Capsaicin-Induced Hyperalgesia and \( \mu \)-Opioid-Induced Antihyperalgesia in Male and Female Fischer 344 Rats

ANDREW C. BARRETT, ERIC S. SMITH, and MITCHELL J. PICKER

Department of Psychology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

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ABSTRACT

The influence of sex in determining responses to opioid analgesics has been well established in rodents and monkeys in assays of short-lasting, phasic pain. The purpose of this investigation was to use a capsaicin model of tonic pain to evaluate sex differences in hyperalgesia and \( \mu \)-opioid-induced antihyperalgesia in Fischer 344 (F344) rats. Capsaicin injected into the tail produced a dose-dependent thermal hyperalgesia in males and females, with the dose required to produce a comparable level of hyperalgesia being 3.0-fold higher in males than in females. These sex differences were modulated by gonadal hormones, inasmuch as gonadectomy increased the potency of capsaicin in males and decreased its potency in females. Morphine, buprenorphine, and dezocine administered by various routes [systemic (s.c.), local (in the tail), and central (i.c.v.)] generally produced marked antihyperalgesic effects in males and females. Although in most instances these opioids were equally potent and effective in males and females, selected doses of local and i.c.v. administered buprenorphine produced greater effects in females. When administered locally, the antihyperalgesic effects of morphine were mediated by peripheral opioid receptors in both males and females, since this effect was not reversed by i.c.v. naloxone methiodide. These data contrast with the finding that \( \mu \)-opioids are more potent in male rodents in assays of phasic pain, thus suggesting that distinct mechanisms underlie male and female sensitivity to opioid antinociception in phasic and tonic pain models. These findings emphasize the need to test male and female rodents in tonic pain assays that may have greater relevance for human pain conditions.

It is becoming increasingly clear that the sex of the organism is a critical determinant of responsiveness to nociceptive stimuli and opioid analgesics. In rodents and primates, for example, males are generally more sensitive to the acute antinociceptive effects of opioids than females in assays of thermal, electrical, and mechanical nociception (Kepler et al., 1991; Cicero et al., 1996; Negus and Mello 1999; Barrett et al., 2002). These findings do, however, contrast with those obtained in humans where the available data indicate that in the postsurgical setting, females are generally more sensitive than males to the antinociceptive effects of opioids (Gear et al., 1996, 1999).

Nonhuman studies of sex differences have utilized acute, phasic pain tests (e.g., tail-flick, hot plate assay), in which a noxious stimulus is applied to a portion of the body for a brief period of time, and the trial is terminated when the animal executes an escape/withdrawal response (for a review, see Mogil et al., 2000). Animal models of tonic pain, in which nociception is prolonged and can be associated with inflammation and tissue damage, have been developed to model the profile of human clinical pain conditions, but not adapted to the study of sex differences in opioid antinociception. In these models, nociception is produced by the administration of one of a number of algogenic agents (e.g., formalin, capsaicin), and pain behaviors or responses to nociceptive stimuli are measured.

In addition to duration, phasic and tonic pain differ in the type of neuronal fibers that transmit afferent nociceptive information. Whereas high intensity, phasic nociceptive stimuli generally activate large-diameter, fast-conducting A\( \delta \) fibers, tonic pain is maintained primarily by activity in slower-conducting C fibers (LaMotte et al., 1992; McCall et al., 1996; Le Bars et al., 2001). Furthermore, tonic and phasic pain tests have distinct neurochemical and anatomical substrates, and can be differentiated behaviorally (e.g., type, duration, and frequency of nociceptive responses) and phar-

ABBREVIATIONS: NLX-M, naloxone methiodide; GDX, gonadectomized; ANOVA, analysis of variance; GTP\( \gamma \)S, guanosine-5’-O-(3-[\( ^{35} \)S]thio) triphosphate.

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macologically (e.g., sensitivity to reversal by N-methyl-d-aspartate receptor antagonists) (Abbott et al., 1982; Trujillo and Akil, 1991; Chaplain et al., 1997; Le Bars et al., 2001). Moreover, in most phasic pain models, opioid analgesics can produce effects via spinal and supraspinal sites, whereas in tonic pain models opioids are also effective when administered peripherally at the site of injury (Yaksh, 1997). Given these differences, testing males and females in assays of tonic pain should provide a more comprehensive understanding of the nature of sex differences.

