Published in final edited form as:

Am J Physiol Regul Integr Comp Physiol. 2006 August ; 291(2): R300–R306. doi:10.1152/ajpregu.00022.2006.

Persistent Pain Model Reveals Sex Difference in Morphine Potency

Xiaoya Wang¹, Richard J. Traub^{2,3}, and Anne Z. Murphy^{1,3}

¹ Department of Biology, Center for Behavioral Neuroscience, Georgia State University, 24 Peachtree Center Ave, 402 Kell Hall, Atlanta, GA 30303-3088

² Department of Biological Sciences, Dental School, University of Maryland, Baltimore, MD 21201

³ Research Center for Neuroendocrine Influences on Pain, University of Maryland, Baltimore, MD 21201

Abstract

Central or systemic administration of agonists directed at the mu or delta opiate receptors generally produce a greater degree of analgesia in males than in females. To date, the majority of studies examining sex based differences in opioid analgesia have employed acute noxious stimuli (i.e. tail-flick and hot plate test); thus, the potential dimorphic response of centrally acting opiates in the alleviation of persistent inflammatory pain is not well established. In the present study, right hindpaw withdrawal latency (PWL) to radiant thermal stimuli was measured in intact male and cycling female Sprague-Dawley rats before and after unilateral hindpaw injection of the inflammatory agent complete Freund's adjuvant (CFA). Control animals received intraplantar injection of saline. Twenty four hours after CFA or saline injection, animals received either saline or morphine bisulfate (0.5 – 15 mg/kg; s.c.). Separate groups of control or inflamed animals were tested on their responsiveness to morphine at 7, 14 and 21 days post-CFA or saline. No sex differences were noted for baseline PWLs, and females displayed slightly less thermal hyperalgesia at 24 hrs post-CFA. At all morphine doses administered, both the antihyperalgesic effects of morphine in the inflamed animals, and the antinociceptive effects of morphine in control animals, were significantly greater in males in comparison to females. Similarly, in males, the antihyperalgesic effects of morphine increased significantly at 7–21 days post-CFA; no significant shift in morphine potency was noted for females. These studies demonstrate sex-based differences in the effects of morphine on thermal hyperalgesia in a model of persistent inflammatory pain.

Keywords

antinociception; antihyperalgesic; inflammation; opioids

Chronic pain, defined as pain lasting more than six months, will affect more than one in three Americans at some point in their life (45). Opioids are the most common therapeutic treatment for pain management, with over 10 billion dollars spent each year on opioid-based analgesics (7,8). Morphine, the most commonly prescribed opiate, is the mainstay for the alleviation of severe pain, both acute (obstetric, postoperative) and chronic (cancer, neuropathic). However, there is a compelling body of data to suggest that the potency of opioids differs in men and women (14,17). In studies of acute somatic or visceral pain,

Correspondence should be addressed to: Anne Z. Murphy, Ph.D., Dept. Biology, Center for Behavioral Neuroscience, Georgia State University, 24 Peachtree Center Ave, 402 Kell Hall, Atlanta, GA 30303-3088, Phone: 404.463.9661, Fax: 404.651.2509, amurphy@gsu.edu.

morphine produces both longer lasting and more profound analgesia in males than in females (5,10–12,28,^{30–32},35,36,39,48).

Sex differences in the analgesic and antinociceptive effects of higher efficacy, receptor-specific agonists have not been as consistent, and it is becoming increasingly clear that opiate receptor specificity, route and dose of drug administration (13,15,16), genetic background (21,37,47,58), hormonal status (56,57) and type of analgesiometric test employed (17) are critical factors in determining the pharmacodynamics of opiate analgesia. Surprisingly, while morphine is primarily prescribed for the alleviation of *chronic* pain, the majority of studies conducted to date examining the sexually dimorphic response of opiates have only employed *acute* noxious stimuli, primarily the tail flick and hot plate tests. Thus, potential sex difference in the response of centrally acting opiates in the alleviation of *persistent* pain is not clearly established (12). Two lines of evidence suggest that persistent pain may have a differential influence on the analgesic/antinociceptive effects of opiates in males and in females. First, a number of studies in male rats have reported that the antihyperalgesic properties of opiates change in the presence of persistent inflammation, becoming more potent over time (26,33,54). Several mechanisms have been implicated, including changes in peripheral (55), spinal (18,49,50) and supraspinal (26,27) opioidergic circuits. Second, persistent pain influences the ovulatory cycle and therefore the hormonal status of females. In particular, following induction of persistent inflammatory pain, female rats display irregular estrous cycles, spending the majority of time in diestrus (12). In female rats, this stage of estrus is associated with significantly lower levels of mu opiate receptor expression in several pain-related areas including the midbrain periaqueductal gray (PAG) (19).

Unfortunately, to date the influence of a persistent pain state on the potency of opioids in females remains unclear. In the present study, we tested the hypothesis that systemic morphine produces a differential degree of analgesia in intact males and cycling females using the intraplantar CFA model of persistent inflammatory pain. We also sought to determine whether the ability of opiates to produce an analgesic response is enhanced during conditions of persistent inflammatory pain in both males *and* females.

