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Sex-related differences in mechanical nociception and antinociception produced by μ- and κ-opioid receptor agonists in rats

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Abstract

Previous studies indicate that in antinociceptive procedures employing thermal, chemical and electrical stimuli, opioids are generally more potent in male than female rodents. The purpose of the present study was to examine nociception and opioid antinociception in male and female rats using a mechanical nociceptive stimulus. Results indicated that males had a higher threshold for nociception, and in tests in which a constant pressure was applied to the hindpaw, the paw withdrawal latencies were consistently longer in males. Opioids with activity at the μ receptor, including levorphanol, morphine, dezocine, buprenorphine, butorphanol and nalbuphine, were generally more potent and/or effective in males. In contrast, sex differences were not consistently observed with the κ-opioid receptor agonists spiradoline, (5,7,8*b*)-*N*-methyl-*N*[2-1(1-pyrrolidinyl),1-oxaspiro[4,5]dec-8-yl benzeneacetamide (U69,593), *trans*-(±)-3,4-dichloro-*N*-methyl-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide (U50,488), enadoline, ethylketocyclazocine, and nalorphine. These findings suggest that males and females differ in their responsiveness to mechanical nociception and that sex differences in sensitivity to κ-, but not μ-, opioid receptor agonists are specific to certain nociceptive stimulus modalities.

Keywords: Sex difference; μ-Opioid receptor agonist; κ-Opioids receptor agonist; Antinociception; Mechanical; (Rat)

1. Introduction

Nociceptive neurons have the ability to respond to mechanical, thermal, electrical and chemical stimuli. There is ample evidence indicating that these stimulus modalities are subserved, in part, by different neuroanatomic pathways and neurochemical mediators (Raja et al., 1999). Moreover, the response to these nociceptive stimuli is influenced by a number of organismic factors, and recent studies suggest that the sex of the individual is a critical determinant of nociceptive responsiveness. In rodents, for example, females display more pain behaviors to chemical nociceptive stimuli and lower thresholds to electrical nociceptive stimuli (Romero et al., 1988; Kepler et al., 1991; Aloisi et al., 1994), with sex differences in responsiveness to thermal and mechanical stimuli being less consistently observed (Kepler et al., 1991; Grisel et al., 1996; Kayser et al., 1996; Bartok and Craft, 1997; Mogil et al., 2000; Barrett et al., 2002). The observation that consistent sex differences are apparent with only a select group of nociceptive stimuli may be a consequence of either the neuroanatomical substrates underlying responsiveness to these stimuli or procedural factors unique to the presentation of each type of nociceptive stimulus. For example, thermal nociceptive procedures typically involve the presentation of a stimulus to the tail or paw and measure latency to withdrawal from the heat source, whereas procedures employing mechanical stimuli typically present a stimulus that increases in intensity and measure the threshold to paw withdrawal or vocalization (Le Bars et al., 2001). Moreover, thermal nociceptive procedures can be confounded by factors such as differential heating of the hindpaw in males compared to females that are smaller in size (Chiari et al., 1999).

In addition to the sexual dimorphism observed with nociception, males and females display a differential sensitivity to the antinociceptive effects of opioids. For example, in rodents and rhesus monkeys, opioids with activity at both μ- and κ-opioid receptors are generally more potent and effective as antinociceptive agents in

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males than females (Kavaliers and Innes, 1987; Kepler et al., 1989; Cicero et al., 1996; Kest et al., 1999; Negus and Mello, 1999; Cook et al., 2000; Craft and Bernal, 2001; Barrett et al., 2002). The majority of these studies have employed thermal nociceptive stimuli, with a limited number using chemical or electrical stimuli. Comparisons across different nociceptive stimulus modalities are of critical importance given recent data in various mouse strains indicating an inverse correlation between baseline responsiveness to thermal and mechanical nociceptive stimuli (Mogil et al., 1999). Similarly, in selective breeding studies, high and low morphine responders in a thermal nociceptive assay are low and high morphine responders, respectively, in a chemical nociceptive assay (Mogil et al., 1996). Sex differences in responsiveness to some non-opioid antinociceptive agents and to opioid-mediated stress-induced antinociception also vary across nociceptive stimulus modalities (Menendez et al., 1994; Tseng and Craft, 2001). Collectively, these findings suggest that sex differences in responsiveness to opioid antinociception may also interact with the type of nociceptive stimulus.

One purpose of the present study was to examine the influence of sex on nociception using a mechanical (paw pressure) nociceptive assay. Two variants of this procedure were used. In one, the paw withdrawal response to an increasing pressure stimulus was measured (threshold procedure), and in the other, the latency to paw withdrawal following the presentation of a fixed intensity stimulus was measured (tolerance procedure). Utilization of these two procedures allow for comparisons to nociception procedures that measure both threshold (e.g., electrical) and tolerance (e.g., thermal) responses. To determine whether the relative contribution of the endogenous opioid system to mechanical nociception is sexually dimorphic, threshold tests were conducted following administration of the opioid receptor antagonist naltrexone (Ryan and Maier, 1988; Ratka and Simpkins, 1990; Mogil and Belknap, 1997). A second purpose of this study was to examine sex differences in opioid antinociception against a mechanical nociceptive stimulus in male and female rats. Tests were conducted with a series of opioids that differ in their selectivity and relative efficacy at the μ- and κ-opioid receptor, and comparisons were made using both the threshold and tolerance procedures.

