotid body activity are not known. However, peripherally chemodenervated dogs have been reported to show only moderate increases in resting end-tidal  $P_{CO_2}$  and moderate or no changes in  $CO_2$  responsiveness or minute ventilation, respectively, when compared to results obtained prior to chemodenervation.<sup>30</sup> This suggests that even in the unlikely event that circulating morphine were to completely block peripheral chemoreceptor activity, the effect on the ventilatory variables measured in the present study would be small. In addition, the presence of hyperoxia in our experiments would be expected to further reduce peripheral chemoreceptor activity and impose an even greater limitation on the ability of morphine to depress ventilation *via* these structures. On the other hand, intravenous morphine is rapidly metabolized, chiefly through hepatic conjugation reactions, yielding morphine-3- and morphine-6-glucuronides. No intracerebral mechanisms for metabolism of morphine have been documented to date.<sup>31</sup> Our own results have demonstrated a gradual increase in the total morphine concentration in CSF over the entire experimental period in the face of a relatively constant CSF free morphine concentration. The most likely explanation for this is a slow penetration across the blood-brain barrier of the highly polar glucuronides.<sup>32</sup> It is tempting to ascribe the gradual, modest deepening of the ventilatory depression (figs. 5, 6) over time, during continuous iv morphine infusion, to an increasing appearance of morphine glucuronides in the brain. However, fourth ventricular administration of morphine-3-glucuronide, the major product of morphine conjugation reactions, results in ventilatory stimulation, not depres-



sion.<sup>33</sup> Conversely, morphine-6-glucuronide has been shown to have a greater ventilatory depressant action than morphine.<sup>33</sup> The relative effect of morphine-6-glucuronide on ventilation during iv morphine administration could be expected to increase over time. However, it is difficult to envisage a contribution of any substantial magnitude considering the rather low plasma levels of this metabolite normally found in association with systemic morphine administration.<sup>34</sup>

In conclusion, present results indicate that the ventilatory depressant actions of intravenous morphine in the dog are a result of interactions with intracerebral  $\mu$ -opiate receptors located not only in superficial brainstem tissue but in deeper brainstem and suprapontine structures as well, where clusters of  $\mu$ -receptors have been identified.<sup>22,35,36</sup> Further evidence that intracerebral sites in addition to superficial brainstem tissue are involved can be taken from our finding that VCP morphine delivery, in contrast to iv delivery, inexplicably produced increases not reductions in respiratory frequency. Suppression of the rhythm generating mechanism may require drug interactions with dorsal pontine centers.<sup>5,37</sup> These neurons may not be accessible to substances delivered into the fourth ventricle or cisterna magna.

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