Inhibition in Spinal Cord of Nociceptive Information by Electrical Stimulation and Morphine Microinjection at Identical Sites in Midbrain of the Cat

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SUMMARY AND CONCLUSIONS

1. The descending inhibition of noxious heat-evoked spinal neuronal excitation produced by morphine (MOR) and electrical brain stimulation (EBS) given at identical sites in the midbrain was quantitatively studied in the anesthetized cat.

2. Fifty-two dorsal horn units driven by electrical stimulation of the posterior tibial and/or superficial peroneal nerves at A- and C-fiber strength and responding to noxious radiant heating (50°C) of the skin of the foot or toepads were studied. All units also responded to mechanical skin stimuli and were located primarily in laminae IV–VI of the dorsal horn.

3. MOR (10–20 µg) was administered at 23 different sites in the midbrain. MOR attenuated the heat-evoked responses of 14 of 18 dorsal horn units studied to a mean 43% of the control heat-evoked response when administered at 18 sites in and immediately surrounding the periaqueductal gray (PAG). MOR administered at four sites in the PAG failed to significantly attenuate spinal nociceptive responses. The 14 sites where MOR was efficacious were distributed throughout the PAG. The efficacy of the MOR-produced inhibition was not correlated significantly to the distance from the cerebral aqueduct for the 18 PAG sites examined nor was there any difference in the spinal inhibitory effects of MOR, whether administered dorsally or ventrally in the PAG. MOR also failed to affect spinal neuronal heat-evoked responses (n = 5) when administered at five sites in the reticular formation ipsilateral to the PAG.

4. EBS at the same 23 sites where MOR was given and at 34 additional sites in the midbrain attenuated the heat-evoked responses of all but one dorsal horn unit studied (mean maximal inhibition to 35% of control). In the PAG, stimulation ventrally inhibited a significantly greater proportion (13/21, 62%) of heat-evoked spinal neuronal responses to ≤25% of the control heat-evoked response than did stimulation in the dorsal PAG (5/15, 33%). The efficacy of the stimulation-produced inhibition was not, however, correlated significantly to the distance from the cerebral aqueduct for the total 57 midbrain sites examined, the 23 sites at which MOR was also tested, or only those EBS sites in and surrounding the PAG (n = 44).

5. Comparison of the efficacy of MOR and EBS given at the same sites in the PAG (n = 18) revealed that there was no significant correlation between the inhibition of spinal units from sites in the PAG where MOR was most efficacious and sites in the PAG from which stimulation was most efficacious in inhibiting the same dorsal horn units.

6. MOR administered in the PAG decreased significantly the slope of the stimulus-response function (SRF) of dorsal horn neurons to graded noxious heating of the skin (42–50°C) without altering the thresholds at which they responded. EBS in the PAG also affected the SRF of dorsal horn neurons in
the same manner, suggesting that MOR and EBS in the PAG influence the same system(s) of descending inhibition by gain reduction in the intensity coding of dorsal horn neurons to noxious heating of the skin (10, 11, 56).

7. MOR administered intravenously (1 mg/kg) attenuated significantly the heat-evoked responses of dorsal horn neurons and decreased significantly their SRFs to graded heating of the skin similarly to MOR administered in the PAG. A lesser intravenous dose of MOR (100 μg) produced no detectable change in the heat-evoked responses of dorsal horn neurons.

8. Naloxone administered intravenously (1–3 mg/kg) often failed to antagonize completely the inhibition produced by MOR administered in the PAG. Naloxone administered in the PAG (10 μg) did, however, completely antagonize the spinal neuronal inhibition produced by MOR previously administered at the same site in the PAG.

9. The results indicate that MOR produces a descending inhibition of nociceptive information by a direct effect in the PAG but that the sites in the PAG at which MOR is most efficacious may be different from those at which EBS is most efficacious.

INTRODUCTION

It has been widely documented that electrical brain stimulation (EBS) and opioids given in the midbrain periaqueductal gray (PAG) produce an antinociception ("analgesia") in a variety of animal species (cf. Refs. 37, 38, 51, 52, 54). Although many parallels between opioid-induced and EBS-produced analgesias have been established (e.g., analgesic tolerance, cross tolerance, naloxone antagonism, etc.; cf. Refs. 31, 37, 38, 51), several recent reports suggest that significant differences exist between the analgesias evoked from the PAG (e.g., Refs. 30, 34, 40).

EBS in the PAG reliably inhibits the nociceptive responses of spinal dorsal horn neurons (10, 11, 22, 35, 43), and it is generally considered that such descending inhibition is necessary to the analgesias evoked from the PAG (cf. Refs. 18, 37, 52). It is not clear, however, whether opioids administered in the PAG also produce a descending inhibition of spinal neuronal nociceptive responses. Bennett and Mayer (5) reported that morphine (MOR) given in the PAG "markedly inhibited" by 25% or more the noxious-evoked responses of less than one-half of 20 wide dynamic range neurons in the rat spinal cord. To the contrary, LeBars and co-workers (31) report that MOR administered in the PAG of the rat failed to inhibit C-fiber-evoked spinal neuronal responses. In a recent study (12), a modest inhibition (mean, 21%) of the heat-evoked excitation of dorsal horn units by relatively large doses of MOR (mean, 93 μg) administered in the PAG of the cat led to the conclusion that the effects produced by large doses of MOR occur after MOR first enters the general circulation. Thus, while opioids given in the PAG reliably produce an analgesia, that MOR administered in the PAG also produces a descending inhibition of spinal neuronal nociceptive responses is not well established.