2. Materials and methods

2.1. Animals

Gonadally intact male and female F344 rats were obtained from Charles River Suppliers (Raleigh, NC, USA). All testing occurred between 3 and 6 months of age and rats were individually housed in a colony maintained on a 12-h light/dark cycle. All rats had unlimited access to food and water.

2.2. Apparatus and testing

Mechanical nociception was applied with the use of an analgesy meter (Ugo Basile, Varese, Italy), a device with a dome-shaped plastic tip (diameter = 1 mm) that applies a linearly increasing pressure (g) to the dorsal surface of the hindpaw, with the tip applied to the region of the paw just proximal to the third digit. In the threshold procedure, each rat was gently restrained by hand and the threshold pressure at which the rat withdrew its paw was recorded. On two occasions prior to the start of testing, each rat was habituated to the testing and injection procedures. To further habituate the rats to the testing protocol, and to provide an initial exposure to drug, the antinociceptive effects of a 10 mg/kg dose of morphine were examined on a third occasion (these data were not used in subsequent analyses). During tests for antinociception, the average of two baseline nociceptive thresholds was recorded, and then one dose of an opioid was administered (i.p.). Thirty minutes later, the nociceptive threshold was determined, with a 500 g cutoff imposed to minimize potential tissue damage. All tests were separated by 4–7 days. The doses of each opioid or opioid combination were tested in a semi-random order in males and females, and each group of rats ($n = 6–12$/group) was generally tested on six to eight occasions.

In addition to tests in which the pressure exerted on the paw increased with time (threshold procedure), tests were also conducted in which the pressure exerted on the paw was fixed, and the latency to paw withdrawal was recorded (tolerance procedure). After determining the relationship between different pressures and paw withdrawal latencies, the 120 and 30 g pressures, which produced comparable baseline latencies (4–5 s) in males and females, respectively, were used for subsequent testing with a select group of μ- and κ-opioid receptor agonists. The habituation, training and testing protocols were identical to that described above, with the exception that a 15-s cutoff was imposed.

2.3. Data analysis

2.3.1. Nociception

To examine sex differences in baseline nociceptive thresholds, repeated measures analysis of variance (ANOVA) was used. To determine whether the relationship governing baseline nociceptive responses to a range of stimuli was similar in males and females, a test for parallelism (procedure 6, Tallarida and Murray, 1987) was conducted on the least-squares regression line of this relationship.

2.3.2. Antinociception

For dose–effect curves using the threshold procedure, paw withdrawal measurements were converted to percentage of the maximum possible effect using the following equation: % antinociceptive effect=[(observed − baseline)/(500 g − baseline)] × 100. A similar equation was used

when tests were conducted with the tolerance procedure, with the exception that a 15-s cutoff was used. When possible, the dose of each drug required to produce a 50% maximal antinociceptive 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. Sex differences in the potency of a drug were considered significant if the 95% confidence intervals did not overlap. For each opioid, a repeated measure 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 (PLSD) test to assess the effect of sex on each dose of an opioid. Because there was a main effect for dose for all opioids tested with the exception of nalbuphine and nalorphine, these statistics are not discussed. For apparent pA_2 analyses, dose ratios were determined by comparing the group ED_{50} of morphine alone to the group ED_{50} of morphine administered in combination with naltrexone. These dose ratios were then analyzed according to the methods of Arunlakshana and Shild (1959) to calculate the apparent pA_2 values for naltrexone (procedure 15, Tallarida and Murray, 1987) in males and females. For tests in which buprenorphine, butorphanol and nalbuphine were administered in combination with morphine, one-way ANOVAs with subsequent PLSD post-hoc tests were conducted to determine if these opioids antagonized the effects of morphine. For statistical analyses using ANOVA, the alpha level was set at 0.05.

2.4. Drugs

The following drugs were used: morphine sulfate, buprenorphine HCl, (+)-amphetamine sulfate (all provided by the National Institute on Drug Abuse), dezocine HCl (generously supplied by AstraZeneca, Montreal, Quebec, Canada), levorphanol tartrate, *trans*-(±)-3,4-dichloro-*N*-methyl-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide methanesulfonate (U50,488) (Research Biomedicals International, Natick, MA, USA), ethylketocyclazocine methanesulfonate (Sterling-Winthrop Research Institute, Rensselaer, NY, USA), enadoline HCl (generously supplied by Parke-Davis Pharmaceuticals, Ann Arbor, MI, USA), spiradoline mesylate, (5,7,8*b*)-*N*-methyl-*N*[2-1(1-pyrrolidinyl),1-oxaspiro[4,5]dec-8-yl benzeneacetamide (U69,593), butorphanol tartrate, nalbuphine HCl, naltrexone HCl and pentobarbital HCl (all from Sigma-Aldrich, St. Louis, MO, USA). Doses for all drugs are expressed in terms of the salts. All drugs were dissolved in distilled water and administered in an injection volume of 0.5–1.0 ml/kg.