Capsaicin, the pungent ingredient in hot chili peppers, is a compound that has been widely used in rodents and humans to study pain mechanisms and to evaluate the analgesic efficacy of various drugs (Holzer, 1991; LaMotte et al., 1992; Park et al., 1995; Ko et al., 1998). Excitation by capsaicin of nociceptors such as C-fibers triggers release of glutamate and neuropeptides from their central terminals in the spinal cord dorsal horn, as well as proinflammatory peptides such as substance P and calcitonin gene-related peptide from their peripheral terminals. These biochemical events are responsible for the hyperalgesia/allodynia, vasodilation, plasma extravasation, and flare commonly observed after administration of capsaicin to human and nonhuman animals (Holzer, 1991; LaMotte et al., 1992). Because these symptoms are consistent across species and similar to those reported in clinical pain conditions produced by inflammation or nerve injury, the use of capsaicin is suitable to model tonic pain.

One purpose of the present investigation was to determine the magnitude and duration of capsaicin-induced thermal hyperalgesia in male and female intact and gonadectomized (GDX) rats. To this end, capsaicin was injected into the distal portion of the rat’s tail, and hyperalgesia was quantified by measuring the tail-withdrawal latency from 45°C water (Ko et al., 2000). A second purpose was to compare the ability of the μ-opioids morphine, buprenorphine and dezocine to reverse this hyperalgesia in male and female rats. The use of the low-efficacy opioids buprenorphine and dezocine was of critical importance, since recent studies suggest that the magnitude of sex differences in opioid antinociception is considerably larger with less efficacious opioids (Barrett et al., 2001).

In models of inflammation, opioids can produce their effects in the periphery or in the central nervous system (Perrot et al., 1999; Ko et al., 2000), and to address this issue, opioids were administered systemically (s.c. in the back), locally (in the tail), and centrally (i.c.v.). To confirm that local administration of morphine was producing antihyperalgesia via peripheral opioid receptors located in the tail, capsaicin was co-injected with morphine in the tail, and naloxone methiodide (NLX-M), an opioid antagonist with limited ability to cross the blood-brain barrier, was administered i.c.v.

Materials and Methods

Subjects. Intact and GDX male and female F344 rats were obtained from Charles River Laboratories, Inc. (Wilmington, MA). Although sex differences have been observed in numerous rat strains, F344 rats were tested for the extremely robust and consistent sex differences observed in this strain in assays of phasic pain (Cook et al., 2000; Barrett et al., 2001). Gonadectomies were performed at the supplier when the rats were 3 months of age, and at least 3 weeks elapsed before any testing was conducted. All testing occurred between 3 and 6 months of age, and rats were individually housed in a colony on a 12-h/12-h light/dark cycle. All rats had unlimited access to food and water.

Apparatus and Testing. A warm-water tail withdrawal procedure, similar to that described by Ko et al. (2000), was used to assess hyperalgesia and opioid-induced antihyperalgesia. Before testing, each rat was habituated to experimenter handling on one occasion. During testing, each rat was lightly restrained under a towel, with the distal 7 cm of the tail immersed in water maintained at 45°C, a relatively innocuous noxious stimulus in rats not treated with capsaicin. Baseline latencies were determined in each rat before administration of any compounds, and rats that failed to maintain their tail in the water for 15 s were excluded from the experiment (this occurred in less than 2% of rats). Immediately after determination of baseline latencies, capsaicin, alone or in combination with an opioid, was injected into the dorsal surface of the tail 3.5 cm from the tip, with only one dose or dose combination tested per experimental session. All injections of capsaicin were made under light halothane anesthesia, with rats recovering from this procedure within 2 to 3 min. Testing occurred approximately once per week, with no rat (n = 6–8/group) being tested on more than five occasions.

Capsaicin-Induced Thermal Hyperalgesia. After determination of baseline latencies in male and female intact and gonadectomized rats, vehicle or capsaicin (0.1–3.0 μg in a 0.1-ml volume) was administered 3.5 cm from the tip of the tail, and tail-withdrawal latencies were redetermined every 15 min for 75 min. To determine the number of occasions in which an individual rat could be tested, selected doses of capsaicin were tested once per week for 4 weeks. Tests were also conducted in separate groups of rats in which capsaicin was injected at 3.5, 5.0 and 6.5 cm from the tip of the tail, and under these conditions the distal 7.0, 8.5, and 10 cm of the tail, respectively, was immersed in water.

