

Sleep Disruption and Increased Apneas after Pontine Microinjection of Morphine

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The medial pontine reticular formation (mPRF) is a cholinceptive brain stem region known to play a key role in regulating rapid eye movement (REM) sleep and state-dependent ventilatory depression. Numerous lines of evidence have shown that opioids inhibit both cholinergic neurotransmission and REM sleep. The present study examined the hypothesis that morphine applied to the cholinceptive mPRF would inhibit REM sleep and alter ventilation. In six cats, guide cannulas were chronically implanted to permit pontine microinjection of morphine sulfate, naloxone, and the cholinergic agonist carbachol. After each mPRF microinjection, 2-h polygraphic recordings quantified respiratory frequency and the percent of time spent in states of wakefulness, non-REM sleep, and REM sleep. The results show that mPRF administration of morphine significantly inhibited REM sleep and that this REM sleep inhibitory effect was blocked by pretreating the mPRF with naloxone. Apneic episodes were increased after injection of morphine alone, and the apneas were decreased by the cholinergic agonist carbachol. The results also demonstrated that the ability of microinjected morphine to inhibit REM sleep was dose-dependent and site-dependent. Considered together, the site-localization, pharmacologic blocking, and dose-response data support the hypothesis that specific regions of the mPRF can contribute to the long-recognized ability of morphine to inhibit REM sleep and alter respiratory control. (Key words: Analgesics, opioid: morphine. Brain, pons: medial pontine reticular formation. Parasympathetic nervous system, agonists: carbachol. Sleep: rapid eye movement.)

MORPHINE, a potent analgesic drug used widely in the perioperative setting, has adverse side effects such as oversedation and respiratory depression. Drowsiness and decreased awareness are commonly observed after opiate administration.¹ Paradoxically, morphine disturbs the normal sleep cycle in humans by increasing the time spent awake and by decreasing the time spent in rapid eye movement (REM) sleep.^{2,3} Postoperative patients treated with morphine display a dose-related decrement in REM sleep and a REM rebound that may be associated with

postoperative complications.⁴ In addition, morphine can exacerbate preexisting sleep-associated respiratory disorders, leading to potentially fatal complications.⁵

The neuronal mechanisms by which morphine alters states of consciousness are incompletely understood, but several lines of evidence suggest that morphine may significantly diminish the cholinergic mediation of arousal, sleep, and ventilation. Morphine has long been known to depress acetylcholine release in various regions of cat brain,^{6,7} and more recent studies using *in vivo* microdialysis have confirmed that systemically administered morphine decreases extracellular concentrations of acetylcholine in the forebrain.⁸

Cholinergic and cholinceptive neurons within the pons are known to mediate states of sleep and arousal.⁹⁻¹¹ For example, administration of microgram quantities of cholinomimetic agents directly into the medial pontine reticular formation (mPRF) reliably produces a state that is behaviorally and polygraphically similar to natural REM sleep.¹²⁻¹⁵ This cholinergically induced state is accompanied by REM sleep-like changes in respiration, such as hypotonia in respiratory muscles of the upper airway,¹⁶ decreased minute ventilation,¹⁷ diminished ventilatory response to hypercapnia,¹⁸ and decreased discharge of pontine respiratory neurons.¹⁹ Both natural REM sleep²⁰ and the cholinergically evoked REM sleep-like state²¹ are characterized by enhanced release of acetylcholine in the pontine reticular formation.

In view of the ability of opioids to depress cholinergic neurotransmission and respiration, and considering the important role of pontine cholinergic systems for generating REM sleep and state-dependent respiratory depression, the current study examined the hypothesis that the mPRF can mediate the ability of morphine to inhibit REM sleep and to alter breathing. To test this hypothesis, morphine sulfate was microinjected unilaterally into the mPRF of intact, unanesthetized cats while states of consciousness and ventilation simultaneously were measured. The results revealed that pontine administration of morphine sulfate inhibited REM sleep and increased apneas. These effects were blocked by microinjection of naloxone into the mPRF, suggesting mediation by an opiate receptor.

Materials and Methods

ANIMAL MODEL AND POLYGRAPHIC RECORDINGS

Adult male cats ($n = 6$) were anesthetized with halothane (1-3% in oxygen), and electrodes for objectively

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scoring states of sleep and wakefulness were surgically implanted. These measures included the electroencephalogram, electrooculogram, electromyogram, and pontogeniculoccipital waves recorded from depth electrodes in the lateral geniculate bodies of the thalamus. As shown in figure 1 and as described in detail elsewhere,^{15,16} two stainless steel guide tubes were aimed stereotaxically for the mPRF according to Berman's atlas.²² After emergence from anesthesia, the cats were allowed to recover for 4–5 weeks, during which time they were trained to sleep in the laboratory. Because states of consciousness and respiration are easily disrupted by physical or emotional discomfort, considerable care was given to the psychological and physical well-being of each cat. The experimental trials began only after the cats demonstrated normal sleep patterns in the laboratory.²³ Cats typically adjusted to the laboratory environment in 4–5 days. This animal model has been used extensively to elucidate cellular-level mechanisms regulating states of consciousness.^{9,11}

The behavioral states of wakefulness, non-REM (NREM) sleep, and REM sleep were scored according to standard polygraphic criteria.^{11,23} The carbachol-induced REM sleep-like state (DCarb) was scored based on its sim-

ilarity to natural REM sleep, as previously defined.^{12–15} Typical polygraphic recordings characterizing wakefulness, NREM sleep, REM sleep, and DCarb are illustrated in figure 2. The REM sleep-like state is referred to as "DCarb" because of the desynchronized (D) electroencephalogram caused by the mixed cholinergic agonist carbachol (Carb). During each experiment, the following dependent variables were recorded for 2 h after microinjection: 1) percent of time spent in wakefulness, NREM sleep, REM sleep, or DCarb; and 2) respiratory frequency measured by a thermistor placed at the nares.