In this report, we examined whether MOR microinjected in the midbrain 1) produces a descending inhibition of spinal dorsal horn neurons and 2) exhibits characteristics similar to the spinal inhibition produced by EBS. These questions were investigated by recording neuronal responses in the spinal dorsal horn to controlled noxious heating of the skin. Inhibitory control of the spinal neuronal responses was quantitatively studied by applying MOR and EBS at identical sites in the midbrain. Portions of these results have been previously reported (21, 57).

METHODS

Experiments were performed on 37 anesthetized, paralyzed cats weighing 2.1–3.5 kg. All animals were initially anesthetized with pentobarbital sodium (40 mg/kg, ip) and venous (jugular or femoral), arterial (carotid or femoral), and tracheal cannulas inserted. When the vertebral laminectomy was begun, paralysis was initiated with pancuronium bromide (0.4 mg/kg, iv) and the animals were artificially ventilated for the remainder of the experiment. Typically, ventilation of the animals with a gaseous mixture of 75% N₂O-25% O₂ was sufficient to maintain adequate anesthesia. When necessary, supplemental doses of pentobarbital (6–12 mg, iv) were given to prevent pupillary dilation and blood pressure changes following hindlimb nerve stimulation at C-fiber strength or noxious heating of the skin. In two experiments, halothane (0.1–0.5%) was added to the breathing mixture to maintain adequate anesthesia. There were no obvious differences in the data obtained from these two cats and all results have been pooled. End-tidal Pco₂,
mean arterial blood pressure, rectal temperature, and central venous pressure were monitored continuously throughout the experiments and maintained within physiologic limits.

The lumbar spinal cord between segments L3 and S1 was exposed by laminectomy. The left posterior tibial (PT) and superficial peroneal (SP) hindlimb nerves were dissected free for electrical stimulation. All exposed nervous tissue was covered by warmed mineral oil. The left hindpaw was fixed in paraffin wax and placed pad upward in a holder.

Glass micropipettes filled with 3 M KCl were used for extracellular single-unit recording in the spinal dorsal horn at the region of maximal input from SP and PT nerves, as determined by measurement of the cord dorsum potential evoked by stimulation of these nerves. Dorsal horn neurons were excited and identified by their responses to PT and SP nerve stimulation at a strength supramaximal for activation of A-fibers (2 V, 0.1 ms). At this stimulus strength, all α- and β-fibers, and most β- and δ-fibers were excited (see Ref. 11). All units showing additional long-latency (≥150 ms) responses to stimulation at C-fiber strength (25 V, 1.0 ms) were tested for responsiveness to noxious heating of the skin. Radiant-heat stimuli were applied with a feedback-controlled quartz halogen lamp focused on the glabrous skin of the foot- or toepad within the unit’s cutaneous receptive field (4). A copper-constantan thermocouple placed in the center of the field of stimulation completed the feedback circuit of the heat stimulator. Heat stimuli were applied at ≥3-min intervals to avoid damage to the skin; spinal neuronal heat-evoked discharges were usually stable within 10% at this interstimulus interval (10, 11).

An opening was made in the skull for the stereotaxic positioning of guide cannulas in the midbrain. Stainless steel needles (0.9 mm OD), through which either a stimulating electrode or an injection cannula could be passed, were positioned medially in the PAG and ipsilaterally (4.5 mm) in the midbrain. For EBS, concentric bipolar stainless steel electrodes (0.2 mm ID; 0.5 mm OD; 0.5 mm tip separation) were employed. Constant-current monophasic cathodal pulses (0.1 ms duration) at a frequency of 100 Hz in trains of 100 ms duration were applied at 300-ms intervals. MOR hydrochloride (35 mM in 130 mM NaCl vehicle = 10 μg morphine base/μl) or vehicle (165 mM NaCl) was administered in volumes of 0.5–1.0 μl via an injection cannula (0.4 mm OD) inserted through the stereotaxically implanted guide cannulas. The stimulating electrode and injection cannula were of identical length and extended 2 mm beyond the tip of the guide cannula. Thus, both MOR and EBS at either the medial or lateral sites in the midbrain could be tested at the same locus. Peristimulus time histograms of unit responses (before and during EBS and after MOR) were permanently recorded on paper and analyzed by computer. At the conclusion of each experiment, electrolytic lesions (1 mA, 6–7 s) were made at both medial and lateral sites in the midbrain. Animals were killed by an overdose of pentobarbital. The brain and lumbar spinal cord were removed and placed in 10% Formalin. Spinal recording sites were determined by coordinating the depth of penetration of the microelectrode from the dorsum of the spinal cord with histologically reconstructed electrode tracks in 20-μm-thick stained sections of the spinal cord; sites in the midbrain where EBS and MOR were applied were determined by the location of lesions drawn from cresyl violet-stained coronal brain sections. Additional experimental details have been provided elsewhere (11). Statistical comparisons were made using Student’s t test for grouped or paired data, P ≤ 0.05 considered significant (two-tailed) (48). Data are presented as mean values ± SE.

