Anesthesiology
71:936–940, 1989

Ventilatory Effects of Fourth Cerebroventricular Infusions of Morphine-6- or Morphine-3-Glucuronide in the Awake Dog

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The ventilatory effects of morphine-6-glucuronide (M-6-G) and morphine-3-glucuronide (M-3-G) were evaluated in awake dogs (n = 10). A fourth ventricle to cisterna magna perfusion (VCP) system was used for drug administration. This permitted a direct comparison of the dose/ventilatory response characteristics of these morphine metabolites to each other and to morphine and obviated the need to consider the blood-brain barrier delay that would complicate analysis of systemic dose *versus* ventilatory response relationships among these drugs. The dose/response pattern for morphine was taken from an earlier study in unanesthetized dogs where the identical mode of drug delivery as in the present report was employed. Morphine-3-glucuronide caused, if anything, a ventilatory stimulation (decreased Pa_{CO_2} and increased CO_2 responsiveness) at the highest infusate concentration studied (50 μg/ml) and no significant ventilatory effects at infusate concentrations at or below 10 μg/ml. On the other hand, M-6-G produced a profound dose-dependent ventilatory depression. Significant increases in Pa_{CO_2} and diminution of CO_2 responsiveness were observed even at the lowest infusate concentration evaluated (0.1 μg/ml). When compared to morphine, M-6-G was found to be about five to ten times more potent as a ventilatory depressant drug. These results imply that M-6-G may play a significant role in the ventilatory depression accompanying systemic morphine administration. (Key words: Brain, ventriculo-cisternal perfusion. Analgesics, opioid: morphine. Biotransformation, morphine: morphine-6-glucuronide; morphine-3-glucuronide. Ventilation, carbon dioxide response.)

THE RESPIRATORY DEPRESSANT action of morphine is well documented. However, little or nothing is known of the contributions of morphine metabolites to respiratory depression. In patients with acquired or pre-existing renal insufficiency, prolonged ventilatory and CNS depression have been reported following morphine.[1,2] While it is unknown whether these effects were due to morphine or its metabolites, patients with renal insufficiency do not excrete morphine glucuronides efficiently.[2,3] The accumulation of these metabolites, therefore, may be responsible for the prolonged ventilatory depression.

The major mechanism of morphine elimination from the body is biotransformation to morphine glucuronides, primarily in the liver, and ultimate excretion of those glucuronides in the urine. One hour following an iv dose of morphine sulfate in humans,[4] monkeys,[5] and dogs,[6] only 10–15% of the total plasma morphine concentration (essentially, free base plus glucuronides) is present as unmetabolized morphine base. In contrast, 75–85% of the total is in the form of morphine-3-glucuronide (M-3-G) with morphine-6-glucuronide (M-6-G) representing 5–10%. Additional metabolites, such as normorphine, are present in yet smaller, if not negligible, fractions of the total.[5,7–9]

In spite of their highly polar nature, M-3-G and M-6-G are capable of penetrating the blood-brain barrier,[6,10] albeit slowly, thus gaining access to the intracerebral sites that mediate opiate-induced ventilatory depression. The majority of these sites appear to be located in the brainstem.[11,12] In the present study, we employed a fourth ventricle to cisterna magna perfusion (VCP) technique, which allows for controlled delivery of drugs to brainstem tissue.[11] Thus, we studied and compared, in unanesthetized dogs, the ventilatory effects of VCP administration of M-3-G and M-6-G. These data were in turn compared with those obtained in an earlier study[11] involving VCP administration of morphine sulfate.

Materials and Methods

The study protocol was approved by the Institutional Animal Care and Use Committee. Adult male mongrel dogs (25–30 kg) were employed (n = 10). The animal selection criteria and surgical procedures were detailed in an earlier report.[11] Briefly, all dogs were prepared with chronic, indwelling femoral arterial and venous catheters, guide cannulae for insertion of 22-G spinal needles into the fourth ventricle and cisterna magna, and a tracheostomy. About 10–14 days recovery were allowed prior to experimentation.