3. Results

3.1. Nociception

Fig. 1 shows average baseline nociceptive thresholds (i.e., the amount of pressure required to produce a paw withdrawal) in males and females. As shown in this figure (panel "A"), males had higher nociceptive thresholds than females [$F(1,15) = 167.82$, $P < 0.05$], with an average pres-

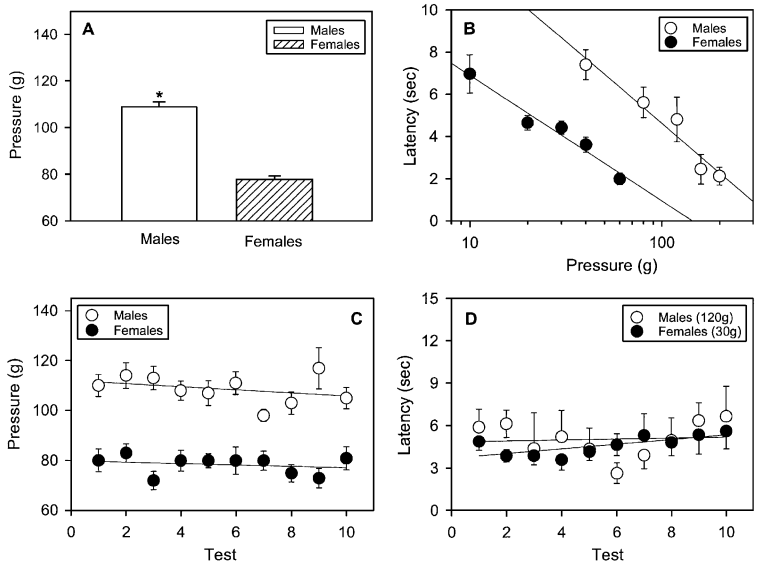

Fig. 1. Effect of sex on baseline nociception. (A) Baseline paw withdrawal thresholds for males and females averaged across 10 test sessions ($n = 16$/group). (B) Relationship between pressure and baseline paw withdrawal latency (tolerance) in males and females ($n = 8$/group). (C) Effect of repeated testing on baseline paw withdrawal thresholds for males and females averaged across 10 test sessions ($n = 16$/group). (D) Effect of repeated testing on baseline paw withdrawal latency (tolerance) for males and females averaged across 10 test sessions ($n = 8$/group). In this procedure, pressures were selected for males (120 g) and females (30 g) such that baseline latencies would be comparable. Vertical bars represent the standard error. Asterisks (*) indicate significant differences ($P < 0.05$) between males and females.

sure required to produce a paw withdrawal of 108 g in males and 79 g in females. Also shown in this figure (panel "B") is that in the tolerance procedure, in which the latency to paw withdrawal following application of various pressures was measured, the pressure required to produce comparable paw withdrawal latencies was consistently larger in males. An analysis of the slopes of the regression lines fitted to these data, however, indicated an absence of sex differences in the relationship between pressure and latency in males and females [$t(76) = 1.22$, $P > 0.05$].

As there is some evidence suggesting that repeated testing can alter sensitivity to a nociceptive stimulus (Milne and Gamble, 1989; Taiwo et al., 1989), tests were conducted on baseline nociceptive thresholds following repeated testing, with each test separated by 4–7 days. Analyses indicated a main effect for sex [$F(1,30) = 83.30$, $P < 0.05$], but no effect for test session [$F(1,30) = 1.25$, $P > 0.05$] and no test × sex interaction [$F(1,30) = 0.20$, $P > 0.05$]. As shown in Fig. 1 (panel "C"), although the baseline thresholds were different in males and females, repeated testing did not systematically increase or decrease baseline nociceptive thresholds in males or females. Similarly, analyses were conducted on the effect of test session on baseline nociceptive tolerance levels (panel "D"), and these analyses indicated neither a main effect for sex [$F(1,14) = 0.11$, $P > 0.05$] nor test session [$F(1,14) = 1.78$, $P > 0.05$], and no test × sex interaction [$F(1,14) = 0.80$, $P > 0.05$].

Fig. 2 shows the percent change from baseline in the mechanical threshold procedure for males and females following administration of several doses of naltrexone. Naltrexone produced dose-dependent decreases in nociceptive thresholds in males and females. Analyses conducted on the effect of naltrexone on baseline nociception indicated a main effect for dose [$F(1,22) = 17.33$, $P < 0.05$] but no effect for sex [$F(1,22) = 0.41$, $P > 0.05$] and no dose × sex interaction [$F(1,22) = 0.14$, $P > 0.05$]. Post-hoc analyses indicated that only the highest dose of naltrexone significantly decreased ($P < 0.05$) nociceptive thresholds in both males and females.

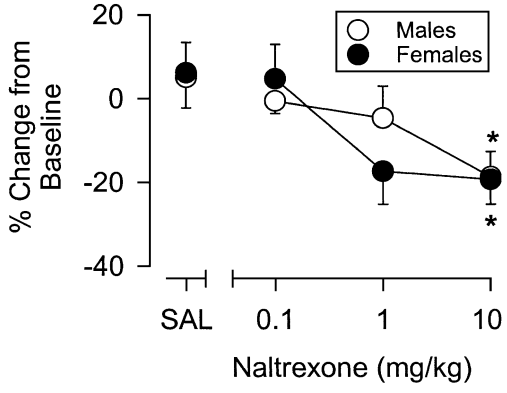

Fig. 2. Effects of naltrexone on paw withdrawal thresholds in males and females ($n = 8$/group). "SAL" represents the effects of a saline control test. Vertical bars represent the standard error. The asterisk (*) indicates a significant difference ($P < 0.05$) between naltrexone and the saline control condition in males and females.