Antihyperalgesic Effects of Opioids. For tests examining the antihyperalgesic effects of opioids, a 3.0-μg dose of capsaicin was used in males and a 1.0-μg dose in females, with these doses producing a comparable peak effect and duration of hyperalgesia in males and females. In tests of the antihyperalgesic effects of opioids, capsaicin was administered in combination with saline or one dose of each opioid injected systemically (s.c.), locally (in the tail), or centrally (i.c.v.). In these tests, tail-withdrawal latencies were determined only 15 min after the capsaicin/opioid injection, since this time point corresponded to the peak effect of capsaicin. A 15-s cutoff latency was implemented, because this corresponded to a maximal antihyperalgesic effect (i.e., nociceptive thresholds returned to baseline levels). To minimize the number of injections and period of anesthesia, during combination tests in which drugs were administered locally in the tail, the opioid and capsaicin were administered in the same syringe. Doses of each opioid were tested in a semirandom order in males and females. To determine whether the antihyperalgesic effects of local morphine were affected by the segmental location of the tail injection, tests were conducted in which the capsaicin/morphine combination was injected at 3.5, 5.0, and 6.5 cm from the tip of the tail, and the distal 7.0, 8.5, and 10 cm of the tail, respectively, was immersed in water.

Peripheral Antihyperalgesic Effects of Morphine. To examine potential peripheral mediation of the effects of locally administered morphine, doses of morphine (1,000 μg) that produced high levels of antihyperalgesia in males and females when administered in the tail, were administered in combination with an i.c.v. dose of 10 μg of NLX-M. This dose of NLX-M has been shown to antagonize completely the antinociceptive effects of systemic morphine in a 52°C warm-water tail-withdrawal procedure (personal observation); in this assay, the antinociceptive effects of morphine are mediated predominantly by central opioid receptors (Yaksh, 1997).

Data Analysis. For time course analyses of the hyperalgesic effects of capsaicin, the area under the curve was estimated using the trapezoidal rule. These calculations were used to conduct repeated measures ANOVAs to assess capsaicin sensitivity in male and fe-
male intact and GDX rats, the effect of once per week testing in males and females, and the effect of injecting capsaicin at various tail locations in males and females. For dose-effect curves examining the antihyperalgesic effects of opioids, latencies to tail withdrawal following administration of the drug were converted to the percentage of the maximum possible effect using the following equation: \( \text{antihyperalgesic effect} = \frac{\text{observed} - \text{baseline}}{15 \text{ s} - \text{baseline}} \times 100 \). Under these conditions, the baseline latency reflected the effect of capsaicin administered in combination with saline. When possible, the dose of each drug required to produce a 50% antihyperalgesic effect (ED\(_{50}\)) was derived mathematically (least-squares method) using log-linear interpolation with at least three doses on the ascending limb of the dose-effect curve. For each opioid, a repeated measures ANOVA was also conducted with sex as the between-groups factor and dose as the repeated measures factor. In instances in which there was a main effect for sex, post hoc tests were conducted using the Fisher’s protected least significant difference test to assess the effect of sex on each dose of an opioid. Because there was a main effect of dose for all opioids tested except i.v. buprenorphine, these statistics are not discussed. Similar repeated measures ANOVAs were conducted to assess the peripheral antihyperalgesic effects of morphine and to assess the effects of morphine injected at different tail locations. For statistical analyses using ANOVA, the alpha level was set at 0.05.

**Drugs.** The following drugs were used: morphine sulfate and buprenorphine HCl (both provided by the National Institute on Drug Abuse), desocine HCl (provided by AstraZeneca, Montreal, QC, Canada), and capsaicin and naloxone methiodide (both purchased from Sigma-Aldrich, St. Louis, MO). Capsaicin was dissolved in a solution of Tween 80/95%/ethanol/saline in a ratio of 1:1:8, and was diluted to lower concentrations with saline. For local injection in the tail, capsaicin was mixed in the same syringe as the opioid in a 0.1-ml volume. For other tests, capsaicin was injected alone in the tail in a 0.1-ml volume, and opioids were administered s.c. in a volume of 0.5 to 1.0 ml/kg, or i.c.v. in a 5-μl volume.

**Intracerebroventricular Injections.** Rats were stereotaxically implanted with a single 22-gauge cannula (Plastics One Inc., Roanoke, VA) into the left lateral ventricle (anterior-posterior +0.9, medial-lateral +1.5, dorsal-ventral −3.2) under anesthesia induced with a 1 ml/kg injection of a 1.1 (v/v) mixture of ketamine (100 mg/ml) and xylazine (20 mg/ml). Coordinates are expressed at millimeters from bregma (Paxinos and Watson, 1986). Rats were given i.c.v. injections of opioids in a 5-μl volume with a 28-gauge (Plastics One Inc.) injector that protruded 1 mm beyond the tip of the cannula. The injector was connected by a length of tubing to a 1 ml/kg injection of a 1:1,100 dilution of angiotensin II before initiation of testing and periodically to lower concentrations with saline. For local injection in the tail, capsaicin was mixed in the same syringe as the opioid in a 0.1-ml volume, and opioids were administered s.c. in a volume of 0.5 to 1.0 ml/kg, or i.c.v. in a 5-μl volume.