RESULTS

Unit sample

Data were obtained from 52 spinal dorsal horn neurons. Recording was restricted to neurons in the dorsal horn as microelectrode penetrations were made only to a depth of 2.5 mm below the dorsum of the spinal cord. Recording sites were primarily in the deeper laminae IV–VI of Rexed (46); two recording sites were in the medial marginal zone (lamina 1). Those units studied had both α- and β- and C-fiber inputs from the SP and/or PT nerves and responded reproducibly to noxious heating of the glabrous skin of the foot- or toepads. An example of a unit’s response to noxious heating of the skin is shown in Fig. 1A. All units studied also responded to mechanical stimuli applied to the foot. Most responded to touch and/or pressure stimuli; about one-half of the units also received input from hair follicle receptors. Thus, the units examined were typical class 2 and class 3 neurons (27).

After characterizing a unit’s response properties and receptive field, heat stimuli (typically 50°C, 10 s duration; see Fig. 1A) were applied once every 3 min. To quantify a unit’s response to noxious heating of the skin, the total number of discharges were counted during the 20-s interval starting with the onset of the heat stimulus or the maximum frequency of the unit’s response was employed. Following establishment of stable control unit responses to heat (three successive responses not varying
FIG. 1. Inhibition by PAGS and MOR of heat-evoked spinal neuronal excitation. A: record of a dorsal horn neuronal response to noxious heating of the footpad (50°C, 10 s). Skin temperature, recorded by a thermocouple, is shown at the bottom; the oscilloscope record of the unit’s response and its peristimulus time histogram (PSTH, 1-s bin width) are shown above. To quantify a unit’s response, the total number of discharges were counted for 20 s beginning with the onset of the heat stimulus or the maximum frequency (Hz) was employed. B: example (PSTHs) of inhibition of another dorsal horn unit by PAGS (450 μA) and morphine administered intracerebrally (ic; 10 μg in 1 μl administered twice, 15 min apart) at the identical site in the PAG. PAGS was started 10 s before the onset of the heat stimulus and lasted for 35 s; the maximal inhibition produced by MOR occurred 10 min following the second administration of MOR in the PAG 2.0 mm distant from the center of the cerebral aqueduct. The control response to noxious heating of the skin is shown above; antagonism of the morphine-induced attenuation by naloxone 11 min after its administration intravenously (iv; 1.3 mg/kg) is shown in the bottom-most PSTH. The period of heating of the skin (50°C, 10 s) is shown below. C: location of 57 sites of EBS (open and filled circles) plotted on representative coronal sections through the midbrain of the cat (A, 1.5 mm—P, 1.5 mm). Filled circles represent those 23 sites where MOR was also microinjected and compared with EBS. Midbrain sites where EBS and MOR were given were ipsilateral to the spinal recording sites.

by more than 10% from the mean; see Ref. 11), the effects of either MOR (10–15 μg) or EBS given in and lateral to the PAG on the heat-evoked discharges were examined. Following the administration of MOR, the unit’s response to heat was tested every 3 min. If MOR’s effect was not significant (i.e., did not attenuate the unit’s heat-evoked response to <75% of the control heat-evoked response), an additional 5–10 μg of MOR was administered at the same site. EBS in the PAG (PAGS) was started 10 s before the onset of the heat stimulus and lasted for 35 s. Examples of the effects of MOR and PAGS on a unit’s response to noxious heating of the skin are given in Fig. 1B; the sites where MOR and EBS were given in the midbrain are portrayed in Fig. 1C.

**MOR inhibition of heat-evoked responses**

The ability of MOR given at 23 midbrain sites to inhibit the heat-evoked discharges of 20 spinal units was examined in 20 cats. MOR was administered both medially and laterally
in the midbrain and its inhibitory efficacy evaluated on the same spinal units in three experiments. When administered at 18 sites in and immediately surrounding the PAG, MOR significantly attenuated the heat-evoked responses of 14 of 18 spinal units studied to a mean 42.8 ± 4.4% of control at a mean 15.5-μg dose of MOR. MOR administered at four sites in the PAG > 2 mm distant from the center of the cerebral aqueduct failed to attenuate significantly the spinal neuronal heat-evoked responses of four spinal units. MOR also did not significantly affect spinal neuronal heat-evoked responses when administered at five midbrain sites in the reticular formation ipsilateral to the PAG (see Fig. 1C). In none of the spinal units examined did MOR produce an enhanced response to noxious skin heating.

Examples of MOR’s descending inhibitory influence are given in Fig. 2. In Fig. 2A, MOR was administered at the edge of the cerebral aqueduct and inhibition of the heat-evoked neuronal response was prompt. In the second example (Fig. 2B), MOR significantly attenuated the heat-evoked response only after it had been administered twice at the ventrolateral edge of the PAG. As suggested by these examples, the time to one-half the maximal spinal neuronal inhibition produced by MOR was significantly correlated (inversely) with the distance from the cerebral aqueduct where MOR was administered (Spearman’s coefficient of rank correlation (48); ƞ = 0.745, ƞ = <0.01). There was no significant correlation, however, between the maximal inhibition of the spinal neuronal heat-evoked response and distance from the cerebral aqueduct for those 14 sites in and adjacent to the PAG where MOR produced a significant descending inhibition (ƞ = 0.072). These data are graphically portrayed as scatter diagrams in Fig. 3. Further, comparison of the inhibitory effects of MOR produced from the dorsal PAG (inhibition to a mean 41.0 ± 4.4% of control, ƞ = 6) with the inhibition produced from the ventral PAG (to a mean 44.2 ± 7.1% of control, ƞ = 8) revealed no differential distribution of loci in the PAG at which MOR produces descending spinal inhibition.

