Opioid inhibition of rapid eye movement sleep by a specific mu receptor agonist

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Summary

Patients receiving opioids report feeling sleepy, but opioids actually inhibit the rapid eye movement phase of sleep (REM). Inhibition of REM sleep is followed by a rebound increase in REM sleep associated with cardiopulmonary complications. The medial pontine reticular formation (mPRF) is a brain region from which morphine can inhibit REM sleep. The present study tested the hypothesis that specific subtypes of opioid receptors within the mPRF mediate inhibition of REM sleep. Synthetic opioid agonists selective for mu, delta and kappa subtypes were microinjected into the mPRF of four awake cats and polygraphic recordings of sleep and breathing were obtained. An enkephalinase inhibitor was microinjected into the mPRF to assess the contribution of endogenous opioids to the control of sleep and breathing. Only the mu agonist significantly inhibited REM sleep, and no opioid depressed breathing. These results demonstrate that opioid-induced REM sleep inhibition is mediated by mu receptor subtypes in the mPRF. (Br. J. Anaesth. 1995; 74: 188-192)

Key words

Disruption of the sleep cycle and respiratory depression are well known but poorly understood opioid side effects. Despite producing sedation, paradoxically morphine alters the sleep–wake cycle by increasing wakefulness and decreasing rapid eye movement (REM) sleep [1, 2]. Opioid–induced respiratory depression can be produced by at least two mechanisms: either directly via effects on chemosensitive and respiratory nuclei in the central nervous system [3, 4] or indirectly via sleep-dependent respiratory depression [5]. REM sleep inhibition alone does not cause respiratory depression, however, REM sleep deprivation in laboratory animals and humans is followed by a rebound increase characterized by respiratory depression [6-9].

The clinical implications of postoperative REM sleep rebound stem from the fact that the profound physiological changes in normal REM sleep are magnified during REM sleep rebound. Relative to wakefulness, REM sleep causes ventilatory depression expressed as hypotonia in the upper airway musculature, hypopnoeas and increased apnoeas [9]. During REM sleep there are also episodic cardiovascular changes, including hypertension, tachycardia and increased coronary artery blood flow [10]. Myocardial stress during REM sleep has been found to be equivalent to that of maximal exercise in some patients [11]. In the perioperative period, inhibition of REM sleep on the first night after operation is followed by an intense REM sleep rebound on the second and third nights after operation [12]. The frequency of hypoxaemic episodes during REM sleep in patients after operation was shown to increase threefold on the second and third nights after operation compared with the preoperative night [8]. Because of the altered physiological regulation during REM sleep, it is not surprising that numerous clinical reports have correlated cardiopulmonary complications specifically with REM sleep rebound [8, 13, 14] and with this postoperative period [15-19].

The brain mechanisms mediating opioid–induced inhibition of REM sleep are poorly understood. In the brain stem, the medial pontine reticular formation (mPRF) is known to play a key role in REM sleep generation [20-22]. Microinjection of morphine directly into the mPRF of the intact, unanaesthetized cat abolished REM sleep [23]. This morphine-induced inhibition of REM sleep was site-specific within the mPRF, dose-dependent and was blocked by administration of naloxone into the mPRF. These studies showed that morphine-induced inhibition of REM sleep can be localized to a specific brain region, the mPRF. The dose-dependence and effect of naloxone support the view that REM sleep inhibition by mPRF morphine is mediated, in part, by opioid receptors. Morphine is known to act at mu (μ), delta (δ) and kappa (κ) receptors [24-26]. Thus inhibition of REM sleep caused by microinjection of morphine into the mPRF [23] could be mediated by subtypes of opioid receptors. The purpose of the present study was to test the hypothesis that specific opioid receptor subtypes in the mPRF cause inhibition of REM sleep. Part of this study has been presented in abstract form [27].
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Materials and methods
Surgical preparations for pontine drug administration and polygraphic recordings

Standard electrodes for objectively quantifying REM sleep, non-REM (NREM) sleep and wakefulness were implanted into four adult male cats under halothane anaesthesia [28] allowing recording of the electro-oculogram (EOG), nuchal electromyogram (EMG), cortical electroencephalogram (EEG) and field potentials from the lateral geniculate bodies of the thalamus. Bilateral stainless steel microinjection guide tubes were implanted above the mPRF using the stereotaxic coordinates of Berman [29], as described previously [30]. Animals were trained to sleep in the laboratory for 1 month after surgery. When normal sleep patterns were observed, experiments were begun.

