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Sex differences in morphine-induced analgesia of visceral pain are supraspinally and peripherally mediated

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Ji, Yaping, Anne Z. Murphy, and Richard J. Traub. Sex differences in morphine-induced analgesia of visceral pain are supraspinally and peripherally mediated. Am J Physiol Regul Integr Comp Physiol 291: R307–R314, 2006. First published March 23, 2006; doi:10.1152/ajpregu.00824.2005.—Increasing evidence suggests there is a sex difference in opioid analgesia of pain arising from somatic tissue. However, the existence of a sex difference in visceral pain and opioid analgesia is unclear. This was examined in the colorectal distention (CRD) model of visceral pain in the current study. The visceromotor response (VMR) to noxious CRD was recorded in gonadally intact male and female rats. Subcutaneous injection of morphine dose-dependently decreased the VMR in both groups without affecting colonic compliance. However, morphine was significantly more potent in male rats than females. Because systemic morphine can act at peripheral tissue and in the central nervous system (CNS), the source of the sex difference in morphine analgesia was determined. The peripherally restricted μ-opioid receptor (MOR) antagonist naloxone methiodide dose-dependently attenuated the effects of systemic morphine. Systemic administration of the peripherally restricted MOR agonist loperamide confirmed peripherally mediated morphine analgesia and revealed greater potency in males compared with females. Spinal administration of morphine dose-dependently attenuated the VMR, but there was no sex difference. Intracerebroventricular administration of morphine also dose-dependently attenuated the VMR with significantly greater potency in male rats. The present study documents a sex difference in morphine analgesia of visceral pain that is both peripherally and supraspinally mediated.

colorectal distention; male; female; μ opioid receptor; visceromotor response; intrathecal; intracerebroventricular

IT IS GENERALLY ACCEPTED THAT females are more sensitive to pain than males (3, 12, 30). Women report lower thresholds, higher pain ratings, and less tolerance to experimental and postsurgical pain compared with men (15, 28). It has also been reported that men have greater activation of the μ-opioid system in the anterior thalamus, ventral basal ganglia, and amygdala than women in response to sustained pain (90), suggesting that sex differences in endogenous opioid receptor activation during painful stimulation might contribute to sex differences in pain.

The existence and direction of sex differences in exogenous opioid-induced analgesia is controversial. Although most animal studies (~66% in rats; see Ref. 22) report that morphine and other μ opioid agonists are more potent in males than females in several tests of acute somatic pain (17, 46, 47, 81), other lines of evidence suggest no difference or differences in the opposite direction. For example, there was no sex difference in the jump test after intracerebroventricular injection of DAMGO, but male rats showed greater analgesic response in the tail flick test (47). Similar studies reported no sex difference in analgesia using systemic fentanyl or buprenorphine in the hot plate or tail withdrawal tests in rats (10) and sex differences in analgesic potency increased when opioid efficacy decreased in the warm water test (81). In persistent pain models, results are mixed. Mu-opioid receptor (MOR) agonists are more potent in male compared with female rats after complete Freund’s adjuvant-induced hyperalgesia (20, 84), but buprenorphine was more potent in females than males during capsaicin induced thermal hyperalgesia (9). The potency of μ agonists also varies across strains in rats and mice (19, 49, 81). Together, this suggests that several factors, including agonist efficacy and administration route, stimulus type, and intensity, as well as strain of subjects, affect the outcome in experimental studies (22, 29, 48).

Sex differences in opioid sensitivity have also been reported in human studies. Clinically, women require less κ opioid receptor agonists after oral surgery (33–35). In contrast, women are reported to use more (5, 15, 61) or less (16) morphine to achieve a similar degree of analgesia after other surgical procedures or during emergency room visits compared with men. However, in experimental pain studies, morphine was either equipotent or more potent in women (31, 67). These reports suggest that morphine may be more effective in men with deep tissue pain but not necessarily during experimental pain.

In comparison to the numerous studies examining sex differences in the potency of opioids in somatic pain, few studies have focused on visceral pain (6, 57). Given that visceral pain such as esophagitis, irritable bowel syndrome, and interstitial cystitis are particularly prevalent in women (12), and opioids are prescribed for alleviation of pain under such circumstances (21, 41), it is important to know whether opioids are more potent in males or females. In the current study, we tested the hypotheses that 1) there is a sex difference in systemic morphine-induced analgesia in a visceral pain model (colorectal distention), and 2) central and/or peripheral μ-opioid receptors...
contribute to the sex difference in morphine sensitivity. Some of these results have appeared in abstract form (39).