CENTRAL ADMINISTRATION OF MORPHINE, NALOXONE, CARBACHOL, AND SALINE

All drugs were injected into the mPRF of awake, unanesthetized cats using a 31-G stainless steel cannula. The microinjection cannula was inserted into the stereotaxically implanted guide tube, and agents were administered in a vehicle of sterile saline that had been filtered through a 0.2- μ m Nalgene filter. Control data were collected using identical procedures for microinjecting saline. Morphine sulfate (0.25 μ l) in concentrations of 0.009, 0.028, and 0.088 M was used to derive a dose-response curve, resulting in administered doses of 1.46, 4.68, and 14.65 μ g. For all other injections, the concentration of drug solutions was 0.088 M. All of these drugs were also delivered in a volume of 0.25 μ l, resulting in the following doses administered for each compound: carbachol 4.0 μ g, morphine sulfate 14.65 μ g, and naloxone 7.97 μ g. Because previous studies have shown that some of these drug effects can persist for more than a day,^{12,13} all of the injection trials in this study were separated by intervals of 3–4 days.

After completion of a series of microinjections, cats were deeply anesthetized with sodium pentobarbital. Brains were perfused *in situ* by cannulating the left ventricle and infusing a 10% formalin solution. Brain stems were frozen, sectioned at 40- μ m thickness, and stained with cresyl violet. Each section containing a lesion from the injector was projected using a computer-driven imaging system and public domain software (National Institutes of Health Image 1.43). The injection sites then were localized using Berman's atlas.²²

STATISTICAL ANALYSES

Drug-induced changes expressed as a percent of the 2-h recording time spent in wakefulness, NREM sleep, REM sleep, or DCarb were assessed by analysis of variance. A statistically significant F value was interpreted to indicate a drug main-effect on the state of consciousness. Differences in the amount of time spent in wakefulness, NREM sleep, REM sleep, or DCarb after mPRF administration of different drugs were evaluated by *t* test comparison.

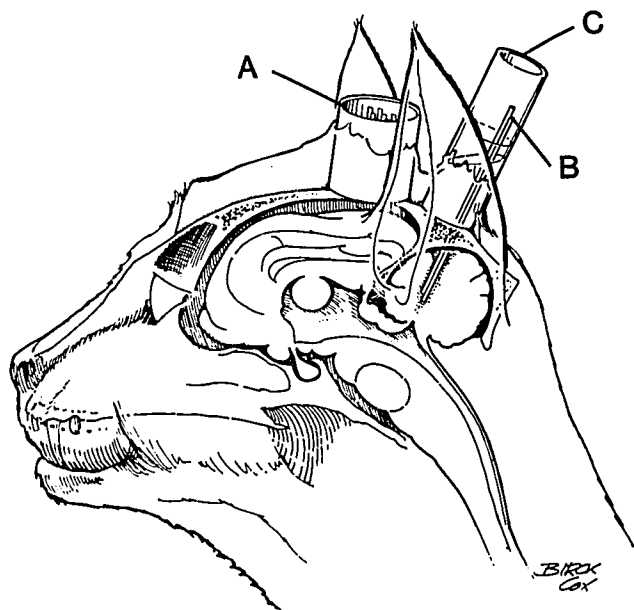
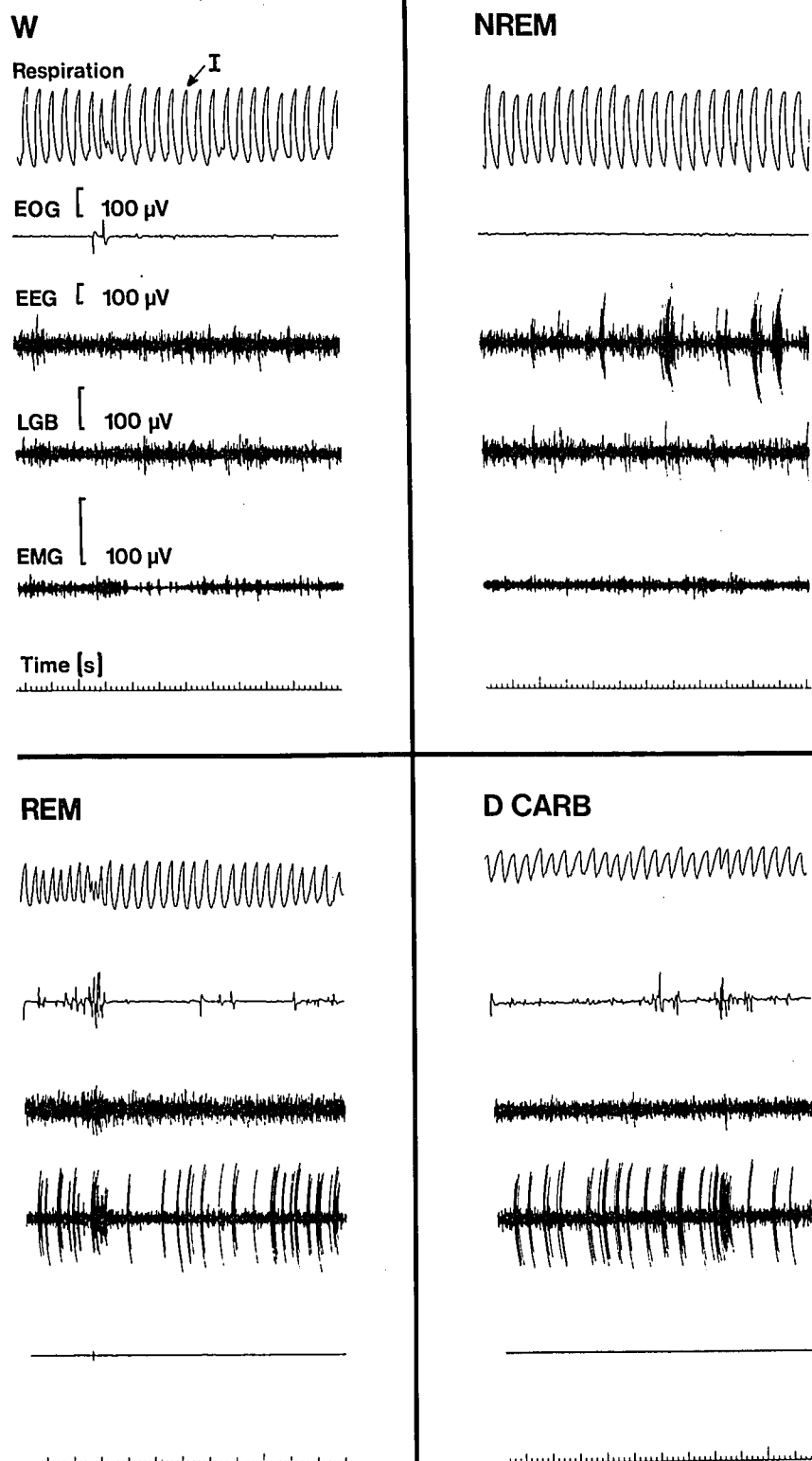