**Results**

**Capsaicin-Induced Thermal Hyperalgesia.** Figure 1 shows the effects of various doses of capsaicin on tail-withdrawal latencies in males and females. Capsaicin produced a dose- and time-dependent hyperalgesia in both males and females. At all doses tested, capsaicin produced peak hyperalgesic effects at 15 min, with tail-withdrawal latencies returning to baseline levels at 45 to 75 min. Also shown in Fig. 1 (inset) is that capsaicin produced dose-dependent increases in the area under the curve in males and females. ANOVAs indicated a main effect of dose \( (F_{1,14} = 647.0, P < 0.05) \), sex \( (F_{1,14} = 48.5, P < 0.05) \), and dose x sex interaction \( (F_{1,14} = 6.6, P < 0.05) \). Post hoc tests revealed that females displayed greater \( (P < 0.05) \) hyperalgesia at each dose (0.1–3.0 μg) of capsaicin tested.

![Figure 1](image_url)  
**Figure 1.** Time course of hyperalgesic responses to various doses of capsaicin (injected 3.5 cm from the tip of the tail) in females \( (n = 8, \text{top panel}) \) and males \( (n = 8, \text{bottom panel}) \) when examined in a warm-water tail-withdrawal procedure in which the distal 7 cm of the tail was immersed in water maintained at 45°C. Time 0 represents baseline responses immediately before capsaicin injection. Inset shows area under the curve analysis for each dose of capsaicin in males and females. Vertical bars represent the standard error; where not indicated, the standard error fell within the data point. Asterisks (*) indicate significant differences \( (P < 0.05) \) between males and females.

Figure 2 shows area under the curve analyses for the effects of capsaicin on tail-withdrawal latencies in intact and GDX males and females. Whereas gonadectomy increased the hyperalgesic response in males, it decreased it in females. Indeed, separate ANOVAs conducted in males and females indicated main effects for treatment \( (\text{males: } F_{1,13} = 17.1, P < 0.05; \text{females: } F_{1,13} = 18.2, P < 0.05) \) and a treatment x dose interaction \( (\text{males: } F_{1,13} = 44.4, P < 0.05; \text{females: } F_{1,13} = 4.7, P < 0.05) \). Post hoc tests revealed that 0.3 to 3.0 μg of capsaicin produced greater \( (P < 0.05) \) hyperalgesia in intact than in GDX females, and the 1.0- and 3.0-μg doses produced greater \( (P < 0.05) \) hyperalgesia in GDX than in intact males.

Because the body weight of males \( (207 ± 6 g) \) was significantly greater than that of females \( (151 ± 5 g) \) \( (t_{10} = 7.3, P < 0.05) \), it was important to assess anatomical tail characteristics and their potential relationship to sex differences in capsaicin-induced hyperalgesia. In particular, the differences in the level of innervation of the tail or tail diameter could influence nociceptive sensitivity. Figure 3 shows tail diameter and tail length in males and females, as well as the hyperalgesic effects of equieffective doses of capsaicin injected into different portions of the distal half of the tail. Whereas the tail was approximately 2 cm longer in males \( (t_{10} = 14.2, P < 0.05) \), the diameter of the tail was comparable \( (F_{1,10} = 2.1, P > 0.05) \) in males and females at measurements taken between 3.5 and 14 cm from the tip of the tail (Fig. 3, left panel). Additional tests were conducted in which capsaicin was injected 3.5, 5.0, and 6.5 cm from the tip of the tail (Ness et al., 1987). Although the area under the curve was generally similar between males and females at each loca-
tion, ANOVAs indicated a test location × sex interaction ($F_{1,14} = 5.7, P < 0.05$), with a significant sex difference obtained only at the 6.5 cm location ($P < 0.05$).