METHODS

Male and female Sprague-Dawley rats (220–250 g) were used in the present study. Rats were housed two per cage in same-sex rooms with free access to food and water, and maintained on a 12:12-h light-dark cycle. All protocols were approved by the University of Maryland Dental School Institutional Animal Care and Use Committee.

Visceromotor response. Five to seven days before recording, electromyogram (EMG) electrodes made from Teflon-coated 32-g stainless-steel wire (Cooner Wire, Chatsworth, CA) were implanted in the lateral abdominal wall to monitor reflexive contraction of the abdominal muscles in response to distending the colon (visceromotor response, vmr) (62, 82). After recovery, animals were single housed in same sex rooms until the time of recording.

Animals were fasted for 24 h before recording. Water was available ad libitum. On the day of the experiment, rats were briefly sedated with halothane, and a 5- to 6-cm balloon, made from the finger of a surgical glove and attached to Tygon tubing, was inserted through the anus into the descending colon and rectum. The secured end of the balloon was maintained 1 cm proximal to the external anal sphincter by taping the tubing to the tail. Rats were loosely restrained in Plexiglas rodent restrainers and allowed 30 min to recover from the halothane. Colorectal distention was produced by inflating the distention balloon with air to 60 mmHg (20-s duration, 3-min interstimulus interval). The pressure was monitored and kept constant by a pressure controller/timing device (Bioengineering, University of Iowa). The rats were first distended 12 times to acclimate to the testing protocol, thereby stabilizing the response (62). Subsequent distentions were run in trials of 6 distentions to establish a baseline vmr. In the rare instance that the mean of the two trials differed by more than 20%, an additional trial was run. After establishment of a stable baseline, morphine was administered and the vmr recorded again.

The EMG data were collected and analyzed with a CED 1401plus using Spike 2 software (Cambridge Electronic Design, Cambridge, UK). The EMG was rectified and the area under the curve (AUC) for the 20-s before distention subtracted from the AUC during the 20-s distention to give the magnitude of the vmr.

Catheter implantation and drug administration. The existence of a sex difference in systemic morphine-induced analgesia to noxious colorectal distension (CRD) was first established. To test whether spinal or supraspinal μ-opioid receptors mediate the sex difference, morphine was injected either intrathecally or intracerebroventricularly. The peripheral component of morphine was examined by using naloxone methiodide and loperamide.

Systemic injection. After insertion of the distention balloon, a polyethylene (PE)-10 catheter was subcutaneously implanted in the back of the neck for drug administration. Multiple doses of morphine (actual doses: 0.1, 0.2, 0.7, 2, and 7 mg/kg sc) or loperamide (1, 2, 7, and 10 mg/kg) were administered, and the vmr to CRD was recorded beginning 20 min after each injection. The responses to six distentions were averaged. The interval between each dose of drug was 40 min.

In separate animals, the half-maximal effective dose (ED50) of morphine was administered subcutaneously, and starting 10 min after morphine administration, the vmr was recorded three times over 10 min. A single dose of naloxone methiodide (0.1, 0.3 or 1.0 mg/kg sc) was administered immediately after the third distention, and the vmr was recorded over the next 20 min. The response to five distentions after naloxone methiodide was averaged.

Intrathecal injection. Catheters were implanted at the same time as the EMG electrodes. The rat was anesthetized with halothane and secured in a head holder. The atlantooccipital membrane was exposed, and an incision was made in the membrane. A catheter made of 32 g polyethylene tubing (RecathCo, Allison Park, PA) was inserted 7.8 cm in the subdural space to reach the lumbosacral spinal cord (L6-S2). The catheter and electrode leads were exteriorized at the back of the neck. Animals displaying any sign of paralysis were immediately removed from the study and euthanized (n = 2). During the experiment, multiple doses of morphine (0.1, 0.2, 0.7, 2, 7 μg, in 5 μl saline) were administered intrathecally over a 2-min period and the vmr to CRD was recorded starting 1 min after each injection. The response to five distentions was averaged. The interval between each dose of drug was 20 min. At the completion of each experiment, Evan’s blue (2 μl) was injected to confirm catheter placement.