FIG. 1. Intact, unanesthetized cat, chronically implanted for recording states of sleep and wakefulness. The macroelectrodes for recording states of consciousness were embedded in dental acrylic on the skull and led to a miniature electronic plug (A). Stereotaxically implanted stainless steel guide tubes (B) made it possible to microinject the medial pontine reticular formation repeatedly by inserting a 31-G needle that extended 5 mm below the guide tube. A protective sleeve (C) also implanted in acrylic helped to hold the guide tubes in place. Thus, following recovery from surgery, it was possible to measure objectively states of consciousness and state-dependent changes in respiration for many months. Modified with permission.¹⁶

FIG. 2. Polygraphic recordings characterizing states of consciousness of intact, unanesthetized cat. Each of the four panels illustrates a 1-min recording from the same animal. From top to bottom, the six channels show respiration (I = inspiration), electrooculogram (EOG) recorded from the orbital sinus, electroencephalogram (EEG) recorded from the frontal cortex, field potential recordings from the lateral geniculate bodies (LGB), electromyographic (EMG) activity recorded from the dorsal neck muscles, and time in seconds (s). The four panels indicate recordings typical of wakefulness (W), non-rapid eye movement (NREM) sleep, rapid eye movement (REM) sleep, and the REM sleep-like state produced by pontine microinjections of carbachol (D CARB). Note that the carbachol-induced REM sleep-like state, like naturally occurring REM sleep, is characterized by rapid eye movements (large deflections in EOG trace), a low-voltage or desynchronized EEG, large field potentials recorded from the LGB, and skeletal muscle atonia (flat EMG trace). Thus, the carbachol-induced state provides a cholinergically elicited model of naturally occurring REM sleep.



Similar analysis of variance and *t* test procedures were used to evaluate changes in respiratory frequency as a function of mPRF drug administration. The α levels for significant *F* and *t* had probabilities of ≤ 0.05 .

Results

Polygraphic recordings for the objective classification of wakefulness, NREM sleep, REM sleep, or DCarb were obtained for 63 injection trials. After each intracranial drug injection, states of consciousness and respiration were recorded for 120 min. Because each study was scored for every 1 min of recording, the results summarize 7,560 min of polygraphic recordings.

LOCALIZATION OF MICROINJECTION SITES

Histologic analyses revealed that all microinjection sites eliciting a REM sleep-like state were within a region of the pontine reticular formation referred to as the gigantocellular tegmental field. This region of the reticular formation is shown on plate 37 of Berman's atlas²² and is synonymously referred to as the mPRF. A typical cresyl violet-stained histologic section is shown in figure 3A, and all of the effective injection sites are summarized schematically in figure 3B. We use the term "mPRF" to refer to the region throughout the remainder of this paper.

EFFECTS OF MEDIAL PONTINE RETICULAR FORMATION DRUG ADMINISTRATION ON SLEEP, WAKEFULNESS, AND RESPIRATION

Figure 4 illustrates a typical series of experiments in one animal. The temporal distribution of wakefulness, NREM sleep, and REM sleep recorded for 2 h after three different saline injections is shown in figure 4A. Normal state transitions in the cat progress from wakefulness, to NREM sleep, to REM sleep, and, most frequently, back to wakefulness. Figure 4A also illustrates the periodic occurrence and duration of REM sleep in the cat. Though highly variable in its temporal distribution and duration, REM sleep in the cat occurs about every 25 min, with an average duration of about 8 min per epoch.²³

Comparing figure 4B to figure 4A reveals that microinjection of carbachol into the mPRF increased the duration and frequency of the REM sleep-like state, and reduced the latency to onset of the first REM sleep-like episode after each of three injections. When morphine was microinjected into the same mPRF sites from which carbachol evoked the REM sleep-like state (fig. 4C), REM sleep was completely inhibited for the 2-h recording period.

The effects on sleep and wakefulness of administering morphine and carbachol into the mPRF are shown in figure 5.