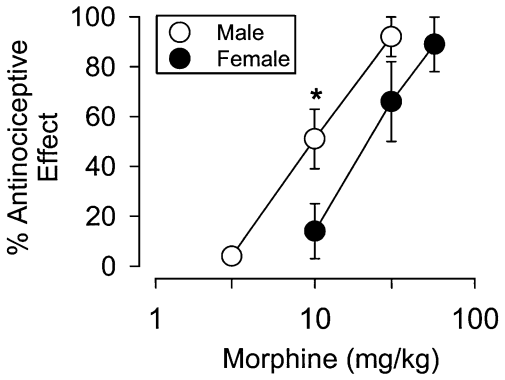

Fig. 3. Antinociceptive effects of morphine ($n = 8$/group) in males and females in the threshold paw pressure procedure, in which nociceptive responses to an increasing pressure stimulus were recorded. Vertical bars represent the standard error. Asterisks (*) indicate significant differences ($P < 0.05$) between males and females.

3.2. Antinociception—threshold procedure

3.2.1. Morphine

Fig. 3 shows the antinociceptive effects of morphine in males and females in the threshold procedure. Morphine produced dose-dependent increases in antinociception in males and females, with near maximal effects obtained at the highest doses tested. A repeated measures ANOVA conducted on the effects of morphine revealed a main effect for sex [$F(1,14) = 14.22$, $P < 0.05$] but no dose × sex interaction [$F(1,14) = 0.21$, $P > 0.05$]. Based on ED_{50} values, morphine was approximately 2.2-fold more potent in males (ED_{50}: 9.9, 95% confidence limits: 7.1–12.9) than females (22.2: 15.6–31.7). When tests were repeated in the same rats, morphine was approximately 2.1-fold more potent in males, suggesting that tolerance did not differentially develop in males or females as a function of repeated morphine testing.

Fig. 4 shows the antinociceptive effects of naltrexone administered in combination with morphine in males and females. Naltrexone produced dose-dependent, parallel, rightward shifts in the morphine dose–effect curve in both groups of rats. Dose ratios using ED_{50} values indicated that the 0.01, 0.1 and 1.0 mg/kg doses of naltrexone shifted the dose–effect curve for morphine 3.6-, 7.1- and 20-fold to the right in males and 2.4-, 6.3- and 31-fold to the right in females. These dose ratios were used to calculate apparent pA_2 values, and these analyses yielded a value of 7.4 (constrained slope) for both males and females.

3.2.2. μ-Opioid receptor agonists

Fig. 5 shows the antinociceptive effects of levorphanol, dezocine, buprenorphine, butorphanol and nalbuphine in males and females tested in the mechanical threshold procedure. As observed with morphine, levorphanol pro-

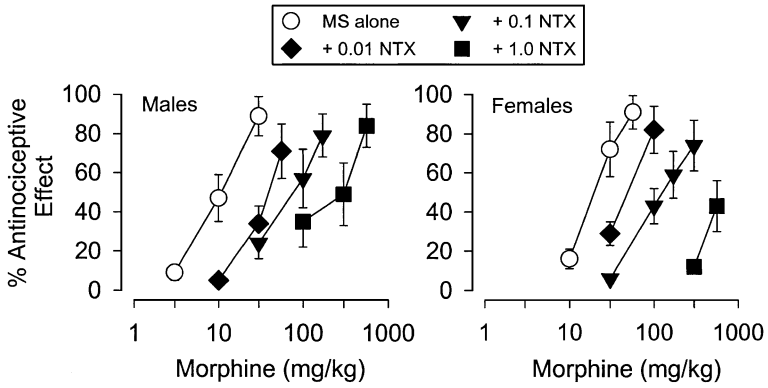

Fig. 4. Antinociceptive effects of morphine in combination with 0.01, 0.1 and 1.0 mg/kg naltrexone in males and females ($n = 7–8$/group) in the threshold paw pressure procedure, in which nociceptive responses to an increasing pressure stimulus were recorded. Vertical bars represent the standard error; where not indicated, the standard error fell within the data point.

duced dose-dependent increases in antinociception in males and females, with near maximal effects obtained at the highest doses tested. A repeated measures ANOVA conducted on the effects of levorphanol revealed a main effect for sex [$F(1,14) = 5.40$, $P < 0.05$] but no dose × sex interaction [$F(1,22) = 0.06$, $P > 0.05$]. Post-hoc tests indicated that the 10 mg/kg dose of levorphanol produced greater antinociception ($P < 0.05$) in males.

Across the dose range examined, dezocine produced dose-dependent increases in antinociception and at the highest dose tested produced a maximal effect of 59% and 54% in males and females, respectively. A repeated measures ANOVA conducted on the effects of dezocine revealed a main effect for sex [$F(1,20) = 5.54$, $P < 0.05$] but no dose × sex interaction [$F(1,20) = 0.05$, $P > 0.05$]. Post-hoc tests revealed that the 10 mg/kg dose of dezocine produced greater antinociception ($P < 0.05$) in males.

Buprenorphine, butorphanol and nalbuphine generally produced dose-dependent increases in antinociception in males, with maximal effects of 31%, 33%, and 15%, respectively. In contrast, these opioids produced only low levels of antinociception in females and in no instance did the maximal effect of these opioids exceed 5%. ANOVA analyses indicated a main effect for sex for buprenorphine [$F(1,18) = 33.05$, $P < 0.05$], butorphanol [$F(1,19) = 11.03$, $P < 0.05$] and nalbuphine [$F(1,16) = 5.25$, $P < 0.05$], as well as a dose × sex interaction for butorphanol [$F(1,19) = 7.27$, $P < 0.05$] and nalbuphine [$F(1,16) = 7.19$, $P < 0.05$]. Post-hoc tests revealed that intermediate to high doses of these opioids produced greater antinociception ($P < 0.05$) in males.