MATERIALS AND METHODS

Animals

Gonadally intact Sprague-Dawley male and female rats (Zivic Miller), weighing between 250–300g, were used in these studies. Rats were housed in same sex pairs in Plexiglas cages with cardboard bedding. Males and females were housed in separate rooms and maintained on a 12:12 hr light-dark cycle at constant ambient temperature (22°C, relative humidity 40–60%). Access to food and water was ad libitum except during testing. Body weight was monitored before testing and throughout the 21 day experimental session. To allow for acclimation to the environment, all animals were housed under the aforementioned conditions for one week prior to experimental testing. All experiments were conducted in strict compliance with the guidelines established by the International Association for the Study of Pain; all protocols were approved by the Institutional Animal Care and Use Committee at Georgia State University.

To characterize the estrous status of female rats, vaginal smears (using the saline lavage technique) were taken daily beginning one week prior to testing and continuing to the end of the experimental session (21d). Proestrus was defined by the presence of nucleated epithelial cells in >90% of the total cell population; estrus was defined by the presence of cornified epithelial cells; diestrus-1 was defined as the presence of both leukocytes and cornified epithelial cells; diestrus-2 was defined as the relative absence of all cell types (23). All

animals were smeared in the morning, approximately 3–4h after lights on. Vaginal smears were conducted a minimum of two hours prior to testing to minimize the potential effects of vaginal stimulation-produced analgesia (38).

Experimental Protocol

After a one-week acclimation period, animals were weighed and paw diameters determined using calibrated calipers applied midpoint across the dorsal to plantar surface of both hindpaws. Paw withdrawal latency to a noxious thermal stimulus was used to measure baseline thermal thresholds using the Paw Thermal Stimulator (Univ. California San Diego). For this test, the rat is placed in a clear Plexiglas box resting on an elevated glass plate maintained at 30°C. Following a one hour acclimation, a radiant beam of light is positioned under the hindpaw and the time for the rat to remove the paw from the thermal stimulus is electronically recorded as the paw withdrawal latency (PWL). Following baseline PWL determination for both hindpaws (average of 3 trials; 3 min inter-trial interval), inflammation and hyperalgesia were induced by injection of 200 µl of CFA (1:1 oil/saline emulsion; Sigma Chemical Co.) into the plantar surface of the right hindpaw using a sterile 25 gauge needle. Control animals received equivolume of sterile saline (0.9%). Following CFA administration, animals were returned to their home cage.

Twenty-four hours later, animals were returned to the testing environment; body weight and paw diameters were determined and the animals acclimated to the test chamber for one hour. After determining PWL for the inflamed paw, animals were injected with either saline or morphine sulfate (obtained from the National Institute on Drug Abuse, Rockville, MD; dissolved in physiological saline). Saline and morphine injections were administered s.c. in a volume of 1.0 ml/kg; doses of morphine (0.0–15.0 mg/kg) were randomly assigned on the day of testing. Separate groups of animals were used for each dosage (n=6–8/dose). PWL's were then determined at 15, 30, 45, 60, 90 and 120 min post-injection. At the conclusion of the test session, animals were given a euthanizing dose of sodium pentobarbital. Separate groups of animals (n=6/sex/timepoint) were tested using this procedure at 7, 14 and 21 days post-CFA to avoid the development of morphine tolerance. Animals of the same sex were tested together (n=6 per session); males and females were tested at the same time of day by the same experimenter.

Data Analysis

Data are expressed as either raw PWL's or percent maximal possible effect (%MPE), defined as $[(\text{PWL} - \text{CFA bsln})/(\text{Maximal PWL} - \text{CFA bsln})]*100$ (29). A maximal PWL of 20 sec was used to prevent excessive tissue damage due to repeated application of a noxious thermal stimulus. Unpaired t-tests were used to assess for significant differences between sex in raw values (bsln and CFA PWL); unpaired comparisons between sex using percentile data were conducted using the non-parametric Mann-Whitney U test. %MPE was calculated for each animal at each time point post-morphine administration. As no significant differences in %MPE were noted for the 30, 45 and 60 minute time points, these values were averaged for derivation of ED₅₀, defined as the dose of morphine that produced 50% of the maximum possible increase in PWL. ED₅₀ determinations and 95% confidence intervals were determined by nonlinear regression analysis. Kruskal Wallance ANOVA was used to assess for differences in %MPE as a function of time post-CFA. All values are reported as Mean ± S.E.M.; $p < 0.05$ was considered statistically significant. Where multiple comparisons were made, p values were adjusted accordingly using Bonferroni. The 'antinociceptive' effects of morphine are in reference to saline treated animals whereas the term 'antihyperalgesic' refers to the actions of morphine in CFA treated animals (26).