PONTINE DRUG ADMINISTRATION AND POLYGRAPHIC RECORDINGS OF SLEEP AND BREATHING

Unilateral microinjections into the mPRF were performed on awake, unmedicated cats by inserting a 31-gauge stainless steel injection cannula into one of the chronically implanted guide tubes. The opioid agonists microinjected into the mPRF included the following: the µ agonist DAGO ([D-Ala², N-Me-Phe⁴,Gly-ol⁵]-enkephalin; 8.95 µg/0.25 µl; 69.6 mmol litre⁻¹), the δ agonist DPDPE (enkephalin, [D-Pen²⁵]-3.92 µg/0.50 µl; 11.5 mmol litre⁻¹) and the κ agonist U50488 (trans-3,4-dichloro-N-methyl-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide methanesulphate salt; 10.2 µg/0.25 µl; 87.3 mmol litre⁻¹). In addition, one animal received three microinjections of kelatorphan (6.45 µg/0.5 µl; 43.4 mmol litre⁻¹), an enkephalinase inhibitor. This cat subsequently received three larger doses of kelatorphan (25 µg/1 µl; 84.7 mmol litre⁻¹). Before initiating any opioid studies the accuracy of guide tube placement was confirmed by microinjecting the cholinergic agonist carbachol (0.4 µg/0.25 µl; 8.8 mmol litre⁻¹), which produces a REM sleep-like state when administered into the mPRF [30-32]. All opioid effects were compared with saline (control), previously shown not to alter sleep or breathing [22]. Ventilatory frequency was monitored by a nasal thermister. Polygraphic recordings of sleep, wakefulness and respiration were obtained for 2 h after each mPRF microinjection, in 1-min epochs, as wakefulness, NREM sleep or REM sleep, according to standard criteria [28]. Analysis of variance and Dunnett’s t test were used to test the hypothesis of a statistically significant drug effect on sleep and breathing.

After completion of the microinjection studies, the cats were anaesthetized deeply with pentobarbitone. The left ventricle was cannulated and the brain perfused with 10% neutral buffered formalin. The brain stem was frozen, sectioned at 40-µm thickness, and stained with cresyl violet for histological localization of the microinjection sites.

Results
The drugs administered and the number of injections (n) were: DAGO (n = 12), DPDPE (n = 18), U50488 (n = 12), kelatorphan (n = 6) and saline (n = 17). These results summarize 7800 minutes of sleep and 5427 minutes of respiratory recordings. Histological analysis confirmed that the microinjection sites were in the mPRF, a brain stem region which corresponds to the gigantocellular tegmental field, illustrated in detail elsewhere [22, 23, 30-32] and on plate 37 of Berman’s atlas [29].

Figure 1 summarizes the effects on sleep and wakefulness of microinjecting opioid agonists into the mPRF. All drug effects were compared with microinjections of saline (control). The µ agonist DAGO caused a statistically significant (P < 0.05) increase (71%) in wakefulness (fig. 1A). Analysis of variance revealed no statistically significant effect of any opioid agonist on the amount of time spent in NREM sleep (fig. 1B). Only DAGO significantly (P < 0.05) reduced the time spent in REM sleep (fig. 1C). The δ agonist DPDPE and the κ agonist U50488 had no significant effects on wakefulness, NREM sleep or REM sleep. Kelatorphan had no statistically significant effect on sleep or wakefulness.
As kelatorphan is not an opioid but an enkephaline inhibitor [33], the kelatorphan results are not shown in figure 1 which illustrates the effects of the opioid agonists on sleep and wakefulness.