On the day of study, unanesthetized dogs were placed in a restraining apparatus that provided support for the torso and immobilized the head. Spinal needles were inserted to depths established during surgery and a tracheal tube was inserted. A rectal thermister was placed for continuous monitoring of body temperature. The temperature remained relatively constant (38–40° C) throughout the study. The arterial pulse and mean arterial pressures (MAP) and end-todal CO_2 (Gould capnograph) were continuously recorded. All drugs were prepared in an artificial cerebrospinal fluid (CSF) solution.[11] Drug perfusions were preceded by a 1-h control VCP with drug-free CSF. The CSF, maintained at 39° C by a heating jacket surrounding the inflow tubing was infused into the fourth ventricle at 0.3 ml/min. The tip of the cisternal outflow

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Received from the Department of Anesthesiology, Michael Reese Hospital and Medical Center, Lake Shore Drive at 31st Street, Chicago, Illinois 60616. Accepted for publication July 29, 1989.
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catheter was held at ear level and the effluent continuously collected. The fourth ventricular pressure was continuously recorded and remained at 5–15 mmHg during the experiments. The animals used in the present study were selected for their tolerance to prolonged restraint. The adequacy of our animal selection process was demonstrated previously, in a series of time-control evaluations in awake dogs, where we reported no appreciable changes in Pa_{CO_2} or CO_2 responsiveness during several hours VCP with a drug-free mock CSF solution.[11]

During the 1-h control and drug perfusion periods, arterial samples were taken every 10 min and analyzed for P_{CO_2}, P_{O_2}, and *p*H. Two CO_2 response evaluations, at 30 and 60 min, were performed employing a CO_2 rebreathing system.[11] We analyzed the 1-s change in inspiratory occlusion pressure (dp/dt) *versus* PA_{CO_2}. Comparisons among animals were facilitated using the dp/dt value at a Pa_{CO_2} = 70 mmHg during rebreathing (dp/dt_{70}). Additional details of the dp/dt_{70} measurements were provided in an earlier report.[11] Following the control period, perfusions of CSF containing M-6-G or M-3-G were initiated. A minimum of three infusate drug concentrations, at increasing concentrations, were studied on a single day. Each drug concentration was maintained for 1 h. For M-6-G, the infusate concentrations were: 0.1, 0.5, 1.0, and 10 μg/ml. In two experiments, cisternal effluent was collected at the end of the last perfusion period for analysis of M-6-G concentrations. Morphine-3-glucuronide was studied at 1.0, 10, and 50 μg/ml. A total of six dogs were employed in the M-6-G evaluations. Four dogs were used for M-3-G experiments. Morphine-3-glucuronide was obtained from Sigma (St. Louis, MO). Morphine-6-glucuronide was supplied to us by the National Institute on Drug Abuse (Research Technology Branch, Division of Research).

Arterial gases and *p*H were measured using an IL 1303 blood gas/*p*H analyzer. Morphine-6-glucuronide concentrations in selected infusate and cisternal effluent samples were analyzed *via* HPLC.[13] For comparisons within animals, a repeated measures analysis of variance, with Bonferonni corrections, was employed. For some comparisons among animals, a nonparametric Mann-Whitney U test was used. To facilitate analysis, the dose/response curves for M-3-G, M-6-G, and morphine (data taken from reference 11—see below) were converted to relationships between linear responses and log concentrations. A linear regression analysis was then performed and the slopes and *y* intercepts (log-concentration = 0) assessed for statistically significant differences.[14]

Results

Control Conditions

The control values for MAP, Pa_{O_2}, Pa_{CO_2}, pH_a, and dp/dt_{70} are given in table 1. No significant differences were observed in any of the physiologic variables when comparing values obtained in the two drug groups during the 1-h control VCP that preceded the drug infusion.

Drug Infusions

No significant changes from control in MAP, Pa_{O_2}, or pH_a were observed at any time during fourth ventricular infusions of either drug. The ventilatory changes related to infusate concentrations (expressed in micromolar units [μM]) are presented in figure 1. The ventilatory changes are expressed as change in Pa_{CO_2} from control (ΔPa_{CO_2}, fig. 1, left) and percent control dp/dt_{70} (fig. 1, right). A single dp/dt_{70} determination was made following 60 min of VCP at each drug level studied. The ΔPa_{CO_2} values were derived from the average of three Pa_{CO_2} measurements obtained over the final 20 min of each period of VCP (taken at 10-min intervals). The latter was based on the overall observation in the present report and our previous study[11] that when Pa_{CO_2} changes did occur in the presence of free morphine or morphine glucuronides, the peak effect was seen by 30–40 min with no further changes thereafter. If anything, M-3-G had a stimulatory influence on ventilation as evidenced by a significant fall in Pa_{CO_2} and increase in dp/dt_{70} from control values ($P < 0.05$) at an M-3-G infusate concentration of 100 μM (50 μg/ml) and log-dose/response slopes of opposite sign to those obtained for the ventilatory depressant morphine (fig. 1). Morphine-6-glucuronide produced a dose-dependent ventilatory depressant effect. However, the effect was more pronounced than that observed in an earlier study in awake dogs where we evaluated the dose-dependency of VCP-administered morphine.[11] It must be emphasized that in the previous study, the animals were evaluated under the identical experimental conditions (with the ex-