As compared with saline-injected controls, morphine significantly increased the percent of total recording time spent in wakefulness ($t = 3.88$; degrees of freedom [df] = 28; $P = 0.001$) and significantly decreased the percent of time spent in both NREM sleep ($t = 2.73$; df = 28; $P = 0.001$) and REM sleep ($t = 4.54$; df = 28; $P < 0.001$). Pontine administration of carbachol caused a 435% increase in the amount of REM sleep as compared to control (saline) amounts. As previously reported,¹²⁻¹⁵ this carbachol-induced increase in REM sleep occurred at the expense of wakefulness and NREM sleep, both of which were significantly reduced after mPRF administration of carbachol ($t = 2.42$; df = 22; $P = 0.024$ for wakefulness and $t = 6.25$; df = 22; $P < 0.001$ for NREM sleep).

The next series of experiments sought to determine whether pretreating the mPRF with naloxone could block the inhibitory effects of morphine on REM sleep. Figure 6 shows the effects on states of consciousness of microinjecting naloxone 15 min before morphine microinjection into the same mPRF sites. After receiving naloxone plus morphine, animals spent significantly more time in REM sleep than they did after mPRF administration of morphine alone (compare figs. 4 and 5; $t = 5.04$; df = 22; $P < 0.001$). The percent of time spent in REM sleep after injection of naloxone plus morphine was not significantly different from control (fig. 5; $t = 0.35$; df = 22; $P = 0.733$). Figure 6 also demonstrates that, as compared with saline-injected controls, mPRF administration of naloxone alone did not significantly alter the amount of wakefulness ($t = 1.00$; df = 22; $P = 0.327$), NREM sleep ($t = 1.68$; df = 22; $P = 0.107$), or REM sleep ($t = 0.89$; df = 22; $P = 0.384$). Thus, these data show that naloxone pretreatment was effective in blocking the inhibition of REM sleep by morphine.

One animal was implanted with microinjection guide tubes stereotactically aimed for a part of the pontine reticular formation not effective in producing the REM sleep-like state. In this same animal, repeated microinjections of morphine sulfate were unable to inhibit natural REM sleep. This finding is consistent with previously published evidence demonstrating that the REM sleep-enhancing effects of cholinergic agonists are site-specific within the pontine reticular formation.^{14,24,25}

We next sought to determine whether REM sleep inhibition by morphine was dependent on the dose of morphine administered. Figure 7 illustrates the effect of administering three doses of morphine on the percent of time spent in REM sleep during the 2-h recording period that followed these injections. The largest dose of morphine (14.65 $\mu\text{g}/0.25 \mu\text{l}$) completely suppressed REM sleep. After a smaller dose of morphine (4.68 $\mu\text{g}/0.25 \mu\text{l}$), REM sleep did occur, but in amounts less than that after control (saline) injections. The smallest dose of mor-

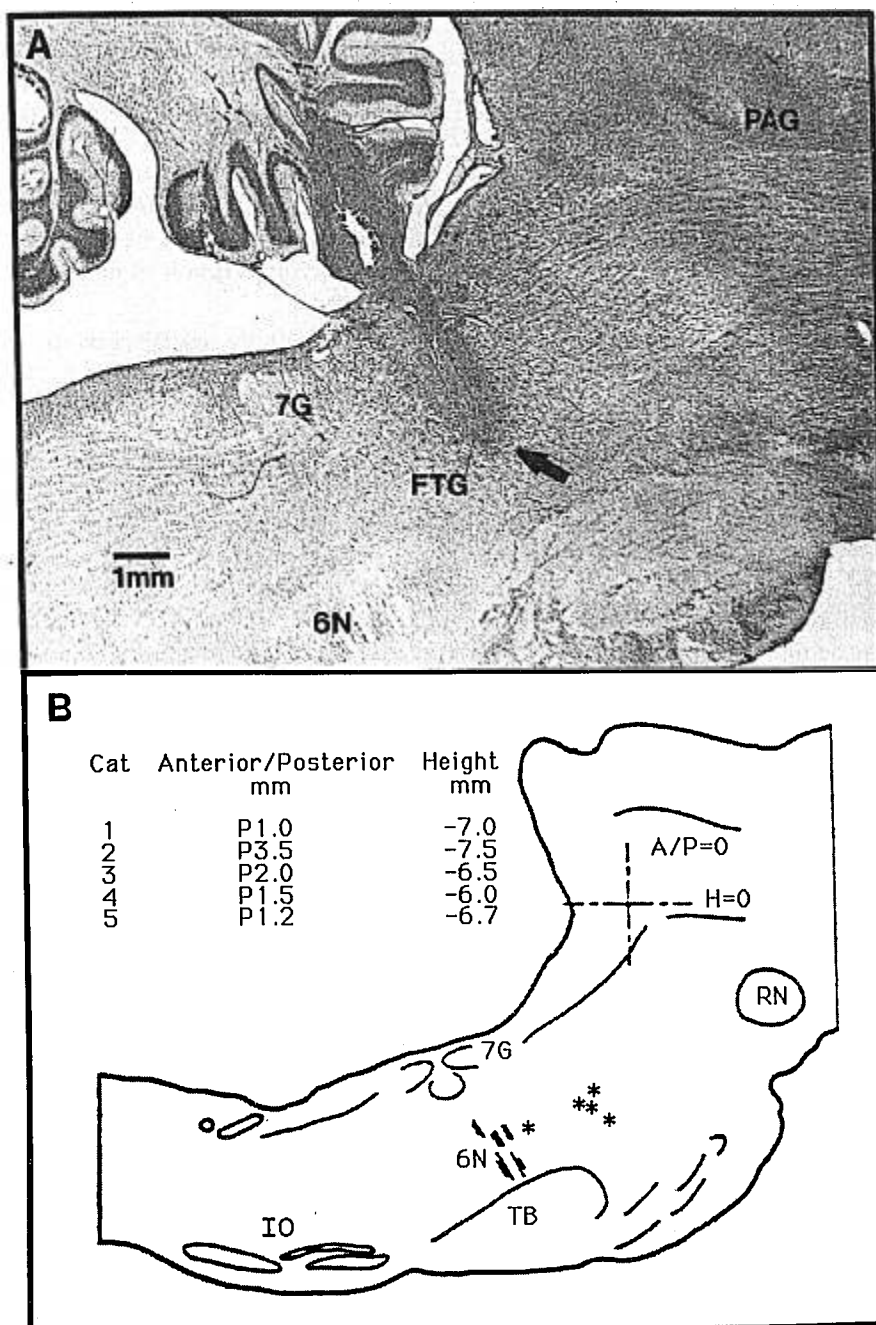
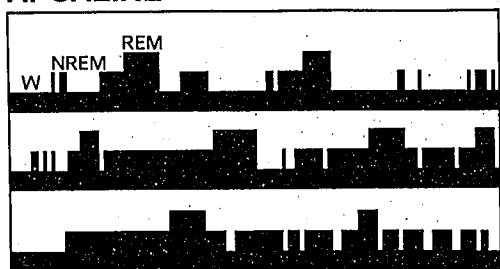