The contribution of mechanical, osmotic, and ionic influences of the microinjection procedure to MOR’s descending inhibitory effects was examined by microinjecting the vehicle solution for MOR in the PAG. There was a slight and insignificant attenuation (to 85.5% of control; ƞ = 3) produced by vehicle administered in the PAG; the efficacy of MOR subsequently given at the same sites was apparently unaffected (MOR-induced inhibition was to a mean 55% of the control heat-evoked response).

MOR’s inhibitory effect on heat-evoked spinal neuronal responses can also be demonstrated following its systemic administration. When administered intravenously (1 mg/kg; ƞ = 4), MOR attenuated spinal nociceptive responses to a mean 51.5 ± 9.5% of control. A lesser 100-μg dose of MOR administered intravenously (i.e., 5- to 10-fold the MOR dose given intracerebrally) failed, however, to affect heat-evoked spinal neuronal responses (ƞ = 3).

Naloxone administered intravenously (1–3 mg/kg) reliably antagonized the inhibition of dorsal horn heat-evoked responses produced by MOR given in the PAG (ƞ = 14; e.g., see Fig. 2). Typically, however, the antagonism by naloxone was incomplete. In two experiments, naloxone (10 μg in 1 μl) was given in the PAG at the same site MOR had been previously administered; MOR’s effect was completely antagonized in these cases (Fig. 4).

Inhibition of heat-evoked responses by EBS, PAGS, and comparison with MOR

The descending inhibitory efficacy of EBS at 57 sites in the midbrain (see Fig. 1C) was examined on the heat-evoked responses of 52 dorsal horn units. In all but one case, EBS significantly attenuated the spinal nociceptive responses (mean maximal inhibition to 34.5 ± 2.7% of control). There was no significant difference between the maximal inhibition of spinal units produced from 44 sites in PAG (mean, 32.6 ± 2.8% of control) and from 13 in the lateral reticular formation (mean, 41.0 ± 6.5% of control). A quantitative comparison between the spinal inhibitory effects of EBS medially and laterally in the midbrain was not carried out (see Ref. 10). However, we did determine that neither the maximal inhibition of the spinal neuronal heat-evoked responses nor the recruitment of inhibition (i.e., increment in inhibition of the heat-evoked response with increasing intensities of EBS) produced from these 57 midbrain sites was significantly correlated with the distance EBS was given...
from the center of the cerebral aqueduct ($r_s = 0.078$ and $r = 0.173$, respectively).

PAGS at 44 sites within 3.0 mm from the center of the aqueduct (see Fig. 1C) was also not significantly correlated to any of the parameters of spinal neuronal inhibition measured. Stimulation at sites ventral in the PAG, however, inhibited a significantly greater proportion of dorsal horn neurons to $\leq 25\%$ of the heat-evoked control response (13/21, 62%) than did stimulation at sites dorsal in the PAG (5/15, 33%; $\chi^2 = 4.114$, $P < 0.005$).

Examination of the efficacy of EBS-produced spinal inhibition from the same 23
FIG. 3. Scatter diagrams plotting of the efficacy of EBS and MOR against distance from the cerebral aqueduct. In A and B, the maximal inhibition produced by EBS and MOR administered intracerebrally on responses of dorsal horn neurons to noxious heating of the skin (50°C, 10 s) is plotted as percent control (total number of impulses in 20 s) on the abscissa against the distance (mm) from the center of the cerebral aqueduct at which EBS and MOR were given (ordinate). A: attenuation by EBS (270–900 μA) of the heat-evoked responses of 52 dorsal horn neurons is diagrammed. There was no significant correlation between distance from the aqueduct and the spinal neuronal inhibition produced by EBS ($r_s = 0.078$). All but one dorsal horn neuron examined was significantly affected by EBS (i.e., its response to 50°C heating of the skin was not attenuated by EBS to <75% of its control heat-evoked response). The 57 midbrain sites at which EBS was given are portrayed in Fig. 1C. B: attenuation by MOR (mean, 15.5-μg dose) administered at 23 sites in the midbrain on the heat-evoked responses of 23 dorsal horn neurons is portrayed. MOR given at five sites > 4.5 mm lateral to the cerebral aqueduct failed to attenuate the heat-evoked response to <75% of the control 50°C heat-evoked response (i.e., did not produce a significant attenuation). There was no significant correlation ($r_s = 0.37; 0.10 > P > 0.05$) between the maximal inhibition produced by MOR and the distance from the cerebral aqueduct at which MOR was administered at the 18 sites in and immediately surrounding the PAG. Four additional sites between 2.0 and 3.0 mm from the cerebral aqueduct also failed to attenuate significantly the heat-evoked spinal neuronal responses; the maximal inhibition produced by MOR on the 14 dorsal horn units affected significantly by MOR applied in the PAG was not correlated significantly to the distance from the aqueduct ($r_s = 0.072$). Sites at which MOR was administered in the brain stem are portrayed in Fig. 1C. C: time to one-half the maximal inhibition produced by MOR (abscissa) is plotted against the distance from the aqueduct at which MOR was administered in and surrounding the PAG (ordinate). The correlation between time and distance for those 14 dorsal horn neurons that were affected significantly by MOR is significant ($r_s = 0.74; P < 0.01$).