Under some conditions of repeated testing, high doses of capsaicin or short intervals between administrations can produce desensitization of the area exposed to capsaicin (for review, see Holzer, 1991). To examine potential sex-dependent changes in sensitivity to capsaicin following repeated testing, the hyperalgesic effects of capsaicin were examined once weekly for 4 weeks. Figure 4 shows the time course (weeks 1 and 4 only) and area under the curve for weekly injections of doses of capsaicin that produced comparable profiles of hyperalgesia in males (3.0 μg) and females (1.0 μg). Results from area under the curve analyses indicated a main effect for test ($F_{1,14} = 14.0, P < 0.05$), but no main effect for sex ($F_{1,14} = 0.002, P > 0.05$), and no sex × test interaction ($F_{1,14} = 2.2, P > 0.05$). Post hoc tests, in which the effect of the test was collapsed across sex, revealed that test 1 differed from test 4 ($P < 0.05$). However, the maximal hyperalgesic effect, which occurred at 15 min, did not differ with repeated testing ($F_{1,14} = 4.2, P > 0.05$) (Fig. 4).

**Antihyperalgesic Effects of Morphine.** In tests examining the antihyperalgesic effects of opioids, equieffective doses of capsaicin were injected 3.5 cm from the tip of the tail in males (3.0 μg) and females (1.0 μg). Figure 5 shows the antihyperalgesic effects of morphine when administered systemically, locally, and centrally. Morphine administered locally and centrally produced dose-dependent increases in antihyperalgesia, with near maximal effects obtained in both sexes. Systemic administration of morphine also produced marked antihyperalgesia. In these tests, the failure to obtain a maximal effect was most likely a consequence of morphine’s slow onset of action, since in tests conducted with a longer pretreatment interval (30 min), systemic morphine produced complete reversal of hyperalgesia in both sexes (data not shown). Table 1 shows ED$_{50}$ values for these dose-effect curves and indicates that there were no sex differences in the antihyperalgesic potency of systemic, local, or central morphine. Moreover, analyses based on ANOVA indicated no main effects for sex (systemic: $F_{1,13} = 0.9, P > 0.05$; local: $F_{1,14} = 1.3, P > 0.05$; central: $F_{1,12} = 0.14, P > 0.05$) and no dose × sex interactions (systemic: $F_{1,13} = 0.2, P > 0.05$; local: $F_{1,14} = 0.3, P > 0.05$; central: $F_{1,12} = 0.08, P > 0.05$). Additional tests were conducted in females in which 3.0 μg of capsaicin (i.e., the dose administered to males) was administered in combination with systemic morphine. The relative potency of morphine in this test was similar to that obtained when females were tested with 1.0 μg of capsaicin and to males tested with 3.0 μg capsaicin (see Table 1).

**Peripheral Antihyperalgesic Effects of Morphine.** Although systemic opioid injections are generally thought to produce their effects by activating opioid receptors in the central nervous system, recent data using assays of inflammatory pain indicate that some opioids can produce antihyperalgesia by activating peripheral opioid receptors (Stein et al., 1989; Ko et al., 1998). The present data, in which morphine was at least 10-fold more potent when administered locally in the tail than systemically, suggests that the effects of local morphine were mediated, at least in part, by peripheral opioid receptors. To investigate this possibility and to determine whether there were sex differences in the peripheral mediation of the antihyperalgesic effects of morphine, Fig. 6 shows data in which local morphine (1,000 μg) was administered in combination with 10 μg of NLX-M (i.c.v.). In both males and females, morphine alone and in combination with i.c.v. NLX-M produced intermediate to high levels of antihyperalgesia. Separate ANOVAs in males and females indicated a main effect of treatment (males: $F_{1,15} = 20.9, P < 0.05$; females: $F_{1,5} = 27.9, P < 0.05$), with morphine alone and in combination with i.c.v. NLX-M differing from i.c.v. NLX-M alone ($P < 0.05$). The antihyperalgesic effects of 1,000 μg of morphine alone were greater in females (65% effect) than in males (44% effect), although this difference failed to reach statistical significance.

**Effects of Morphine at Different Tail Locations.** Given that the effects of local morphine were mediated by opioid-sensitive mechanisms in the tail, tests were conducted to determine whether the injection site on the tail differentially affected antihyperalgesic sensitivity in males and females. Figure 7 shows the antihyperalgesic effects of 1,000 μg of morphine injected at sites 3.5, 5.0, and 6.5 cm from the tip of the tail. In both males and females, local morphine produced a significant antihyperalgesic effect at each test location, and ANOVA results indicated no main effect for test location ($F_{1,12} = 0.001, P > 0.05$) or sex ($F_{1,12} = 1.4, P > 0.05$), and no sex × test location interaction ($F_{1,12} = 0.03, P > 0.05$). Although there was no main effect for sex, the effects produced by morphine were slightly greater in females (average effect, 61%) than males (average effect, 48%) at each injection location.