Intracerebroventricular injection. The rat was anesthetized with a mixture of 55 mg/kg ketamine, 5.5 mg/kg xylazine, and 1.1 mg/kg acepromazine. After EMG electrode placement, the rat was placed into a stereotaxic apparatus. A craniotomy was made dorsal to the lateral ventricle and a stainless steel guide cannula (22 G, C313G, PlasticOne, Roanoke, VA) was implanted at the following coordinates (mm) [bregma: −1.0; ML: 1.2; dorsoventral (DV): 3.2] (64). Two small screws were placed on either side of the sagittal suture to hold the guide cannula in place with cranioplastic cement. During the experiment, increasing doses of morphine (0.1, 0.2, 0.7, 2, 7, and 20 μg, in 2 μl saline) were administered to the ventricle through a 30/32 gauge inner catheter over a 2-min period, and the vmr to CRD was recorded starting 1 min after each injection. The response to five distentions was averaged. The interval between each dose of drug was 20 min. Evan’s blue (1 μl) was injected to confirm injection into the ventricle at the completion of each experiment.

Compliance. The effect of morphine on colonic compliance was measured in awake rats. The volume in the distention balloon was increased from 0–8 ml in 0.5-ml increments, and the pressure was recorded at each volume. Rats were then given 10 mg/ml morphine (sc), and the compliance was measured again 15 min later.

Drugs. Morphine sulfate (supplied by the National Institute on Drug Abuse gift supply program) and naloxone methiodide (Sigma, St. Louis, MO) were dissolved in saline to desired concentrations. Doses used in the present experiment were selected according to previous studies (18, 46, 71). Loperamide (Sigma) was first dissolved in DMSO and brought to the desired concentration with saline (the final concentration of DMSO was 20%). Actual doses administered are listed in the appropriate section of Methods, cumulative doses are shown in the figures when appropriate.

Statistics. The vmr data are reported as AUC, percent baseline response (%baseline) or percent maximum possible effect (%MPE = [postdrug AUC-predrug AUC]/[0-predrug AUC] × 100%), as appropriate and expressed as means ± SE. The dose that reduced the magnitude of the vmr to 50% of baseline (ED50) was determined by nonlinear regression carried out in PRISM (GraphPad Software, San Diego, CA). Data were analyzed using the Student’s t-test or one or two-way repeated-measures (RM) ANOVA followed by Student-Newman-Keuls test for multiple comparisons as appropriate. The compliance data were analyzed by three-way RM ANOVA in SPSS. P < 0.05 was considered significant.

RESULTS

Baseline response to CRD. In a pilot study, we collected data from a group of female rats for which the stage of the estrous cycle was determined by taking vaginal smears for at least 2 cycles. Because no difference in the magnitude of vmr for rats in proestrus, estrus, and metestrus/diestrus stages was observed (48.8 ± 9.4, 47.6 ± 8.7, and 45.3 ± 4.5, respectively, n = 3–6/phase, one-way ANOVA, P = 0.95), the estrous phases for female rats in the present study were not measured.

The baseline vmr was compared between the male and female rats. There were no differences in the baseline responses from same-sex animals in the different experimental paradigms, so the data were pooled (male n = 38, female n =
The mean baseline vmr (AUC) for the female rats was significantly greater than male rats (47.6 ± 2.3 vs. 36.4 ± 1.9, Student’s t-test, P < 0.001).

Effect of systemic morphine. Systemic morphine dose-dependently decreased the vmr in both male (n = 9) and female (n = 6) rats (Fig. 1A; one-way RM ANOVA, P < 0.001, dose for each sex). The potency of morphine was significantly greater in male rats compared with female rats, as reflected by the difference in ED\textsubscript{50}s (Table 1).

There was no difference in colonic compliance between male and female rats (n = 4 each) before administering morphine. Morphine (10 mg/kg sc) did not significantly alter compliance in either the male or female rats (Fig. 1B).