FIG. 3. Histologic localization of injection sites. A: Photomicrograph of a sagittal section through the cat brainstem, stained with cresyl violet. Rostral is to the right, and caudal is to the left. Glial scar (arrow) indicates site of drug administration within the medial pontine reticular formation. Other landmarks: 6N = abducens nerve; 7G = genu of the facial nerve; FTG = gigantocellular tegmental field; PAG = periaqueductal grey. B: Injection sites in the current study. Each asterisk marks the deepest point of an injection site identified as in A. This area of the pons corresponds to Plate 37 from the atlas of Berman.²² Rostral is to the right. The cross bars mark stereotaxic zero in the anterior/posterior (A/P) plane and in the horizontal (H) plane. Note that as in A, the brainstem region injected is in front of 6N and below 7G. The table gives the posterior (P) and horizontal (height) coordinates for the five injection sites in each of five cats used in this study. 6N = abducens nerve; 7G = genu of the facial nerve; IO = inferior olive; RN = red nucleus; TB = trapezoid body.

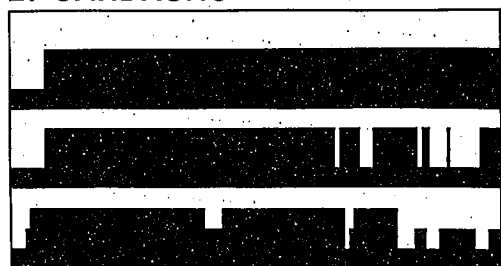
phine (1.465 $\mu\text{g}/0.25 \mu\text{l}$) injected into the mPRF resulted in amounts of REM sleep that were not different from those recorded after saline control injections (fig. 5).

The effects of mPRF drug administration on breathing are shown in figure 8. After microinjection of morphine, the number of apneas was significantly greater than the

A. SALINE



B. CARBACHOL



C. MORPHINE

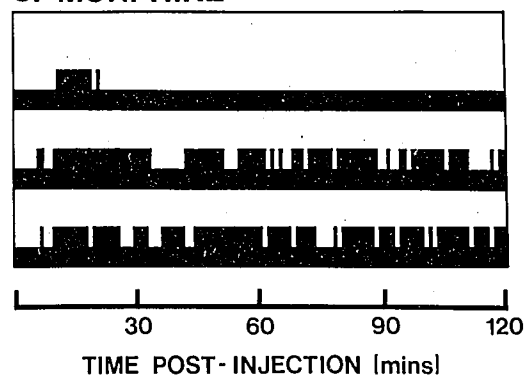


FIG. 4. Temporal distribution of states of consciousness after medial pontine reticular formation microinjections. Each horizontal trace plots the occurrence and duration of states of consciousness for 120 min of polygraphic recording after three microinjections of saline (A), carbachol (B), and morphine (C) into the same medial pontine reticular formation site in one animal. The shortest histograms indicate wakefulness (W); the medium height histograms indicate non-rapid eye movement (NREM) sleep; and the tallest histograms indicate natural rapid eye movement (REM) sleep (A) or the carbachol-induced REM sleep-like state (B). In B, note that for each of the three injections illustrated, carbachol increased the REM sleep-like state and decreased the amount of time spent in wakefulness and NREM sleep. In contrast, C shows that pontine microinjections of morphine sulfate completely blocked the occurrence of REM sleep. All intracranial drug administration was performed on intact, unanesthetized cats that were in states of quiet wakefulness at the beginning of the injection.

number of apneas after mPRF administration of carbachol, but not significantly different from the number of apneas after microinjection of saline. Respiratory frequency after mPRF administration of morphine was com-

pared to the frequency after saline injection, independent of arousal state. Respiratory frequency (mean \pm SD) after microinjection of morphine (28.4 ± 9.8 breaths/min) was not significantly different from control (26.8 ± 8.5 breaths/min). Latency to onset of the first apnea, measured from the time of pontine drug administration, was not significantly altered by morphine. There was no systematic dose response in the number of apnea events after mPRF microinjection with morphine.