As it was possible that the limited effectiveness of buprenorphine, butorphanol and nalbuphine in males and females was a consequence of their low efficacy at the μ-opioid receptor, antagonism tests were conducted in which these opioids were administered in combination with morphine. Fig. 6 shows the effects of a relatively high dose of buprenorphine, butorphanol and nalbuphine when administered in

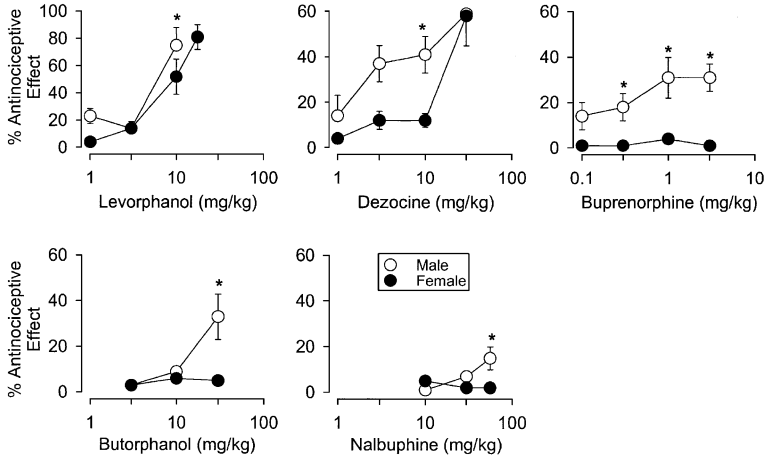

Fig. 5. Antinociceptive effects of levorphanol ($n = 8$/group), dezocine ($n = 11$/group), buprenorphine ($n = 10$/group), butorphanol ($n = 10–11$/group) and nalbuphine ($n = 8–10$/group) in males and females in the threshold paw pressure procedure, in which nociceptive responses to an increasing pressure stimulus were recorded. Vertical bars represent the standard error. Asterisks (*) indicate significant differences ($P < 0.05$) between males and females.

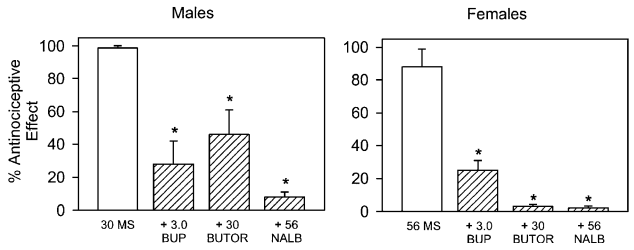

Fig. 6. Antinociceptive effects of morphine in combination with buprenorphine, butorphanol and nalbuphine ($n = 8$/group) in male and female rats in the threshold paw pressure procedure, in which nociceptive responses to an increasing pressure stimulus were recorded. The doses of morphine chosen for males (30 mg/kg) and females (56 mg/kg) produced maximal levels of antinociception in the dose–effect curve for morphine in each sex. Vertical bars represent the standard error. Asterisks (*) indicate significant antagonism ($P < 0.05$) of the antinociceptive effects of morphine. MS = morphine, BUP = buprenorphine, BUT = butorphanol, NALB = nalbuphine. All doses were administered on a mg/kg basis.

combination with a dose of morphine that produced maximal effects in males and females. An ANOVA indicated a main effect for treatment in males [$F(3,31) = 14.76$, $P < 0.05$] and females [$F(3,31) = 40.69$, $P < 0.05$], with post-hoc analyses indicating that each of these opioids antagonized ($P < 0.05$) the effects of morphine in both males and females.

3.2.3. κ-Opioid receptor agonists

Fig. 7 shows the antinociceptive effects of various κ-opioid receptor agonists. In both males and females, spiradoline, U69,593, U50,488, enadoline and ethylketocyclazocine produced dose-dependent increases in antinociception with the highest doses tested producing intermediate to high levels of antinociception. In contrast to these κ-opioid receptor agonists, nalorphine failed to produce an antinociceptive effect in either males or females. ANOVA analyses indicated a main effect for sex for U69,593 [$F(1,14) = 4.70$, $P < 0.05$] and ethylketocyclazocine [$F(1,13) = 7.89$, $P < 0.05$], but no other main effects or interactions for any other drugs. Post-hoc tests revealed that 3.0 mg/kg ethylketocyclazocine produced greater antinociception ($P < 0.05$) in males, whereas 3.0 mg/kg U69,593 produced greater antinociception ($P < 0.05$) in females. Based on ED_{50} values (95% confidence limits), the potency of spiradoline, U69,593, U50,488, and enadoline was not significantly different in males and females, whereas ethylketocyclazocine was approximately 2.2-fold more potent in males (2.2: 1.7–2.9) than females (4.9: 3.2–7.4).