Table 1. ED\textsubscript{50}s of morphine given through different routes in male and female rats

<table>
<thead>
<tr>
<th>Route</th>
<th>Male (mg/kg)</th>
<th>Female (mg/kg)</th>
</tr>
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<tbody>
<tr>
<td>Systemic, sc</td>
<td>1.46 (1.06–2.0)</td>
<td>3.84 (2.71–5.44)*</td>
</tr>
<tr>
<td>Intrathecal, µg</td>
<td>0.18 (0.04–0.94)</td>
<td>0.18 (0.01–2.34)</td>
</tr>
<tr>
<td>Intracerebroventricular, µg</td>
<td>0.7 (0.1–4.66)</td>
<td>7.46 (3.34–16.68)*</td>
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The half-maximal effective dose (ED\textsubscript{50}) calculated with nonlinear regression of the % maximum possible effect data. The 95% confidence limits are listed in parentheses. *P < 0.001 vs. male.

Fig. 1. A: Dose-response curves for male and female rats receiving subcutaneous injections of morphine in a cumulative dosing paradigm. Starting 20 min after each dose of morphine, the visceromotor response (vmr) to colorectal distention (CRD) was recorded. The interval between each injection of morphine was 40 min. B: baseline response before morphine administration. The half-maximal effective dose (ED\textsubscript{50}) for the female rats was significantly higher than for the male rats (see Table 1).

Effect of systemic morphine. To determine the appropriate time period during which the peripheral component of systemic morphine could be compared between the two groups, the time course of the ED\textsubscript{50} dose (sc) of morphine was obtained. The ED\textsubscript{50} calculated from the previous experiment was given to the rats, and the vmr to CRD was recorded during the next 90 min (Fig. 2A, n = 5–6 per group). In both groups, the effect of morphine peaked by 10–20 min after the injection.

Fig. 2. The contribution of peripheral µ-opioid receptors to morphine-induced analgesia. A: time course for analgesia produced by the ED\textsubscript{50} dose of morphine in male and female rats. The vmr to CRD was recorded starting 10 min after morphine administration. There was no difference in the percent maximal possible effect (%MPE) for 45 min after morphine injection during which the naloxone methiodide study was done. B: dose-response curves showing naloxone methiodide reversing the effect of the ED\textsubscript{50} dose of morphine in male and female rats, suggesting there is a peripheral component to morphine analgesia of the vmr. C: dose-response curves for subcutaneous loperamide in male and female rats, showing a sex difference in the peripheral activation of µ-opioid receptors.
Twenty minutes after administering the ED50 dose of morphine, the vmr to CRD was 50.2 ± 6.8% and 46.8 ± 7.5% of their baseline responses in male and female rats, respectively, validating the ED50 values derived from the previous cumulative dose experiment. The vmr to CRD remained at 50% of baseline for 45 min after morphine injection, and no difference was noted between the two sexes during this time (two-way RM ANOVA; sex, dose; \(P = 0.621\)). Approximately 50 min after morphine administration, the analgesic effect began to decrease in both groups. The vmr in male rats returned to baseline by 70 min; in contrast, females had not returned to baseline by 90 min when the experiment was terminated. Subsequent subcutaneous morphine studies were conducted within the 40-min window in which the analgesic effect of morphine was constant in both males and females.

**Effect of naloxone methiodide.** Naloxone methiodide (NalMet), a \(\mu\) opioid receptor preferring quaternary salt that has limited accessibility to the central nervous system (CNS) if given systemically, was used to determine the peripheral component of the analgesic effect of subcutaneous morphine. In this experiment, the vmr was measured before and after subcutaneous administration of the ED50 dose of morphine (\(n = 4\) or 5 per sex/dose) and then following subcutaneous NalMet administration. Each rat received one dose of NalMet. In both male and female rats, NalMet at 0.1 mg/kg did not affect morphine antinociception to noxious CRD (Fig. 2B). NalMet at 0.3 and 1.0 mg/kg dose-dependently reversed the effect of morphine in both sexes with greater potency in males compared with females (Fig. 2B; Two way ANOVA; sex, dose; \(P < 0.05\)). As a control, 1 mg/kg NalMet alone had no effect on the vmr (\(n = 4/sex, \text{data not shown}\)).