RESULTS

CFA-induced thermal hyperalgesia

No sex differences were noted in PWL's prior to CFA administration (male PWL, 7.30 ± 0.21 seconds ($n = 54$) versus female PWL, 7.06 ± 0.18 seconds ($n = 54$); $t = 0.873$, $df=106$, $p>0.05$). Intraplantar injection of CFA-induced a significant degree of inflammation and thermal hyperalgesia that was restricted to the injected paw. Twenty-four hours post-CFA administration, paw diameters for the injected paw increased on average from 5.79mm to 10.57mm. This percent increase in paw diameter was slightly greater in females ($190 \pm 2\%$) in comparison to males ($176 \pm 2\%$) (MWU, $Z = -4.45$, $p<0.01$; Figure 1). By contrast, no change in paw diameter was noted for either males or females 24hr after intraplantar injection of saline (data not shown). In both males and females, intraplantar CFA-induced a significant degree of thermal hyperalgesia, as reflected by a decrease in PWL's. In both males and females, PWL's decreased from 7.1 sec to approximately 3 sec (Figure 1). The percent change in PWL following CFA administration was slightly higher in females ($-59 \pm 1\%$) in comparison to males ($-53 \pm 2\%$). This small sex difference in % change in baseline was statistically significant (MWU, $Z = -2.67$, $p<0.05$). No change in PWL was noted for the saline control group ($t=0.17$; $df=45$; $p>0.05$); similarly, no significant differences were noted in PWL's for intraplantar saline treated animals in comparison to uninjected controls ($t=0.19$, $df=24$, $p>0.05$) indicating that intraplantar administration of saline had no effect on thermal sensory thresholds.

Hormonal Influences—Estrous cycle status was determined for all female animals via vaginal lavage for a minimum of one week prior to the onset of testing. There were no significant differences in any of the indices examined for diestrus 1 and diestrus 2 so these data are combined. Changes in hormone status had no significant impact on baseline PWL's ($F_{(2,39)}=1.51$, $p>0.05$). Similarly, no significant differences were noted in either the degree of edema, defined as a percent change in paw diameter produced by intraplantar CFA (KW, $H=1.54$, $df=2$, $p>0.05$) or in the degree of thermal hyperalgesia at 24 hours post-CFA, defined as a decrease in PWL (KW, $H=2.79$, $df=2$, $p>0.05$). Together, these data indicate that in females, changes in gonadal steroid levels had no impact on baseline nociceptive thresholds or in CFA-induced thermal hyperalgesia.

Effect of Morphine in 24 hour CFA treated rats

Systemic administration of morphine (0.05–15.0 mg/kg; s.c.) at 24h post-CFA produced dose-dependent increases in PWL. There were no significant differences in %MPE for the 30, 45 and 60 min post-morphine measurements across all doses examined; therefore, these data are averaged. Significant sex differences were noted in the antihyperalgesic effect of morphine; at all doses tested, males consistently had a significantly higher %MPE in comparison to females ($p<0.05$; Figure 2). This sexually dimorphic effect was most evident at the higher doses of morphine. For example, administration of the 8.0 mg/kg dose produced an $80 \pm 12\%$ MPE in males; by contrast, in females, the %MPE was only $37 \pm 11\%$ (MWU; $p<0.01$). Similar sexually dimorphic responses were noted for the 12.0 mg/kg dose, where all males were at 100% MPE; in females, %MPE was $71 \pm 13\%$ (MWU; $p<0.05$; Figure 2). A maximum antihyperalgesic response in females was not noted until the 15 mg/kg dose; this dose of morphine was fatal to all males tested. An example of the sexually dimorphic effect of morphine as a function of time post-injection is shown in Figure 3. ED₅₀ values, determined using sigmoidal dose-response function (variable slope) were 5.93 in males (95% confidence interval of 4.40–8.0) versus 9.4 in females (95% CI = 7.73–11.24) ($p<0.01$). Unfortunately, the influence of gonadal steroids on morphine-induced antihyperalgesia could not be discerned in this study; at 24 hrs post-CFA, the majority of female animals stopped cycling (i.e. females were recorded to be in diestrus 1/2).

Effect of Morphine in Saline treated rats

Morphine (4.0–12.0 mg/kg; s.c.) also produced a significantly greater analgesic response in saline treated males in comparison to females. As shown in Figure 4, significant sex differences in the antinociceptive potency of morphine were noted at all doses examined. Administration of morphine at 8.0 or 12.0 mg/kg produced 100%MPE in all males tested; by contrast, %MPE in females was $37\pm 16\%$ and $58\pm 12\%$, respectively (MWU; $p<0.01$).

Effect of Persistent Pain on Morphine Antihyperalgesia

The antihyperalgesic effect of morphine (8.0 mg/kg, s.c.) was determined at 24h, 7d, 14d and 21d post-CFA or saline ($n=6/\text{sex}/\text{days}$ post-injection). No significant differences were noted in body weight for CFA versus saline injected animals (males, $F_{(1,234)}=2.068$, $p>0.05$; females, $F_{(1,240)}=.27$, $p>0.05$). Figure 5A shows the time course for CFA-induced edema. Paw edema decreased as a function of time post-CFA injection from peak values observed at 24 hr; at 7 days post-CFA, injected paw diameters were 139% of control paw diameter in both males and females. At 14 and 21d post-CFA, paw diameters for the injected paw remained at approximately 131% of the uninjected paw, indicating persistent inflammation. There were no significant sex differences in paw diameter at 7, 14, and 21 days post-CFA (MWU, $p>0.05$). No changes in left paw diameter were noted at any time point in CFA treated animals indicating that CFA-induced edema was limited to the injected paw, and that at 21d post-injection, the inflammation had not spread to the contralateral paw.