Discussion

This study demonstrated for the first time that microinjection of morphine into the mPRF decreased REM sleep by 87% compared to control. The REM sleep inhibitory effect of morphine was dose-dependent and site-specific within the pons. Pretreating the mPRF with naloxone blocked the ability of morphine to inhibit REM sleep. Taken together, these results support the hypothesis that the REM sleep inhibiting effect of morphine is mediated by an opiate receptor and localized, in part, to the mPRF. The findings are discussed with regard to pontine cholinergic systems known to regulate sleep and breathing.

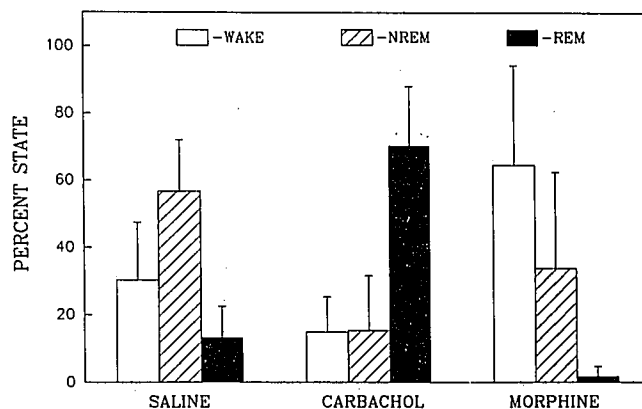


FIG. 5. Effects of carbachol and morphine on percent state. Each histogram plots the mean and standard deviation for the percent of the 2-h recording period spent in wakefulness (open bars), non-rapid eye movement (NREM) sleep (hatched bars), and rapid eye movement (REM) sleep or the carbachol-induced REM sleep-like state (solid bars). These histograms summarize the results from 15 saline injections ($0.25 \mu\text{l}$), 9 carbachol injections ($4.0 \mu\text{g}/0.25 \mu\text{l}$), and 15 morphine sulfate injections ($14.65 \mu\text{g}/0.25 \mu\text{l}$) performed in five animals. Analysis of variance evaluating 57 injections for all five drugs (figs. 4 and 5) revealed a significant drug main effect on the percent of time spent in wakefulness ($F = 12.89$; $df = 4,52$; $P < 0.001$), NREM sleep ($F = 12.70$; $df = 4,52$; $P = 0.000$), and REM sleep ($F = 84.09$; $df = 4,52$; $P < 0.001$). The percent of time spent in each state after control (saline) injections is consistent with previously reported values.¹²⁻¹⁵ Note that microinjection of morphine into the same medial pontine reticular formation sites from which carbachol evoked the REM sleep-like state virtually eliminated REM sleep. (See text for individual *t* test comparisons.)

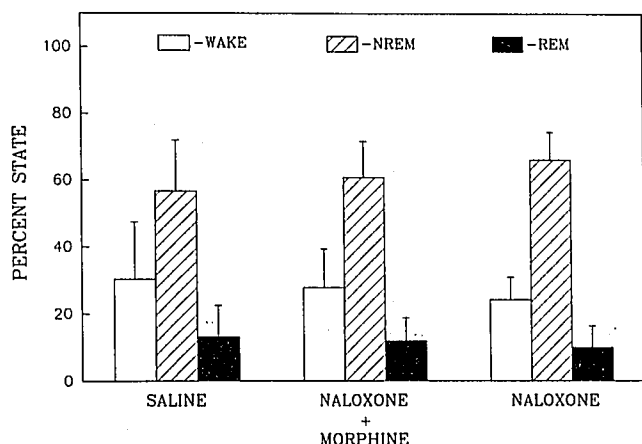


FIG. 6. Centrally administered naloxone blocked the rapid eye movement (REM) inhibitory effect of morphine. Each histogram plots the mean and standard deviation for the percent of the 2-h recording period spent in wakefulness, non-rapid eye movement (NREM) sleep, and REM sleep. The histograms derived from five cats summarize 15 microinjections of saline (0.25 μ l; these are the same saline data shown in fig. 4), 9 microinjections of naloxone (7.97 μ g/0.25 μ l) plus morphine (14.65 μ g/0.25 μ l), and 9 microinjections of naloxone alone (7.97 μ g/0.25 μ l). Naloxone was injected into the medial pontine reticular formation 15 min before an equimolar dose of morphine sulfate was injected. This figure shows that there were no significant differences in the amount of time spent in wakefulness, NREM sleep, and REM sleep after injections of saline, naloxone plus morphine, or naloxone alone.

The cholinergic model of REM sleep provides an important conceptual framework for interpreting the central nervous system sites and mechanisms through which morphine inhibits REM sleep. Although this cholinergic model has greatly advanced the field of sleep neurobiology,^{9,11} it has not yet been widely used as a tool for studying the mechanisms by which anesthetics cause altered states of consciousness and state-dependent changes in autonomic physiology.²⁶ The current results and the productive use of microinjection techniques by others^{27,28} suggest that the cholinergic model shows great potential for future studies of the neuronal mechanism generating states of consciousness and state-dependent changes in autonomic physiology.¹⁰

INHIBITION OF RAPID EYE MOVEMENT SLEEP BY MORPHINE IS SITE-DEPENDENT AND DOSE-DEPENDENT

The results shown in figures 3–5 demonstrate that mPRF microinjections of morphine sulfate significantly depressed REM sleep; that this REM sleep inhibition was blocked by pretreating the mPRF with the opiate antagonist naloxone; and that morphine's inhibition of REM sleep was site-specific within the pons. Furthermore, microinjecting morphine into the mPRF of intact, unanesthetized cats significantly inhibited REM sleep without

causing behavioral excitement or excessive motor activity. Systemic or intraventricular administration of morphine has long been known to suppress REM sleep,²⁹ but the current results are the first to localize the REM sleep inhibitory effects of morphine to the mPRF. This finding in the cat parallels the REM sleep inhibition reported for normal human volunteers,³ former opioid addicts,² many nonhuman animals,³⁰ and postoperative patients receiving morphine.^{3,4} The morphine-induced REM sleep inhibition seen in the current study was site-specific within the mPRF. Morphine caused REM sleep inhibition only when administered into mPRF regions that had been demonstrated to be effective for the cholinergic enhancement of REM sleep (fig. 3). Morphine was unable to inhibit REM sleep when microinjected into pontine sites from which carbachol failed to evoke the REM sleep-like state. The current results encourage future mapping studies designed to define the extent of the mPRF region from which REM sleep inhibitory effects can be elicited by administering morphine.