**Effect of loperamide.** Loperamide, a peripherally active \(\mu\)-opioid agonist, was used to determine whether there is a sex difference in peripheral \(\mu\)-opioid receptor activity. Loperamide dose-dependently attenuated the vmr to CRD in both male (\(n = 6\)) and female (\(n = 8\)) rats (Fig. 2C; one-way RM ANOVA, \(P < 0.01, \text{dose for each sex}\)). At the highest dose tested, the vmr was attenuated by 77% in male rats, but only 42% in female rats.

**Effect of intrathecal morphine.** To determine whether the sex difference in the potency of systemic morphine was mediated by spinal \(\mu\)-opioid receptors, the effect of intrathecal morphine was compared in male (\(n = 5\)) and female (\(n = 5\)) rats. Intrathecal morphine dose-dependently decreased the vmr to CRD in both male and female rats (Fig. 3A; one-way RM ANOVA, \(P < 0.001, \text{dose for each sex}\)), but no sex difference in the ED50 dose of morphine was observed (Table 1).

In a separate group of animals (\(n = 2–3\) per sex), 1 mg/kg naloxone methiodide (sc) was administered 20 min after 10 \(\mu\)g morphine (it). NalMet did not reverse the effect of morphine, confirming the effect of morphine was restricted in the CNS and NalMet acted peripherally.

**Effect of intracerebroventricular morphine.** To determine whether the difference in the potency of systemic morphine was mediated by supraspinal \(\mu\)-opioid receptors, the effect of intracerebroventricular morphine was compared in male (\(n = 5\)) and female (\(n = 5\)) rats. Intracerebroventricular morphine dose-dependently decreased the vmr to CRD in each group (Fig. 3B; one-way RM ANOVA, \(P < 0.001, \text{dose for each sex}\)). Morphine was more potent in male rats compared with female rats, as reflected by the significant difference in the ED50 dose of morphine (Table 1).

The effects of intrathecal and intracerebroventricular morphine were compared within the same sex group. The baseline AUC for male rats was not different between the intracerebroventricular and intrathecal injection groups (36.5 ± 6.9 vs. 39.1 ± 4.6, \(t\)-test, \(P = 0.76\)). Similarly, no difference in baseline AUC was found in female rats receiving intracerebroventricular or intrathecal injection of morphine (49.1 ± 8.4 vs.
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42.9 ± 5.5, Student’s t-test, P = 0.55). However, intracerebroventricular morphine was less potent in attenuating the vmr compared with intrathecal injection for both sexes (two-way RM ANOVA; male, P < 0.05; female, P < 0.001; Fig. 3C).

DISCUSSION

The present study documents a sex difference in the analgesic response to systemic morphine in a visceral pain model. More importantly, our experiments reveal that the sex difference in the potency of systemic morphine is mediated by peripheral and supraspinal, but not spinal, MOR.

Sex differences in the analgesic potency of morphine to visceral pain. Controversial results have been reported as to whether there is a sex difference in the analgesic effect of morphine. Investigators using different strains of animals and pain assays report males being more sensitive (5, 6, 15, 17, 20, 46, 47, 84), equally sensitive (10, 47), or less sensitive (49) than females to the antinociceptive effect of morphine. The first part of our study, using colorectal distention as a model of visceral pain, shows a clear sex difference in the potency of systemically administered morphine. This is consistent with findings using the hot-plate, tail-flick, and writhing tests (6, 17, 47). Previous studies report no difference in the peak concentration of morphine in the blood and brain and the half-life (t1/2) in male and female rats after subcutaneous injection of morphine (18) or in concentrations of morphine or its metabolites in men and women after intravenous injection (67), suggesting that sex-related differences in the antinociceptive activity of morphine were not due to the difference in absorption or distribution. On the other hand, female rats are reported to have a higher morphine-3-glucuronide (M3G)/morphine ratio after subcutaneous injection of morphine (7). Given that M3G could act as a functional antagonist to the actions of morphine (32, 36, 72), sex differences in plasma M3G/morphine ratio after systemic injection may play a role in sex differences in morphine antinociception.

It should be noted that although the ED₅₀ of systemic morphine in male rats was much lower than that in female rats, the analgesic effect of the ED₅₀ morphine had a shorter duration in the male rats. However, when the same dose of morphine was given to female and male rats, the effect of morphine lasted longer in the males (17). Therefore, it is important to consider both the dose and time elapsed after injection when comparing the analgesic potency of morphine between the sexes.