3.3. Antinociception—tolerance procedure

Fig. 8 shows the antinociceptive effects of the μ-opioid receptor agonists morphine and buprenorphine, as well as the κ-opioid receptor agonists spiradoline and enadoline in males and females using the tolerance procedure in which the pressure exerted on the paw was fixed, and the latency to paw withdrawal was recorded. Stimulus intensities were selected for males and females such that baseline latencies were similar (4–5 s). Results from these analyses were similar to those obtained with the threshold procedure. For example, morphine was 3.4-fold more potent in males (ED_{50}: 11.4; 95% confidence limits: 8.5–15.3) than females (34.1: 24.8–46.7) and when tests were repeated in the same rats, morphine was 2.3-fold more potent in males. Similarly, buprenorphine was more effective in males than females, producing a 61% effect in males and only a 5% in females. Statistical analyses indicated a main effect for sex for

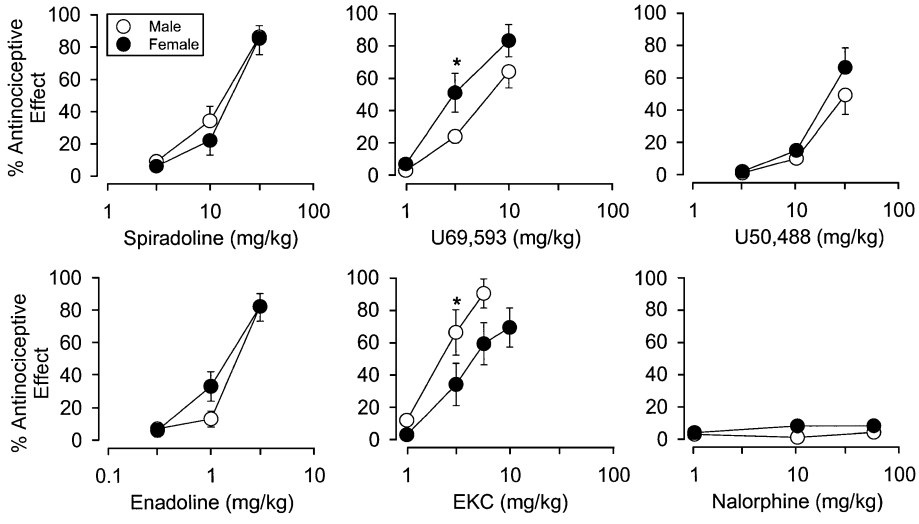

Fig. 7. Antinociceptive effects of spiradoline ($n = 8$/group), enadoline ($n = 8$/group) U69,593 ($n = 9$/group), ethylketocyclazocine ($n = 8$/group), U50,488 ($n = 8$/group) and nalorphine ($n = 6$/group) in males and females in the threshold paw pressure procedure, in which nociceptive responses to an increasing pressure stimulus were recorded. 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.

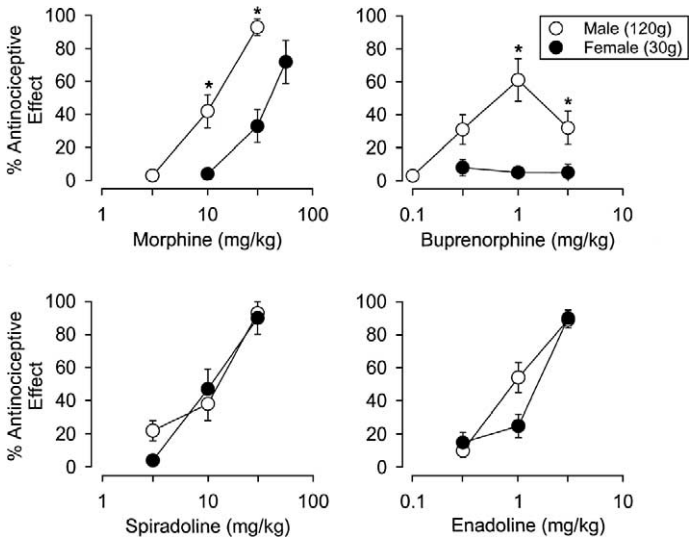

Fig. 8. Antinociceptive effects of morphine ($n = 8–10$/group), buprenorphine ($n = 8–10$/group), spiradoline ($n = 8$/group) and enadoline ($n = 8$/group) using the tolerance version of the paw pressure procedure in which fixed intensity pressures were selected for males (120 g) and females (30 g) to ensure comparable baseline paw withdrawal latencies (4–5 s). 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.

morphine [$F(1,15) = 25.63$, $P < 0.05$] and buprenorphine [$F(1,16) = 21.95$, $P < 0.05$], as well as a dose × sex interaction for buprenorphine [$F(1,16) = 6.52$, $P < 0.05$]. Post-hoc analyses indicated that doses of 10 and 30 mg/kg morphine and 1.0 and 3.0 mg/kg buprenorphine produced greater antinociception ($P < 0.05$) in males than females. In contrast to these μ-opioid receptor agonists, there were no sex differences in the potency and effectiveness of spiradoline and enadoline, and statistical analyses indicated that there were no main effects or interactions for either drug.

3.4. Control tests with (+)-amphetamine and pentobarbital

If the locomotor and/or sedative effects of opioids were influencing the observed effects, then drugs that increase locomotor activity would be expected to decrease nociceptive thresholds and drugs that produce sedation would be expected to increase nociceptive thresholds. Fig. 9 shows results from tests conducted with (+)-amphetamine and pentobarbital, drugs that increase locomotor activity and produce sedation, respectively, but are devoid of antinociceptive effects. In both males and females, doses of (+)-amphetamine and pentobarbital that produced visible alterations in behavior (e.g., hyperactivity, sedation) failed to markedly alter nociceptive thresholds. Analyses on the effects of (+)-amphetamine and pentobarbital, respectively, failed to indicate a main effect for dose [$F(1,14) = 0.09$, $P > 0.05$; $F(1,14) = 0.04$, $P > 0.05$] sex [$F(1,14) = 0.90$, $P > 0.05$; $F(1,14) = 0.33$, $P > 0.05$], or a dose × sex interaction [$F(1,14) = 0.04$, $P > 0.05$; $F(1,14) = 2.89$, $P > 0.05$].