FIG. 4. Antagonism by naloxone of MOR's attenuation of a dorsal horn unit's responses to noxious heating of the skin (50°C, 10 s). The unit's response (total number of impulses in 20 s) is plotted against time (min) along the abscissa. MOR (10 μg) was administered initially at time zero and once again 15 min later. Naloxone (1 mg/kg, iv) given 3 times in succession only partially and temporarily reversed the MOR-induced attenuation of the unit's response to heating of the skin. Naloxone (10 μg) administered in the PAG completely reversed MOR's effect. The site of administration of MOR and naloxone in the PAG drawn from a histologic section through the midbrain from this experiment is indicated at the right.
midbrain sites where MOR was administered revealed no significant correlations between either the maximal inhibition produced or the recruitment of inhibition and distance from the cerebral aqueduct. Further, comparison of inhibition of the same spinal units produced by MOR and EBS given at identical midbrain sites also revealed no significant correlations between the efficacy of MOR and EBS, either from 18 sites in the PAG (r_s = 0.061; excluding the 5 sites in the lateral reticular formation where MOR was ineffective) or from 14 of the 18 PAG sites (r_s = -0.104, excluding those 4 PAG sites where MOR was ineffective). That is, when given at identical sites in the PAG and examined on the same dorsal horn neurons, the sites at which MOR produces its most efficacious descending spinal nociceptive inhibition are different from those sites where PAGS is most efficacious.

**MOR inhibition of responses to graded skin heating**

While there is little correlation in the PAG of sites at which MOR and PAGS are most efficacious in affecting the heat-evoked re-

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**FIG. 5.** Effects of MOR and PAGS administered at the same site in the PAG on the SRF of a dorsal horn neuron to graded noxious heating of the skin. A: response to heating of the skin (42-50°C) is plotted on the ordinate (total number of impulses in 20 s) against time (min) on the abscissa. Control responses to heating (●), the effect of PAGS (550 μA; ○), and responses following the intracerebral (ic) administration of MOR (15 μg; ▲) are represented. The broken line connects the control responses to 48°C heating of the skin. The unit’s response to 48°C heating was unchanged following repeated PAGS. The maximal inhibition produced by MOR was achieved approximately 14 min after its administration and the unit’s response to 48°C heating of the skin was unchanged following the approximate 16-min period during which MOR’s influence on the SRF was determined. B: responses (total number of impulses in 20 s) of the same dorsal horn neuron in A are plotted against temperatures of heating the skin: control (●), during PAGS (550 μA; ○), and after MOR’s (15 μg) effect was maximal (▲). Note the low variability of the control responses at 46 and 48°C, determined 18 and 21 min apart, respectively. C: location of the site at which MOR and PAGS were given drawn from a histological section through the midbrain from this experiment.
FIG. 6. Effect of MOR administered in the PAG and intravenously on the SRF of dorsal horn neurons to graded noxious heating of the skin. A: mean responses (total number of impulses in 20 s) of five dorsal horn neurons are plotted against temperatures of heating the skin. In all five experiments, control (○) responses to graded heating of the skin were determined prior to the administration of MOR (10–15 μg; ▲) in the PAG. Effects of MOR on the SRF were assessed when MOR's effect was maximal and stable (see Fig. 5A). Each data point (○ and ▲) represents the mean response of five dorsal horn units from five different experiments; data for the one unit portrayed in Fig. 5 is included among the five units in A above. B: histological localization of the five sites of administration of MOR in the PAG are diagrammed on representative sections through the midbrain. C: mean responses (maximum frequency; Hz) of four dorsal horn neurons are plotted against temperatures of heating the skin. Control (○) responses to graded heating of the skin were determined before the intravenous (iv) administration of MOR (1 mg/kg; ▲). Effects of MOR on the SRF were determined when MOR's effect was maximal and stable. Each data point represents the mean response of four dorsal horn neurons from four different experiments.

Responses of the same spinal units, both MOR and PAGS produce the same effect on the stimulus-response function (SRF) of dorsal horn neurons to graded noxious heating of the skin. An example of the influence of MOR and PAGS on the SRF of the same dorsal horn neuron is given in Fig. 5. Both MOR and PAGS decrease the slope of the SRF without significantly changing the threshold at which the neuron responds to heat applied to the footpad. The effect of MOR on the SRF of five dorsal horn neurons is presented in Fig. 6A. When administered intracerebrally, MOR decreased significantly (t = 3.82, P < 0.02 at 4 df) the slope of the SRF to a mean 46.2 ± 5.3% of the control SRF. Likewise, MOR administered intravenously (1 mg/kg) affects the SRF of dorsal horn neurons in the same manner (Fig. 6C); MOR decreased significantly the slope of the SRF to 60.9 ± 5.2% of control (t = 4.29, P < 0.05 at 3 df).