TABLE 1. Control Ventilatory and Arterial Blood Variables

	MAP (mmHg)	Pa_{O_2} (mmHg)	Pa_{CO_2} (mmHg)	pH_a	dp/dt_{70} (mmHg/sec)
M-6-G (n = 6)	122 ± 5	224 ± 20	37.8 ± 2.1	7.335 ± 0.012	83.3 ± 16.8
M-3-G (n = 4)	130 ± 2	205 ± 8	39.0 ± 0.3	7.341 ± 0.008	45.7 ± 7.7

All values are mean ± SE.

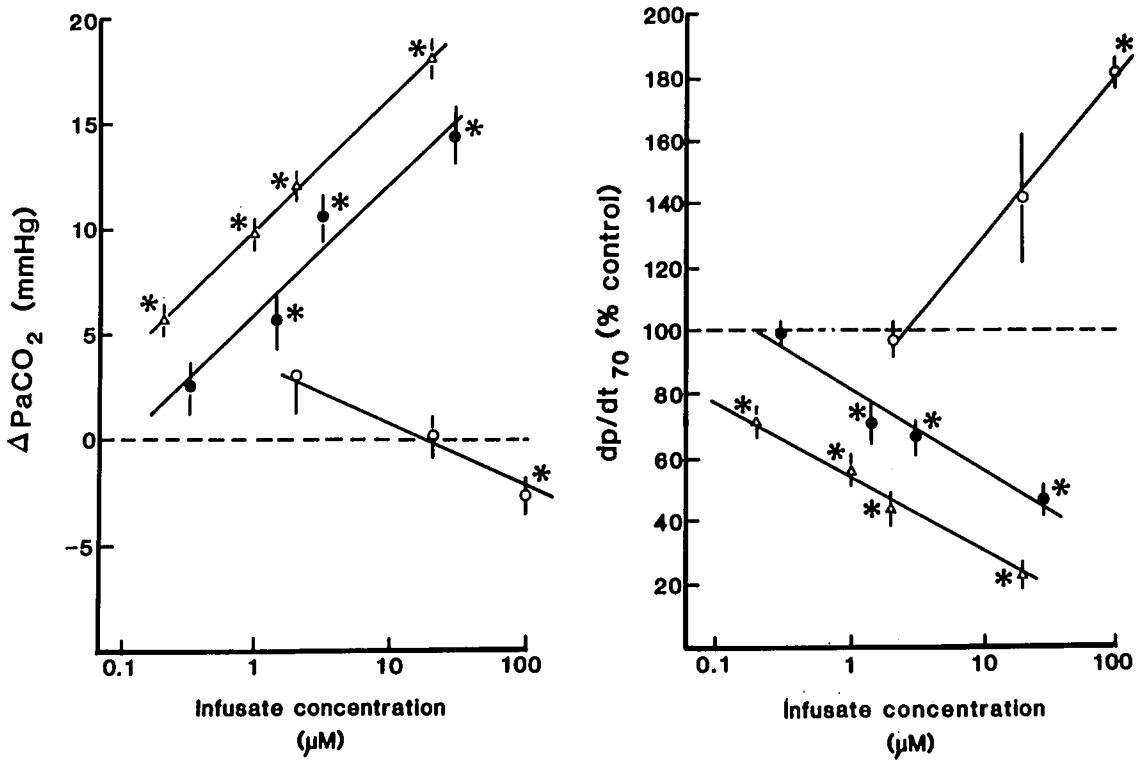

FIG. 1. Changes in arterial P_{CO_2} from control (ΔPa_{CO_2}, left portion) and percent control dp/dt_{70} (right portion) in the presence of fourth ventricular infusions of varying concentrations of morphine sulfate (closed circles) (data taken from ref. 11), M-6-G (open triangles), or M-3-G (open circles). All values are means ± SE. The ΔPa_{CO_2} *versus* log-concentration slopes (±95% confidence limits) for morphine sulfate and M-6-G were, respectively, 6.0 (±2.6) and 6.1 (±0.5). For the dp/dt_{70} (percent control) analyses, the slopes were, respectively, −25.5 (±7.5) and −23.6 (±4.9). No significant differences were found when comparing the appropriate slopes for morphine sulfate and M-6-G. The *y* intercept (log-concentration = 0 [*i.e.*, 1 μM]) values for the ΔPa_{CO_2} analyses comparing morphine sulfate and M-6-G were, respectively, 5.9 (±1.9) mmHg and 10.1 (±0.3) mmHg. The dp/dt_{70} (percent control) intercepts were, respectively, 81.0 (±10.1)% and 54.3 (±3.2)%. With both comparisons, highly significant differences were present ($P < 0.001$). Analysis of the dose/response patterns for M-3-G yielded slopes of −4.7 (±3.3) for the ΔPa_{CO_2} evaluation and 50.5 (±39.5) for the dp/dt_{70} evaluation ($P < 0.001$ in both cases compared to morphine sulfate and M-6-G). The *y* intercept values were 5.3 (±4.7) (ΔPa_{CO_2}) and 78.7 (±57.8) (dp/dt_{70}). *$P < 0.05$, compared to control.

ception of infusate drug composition) as in the present study. In the presence of M-6-G, significant increases in Pa_{CO_2} and reductions in dp/dt_{70} ($P < 0.05$) were observed at all infusate concentrations, including the lowest infusate dose studied −0.2 μM (0.1 μg/ml). In contrast, the lowest infusate concentration of morphine (0.3 μM), despite being 50% higher than the above M-6-G dose, produced no significant changes in Pa_{CO_2} or dp/dt_{70}. Furthermore, significant