Comparisons of ventilatory frequency across sleep–wake states showed that it slowed during NREM sleep compared with wakefulness and REM sleep (table 1). No opioid agonist significantly decreased ventilatory frequency below control (saline) values during any sleep–wake state (table 1).

**Discussion**

This study has shown, for the first time, that inhibition of REM sleep produced by opioid microinjection into the mPRF was mediated selectively by the \( \mu \) receptor subtype. The finding that the \( \mu \) agonist DAGO caused a statistically significant decrease in REM and an increase in wakefulness corresponds closely to the effect of mPRF morphine administration in cat [23]. The results support the hypothesis that inhibition of REM sleep caused by systemic opioid administration is mediated, at least in part, by \( \mu \) receptors in the mPRF. Additionally, the absence of opioid-induced decrease in ventilatory frequency suggests that the reduction in the rate of breathing caused by systemically administered opioid is not mediated by a single opioid receptor subtype within the mPRF.

Enkephalinas rapidly terminate the pharmacological actions of enkephalins [33]. Intracerebroventricular (i.c.v.) administration of the enkephalinase inhibitor kelatorphan has been shown to enhance analgesia in the rat [33]. Therefore, we wondered if increasing or prolonging the action of endogenous enkephalins in the mPRF would cause inhibition of REM sleep. In the present study, microinjection of kelatorphan into the mPRF did not inhibit REM sleep. It is not clear if the absence of a kelatorphan effect on REM sleep was caused by methodological differences between the previous report [33] and the present study. These differences include species (rat vs cat), routes of administration (i.c.v. vs mPRF microinjection), maximum doses (50 \( \mu \)g vs 25 \( \mu \)g) and different dependent measures (analgesia vs REM sleep).

Microinjection studies in the intact, unanaesthetized cat have demonstrated cholinergic control of REM sleep and breathing by the mPRF [22, 34]. Carbachol, a cholinergic agonist, produces a REM sleep-like state when microinjected into the mPRF [21, 30–32]. Conversely, administration of atropine into the mPRF inhibits both natural REM sleep [35] and the REM sleep-like state caused by administration of carbachol into the mPRF [21, 30]. Opioids, because of their ability to inhibit release of acetylcholine [36, 37], might logically be expected to modulate cholinergic control of sleep and breathing. Opioid-induced inhibition of release of acetylcholine is dependent on receptor subtype, brain region and species. For example, in rabbit hippocampal slices, \( \mu \), \( \delta \) and \( \kappa \) agonists inhibited acetylcholine release [38]; in rat striatal slices, only \( \delta \) receptor agonists inhibited acetylcholine release; and in rat frontal cortex, opioids were ineffective [39]. Microdialysis of the mPRF in the barbiturate anaesthetized cat showed a decrease in acetylcholine release when morphine was administered systemically [40]. Collectively these results suggest that opioid-induced inhibition of both REM sleep [23] and acetylcholine release [40] is mediated by \( \mu \) opioid receptors in the mPRF. The ineffectiveness of kelatorphan may indicate that endogenous enkephalins in the mPRF are not significantly involved in the regulation of sleep.

In the cat, systemic administration of opioids is known to cause behavioural excitement. Microinjection of morphine directly into the mPRF of intact, unanaesthetized cats inhibited REM sleep without causing behavioural excitement or excessive motor activity [23]. In the present study, pontine administration of DAGO did not cause excess movement or behavioural excitement. While the potential for species-specific effects must be considered, it is clear that the basic and clinical understanding of opioids has been significantly advanced by studies on the cat [4].