**Antihyperalgesic Effects of Buprenorphine and Dezoicine.** In phasic pain models, sex differences in antino-
ciceptive potency of high-efficacy opioids, such as morphine, are generally small relative to that produced by low-efficacy opioids, such as dezocine (Cook et al., 2000; Barrett et al., 2001). Thus, the failure to observe sex differences with morphine could be a consequence of its high relative efficacy or the lack of underlying sex differences in opioid sensitivity in this assay. To test these hypotheses, Fig. 8 shows the antihyperalgesic effects of the low-efficacy opioids buprenorphine and dezocine administered systemically and locally, with additional tests conducted with buprenorphine administered centrally. Buprenorphine and dezocine produced high levels of antihyperalgesia in both sexes, although there were no sex differences in their ED_{50} values (Table 1). Tests based on ANOVA, however, indicated a main effect of sex for locally administered buprenorphine (F_{1,14} = 4.6, P < 0.05), with post hoc analyses indicating that the 30-μg dose produced greater (P < 0.05) antihyperalgesia in females than in males. Buprenorphine administered centrally produced marked an-
TABLE 1
ED_{50} values (95% confidence limits) for males \((n = 6–8)\) and females \((n = 6–8)\) in tests conducted with morphine, buprenorphine, and dezocine
when administered systemically (s.c.), locally (tail), and centrally (i.c.v.).
With one exception \((a)\), males \((3.0 \mu g)\) and females \((1.0 \mu g)\) were administered equieffective doses of capsaicin.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Route of Administration</th>
<th>Subcutaneous</th>
<th>Tail</th>
<th>Intracerebroventricular</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg/kg</td>
<td>µg</td>
<td>µg</td>
</tr>
<tr>
<td>Morphine</td>
<td>Male</td>
<td>20.6 (14.4–29.5)</td>
<td>195.8 (112.2–241.5)</td>
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<td>303.1 (175.9–522.3)</td>
<td>3.0 (2.2–4.1)</td>
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<tr>
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<td>Female*</td>
<td>12.6 (7.1–22.5)</td>
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<td>_b</td>
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<tr>
<td>Buprenorphine</td>
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<td>41.4 (31.9–53.8)</td>
<td>_c</td>
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<tr>
<td></td>
<td>Female</td>
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<td>27.3 (17.7–42.2)</td>
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<tr>
<td>Dezocine</td>
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</tr>
<tr>
<td></td>
<td>Female</td>
<td>0.26 (0.18–0.38)</td>
<td>207.7 (131.3–328.5)</td>
<td>_b</td>
</tr>
</tbody>
</table>

* Females administered 3.0 µg of capsaicin in combination with morphine.

b, not tested.

* Maximal effect less than 50%.

Fig. 6. Antihyperalgesic effects of local (3.5 cm from the tip of the tail) injections of morphine \((1,000 \mu g)\) alone and in combination with i.c.v. NLX-M \((10 \mu g)\) in males \((n = 6)\) and females \((n = 6)\) when examined in a warm-water tail-withdrawal procedure in which the distal 7 cm of the tail was immersed in water maintained at 45°C. Equieffective doses of capsaicin (females, 1.0 µg; males, 3.0 µg) were injected 3.5 cm from the tip of the tail, with tests conducted 15 min after capsaicin injection. Vertical bars represent the standard error; where not indicated, the standard error fell within the data point. Asterisks (*) indicate significant differences \((P < 0.05)\) compared with the effect of i.c.v. NLX-M administered alone.

Fig. 7. Antihyperalgesic effects of local (tail) injections of morphine \((1,000 \mu g)\) administered at various regions of the tail in males \((n = 8)\) and females \((n = 8)\) when examined in a warm-water tail-withdrawal procedure in which the distal portion of the tail was immersed in water maintained at 45°C. Equieffective doses of capsaicin (females, 1.0 µg; males, 3.0 µg) were injected 3.5 cm from the tip of the tail, with tests conducted 15 min after capsaicin injection. Vertical bars represent the standard error.

tihyperalgesia in females, but only low levels of antihyperalgesia in males. ANOVA results indicated a main effect for sex \((F_{1,12} = 31.2, P < 0.05)\), with post hoc analyses indicating that doses of 1.0 and 3.0 µg produced greater \((P < 0.05)\) antihyperalgesia in females than in males. The low solubility of dezocine, and the inability to administer large volumes directly into the cerebral ventricles, precluded testing i.c.v. dezocine.