4. Discussion

The present findings indicate that sex is an important determinant of mechanical nociception, with males displaying an approximately 37% higher threshold than females. These sex differences extend an emerging literature documenting a sexual dimorphism in nociceptive sensitivity observed in both humans and nonhumans in tests using

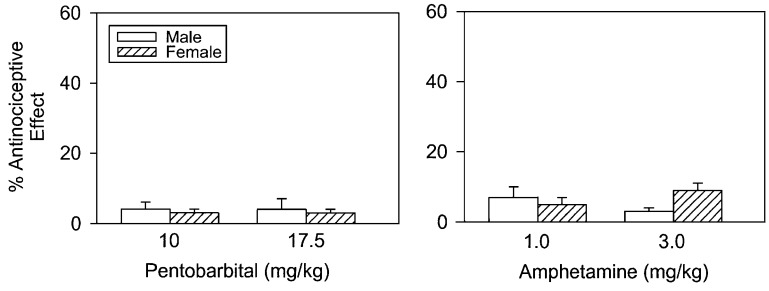

Fig. 9. Effects of (+)-amphetamine and pentobarbital ($n = 8$/group) on paw withdrawal thresholds in males and females using the threshold paw pressure procedure, in which nociceptive responses to an increasing pressure stimulus, were recorded. Vertical bars represent the standard error.

thermal, chemical and electrical nociceptive stimuli (for reviews, see Mogil et al., 2000; Fillingim and Ness, 2000). As observed in the threshold procedure, there were marked sex differences in the tolerance procedure, with paw withdrawal latencies being consistently longer in males. The function relating pressure to paw withdrawal latency, however, was characterized by a similar slope in males and females. These sex differences observed in baseline nociception did not appear to be a consequence of differential involvement of the endogenous opioid systems, as the opioid receptor antagonist naltrexone produced equal decreases in nociceptive thresholds in both males and females.

The present data contrast with recent studies indicating that paw pressure thresholds were similar in Sprague–Dawley males and females (Tseng and Craft, 2001), whereas vocalization thresholds were higher in females (Kayser et al., 1996). Baseline paw pressure and vocalization thresholds in these studies, however, were greater than 200 g in both males and females, compared to the 108 and 79 g observed in the present investigation with males and females, respectively. Thus, it is possible that sex differences in nociception are strain-dependent, and there is evidence in both rat and mouse strains consistent with this observation (Kest et al., 1999; Cook et al., 2000; Mogil et al., 2000; Barrett et al., 2002; Terner et al., 2002).

In the mechanical nociceptive procedure used in the present investigation, levorphanol and morphine were less potent and dezocine, buprenorphine, butorphanol and nalbuphine less effective than that reported in thermal nociceptive assays (Cicero et al., 1996; Bartok and Craft, 1997; Cook et al., 2000). Although the apparent potency and effectiveness of opioids can be altered by the arbitrary cutoff value, the cutoff value used in the present experiment (500 g) did not appear to play a critical role, as in many instances, these opioids failed to increase nociceptive thresholds above baseline values. Whether the μ-opioid receptor agonists produced minimal or maximal antinociceptive effects, there were marked sex differences in their potency and/or effectiveness. For example, morphine was more potent in males and the less effective μ-opioid receptor agonists dezocine and buprenorphine, as well as the mixed-action opioids butorphanol and nalbuphine, were more effective in males. These data are consistent with results obtained with other nociceptive modalities, and suggest that males are uniformly more sensitive to the antinociceptive effects of opioids with prominent activity at the μ-opioid receptor (Kepler et al., 1989; Cicero et al., 1996; Bartok and Craft, 1997; Cook et al., 2000). Whereas it is possible that the sex differences observed with morphine may be related to the presence of active metabolites or their differential metabolism to morphine-3-glucuronide (South et al., 2001; Baker and Ratka, 2002), that sex differences were observed with μ-opioid receptor agonists other than morphine is consistent with evidence that opioid pharmacokinetics cannot sufficiently explain this sexual dimorphism (Kepler et al., 1991; Candido et al., 1992).

It was unlikely that the sex differences observed in the mechanical nociceptive procedure were a consequence of activity in receptor systems other than the μ-opioid system or a nonspecific behavioral response to the opioids. First, where comparisons could be made, the relative rank order of potency and effectiveness of these opioids was generally consistent with other assays sensitive to the effects of μ-opioid receptor agonists, a finding suggestive of a common mechanism of action (Young et al., 1984; Tiano et al., 1998; Morgan et al., 1999a; Cook et al., 2000). Additionally, that buprenorphine, butorphanol and nalbuphine antagonized the effects of morphine indicates that these opioids are active at the μ-opioid receptor but have low efficacy (Hayes et al., 1987; Walker et al., 1994; Morgan et al., 1999b). Secondly, the apparent pA_2 analyses calculated from the naltrexone antagonism of morphine yielded a value of 7.4 in males and females, which is within the range previously reported for naltrexone against the effects of μ-opioid receptor agonists (Walker et al., 1994,1999; Garner et al., 1997). The finding that morphine produces its effects via pharmacologically similar receptor populations in males and females is consistent with a recent report employing receptor-selective antagonists, in which beta-funaltrexamine, but not norbinaltorphimine or naltrindole, dose-dependently antagonized the antinociceptive effects of morphine in both males and females (Craft et al., 2001). Finally, it is unlikely that sex-specific differences in sensitivity to the locomotor activating and sedative effects of opioids contributed to sex differences in opioid antinociception (Craft et al., 1996; Boyer et al., 1998), as tests conducted with (+)-amphetamine and pentobarbital, drugs with marked locomotor activating and sedative effects, respectively, failed to alter nociceptive thresholds in either males or females.