differences ($P < 0.05$) were found when comparing the ΔPa_{CO_2} and dp/dt_{70} (percent control) results for these two drugs at the above infusate concentrations. When comparing the M-6-G dose/response curve to that obtained for morphine (fig. 1), one finds the slopes of the regression lines relating Pa_{CO_2} or dp/dt_{70} to log concentration to be identical. However, the M-6-G and morphine lines in both the left and right portions of figure 1 are significantly displaced from one another ($P < 0.001$), as indicated by *y* intercept (log concentration = 0) analysis. In quantitative terms, a fivefold or elevenfold greater morphine over M-6-G infusate concentration is required to achieve, respectively, the same level of Pa_{CO_2} elevation or dp/dt_{70} depression.

In the two experiments where the cisternal effluent/infusate M-6-G concentrations were analyzed, an average ratio of 0.75 was measured. The significance of this observation is discussed below.

Discussion

The results of this study have shown that M-3-G, when presented to brainstem receptors, stimulates rather than depresses ventilation and M-6-G has a greater ventilatory depressant effect than morphine. These findings agree with previous results demonstrating a substantial analgesic potency of M-6-G,[3,15] exceeding even that of morphine in mice,[15] and no analgesic activity of M-3-G.[15] In fact, M-3-G has been reported to produce a CNS excitation that is probably not mediated by opiate receptors.[16-18]

These observations are not surprising considering the strong cerebral opiate receptor binding characteristics of M-6-G and the relative lack of any affinity of M-3-G for these same receptors.[19] In that morphine-induced ventilatory depression and analgesia are mediated by μ-opiate receptors,[20] it might be concluded that morphine μ receptor activity is blocked with conjugation in the three position, but is enhanced with conjugation in the six position. Furthermore, these results imply, as others have suggested,[19,21] that proper interaction of morphine or its analogues with the μ receptor requires a free phenolic (three position) hydroxyl group or at least an absence of conjugation in the three position.