Discussion
Capsaicin-Induced Thermal Hyperalgesia. Capsaicin alone produced dose- and time-dependent hyperalgesia, with males being less sensitive than females to all doses of capsaicin tested. To achieve comparable profiles of hyperalgesia, males required a dose of capsaicin approximately 3-fold higher than that in females. Although the influence of sex on sensitivity to tonic nociception has not been systematically evaluated, there are reports indicating that hindpaw injections of formalin produce a greater nociceptive response in females, as evidenced by more frequent licking, flexing, and jerking of the paw (Aloisi et al., 1994; Gaumond et al., 2002). These results are consistent with a decreased nociceptive sensitivity in males in phasic nociception assays employing thermal, mechanical, and electrical nociceptive stimuli and, taken together, indicate sex differences across a broad range of nociceptive stimuli that vary in their type (thermal versus mechanical), duration (phasic versus tonic), and intensity (for review, see Mogil et al., 2000).

Sex differences in capsaicin-induced hyperalgesia were dependent on intact gonadal hormone status in both males and females, since GDX males were more sensitive to capsaicin than intact males, and GDX females were less sensitive than intact females. The importance of gonadal hormone status has also been demonstrated in other inflammatory states, such as that produced by bradykinin, prostaglandin E_{2}, and complete Freund’s adjuvant (e.g., Green et al., 1999; Aloisi and Ceccarelli, 2000; Bradshaw et al., 2000). For example, the higher frequency and duration of formalin-induced nociceptive behaviors are reduced following gonadectomy in females, and enhanced in males (Gaumond et al., 2002). The localization of estrogen receptors and estrogen mRNA on peripheral sensory neurons and in the spinal cord suggests a mechanism, in females at least, whereby estrogen could modulate responses to inflammatory nociceptive stimuli (Morrell et al., 1982; Papka et al., 1997).
Injecting a fixed amount of capsaicin in the tail may produce effects that reflect either the level of innervation or the tail diameter at the injection site, both of which could contribute to sex differences in capsaicin-induced hyperalgesia. Measurements of the tail, however, indicated that there were no sex differences in diameter across the distal 14 cm of the tail. Moreover, capsaicin injections at various locations of the tail generally produced similar hyperalgesic responses in males and females. These findings suggest that tail anatomy did not contribute significantly to the analysis of sex differences in hyperalgesia.

**Opioid-Induced Antihyperalgesia.** Morphine, buprenorphine, and dezocine produced marked attenuation of hyperalgesia in males and females when administered systemically, locally, and centrally. The rank order of systemic potency of these opioids (buprenorphine > dezocine > morphine) was comparable in males and females, and similar to that obtained in other assays sensitive to the effects of μ-opioids (Young et al., 1984; Cook et al., 2000). Such findings suggest that in both males and females these opioids produced their effects by a common mechanism, most likely activity at the μ-opioid receptor.

Despite the marked sex differences in hyperalgesia, males and females were generally equally sensitive to the antihyperalgesic effects of morphine, buprenorphine, and dezocine. However, in two treatment conditions, selected doses of local and i.c.v. buprenorphine produced greater antihyperalgesic effects in females. These findings contrast sharply with a wealth of data in thermal and mechanical assays of phasic pain, in which acutely administered μ-opioids are markedly more potent and effective in males, with the magnitude of this effect being larger with the less efficacious opioids (Kepler et al., 1991; Cicero et al., 1996; Negus and Mello, 1999; Cook et al., 2000; Barrett et al., 2002). A number of studies have documented important differences between assays of tonic and phasic pain and their modulation by opioids. For example, lesioning the dorsolateral funiculus spinal pathway, a major pathway involved in descending pain inhibition, attenuates morphine antinoceception in the tail-flick, but not formalin, assay (Ryan et al., 1985). Pharmacological data also indicate a dissociation between these two pain types, since chronic morphine treatment produces little tolerance in the formalin test under conditions in which marked tolerance is observed in the tail-flick test (Abbott et al., 1981). As such, these findings suggest that pain duration (phasic versus tonic) interacts with sex to determine the direction of sex differences in antinociception/antihyperalgesia.