The time course for CFA-induced thermal hyperalgesia is shown in Figure 5B. In males, paw withdrawal latencies were significantly different from baseline at 1, 7 and 14 days post-CFA ($p<0.05$). In females, paw withdrawal latencies were significantly shorter than baseline at all four time points ($p<0.05$). No significant differences were noted in percent change in baseline for males versus females at any of the time points examined ($p>0.05$).

The effects of persistent pain on morphine-induced antihyperalgesia are shown in Figure 6. In males, the %MPE induced by morphine (8 mg/kg; s.c.) significantly increased as a function of time post-CFA (KW, $H=13.12$, $p<0.01$). By contrast, in females, the duration of inflammation had no significant effect on morphine-induced antihyperalgesia ($H=2.67$, $df=3$, $p>0.05$; Figure 6). No differences in %MPE were noted for the 30, 45 and 60 min time periods so these data are averaged in Figure 6C. In females, the average %MPE following 8 mg/kg morphine increased from $45\pm 11\%$ on day 1 post-CFA to approximately 59% on days 7 and 14, to $80\pm 7\%$ at day 21 post-CFA. In males, the average %MPE increased from $69\pm 13\%$ on day 1 post-CFA to $100\pm 0\%$ at day 7; %MPE remained at 100% at 14 and 21 days post-CFA.

No changes in the antinociceptive effects of morphine were noted in saline treated animals (Figure 7). In males, %MPE ranged from $90\pm 10\%$ to $100\pm 0\%$ (KW, $H=3.91$, $df=3$, $p>0.05$) suggesting a ceiling effect; in females, %MPE ranged from $14\pm 4\%$ to $49\pm 27\%$ (KW, $H=4.44$, $df=3$, $p>0.05$). The degree of antinociception produced by 8.0 mg/kg of morphine was significantly greater in males in comparison to females at all time points examined (MWU, $p<0.01$).

DISCUSSION

The results of these studies clearly demonstrate that morphine produces a differential degree of antihyperalgesia in response to thermal stimuli in a model of persistent inflammatory pain. At all doses tested, the antihyperalgesic effect of morphine in CFA treated animals was significantly greater in males than in females. Similar results were noted in saline treated animals, where morphine produced a significantly greater antinociceptive effect in males in comparison to females. The results of these studies also demonstrate that in males, the

antihyperalgesic effects of morphine are significantly enhanced in the presence of persistent inflammation. In contrast, in females the presence of persistent inflammation had no influence on morphine potency. In the present study, sensory thresholds were assayed using a thermal noxious stimulus; however, as mechanical hypersensitivity and thermal hypersensitivity are genetically distinct (42), future studies employing a mechanical stimulus are warranted.

The observed sex differences in the antihyperalgesic and antinociceptive effects of morphine are not due to sex differences in baseline sensory thresholds, CFA-induced edema, or in the degree of thermal hyperalgesia produced by intraplantar CFA; rather, these results suggest that there is something inherently different between males and females in the mechanisms of morphine action in the presence of persistent inflammatory pain. Morphine's actions are mediated primarily by the mu opioid receptor (46,52), a member of the inhibitory G protein coupled receptor superfamily. Kelly et al. (34) reported that in hypothalamic slices, estrogen rapidly uncouples the mu opioid receptor (MOR) from G-protein-gated inwardly rectifying potassium channels, thereby significantly decreasing MOR agonist-induced hyperpolarization (44). Estrogen has also been shown to result in MOR internalization in the rat hypothalamus, thereby limiting the amount of receptor available for exogenous ligand binding (20). These results parallel studies using acute pain assays in which high estrogen levels were associated with decreased opioid potency (56). In the present study, the effects of estrogen on morphine potency in animals with persistent inflammatory pain could not be discerned; within 24 hrs of intraplantar CFA, most females were in diestrus and remained there for the duration of the study. Interestingly, during diestrus, plasma estrogen levels are low; therefore, the maintenance of our sex difference in opioid antihyperalgesia suggests that the levels of estrogen present during diestrus may be sufficient to influence morphine binding to the mu receptor and/or the subsequent signaling cascades. Alternatively, these results may also implicate additional mechanisms outside of the 'activational' effects of gonadal steroids as contributing to the observed sex difference in morphine antihyperalgesia (43).