Postoperative patients⁴ and normal volunteers³ treated with morphine exhibit a decrement in REM sleep that is dose-related. In the current study, the amount of REM sleep recorded for 2 h after mPRF administration of morphine also was dependent on the morphine dose (fig. 7). This dose-dependence is consistent with the hypothesis that REM sleep inhibition caused by mPRF administration of morphine is receptor-mediated.

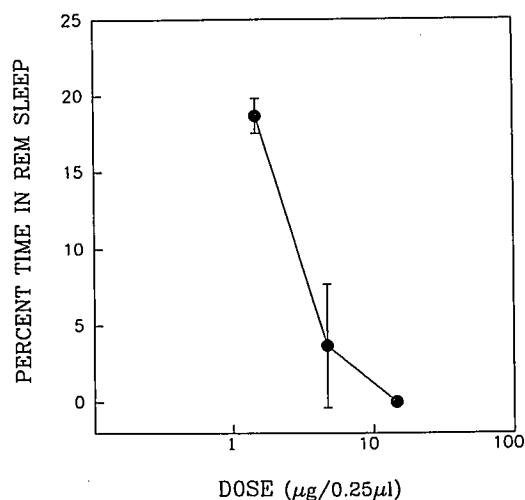


FIG. 7. Dose-dependent suppression of rapid eye movement (REM) sleep by injection of morphine in the medial pontine reticular formation. Each point represents the mean \pm standard deviation of three injections each in one cat. The smallest dose of morphine (1.46 μ g/0.25 μ l) had no effect on percent of time spent in REM sleep. The middle dose of morphine (4.68 μ g/0.25 μ l) caused a 72% decrease below control levels of REM sleep. The largest dose of morphine (14.65 μ g/0.25 μ l) totally eliminated the occurrence of REM sleep during the three recordings, each 2 h in duration.

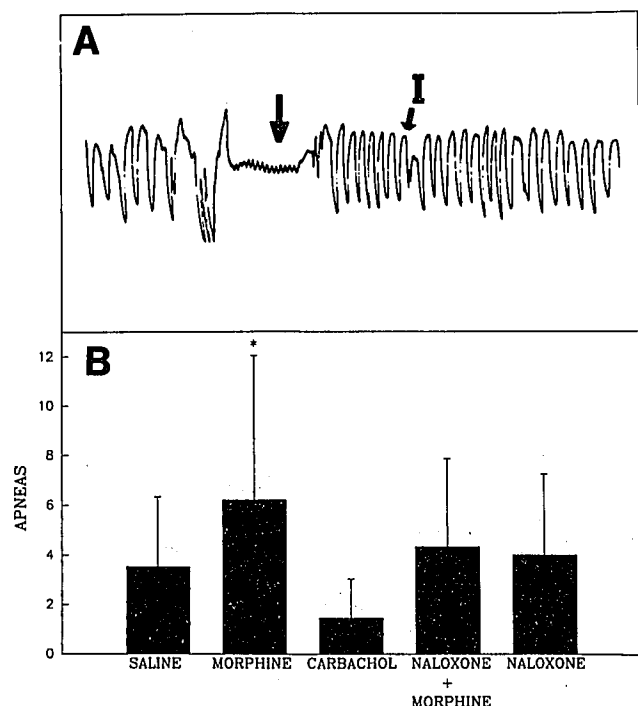


FIG. 8. Increased apneas caused by microinjection in the medial pontine reticular formation. A: A typical respiratory recording. Large arrow = An apnea, defined here as a respiratory pause more than two times the duration of the respiratory cycle (T_{tot}) for the preceding five breaths. Small arrow = inspiration (I). The histograms in B summarize the mean (and standard deviation) number of apneas that occurred after microinjection of each drug into the medial pontine reticular formation. The largest number of apneas per recording interval occurred after microinjection of morphine into the medial pontine reticular formation. The coefficient of variation as an index of variability for the apnea measure was also greatest after morphine. *Comparison by t test revealed a statistically significant difference in apnea frequency between morphine and carbachol injections ($t = 2.37$; $df = 22$; $P = 0.027$). Thus, although the medial pontine reticular formation contains no major clusters of respiratory neurons, it is an area of the reticular formation that can cause state-dependent respiratory depression.

PONTINE ADMINISTRATION OF NALOXONE BLOCKS RAPID EYE MOVEMENT SLEEP INHIBITION BY MORPHINE

Pretreating the mPRF with naloxone effectively blocked the REM sleep inhibition produced by mPRF administration of morphine (fig. 6). This naloxone-blocking result also supports our working hypothesis that REM sleep inhibition caused by mPRF morphine is a receptor-mediated phenomenon. It is not possible from the current results to specify which receptors or endogenous neurotransmitters mediate the REM sleep inhibition caused by mPRF morphine. REM sleep is generated in part by pontine cholinergic mechanisms, and morphine has been shown to depress acetylcholine release in lateral ventricle perfusates⁶ and in individual brain structures measured

both *in vivo*^{7,8} and *in vitro*.³¹ The ability of naloxone to block morphine-induced inhibition of REM sleep is also compatible with the hypothesis of cholinergic mediation, since naloxone has been shown to increase acetylcholine release *in vitro*.³² The current data suggest future studies that aim to clarify opiate and cholinergic receptor interactions within regions of the mPRF known to be involved in generating REM sleep.