The mPRF is involved in the regulation of REM sleep [22], but it has not traditionally been regarded as a brain region influencing breathing [41]. Recent findings, however, have revealed that the mPRF may alter breathing during sleep [34], particularly respiratory depression of REM sleep. Ventilatory frequency is similar during wakefulness and REM sleep [9] (table 1), but breathing during REM sleep is characterized by a disordered respiratory pattern, decreased minute ventilation, decreased carbon dioxide responsiveness, decreased upper airway muscle tone and decreased discharge of pontine respiratory neurones [42–44].

By what mechanisms might the mPRF influence brain stem nuclei controlling breathing? Neuroanatomical studies showed that the mPRF projects to, and receives projections from, brain stem respiratory nuclei and respiratory muscles. Retrograde labelling of the mPRF by pseudorabies virus injection into the posterior cricoarytenoid muscle of the larynx demonstrated a multisynaptic pathway between the mPRF and this upper airway muscle [45]. Fluorescent tracing techniques revealed bi-directional pathways between the mPRF and the pontine and medullary respiratory groups [35]. Thus there are neuronal pathways through which regions of the mPRF known to regulate sleep can influence breathing.
Although sleep has long been known to exacerbate opioid-induced respiratory depression [46], the present study demonstrated that the primary effect of microinjecting the μ opioid agonist DAGO into the reticular formation was inhibition of REM sleep (fig. 1) and not depression of ventilatory frequency (table 1). During wakefulness all three agonists increased ventilatory frequency. In NREM sleep, DPDPE and U50488 increased ventilatory frequency. The mechanisms underlying opioid-induced increases in ventilatory frequency are not known but similar findings have been reported by others [4].

The present study supports the conclusion that μ opioid receptors in the mPRF mediate opioid-induced inhibition of REM sleep. It is unclear if this resulted from transduction properties of the μ receptor itself, the relative number of μ, δ and κ receptors in the mPRF or the binding affinities of the opioid receptor subtypes within the mPRF. Autoradiographic mapping of opioid receptor subtypes in the brain stem has been performed [47, 48], but the precise localization of opioid receptor subtypes in the mPRF of the cat remains to be determined.

Another potential limitation of the present study is the concentration of opioid agonists and the potential for DAGO to act at δ and κ receptors. It can be noted, however, that DPDPE and U50488 did not produce DAGO-like effects (fig. 1).

Synergistic or inhibitory interactions between receptors for different opioid subtypes have been documented (e.g. the μ–δ receptor complex [49]). Thus it is important for future studies to examine interactions between opioid receptor subtypes with regard to opioid-induced inhibition of REM sleep and respiratory depression. Morphine injected directly into the mPRF caused dose-dependent inhibition of REM sleep [23]. In addition, future studies designed to characterize the dose–response relationship between DAGO, DPDPE, U50488 and the amount to which REM sleep is inhibited are advocated.

This study has clinical implications concerning two major opioid side effects for which there is no effective specific therapy: disruption of REM sleep and respiratory depression. For the anaesthetist, the clinical importance of opioid-induced respiratory depression is clear [46, 50, 51]. Less obvious, but gaining increasing recognition, is the significance of opioid-induced inhibition of REM sleep and the intense REM sleep rebound seen after discontinuation of opioid therapy [8, 12–14]. The physiological effects of REM sleep, such as episodic tachycardia, hypertension, apnoea and ventilatory depression, are poorly tolerated by the postoperative patient with impaired ventilatory function and increased cardiovascular demand.

It is also important to determine the severity of opioid-induced REM sleep rebound leading to apnoea. Recent epidemiological studies [52] found that 9% of women and 24% of men have at least five episodes of abnormal breathing during each hour of sleep and that 2% of women and 4% of men have a sleep apnoea syndrome. The fact that this prevalence was observed in a population with no prior diagnosis of sleep apnoea [52] demonstrates that it is not a rare disease. In the perioperative setting, a more complete understanding is needed of the three-way interaction between opioid agonists, REM sleep inhibition and cardiopulmonary regulation before any therapeutic intervention can be designed. The possibility exists that opioid analgesia can be achieved while avoiding disruption of REM sleep and respiratory depression [53, 54].

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References