Do peripheral µ-opioid receptors contribute to sex differences in morphine analgesia? In vivo and in vitro studies report modest or no effect of µ-opioid receptor agonists on resting activity of primary afferent fibers (2). Under painful stimulation (noxious urinary bladder distention), neither morphine nor fentanyl altered the pelvic nerve afferent fiber activity (80). These observations seem to support the widely accepted notion that the analgesic effects of opioids are exclusively mediated by actions in the CNS. However, peripheral opioid receptor activity is regulated by the status of the peripheral tissue/nerve, based on the fact that these receptors become functional after the induction of inflammation (42, 74–76).

The functions of peripheral µ-opioid receptors and the existence of sex differences in their functions in visceral pain have not been well documented, partly because of the location of the visceral organs that makes it hard for local application of a precise amount of drugs. In our study, we used a peripherally restricted µ-opioid receptor-prefering antagonist, naloxone methiodide, and showed that it dose-dependently reversed the effect of systemic morphine, suggesting peripheral µ-opioid receptors are involved in morphine-induced analgesia to nociceptive CRD. This was further supported by the finding that loperamide, a peripherally active µ-opioid agonist that has minimal access to the CNS (26, 69), also dose-dependently attenuated the vmr. These observations are in line with several recent studies demonstrating peripheral antinociceptive effects of opioids in the absence of peripheral inflammation (51, 52) (for reviews, see Refs. 8, 74, 75). This notion is further supported by the findings that: 1) µ-opioid receptors are present in normal dorsal root ganglia (DRG) neurons (38), including those innervating the colon (unpublished observations), as well as nerve endings in human skin and gastrointestinal tract (73, 77); 2) The µ agonist DAMGO-induced MOR G-protein coupling in DRG of noninflamed animals (38, 89); 3) in vitro calcium imaging shows that morphine inhibited the increase in the free intracellular Ca²⁺ concentration evoked by depolarization of DRG neurons (50). However, our observations are in contrast to some of the previous studies showing no effect of intra-arterial morphine on gastric vagal afferent fiber responses to gastric distention or pelvic nerve fiber responses to urinary bladder or colorectal distention (63, 70, 79). The discrepancy between our observation and others might be due to the different route of morphine administration and different experimental preparation. Morphine, when given through the artery, has been reported to have no effect on the somatocardiac sympathetic reflexes, whereas application of morphine into the femoral vein was effective (1). Therefore, it is possible that intra-arterially administered morphine may be quickly removed from the body before it gets to the peripheral µ-opioid receptors. Furthermore, cutting dorsal roots to record from teased fibers may cause changes in peripheral expression of µ-opioid receptors (78, 88), which may explain the ineffectiveness of morphine in neuropathic pain models (25).

Differences in the magnitude of reversal of morphine analgesia by naloxone methiodide was noted between the sexes, suggesting that peripheral µ-opioid receptors contribute to sex differences in visceral analgesia induced by systemic morphine. This was confirmed by the difference in the dose-response curves for systemic loperamide. Because there was no difference in colonic compliance or colon plasma extravasation between male and female rats (40, 83), the sex difference in the peripheral effect of morphine was not due to differences in the peripheral tissue and likely resulted from differences in the colonic afferent neurons. In contrast, a recent study demonstrated loperamide had no effect on paw withdrawal pressure threshold in both male and female rats (20). This difference suggests that peripheral morphine analgesia, in general, and sex differences, in particular, may differ by organ and experimental design.

Do spinal µ-opioid receptors contribute to sex differences in morphine analgesia? We next determined whether the sex difference in the response to systemic morphine was mediated by opioid receptors in the CNS. As a first step, morphine was administered intrathecally, and a potent antinociceptive effect was achieved in both male and female rats with no sex difference. These observations are consistent with the findings...
that MOR immunoreactivity is enriched in the superficial laminae of the dorsal horn (4), and studies reporting intrathecal morphine to be potent in eliciting antinociceptive effects in both male and female subjects (23, 58, 68). Given there is no difference in the dose-response curves to intrathecal morphine between ovariectomized and estrogen replacement rats (39) when the effects of estrogen would be expected to be greatest, the lack of a sex difference is not surprising. Similarly, no sex difference or gonadal hormone-regulated effect of intrathecal morphine has been observed in other somatic or visceral pain models (24, 53, 71).