**Central Versus Peripheral Antihyperalgesic Effects of Morphine.** The greater potency of morphine when administered locally versus systemically, as well as the inability of i.c.v. NLX-M to attenuate the effects of local morphine, suggest that morphine produced its antihyperalgesic effects by activation of peripheral opioid receptors (see Ko et al., 1998). The hydrophilic properties of morphine, which tend to limit redistribution of local injections to general circulation, may account for its peripheral antihyperalgesic effects (Herz and Teschemacher, 1971). In contrast, buprenorphine, which is highly lipophilic, was equally potent when administered systemically and locally, and thus it would be extremely difficult to demonstrate that its effects are mediated specifically by peripheral opioid receptors (Dickenson et al., 1990). Nevertheless, the present findings with morphine are congruent with a variety of reports demonstrating the utility of small, local doses of opioids in alleviating hyperalgesic states, and extend these findings to female rats (Stein et al., 1989; Perrot et al., 1999).

The lack of sex differences with morphine was not a consequence of its peripheral opioid activity, since similar effects were obtained when morphine was administered centrally. In contrast, data from assays of phasic pain, in which nociception is mediated by spinal and supraspinal activity, indicate that i.c.v. and site-specific brain injections of morphine produce greater effects in males than in females (Boyer et al., 1998; Kest et al., 1999). These findings imply that the specific pain model, and not the site of opioid activity, determines the magnitude and direction of sex differences in antinociception. As such, sex differences may not be a consequence of opioid activation of descending pain pathways, but the manner in which opioids interact with the neural substrates specific to each pain assay (Cicero et al., 1996). Even among tonic pain models, there are numerous techniques to induce nociception (e.g., nerve ligation, surgical incisions, algogenic compounds), and each can be differentiated in terms of their neurochemical mediation, N-methyl-D-aspartate receptor in-
volvement, behavioral response profile, and sensitivity to opioids (e.g., Chaplan et al., 1997; Zahn and Brennan, 1998). Thus, it is unclear as to whether these differences across assays are a consequence of the duration of the nociceptive stimulus, type of neuronal fibers that transmit theafferent nociceptive information, or the neurochemical substrates mediating nociception.

**Relation to Human Studies and Implications.** The available data from human studies of sex differences are based on postsurgical models of tonic pain and, similar to the present data, indicate that low-efficacy opioids produce greater pain relief in females compared with males (Gear et al., 1996, 1999). In contrast, studies in nonhumans have focused primarily on phasic pain models and have reported that opioids are more potent in males. The type of pain assay employed could account for these discrepant results, although other factors such as species differences cannot be eliminated. However, even if qualitatively similar tests are employed in humans and nonhumans, direct comparisons will be complicated by critical procedural differences, or the potential contribution of sex-dependent psychological factors in humans.

The present findings, coupled with prior data, have a number of important implications for understanding sex differences in opioid antinociception/antihyperalgesia. First, the finding that the magnitude and direction of sex differences depends on the nociceptive assay suggests that sex differences are determined not by global differences in opioid signal transduction mechanisms, but by unique interactions between each nociceptive modality (e.g., phasic versus tonic) and the opioid system. Consistent with this notion is a recent [35S]GTPγS binding study by Selley et al. (2003), in which equal magnitudes of agonist-induced [35S]GTPγS binding were observed in discrete brain regions obtained from male and female F344 and Lewis rats. Second, given the similarity of sex differences in rodents and humans in models of phasic pain, rodents would appear to afford a useful species to model human clinical pain conditions and examine underlying mechanisms of sex differences. To glean a better understanding of sex differences in antihyperalgesia and their potential relationship to humans, it will be informative to test males and females in other models of tonic pain, some of which may be even more analogous to human chronic pain in that they produce hyperalgesic states that last for days or weeks (e.g., Freund’s adjuvant arthritis model).

**Summary.** These data indicate that capsaicin-induced thermal hyperalgesic responses are determined by sex and gonadal hormone status. In most instances the potency and effectiveness of opioids in alleviating this hyperalgesia were comparable across the sexes, although there were instances in which buprenorphine produced greater antihyperalgesic effects in females. These data contrast with numerous rodent studies employing assays of phasic pain, in which opioids are markedly more potent and effective in males. The differences across assays emphasize the need to systematically compare males and females across different models of tonic pain.

References

- Cicero TJ, Nock B, and Meyer ER (1996) Gender-related differences in supraspinal morphine antinociception/antihyperalgesia. First, the type of pain assay employed could account for these discrepant results, although other factors such as species differences cannot be eliminated. However, even if qualitatively similar tests are employed in humans and nonhumans, direct comparisons will be complicated by critical procedural differences, or the potential contribution of sex-dependent psychological factors in humans.

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References


**Address correspondence to:** Andrew C. Barrett, Department of Psychology, Davie Hall, CB #3270, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599. E-mail address: barrett3@email.unc.edu