Spinal inhibition by MOR plus PAGS

When the inhibition of spinal neuronal heat-evoked responses produced by MOR administered in the PAG is maximal, can additional inhibition be produced by PAGS applied at the identical site? In six experiments where this question was examined, MOR attenuated the spinal neuronal heat-evoked responses to a mean 48.8 ± 8.2% of control. In these experiments, PAGS (270–900 μA) given while MOR was maximally effective further attenuated the responses of the same units to a mean 34.8 ± 8.2% of control. In two experiments, PAGS did not further inhibit the MOR-attenuated heat-evoked response; in both cases, MOR attenuated the spinal neuronal responses to a greater extent (to 45% of control) than did PAGS (to 65% of control) prior to the administration of MOR. In the other four experiments, PAGS after MOR was microinjected further attenuated the heat-evoked response but not to a significantly greater extent than PAGS did before the administration of MOR (i.e., to a mean 29.5 ± 5.9% of control before MOR and to a mean 29.7 ± 9.4% of control in the presence of MOR). Thus, PAGS can further attenuate heat-evoked spinal neuronal responses in the presence of MOR but not apparently in an additive fashion.

DISCUSSION

Descending inhibition by MOR from PAG

While it has been widely documented that systemically administered MOR attenuates
significantly responses of spinal nociceptive neurons (6, 7, 14, 16, 32), these data clearly establish that MOR administered directly in the PAG produces a descending inhibition of spinal neurons. Support for this conclusion is provided by comparison of MOR’s inhibitory effect when administered medially and laterally in the midbrain, comparison of MOR’s effects when administered intracerebrally and systemically, and antagonism of MOR’s inhibitory effect by naloxone.

Only when MOR was administered in or immediately surrounding the PAG were the spinal neuronal responses evoked by noxious skin heating attenuated significantly. MOR was efficacious when administered at 14 of 18 sites in and adjacent to the PAG; MOR was ineffective when given at 5 sites in the reticular formation well lateral from the PAG. Thus, MOR’s descending inhibition of spinal neurons was produced only from a circumscribed area in the midbrain.

When given intravenously in a dose of 1 mg/kg, MOR also attenuated significantly spinal neuronal heat-evoked responses. Consequently, as has been demonstrated for the highly lipophilic opioid etorphine (13), it can be argued that when administered intracerebrally, MOR produced the spinal inhibition we observed by passing to the blood and thence to the spinal cord. This argument can be discounted, since 100 μg of MOR given intravenously, which was 5- to 10-fold that given in the PAG, did not affect spinal neuronal nociceptive responses. While diffusion to blood may not account for MOR’s inhibitory efficacy when given in the PAG, the data do suggest that access to the cerebral aqueduct is important, at least with respect to the latency to onset of MOR’s action. The correlation between distance from the aqueduct where MOR was administered in the PAG and the maximal inhibition produced was not statistically significant; the correlation between distance and the time to one-half the maximal inhibition, however, was significant.

The distance MOR diffuses following its intracerebral injection and the specificity of the PAG as a MOR-sensitive locus of antinociceptive action have been previously considered (cf. Ref. 54). It has been determined that nearly 90% of an intracerebral dose of MOR (1.0 μl) is retained within 1.0 mm of the site of injection 30-60 min after its administration (36) and 2 less than 10% of a dose of MOR injected in the PAG enters the cerebral aqueduct during the first hour after its injection (see discussion in Ref. 54). Further, if MOR were producing its inhibitory influence on dorsal horn neurons by diffusion to the aqueduct, it would be expected that lesser doses of MOR administered either in the aqueduct or ventricles would be required to produce effects equivalent to those observed following MOR’s administration in the PAG. Studies in the monkey and rat reveal that the effective antinociceptive dose for MOR when administered in the ventricular system is greater than the effective dose when administered in the PAG (45, 50, 54). In four of our experiments, MOR was administered directly into the cerebral aqueduct or at its edge. The attenuation of the heat-evoked response (to a mean 43.5% of control) did not distinguish these 4 loci from the other 10 loci in and immediately surrounding the PAG where MOR was efficacious. Five other injection sites 1.6-3.0 mm distant from the center of the cerebral aqueduct were more effective in attenuating spinal neuronal heat-evoked responses.

Unlike previous behavioral studies identifying the ventral-ventrolateral PAG as most sensitive to MOR’s antinociceptive effect (33, 34, 55), we found no difference between the spinal inhibition produced by MOR from either the dorsal or ventral PAG. Thus, while there was no apparent dorsoventral or radial gradient in the PAG for spinal neuronal inhibition produced by MOR, we did observe that the latency to onset of MOR’s spinal inhibition was correlated significantly to the radial distance MOR was administered from the cerebral aqueduct.

The final argument in support of MOR’s descending inhibitory effect arising directly from the PAG is provided by consideration of the antagonism of MOR’s action by naloxone. When given intravenously, naloxone always antagonized MOR’s inhibition of spinal nociceptive responses. However, this antagonism was frequently incomplete and short-lived; additional naloxone often had to be given intravenously (up to 3 mg/kg) or had to be given in the PAG (see Figs. 2 and 4). This pattern of antagonism by naloxone is
understandable in terms of receptor kinetics and is consistent with a direct action by MOR in the PAG.