Although μ , δ , and κ opiate receptors have been demonstrated to be differentially localized within the mid-brain, the distribution of opiate receptor subtypes in the feline pontine reticular formation is not known.^{33,34} In addition, it should be noted that although the mPRF is cholinceptive (*i.e.*, contains cholinergic receptors), the mPRF is not cholinergic (*i.e.*, mPRF neurons do not produce acetylcholine). All of the acetylcholine within the mPRF comes from the pedunculopontine and laterodorsal tegmental nuclei, located dorsal and lateral to the mPRF.¹¹ Recent intracellular recordings have shown that opioid peptides cause a dose-dependent hyperpolarization and a reduction in the spontaneous discharge of pedunculopontine neurons.³⁵ Thus, it will be important for future studies to test the hypothesis that morphine can block the release of acetylcholine from pedunculopontine and laterodorsal terminals within the mPRF.

THE PONTINE RETICULAR FORMATION CONTRIBUTES TO STATE-DEPENDENT RESPIRATORY DEPRESSION

A recent series of basic studies has shown that the cholinceptive mPRF region illustrated by figure 3 can also mediate state-dependent respiratory depression. These previous studies showed that in the intact, unanesthetized cat, many respiratory measures during REM sleep were mimicked during the cholinergically induced REM sleep-like state. As with natural REM sleep, the cholinergically induced REM sleep-like state is accompanied by upper airway muscle hypotonia,¹⁶ a significantly depressed minute ventilation,¹⁷ a diminished discharge of some pontine respiratory neurons,¹⁹ and a depressed ventilatory response to hyperoxic hypercapnia.^{18,36} Clearly, some of the same cholinceptive reticular regions known to be involved in REM sleep generation can also mediate state-dependent respiratory depression. A functional interaction between opioid-induced respiratory depression and cholinergic systems also has been observed in humans³⁷⁻³⁹ and has been shown in animal experiments to be localized to the brain stem.⁴⁰⁻⁴²

In view of these earlier data, the current finding of an increased number of respiratory pauses (apneas) produced by mPRF microinjection of morphine (fig. 7) is of considerable interest. This increased frequency of central apneas is reminiscent of the central apneas produced by intra-

venous morphine in human subjects.⁴³ Thus, in both cats (fig. 8) and humans there are data showing that morphine disrupts respiratory pattern generation. The cellular mechanism causing opioid alteration of respiratory pattern generation is not completely understood. Opioid alteration of breathing is a complex process mediated at sites in both the central and peripheral nervous systems⁴⁴ by a variety of opiate receptors.³⁹ Previous studies using opioid administration in fetal sheep show that the opioid effect on breathing can be biphasic. Low doses stimulated and high doses depressed respiratory rhythm, and these effects were suggested to be mediated by μ_1 receptors and to involve central muscarinic pathways.^{45,46}

Similar complexities concerning central respiratory pattern generation also were observed in the current study. For example, although there were increased apneas after mPRF administration of morphine, respiratory frequency after morphine was not significantly different from respiratory rates that followed mPRF injections of saline. Although the current data cannot address the mechanisms of apnea generation, the results do encourage future electrophysiologic studies of pontine respiratory neuron discharge after mPRF administration of morphine.¹⁹

LIMITATIONS, CONCLUSIONS, AND POTENTIAL CLINICAL RELEVANCE

The potential for species-specific differences is an acknowledged limitation for investigations such as this, which aim to elucidate basic mechanisms causing morphine-induced disruptions of REM sleep and respiratory control. The respiratory depression and REM sleep inhibition associated with morphine use in humans also occurs in cats,^{29,47} and the data reviewed above support our hypothesis that this respiratory depression is, at least in part, cholinergically mediated.

The potential clinical relevance of the current investigation derives from the ability of morphine not only to depress respiration directly, but also to alter respiratory control adversely by inhibiting REM sleep. When clinically administered morphine is discontinued, one can anticipate a homeostatic rebound in the amount of time spent in REM sleep.⁴ This REM rebound can have serious negative consequences for patients with central or obstructive sleep apnea because motor atonia and airflow occlusions are known to be exacerbated during REM sleep.²⁶ Clearly, one cannot directly extrapolate from the current study to sleep-related postoperative complications documented for some patients who have been administered opioids.^{4,5} There is an unquestionable need, however, to understand the basic mechanisms by which morphine alters sleep and accentuates state-dependent respiratory depression.

A limitation of the current study is that it is not possible from these data to specify the neuronal mechanisms by

which morphine inhibits REM sleep generation. To the best of our knowledge, however, the current data provide the first demonstration that the long-recognized REM sleep inhibitory effects of morphine in the cat can be localized, at least in part, to the mPRF. The findings that the REM sleep inhibitory effects were dose-dependent and blocked by naloxone are consistent with the hypothesis that inhibition of REM sleep by morphine is a receptor-mediated phenomenon. Therefore, these new data represent an essential first step toward specifying the cellular and receptor-level mechanisms within the mPRF through which morphine inhibits REM sleep. Based on the evidence reviewed above, we hypothesize that morphine in the mPRF inhibits REM sleep by disrupting cholinergic neurotransmission. This is a hypothesis open to future experimental tests.

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