While morphine has been shown to act at a variety of central and peripheral sites, several lines of evidence suggest that the midbrain periaqueductal gray (PAG) is a primary site of action for systemic morphine. Lesions of the PAG, or intra-PAG administration of MOR antagonists significantly attenuate the antinociceptive effects of systemic morphine (22,41). There are no direct projections from the PAG to the dorsal horn of the spinal cord, the site of primary afferent termination. Rather, PAG efferents terminate directly onto spinally-projecting neurons within the rostral ventromedial medulla (RVM), and this PAG-RVM-spinal cord circuit has been shown to be a primary circuit for both endogenous pain modulation and opioid-based analgesia (2–4). Recent anatomical studies in the rat have demonstrated that the PAG-RVM circuit is sexually dimorphic both in its anatomical organization as well as in its activation during persistent inflammatory pain (43). In addition, systemic morphine preferentially suppresses the activation of this circuit in males, but not females. More recently, we have reported significant sex differences in MOR protein levels within the caudal ventrolateral PAG, with intact males having 11% higher expression levels than diestrus females (19). Together, these data suggest that the PAG, and its descending projections to the RVM and spinal cord, may provide the anatomical substrate for the observed sex differences in the effects of morphine.

The results of the present study demonstrating a significant sex difference in morphine potency are consistent with previous studies using acute pain models in which morphine was injected either systemically (1,11,31,32,36,48), or directly into the RVM (6) or PAG (39,40). The results of animal studies parallel recent findings in humans indicating that females require 30% more morphine than males to achieve a comparable level of analgesia

for relief of post-surgical pain (9). Recently, using the CFA adjuvant-induced arthritis model, Cook and Nickerson (12) reported that the antihyperalgesic effects of morphine, as well as the mu agonists oxycodone and butorphanol, were significantly greater in males in comparison to females. Similar to the present results, the authors also reported an increase in morphine potency as a function of post-arthritic time in males, with no corresponding change in ED₅₀ values in vehicle-treated animals or CFA-treated females. Interestingly, these investigators also reported that female rats ceased to cycle normally following CFA administration, suggesting that chronic inflammation interferes with the normal estrus cycle in females. These results together suggest serious implications for the reproductive health of women suffering from chronic pain conditions.

In males, the antihyperalgesic effects of morphine, assessed using a thermal stimulus, were potentiated as a function of time post-inflammatory pain. No change in morphine antinociception was noted in saline treated animals, suggesting that the observed increase in morphine potency was due to the presence of persistent inflammation. Similar to the results obtained in the present study, Hurley and Hammond (27), also using a thermal stimulus, reported a significant increase in the antihyperalgesic potency of the mu agonist DAMGO, administered directly into the RVM, as a function of time post-inflammation. More importantly, they reported that the antinociceptive potency of DAMGO, determined for the contralateral, uninflamed paw, was also progressively enhanced as a function of time post-injury, with the magnitude of enhancement paralleling the chronicity of the inflammation. Together with the present findings, this suggests that in males, persistent inflammation alters the responsiveness of opioid-sensitive CNS circuits to exogenous opioid administration. No significant change in opioid potency was observed in females as a function of time post-CFA. However, a slight increase in the antihyperalgesic effect of morphine was observed at 21 days post-CFA, suggesting that a leftward shift in morphine potency may have been observed given a longer inflammatory time.

Several mechanisms have been proposed to account for persistent inflammation induced changes in opioid potency, including upregulation glutamate receptors in the RVM (24,25), increased MOR expression and second messenger coupling in the lumbar dorsal root ganglia (51,60) and increased release of endogenous opioids in several supraspinal sites, including the PAG and RVM (27,53,59). These results together suggest that in males, multiple mechanisms contribute to persistent pain induced changes in opioid potency. In females, an increase in morphine potency was not observed until 21 days post-CFA; this suggests that either the aforementioned changes are not induced in females, or there are other compensatory mechanisms occurring, such that the changes at one site are countered by changes at another.

In summary, these results indicate that morphine produces a greater degree of antihyperalgesia and antinociception in males in comparison to females in a model of persistent inflammatory pain. The results also demonstrate that the persistent inflammation-induced enhancement of opioid potency, as measured by thermal hyperalgesia, is restricted to males. Studies are currently underway examining the potential mechanisms underlying the observed sex differences in the effects of morphine.

Acknowledgments

We would like to thank Michael Gold for comments on an earlier version of this manuscript. We would also like to thank Ryan Castiniera for technical assistance.

Grants: This work was supported by NIH DA16272 and AR69555 awarded to AZM.