**MOR reduces gain of SRF to skin heating**

Class 2 and class 3 dorsal horn neurons respond to graded heating of the skin (40–55°C) in a monotonic linear fashion (56). PAGS has been previously demonstrated to reduce the amplification or gain of the SRF of dorsal horn neurons to noxious heating of the skin (10, 22, 56). It has been suggested that such a gain reduction represents a multiplicative interaction of excitatory and inhibitory synapses at sites in close proximity, including pre- and postsynaptic inhibitory mechanisms (10). In this report, MOR administered in the PAG was also demonstrated to exert a gain control on dorsal horn neurons, decreasing significantly the slope of the SRF to noxious heating of the skin. MOR administered intravenously similarly affected the slope of the SRFs of spinal neuronal heat-evoked responses. It does not necessarily follow, however, that systemically administered MOR exerts its effects on spinal neurons via the PAG. Opiates have a direct effect on the spinal cord (cf. Ref. 53) and MOR applied directly on the exposed spinal cord attenuates significantly spinal neuronal heat-evoked responses (28).

**Spinal inhibition by PAGS and comparison with MOR**

In addition to evaluating quantitatively the spinal neuronal inhibition produced by MOR given in the PAG, another objective of this study was to compare the descending inhibitions produced by MOR and EBS given at identical sites in the midbrain. It is not likely that we stimulated or microinjected MOR at the most sensitive site in the PAG for either MOR or EBS in each experiment, but the collective weight of the data allows comparisons between PAGS and MOR. Unlike MOR, EBS attenuated significantly spinal neuronal nociceptive responses at both medial and lateral midbrain sites. This has been previously reported (10) and, although there are quantitative differences between the effects of EBS produced medially and laterally in the midbrain, the inhibition of spinal neuronal nociceptive responses from the midbrain is not limited to a single circumscribed area as, apparently, is MOR.

There was no significant correlation between either the efficacy of EBS or the recruitment of inhibition and the distance radially from the aduct where EBS was given for all 57 midbrain sites tested, only those sites in and around the PAG, or only those sites at which MOR was also administered. There was, however, a difference in the spinal inhibition by PAGS from dorsal and ventral PAG. PAGS at 18 sites attenuated heat-evoked spinal neuronal responses to ≤25% of control and 13 of these “most efficacious” loci were in the ventral PAG, a result different than found regarding MOR.

Direct comparison of the spinal inhibitory effects produced by MOR and PAGS when given at the identical PAG site revealed another difference between MOR and PAGS. When examined on the same spinal neuron, those sites where MOR produced its most efficacious descending inhibition were not correlated significantly with those sites from which PAGS was most efficacious. While MOR and PAGS both significantly attenuate spinal nociceptive responses and affect the SRF similarly, that MOR and PAGS are most efficacious from different sites in the PAG raises the possibility that they may be affecting different systems of descending inhibition. This possibility is supported by results obtained employing other experimental paradigms (30, 33, 34, 39, 40). For example, the depletion of both serotonin and norepinephrine in the spinal cord attenuates the antinociceptive efficacy of MOR, but not of PAGS, when given in the PAG (30). So, too, do bilateral electrolytic lesions in the gigantocellular fields lateral to nucleus raphe magnus (NRM) block the antinociception produced by MOR given in the PAG without affecting that produced by PAGS at the same site (40). Additionally, naloxone fails to antagonize PAGS-induced antinociception (24) or PAGS-produced descending inhibition of spinal nociceptive responses (9) in cats. Naloxone's effect in this regard is controversial and may be related to the location in the PAG stimulated (8).

Any conclusion drawn solely from these data relative to whether MOR and PAGS affect the same or different systems of descend-
ing inhibition must be cautiously made, however. MOR influences neurons in the PAG, producing a descending inhibition of spinal neuronal nociceptive responses. PAGS, given at the identical site, may affect the same and/or other neurons as well as descending and ascending fibers of passage. Moreover, the area of neural tissue affected by both may be different. As indicated above, MOR probably did not affect significantly neurons at a distance greater than 1 mm from the locus of microinjection (36). Coaxial bipolar stimulation with electrodes having a 0.5-mm tip separation (cathode most ventral) would produce an ellipsoid current field, which, at increasingly greater current intensities, may affect a different area of tissue than MOR. When comparing sites at which MOR and PAGS were most effective in attenuating the spinal neuronal heat-evoked responses, these factors may contribute in an unknown way to the effects observed. Alternatively, considering the similarities between the effects of MOR and PAGS on spinal neuronal heat-evoked responses, whatever additional or different neural elements possibly affected by PAGS do not appear to contribute significantly to the effects observed.

That PAGS can further inhibit responses of spinal units previously inhibited by MOR previously administered at the same site could be interpreted to support the hypothesis that MOR and PAGS affect different descending systems. Since we did not do a dose-response study with MOR, however, we do not know whether PAGS can further attenuate heat-evoked spinal neuronal responses when the dorsal horn units are inhibited to the asymptotic limit of MOR's efficacy. Our data might be considered to be consistent with other reports suggesting that MOR and PAGS may affect different systems of antinociception and descending inhibition (30, 33, 34, 39, 40); they are, however, not conclusive on this point.