**Supraspinal action.** Mu-opioid receptors are present in a number of brain areas, including the cerebral cortex, striatum, hippocampus, locus coeruleus, parabrachial nuclei, rostroventromedial medulla (RVM), and periaqueductal gray (PAG) (4, 43), and several of these regions show sexually dimorphic expression of MOR (27). Accordingly, a sex difference in the analgesic effect of supraspinally administered morphine has been reported with tests on somatic tissue (6, 46). Injection of μ-opioid receptor agonists into the RVM and PAG elicited a greater magnitude of antinociception on the tail flick and jump tests in male than female rats (13, 54). After hindpaw inflammation, morphine injected into the PAG is more potent in male compared with female rats (59). To date, however, the effect of intracerebroventricular morphine on visceral pain has not been examined. The result of our study shows for the first time that activation of supraspinal μ-opioid receptors produces a profound sex difference in morphine-induced analgesia in a visceral pain model. No sex difference was noted in the analgesic potency of spinal morphine suggesting a supraspinal mode of action for intracerebroventricular morphine. Further studies need to be carried out to reveal the brain areas where sex differences in the antinociceptive potency of morphine is mediated.

**Spinal vs. supraspinal morphine.** Although both spinal and supraspinal administration of μ-opioid receptor agonists produce potent analgesia in male and female rats, supraspinal morphine was significantly less potent than spinal morphine. This difference likely reflects the difference in the mechanism of action of spinal and supraspinal morphine. Supraspinal morphine alleviates pain indirectly by activating a descending inhibitory system. Morphine in the PAG or RVM inhibits “ON” cells and indirectly excites “OFF” cells leading to antinociception (37). In contrast, intrathecal morphine acts directly on receptors localized in the spinal cord, inhibiting post synaptic neurons and decreasing transmitter release pre-synaptically (60). Given this, it is not surprising to observe synergistic antinociceptive effects of spinal and supraspinal morphine (86, 87).

Possible mechanisms underlying sex differences in μ-opioid receptor-mediated antinociception. Laboratory experiments point to a biological basis for sex differences in opioid analgesia. Opioid receptor density in several pain-related areas [PAG, parabrachial nucleus (PBN)] is significantly lower in female rats during proestrus compared with diestrus and metestrus phases or male rats (27). Because rats in proestrus have the greatest plasma estrogen concentration than the other phases (14), a possible role of 17β-estradiol in modulating μ-opioid receptor expression has been proposed. In fact, short-term treatment with estrogen in ovariectomized rats decreased the number of opioid binding sites in the brain, decreasing the analgesic effects of morphine (11, 66, 85). In addition, estrogen modulates the potency of morphine by influencing G protein coupling (44, 45, 55, 66, 65). Unpublished data from our lab show ovariectomized rats had similar dose-response curves to male rats when morphine was injected systemically, intrathecally, or intracerebroventricularly. Furthermore, morphine is more potent in ovariectomized rats compared with ovariectomized rats with estrogen replacement (39). These data suggest that estrogen decreases MOR function by decreasing available receptors and/or altering receptor coupling to G proteins and may underlie the sex difference in morphine-induced analgesia.

Alternatively, the sex difference in morphine potency may be caused by the higher nociceptive responses to CRD in female rats. However, this seems unlikely since the difference in the baseline response were small (~25%) but statistically significant. In contrast the ED50 dose of morphine differed by more than twofold, suggesting that differences in the baseline nociceptive response likely does not fully account for the differences in morphine potency.

In conclusion, the present experiment reveals a sex difference in the attenuation of colorectal pain produced by systemic morphine. We show clear evidence that morphine analgesia is mediated by peripheral, spinal, and supraspinal μ-opioid receptors. However, the sex difference is supraspinally, and to a lesser extent, peripherally mediated. Although activation of spinal MORs is the most potent site for morphine analgesia, it does not contribute to the sex difference in the analgesic effects of morphine.

**GRANTS**

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Sex Differences in Morphine Analgesia of Visceral Pain