References

1. Bartok RE, Craft RM. Sex differences in opioid antinociception. *J Pharmacol Exp Ther* 1997;282:769–778. [PubMed: 9262340]
2. Basbaum AI, Clanton CH, Fields HL. Three bulbospinal pathways from the rostral medulla of the cat: an autoradiographic study of pain modulating systems. *J Comp Neurol* 1978;178:209–224. [PubMed: 627624]
3. Basbaum AI, Fields HL. Endogenous pain control mechanisms: review and hypothesis. *Ann Neurol* 1978;4:451–462. [PubMed: 216303]
4. Basbaum AI, Fields HL. Endogenous pain control systems: Brainstem spinal pathways and endorphin circuitry. *Ann Rev Neurosci* 1984;7:309–338. [PubMed: 6143527]
5. Bodnar RJ, Romero MT, Kramer E. Organismic variables and pain inhibition: roles of gender and aging. *Brain Res Bull* 1988;21:947–953. [PubMed: 3066444]
6. Boyer JS, Morgan MM, Craft RM. Microinjection of morphine into the rostral ventromedial medulla produces greater antinociception in male compared to female rats. *Brain Res* 1998;796:315–318. [PubMed: 9689486]
7. Brookoff D. Chronic pain: 1. A new disease? *Hosp Pract (Off Ed)* 2000;35:45–52. 59.
8. Brookoff D. Chronic pain: 2. The case for opioids. *Hosp Pract (Off Ed)* 2000;35:69–72. 75–66, 81–64.
9. Cepeda MS, Carr DB. Women experience more pain and require more morphine than men to achieve a similar degree of analgesia. *Anesth Analg* 2003;97:1464–1468. [PubMed: 14570666]
10. Cicero TJ, Nock B, Meyer ER. Gender-related differences in the antinociceptive properties of morphine. *J Pharmacol Exp Ther* 1996;279:767–773. [PubMed: 8930182]
11. Cicero TJ, Nock B, Meyer ER. Sex-related differences in morphine's antinociceptive activity: relationship to serum and brain morphine concentrations. *J Pharmacol Exp Ther* 1997;282:939–944. [PubMed: 9262361]
12. Cook CD, Nickerson MD. Nociceptive sensitivity and opioid antinociception and antihyperalgesia in Freund's adjuvant-induced arthritic male and female rats. *J Pharmacol Exp Ther* 2005;313:449–459. [PubMed: 15608071]
13. Craft RM. Sex differences in drug- and non-drug-induced analgesia. *Life Sci* 2003;72:2675–2688. [PubMed: 12679185]
14. Craft RM. Sex differences in opioid analgesia: "from mouse to man". *Clin J Pain* 2003;19:175–186. [PubMed: 12792556]
15. Craft RM, Bernal SA. Sex differences in opioid antinociception: kappa and 'mixed action' agonists. *Drug Alcohol Depend* 2001;63:215–228. [PubMed: 11418226]
16. Craft RM, Kruzich PJ, Boyer JS, Harding JW, Hanesworth JM. Sex differences in discriminative stimulus and diuretic effects of the kappa opioid agonist U69,593 in the rat. *Pharmacol Biochem Behav* 1998;61:395–403. [PubMed: 9802834]
17. Craft RM, Mogil JS, Aloisi AM. Sex differences in pain and analgesia: the role of gonadal hormones. *Eur J Pain* 2004;8:397–411. [PubMed: 15324772]
18. Dubner R, Ruda MA. Activity-dependent neuronal plasticity following tissue injury and inflammation. *Trends Neurosci* 1992;15:96–103. [PubMed: 1373925]
19. Duncan KA, Murphy AZ. Sex-linked differences in mu opiate receptor distribution in the rat brain. *Soc Neuroscience Abstr.* 2005
20. Eckersell CB, Popper P, Micevych PE. Estrogen-induced alteration of mu-opioid receptor immunoreactivity in the medial preoptic nucleus and medial amygdala. *J Neurosci* 1998;18:3967–3976. [PubMed: 9570823]
21. Flores CM, Mogil JS. The pharmacogenetics of analgesia: toward a genetically-based approach to pain management. *Pharmacogenomics* 2001;2:177–194. [PubMed: 11535108]
22. Flores JA, El Banoua F, Galan-Rodriguez B, Fernandez-Espejo E. Opiate anti-nociception is attenuated following lesion of large dopamine neurons of the periaqueductal grey: critical role for D1 (not D2) dopamine receptors. *Pain* 2004;110:205–214. [PubMed: 15275769]