Descending pathways

While it is clear from many studies that MOR and PAGS evoke a powerful antinociception from the PAG (e.g., Refs. 17, 24, 33–35, 38–41, 43), it is not clear what features of the PAG are important and unique. We are not aware of any reports describing the distribution of opiate receptors in the cat mesencephalon. In the rat and monkey, however, the distribution of opiate receptors and endogenous opioids in and surrounding the PAG is relatively homogenous and their concentration "moderate" compared to other areas in the brain (cf. Ref. 49). The distribution of leucine enkephalin in the cat PAG has recently been examined (42), but whether the heterogeneously distributed clusters of enkephalin-labeled cells and terminals in the PAG relate to differences between MOR- and stimulation-produced effects remains to be determined.

Hamilton (25, 26) described cytoarchitectural subdivisions of the PAG and their projections, emphasizing that the PAG is not a homogenous structure (see also Ref. 42). The sites in this and other studies when MOR and PAGS are most efficacious are located primarily in the PAG nuclei medialis and lateralis (25), both of which send projections in a radiating pattern dorsally and ventrally to the surrounding tectum and tegmentum. Ascending and descending fibers have also been described (26, 47), the more important considered to be the projection from the ventrolateral PAG caudally to the midline medullary nuclei raphe magnus, pallidus, and obscurus (19, 47). Since the NRM is generally held to be particularly important to an endogenous descending antinociceptive system (cf. Refs. 2, 18), it is considered that the antinociception produced by MOR and PAGS given in the PAG is effected via this medullary link (for which there are supporting data; e.g., Refs. 1, 3). However, there are recent data indicating that the NRM and PAG are not necessarily so linked in a descending antinociceptive system, suggesting that there are multiple paths through the medulla from the PAG to the spinal cord (20, 22, 23). In a quantitative study of the effects of EBS in the NRM and PAG on dorsal horn neurons in the cat, significant differences were found between the effects of EBS at the two sites on the responses of the same dorsal horn neurons to noxious heating of the skin (22). Further, when the NRM was reversibly blocked by the microinjection of lidocaine, the efficacy of stimulation in either the PAG or reticular formation lateral to the PAG was unaffected (23).

Antinociception and descending spinal inhibition

It is generally believed that the descending inhibition evoked by MOR and PAGS is nec-
ecessary for the analgesias produced in the PAG. Indeed, both PAGS (24) and MOR (44) applied in the PAG of the cat significantly attenuate behavioral responses to noxious stimuli. However, it has long been recognized that EBS in the midbrain of the cat produces a descending inhibition of dorsal horn neurons at sites that do not affect behavioral responses to noxious stimulation (35). Further, EBS in the dorsal PAG is frequently associated with affective and/or aversive effects (e.g., Refs. 29, 43), while EBS in the ventral and ventrolateral PAG produces an antinociception generally free of affective or aversite effects (e.g., Refs. 24, 43). While stimulation in the ventral PAG in this study inhibited a significantly greater proportion of spinal neurons to \( \leq 25\% \) of the control noxious heat-evoked response than did stimulation in the dorsal PAG, stimulation throughout the PAG is clearly efficacious in inhibiting noxious-evoked spinal neuronal responses (e.g., Refs. 10, 11, 22, 35). Regarding MOR, we observed significant inhibition of noxious heat-evoked spinal neuronal activity from 14 of 18 sites of administration in the dorsal and ventral PAG. An antinociception can be demonstrated in the awake cat when MOR is administered systemically (e.g., Ref. 24) or intracerebrally (44).

In a report where the antinociceptive efficacy of MOR and PAGS was established prior to examining descending inhibition subsequently in the same animals (rat), MOR and PAGS inhibited the noxious-evoked responses of 45–60\% of the wide dynamic range spinal neurons studied (5). The effects of MOR and PAGS were not studied on the same neurons, however, and sites in the midbrain that did not produce an antinociception when either MOR or PAGS were administered were not studied. In other investigations where the antinociceptive efficacy of MOR (39) and PAGS (39, 41) was established and MOR and PAGS that were not antinociceptive also examined, the interrelationship between antinociception and inhibition of noxious-evoked neuronal responses in the medullary reticular formation was highly significant. A similar evaluation of descending inhibition of spinal neuronal noxious-evoked responses has yet to be reported.

In summary, these results clearly demonstrate that MOR can produce a descending inhibitory effect on spinal nociceptive neurons by a direct action in the PAG. MOR administered in the reticular formation lateral to the PAG failed similarly to inhibit spinal neuronal heat-evoked responses. Like PAGS, MOR given in the PAG also decreases significantly the slope of the SRF of dorsal horn neurons to graded heating of the skin, suggesting that both MOR and stimulation in the PAG similarly affect the same system of descending inhibition. However, comparison of inhibition of the same spinal neurons produced by MOR with that produced by PAGS given at the identical site in the PAG suggest that the sites in the PAG where MOR and stimulation produce their most efficacious spinal inhibition may be different. This raises the possibility, alternatively, that MOR and PAGS may affect different systems of descending inhibition.

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