There are two potential complications related to our study design that merit some consideration. The first is associated with the use of a drug infusion regimen of increasing concentrations. The possibility exists that this particular protocol may be accompanied by factors such as drug tolerance and/or accumulation, thus diminishing the accuracy of our results. However, our previous work with morphine speaks against this possibility. Thus, the same level of ventilatory depression was observed whether a 10 μg/ml infusate dose was given initially or following a sequence of increasing concentrations (as in the present study). Furthermore, no additional ventilatory changes occurred when the period of VCP at 100 μg/ml was extended from 1 to 3 h. Taken together, these results indicate the absence of any significant influence from acute drug tolerance or drug accumulation in our experiments. Although direct evidence is lacking, the same conclusion can probably be made regarding M-6-G. This contention is supported by our finding of a constant log-dose/response relationship between morphine and M-6-G with increasing infusate concentrations (fig. 1). The existence of identical, although displaced, log-dose/response slopes for intracerebrally administered morphine and M-6-G (analgesic effects), in the absence of tolerance and accumulation effects, was established in an earlier study in mice.[15] If these factors had influenced M-6-G and not morphine-induced responses, then the parallel nature of the dose/response curves could not have been maintained at the higher concentrations.

The second potential complication comes from the employment of infusate rather than cisternal concentrations in our comparison of the dose-response characteristics of morphine *versus* M-6-G (fig. 1). We have previously reported that the ventilatory changes accompanying VCP administration of morphine (constant inflow concentration) follow closely the changes in morphine concentration in the cisternal effluent.[11] Thus, both the ΔPa_{CO_2} and cisternal CSF morphine concentrations show increases up to 30 min, following initiation of morphine delivery *via* VCP, with both leveling off thereafter. This indicates that the drug concentration responsible for a given ventilatory response in VCP experimentation is much more accurately represented by the cisternal effluent rather than the inflow solution. From our previous work,[11] we have found the cisternal outflow/infusate concentration ratio for morphine to achieve a value of 0.62 at 60 min of VCP at a fixed infusate concentration. Based on limited data, the outflow/infusate concentration ratio for M-6-G was 0.75. The finding of a higher ratio with M-6-G is consistent with our earlier demonstration that the ratio is inversely related to drug lipophilicity.[11] This is probably a reflection of the fact that the more lipophilic a substance is the greater will be its rate of loss to the blood.[22] There is no question that highly polar M-6-G is less lipophilic than morphine.[10] In any case, when applying a 20% correction to the M-6-G dose-response curves of figure 1, to reflect this difference in relative cisternal drug concentrations, the M-6-G curve is shifted to the right by a small degree in relation to morphine. This change only slightly alters the relative potency of M-6-G from five- to elevenfold greater than morphine to four- to ninefold greater. The conclusion of a substantially more pronounced ventilatory depressant action of M-6-G, compared to morphine, does not need to be altered.

Does M-6-G play a significant role in the respiratory depression accompanying systemic morphine administration? Present results indicate that at the brainstem level the concentration of M-6-G needed to produce a specific level of ventilatory depression is about 10–20% that of morphine. Under relatively normal conditions, following systemic morphine administration, a CSF M-6-G to morphine concentration ratio in the range of 0.1–0.2 is achieved within 90 min in humans[23] and less than 45 min in rats.[10] Indirect evidence in dogs[11] suggests that perhaps 2–3 h are needed to reach this ratio in the CSF during iv morphine administration. In humans, this relative M-6-G concentration occurs, following a single iv or im injection of morphine, in the face of a minimum concentration ratio in the blood of 0.3–0.5.[4,5,23,24] This relationship in the blood is attained within 1 h and is maintained for up to 12 h or more. Considering that M-6-G penetrates into the brain more slowly than morphine,[10] one could expect the cerebral M-6-G to free-morphine concentration ratio to increase further with time from the ratio measured at 1–2 h. The possibility therefore exists that a substantial portion, perhaps 50% or more, of the respiratory depression observed by 1 h following systemic morphine administration is due to M-6-G, with the relative M-6-G contribution increasing over time. The above takes on yet greater significance under conditions where the relative plasma M-6-G concentration approaches or even exceeds that of free morphine. This has been reported to occur in association with long-term or sustained-released oral

morphine administration[23,25] and, following only a single parenteral dose of morphine, in patients with renal failure.[2,4,24]

In conclusion, present findings have demonstrated that M-6-G, but not M-3-G, is a ventilatory depressant, exhibiting a potency substantially in excess of that of free morphine. Additional issues such as the ventilatory effects of other morphine metabolites or the possible role of suspected CNS conjugation reactions[18] await resolution in future investigations.

The authors wish to thank Richard Ripper, George Dominquez, and Fatima Ali for expert technical assistance, and Lorraine Butler for typing this manuscript.

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