23. Freeman, M. The neuroendocrine control of the ovarian cycle of the rat. In: Knobil, E.; Neill, J., editors. *The Physiology of Reproduction*. New York: Raven Press Ltd; 1988. p. 1893-1928.
24. Guan Y, Guo W, Zou SP, Dubner R, Ren K. Inflammation-induced upregulation of AMPA receptor subunit expression in brain stem pain modulatory circuitry. *Pain* 2003;104:401-413. [PubMed: 12855351]
25. Guan Y, Terayama R, Dubner R, Ren K. Plasticity in excitatory amino acid receptor-mediated descending pain modulation after inflammation. *J Pharmacol Exp Ther* 2002;300:513-520. [PubMed: 11805211]
26. Hurley RW, Hammond DL. The analgesic effects of supraspinal mu and delta opioid receptor agonists are potentiated during persistent inflammation. *J Neurosci* 2000;20:1249-1259. [PubMed: 10648729]
27. Hurley RW, Hammond DL. Contribution of endogenous enkephalins to the enhanced analgesic effects of supraspinal mu opioid receptor agonists after inflammatory injury. *J Neurosci* 2001;21:2536-2545. [PubMed: 11264327]
28. Islam AK, Cooper ML, Bodnar RJ. Interactions among aging, gender, and gonadectomy effects upon morphine antinociception in rats. *Physiol Behav* 1993;54:45-53. [PubMed: 8392209]
29. Jensen TS, Yaksh TL III. Comparison of the antinociceptive action of mu and delta opioid receptor ligands in the periaqueductal gray matter, medial and paramedial ventral medulla in the rat as studied by microinjection technique. *Brain Res* 1986;372:301-312. [PubMed: 2871901]
30. Ji Y, Murphy AZ, Traub RJ. Estrogen modulates the visceromotor reflex and responses of spinal dorsal horn neurons to colorectal stimulation in the rat. *J Neurosci* 2003;23:3908-3915. [PubMed: 12736360]
31. Kavaliers M, Innes D. Stress-induced opioid analgesia and activity in deer mice: sex and population differences. *Brain Res* 1987;425:49-56. [PubMed: 3427425]
32. Kavaliers M, Innes DG. Sex and day-night differences in opiate-induced responses of insular wild deer mice, *Peromyscus maniculatus triangularis*. *Pharmacol Biochem Behav* 1987;27:477-482. [PubMed: 3659070]
33. Kayser V, Guilbaud G. The analgesic effects of morphine, but not those of the enkephalinase inhibitor thiorphan, are enhanced in arthritic rats. *Brain Res* 1983;267:131-138. [PubMed: 6574799]
34. Kelly MJ, Qiu J, Ronnekleiv OK. Estrogen modulation of G-protein-coupled receptor activation of potassium channels in the central nervous system. *Ann N Y Acad Sci* 2003;1007:6-16. [PubMed: 14993035]
35. Kepler KL, Kest B, Kiehl JM, Cooper ML, Bodnar RJ. Roles of gender, gonadectomy and estrous phase in the analgesic effects of intracerebroventricular morphine in rats. *Pharmacol Biochem Behav* 1989;34:119-127. [PubMed: 2626443]
36. Kepler KL, Standifer KM, Paul D, Kest B, Pasternak GW, Bodnar RJ. Gender effects and central opioid analgesia. *Pain* 1991;45:87-94. [PubMed: 1677751]
37. Kest B, Wilson SG, Mogil JS. Sex differences in supraspinal morphine analgesia are dependent on genotype. *J Pharmacol Exp Ther* 1999;289:1370-1375. [PubMed: 10336528]
38. Komisaruk BR. Antinociceptive effects of vaginal stimulation in rats: neurophysiological and behavioral studies. *Brain Res* 1977;137:85-107. [PubMed: 922508]
39. Krzanowska EK, Bodnar RJ. Analysis of sex and gonadectomy differences in B-endorphin antinociception elicited from the ventrolateral periaqueductal gray in rats. *Eur J Pharm* 2000;392:157-161.
40. Krzanowska EK, Bodnar RJ. Morphine antinociception elicited from the ventrolateral periaqueductal gray is sensitive to sex and gonadectomy differences in rats. *Brain Res* 1999;821:224-230. [PubMed: 10064807]
41. Lane DA, Patel PA, Morgan MM. Evidence for an intrinsic mechanism of antinociceptive tolerance within the ventrolateral periaqueductal gray of rats. *Neuroscience* 2005;135:227-234. [PubMed: 16084660]
42. Lariviere WR, Wilson SG, Laughlin TM, Kokayeff A, West EE, Adhikari SM, Wan Y, Mogil JS. Heritability of nociception. III. Genetic relationships among commonly used assays of nociception and hypersensitivity. *Pain* 2002;97:75-86. [PubMed: 12031781]

43. Loyd D, Murphy AZ. Sex differences in the anatomical and functional organization of the periaqueductal gray-rostral ventromedial medullary pathway in the rat: A potential circuit mediating the sexually dimorphic actions of morphine. *J Comp Neurol*. 2006 in press.
44. Malyala A, Kelly MJ, Ronnekleiv OK. Estrogen modulation of hypothalamic neurons: Activation of multiple signaling pathways and gene expression changes. *Steroids* 2005;70:397–406. [PubMed: 15862823]
45. Martin, PR. Headaches. In: King, NJ.; Remenyi, A., editors. *Health Care: A Behavioral Approach*. Sydney, Australia: Grune & Stratton; 1986. p. 145-157.
46. Matthes HW, Maldonado R, Simonin F, Valverde O, Slowe S, Kitchen I, Befort K, Dierich A, Le Meur M, Dolle P, Tzavara E, Hanoune J, Roques BP, Kieffer BL. Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene. *Nature* 1996;383:819–823. [PubMed: 8893006]
47. Mogil JS, Chesler EJ, Wilson SG, Juraska JM, Sternberg WF. Sex differences in thermal nociception and morphine antinociception in rodents depend on genotype. *Neurosci Biobehav Rev* 2000;24:375–389. [PubMed: 10781697]
48. Negus SS, Mello NK. Opioid antinociception in ovariectomized monkeys: comparison with antinociception in males and effects of estradiol replacement. *J Pharmacol Exp Ther* 1999;290:1132–1140. [PubMed: 10454487]
49. Ossipov MH, Kovelowski CJ, Porreca F. The increase in morphine antinociceptive potency produced by carrageenan-induced hindpaw inflammation is blocked by naltrindole, a selective delta-opioid antagonist. *Neurosci Lett* 1995;184:173–176. [PubMed: 7715840]
50. Parra MC, Nguyen TN, Hurley RW, Hammond DL. Persistent inflammatory nociception increases levels of dynorphin 1–17 in the spinal cord, but not in supraspinal nuclei involved in pain modulation. *J Pain* 2002;3:330–336. [PubMed: 14622757]
51. Shaqura MA, Zollner C, Mousa SA, Stein C, Schafer M. Characterization of mu opioid receptor binding and G protein coupling in rat hypothalamus, spinal cord, and primary afferent neurons during inflammatory pain. *J Pharmacol Exp Ther* 2004;308:712–718. [PubMed: 14593084]
52. Sora I, Takahashi N, Funada M, Ujike H, Revay RS, Donovan DM, Miner LL, Uhl GR. Opiate receptor knockout mice define mu receptor roles in endogenous nociceptive responses and morphine-induced analgesia. *Proc Natl Acad Sci U S A* 1997;94:1544–1549. [PubMed: 9037090]
53. Spetea M, Rydelius G, Nylander I, Ahmed M, Bileviciute-Ljungar I, Lundeborg T, Svensson S, Kreicbergs A. Alteration in endogenous opioid systems due to chronic inflammatory pain conditions. *Eur J Pharmacol* 2002;435:245–252. [PubMed: 11821033]
54. Stein C, Millan MJ, Herz A. Unilateral inflammation of the hindpaw in rats as a model of prolonged noxious stimulation: alterations in behavior and nociceptive thresholds. *Pharmacol Biochem Behav* 1988;31:455–451. [PubMed: 3244721]
55. Stein C, Millan MJ, Yassouridis A, Herz A. Antinociceptive effects of mu- and kappa-agonists in inflammation are enhanced by a peripheral opioid receptor-specific mechanism. *Eur J Pharmacol* 1988;155:255–264. [PubMed: 2853065]
56. Stoffel EC, Ulibarri CM, Craft RM. Gonadal steroid hormone modulation of nociception, morphine antinociception and reproductive indices in male and female rats. *Pain* 2003;103:285–302. [PubMed: 12791435]
57. Stoffel EC, Ulibarri CM, Folk JE, Rice KC, Craft RM. Gonadal hormone modulation of mu, kappa, and delta opioid antinociception in male and female rats. *J Pain* 2005;6:261–274. [PubMed: 15820914]
58. Turner JM, Lomas LM, Picker MJ. Influence of estrous cycle and gonadal hormone depletion on nociception and opioid antinociception in female rats of four strains. *J Pain* 2005;6:372–383. [PubMed: 15943959]
59. Williams FG, Mullet MA, Beitz AJ. Basal release of Met-enkephalin and neurotensin in the ventrolateral periaqueductal gray matter of the rat: a microdialysis study of antinociceptive circuits. *Brain Res* 1995;690:207–216. [PubMed: 8535838]
60. Zollner C, Shaqura MA, Bopaiah CP, Mousa S, Stein C, Schafer M. Painful inflammation-induced increase in mu-opioid receptor binding and G-protein coupling in primary afferent neurons. *Mol Pharmacol* 2003;64:202–210. [PubMed: 12869624]

Figure 1.

(A) Mean paw diameter for the uninjected (left) and CFA-injected paw (right) in male and female rats. The percent change in paw diameter is shown on the right. (B) Paw withdrawal latencies (PWL) for male and female rats following intraplantar CFA. The percent change in baseline is shown on the right.

Figure 2. Morphine-induced antihyperalgesia in male and female CFA-treated animals. Data are plotted as mean percent maximal possible effect (%MPE) as a function of morphine dose. * denotes $p < 0.05$ for male-female comparison.

Figure 3.

Time course for morphine-induced antihyperalgesia (8.0 mg/kg dose) in CFA-treated male and female rats. Data are plotted as percent maximal possible effect as a function of time post-injection.

Figure 4. Morphine-induced antinociception in saline-treated male and female rats. Data are plotted as mean percent maximal possible effect (%MPE) as a function of morphine dose.

Figure 5.
Time course for changes in paw diameter (A) and paw withdrawal latencies (B) as a function of time-post intraplantar CFA administration.

Figure 6.

The effects of persistent inflammatory pain on the antihyperalgesic actions of morphine (8.0 mg/kg) in male (A) and female (B) rats. DPI = days post-CFA injection. Data are plotted as percent maximal possible effect (%MPE) as a function of time post-morphine administration. (C) Data from the 30, 45 and 60 minutes post-morphine time points were averaged and are plotted as mean %MPE as a function of time post-CFA.

Figure 7.
The effects of intraplantar saline on the antinociceptive actions of morphine (8.0 mg/kg) in male and female rats. Data are plotted as mean %MPE as a function of time post-saline.