Given the sex-related differences in baseline nociception, it is possible that these differences contributed to the sex differences observed with the μ-opioid receptor agonists. To control for sex differences in baseline nociceptive thresholds, the paw pressure procedure was modified to exert a fixed intensity pressure on the paw, with pressures selected separately for males and females to ensure comparable paw withdrawal latencies (tolerance procedure). As observed in the threshold procedure, under these conditions marked sex differences were apparent with morphine and buprenorphine. That baseline levels of nociception could not sufficiently account for sex differences in opioid antinociception has also been reported in thermal nociceptive assays (Cook et al., 2000; Barrett et al., 2002; Terner et al., 2002).

The potential importance of gonadal hormones in accounting for sex differences in μ opioid-induced antinociception has been examined in numerous investigations. Although there are discrepancies in the literature, a number of reports suggest that gonadal hormones play a clear role in opioid antinociception (Kepler et al., 1989; Ali et al., 1995; Mogil et al., 2000; Cicero et al., 2002). For example, in a recent study using a thermal nociceptive assay in F344 and Sprague–Dawley rats, the potency and/or effectiveness of

μ-opioid receptor agonists were increased by gonadectomy in females. The opposite effect was obtained in males, as gonadectomy decreased the potency and/or effectiveness of μ-opioid receptor agonists. In both males and females, the effect of gonadectomy on antinociception depended on the relative effectiveness of the opioid, such that larger effects were observed with less effective opioids. Gonadectomy also markedly altered baseline nociception, such that nociceptive latencies were increased in females and decreased in males (Terner et al., 2002). That the profile of sex differences in nociception and μ-opioid antinociception in the present investigation is similar to other reports suggests that gonadal hormones also play a role in mediating sex differences in assays of mechanical nociception.

The κ-opioid receptor agonists spiradoline, U69,593, U50,488, ethylketocyclazocine and enadoline produced intermediate to high levels of antinociception in both males and females, a finding similar to those reported in other procedures employing thermal and mechanical nociceptive stimuli (Hayes et al., 1987; Millan, 1989; Butelman et al., 1993; Barrett et al., 2002). In contrast to the marked sex differences observed with the μ-opioid receptor agonists, there were small and inconsistent sex differences with the κ-opioid receptor agonists. For example, sex differences were not observed in the threshold procedure with spiradoline, enadoline, U50,488 and nalorphine or in the tolerance procedure with spiradoline and enadoline. In the threshold procedure, however, ethylketocyclazocine was more potent in males and a single dose of U69,593 was more effective in females. Although the factors that account for these discrepancies are unclear, it is known that ethylketocyclazocine has activity at both μ- and κ-opioid receptors (Hayes et al., 1986; Picker et al., 1990; Broadbear et al., 1994) and thus it is possible that its greater potency in males reflects its activity at the μ-opioid receptor.

The absence of consistent sex differences in κ-opioid antinociception with F344 rats contrasts with previous results obtained in thermal nociceptive assays, in which F344 males are more sensitive to the antinociceptive effects of κ-opioid receptor agonists (Barrett et al., 2002). The failure to observe sex differences with these opioids was not a consequence of procedural factors specific to each variation of the paw pressure procedure or to differences in baseline nociceptive thresholds, as similar results were obtained with both the threshold and tolerance procedures. Whether such modality-specific sex differences with κ-opioid receptor agonists exists for other strains of rat or other species remains to be determined. Nevertheless, the importance of nociceptive modality has also been emphasized with other antinociceptive agents such as cannabinoids, in which sex differences with selected compounds are apparent in a thermal but not a mechanical nociceptive assay (Tseng and Craft, 2001). Similarly, in a form of stress-induced antinociception, sex differences are apparent in a thermal but not a chemical nociceptive assay (Menendez et al., 1994). These data suggest that the convergence of antinociceptive systems and nociceptive pathways is unique to each nociceptive modality, and sensitivity in one assay is not necessarily predictive of sensitivity in another assay.

The mechanism(s) underlying these modality-specific sex differences is unclear. Some data suggest, however, that the relative contribution of neurotransmitter systems in the spinal cord may be involved. For example, whereas norepinephrine depletion in the spinal cord attenuates the effects of morphine in a mechanical but not thermal nociceptive assay, serotonin depletion in the spinal cord attenuates the effects of morphine in a thermal but not mechanical nociceptive assay (Kuraishi et al., 1983, 1985; Giordano and Barr, 1988). Such findings suggest that spinal norepinephrine may be more critical for mechanical antinociception, whereas spinal serotonin may be more critical for thermal antinociception. As such, determining the relative contribution of spinal norepinephrine and serotonin to opioid-induced antinociception in males and females may lend insight into these modality-specific sex differences.

Alternatively, these discrepancies across modalities may be a consequence of the specific opioids tested, as previous studies indicate that sex differences with κ-opioid receptor agonists are most apparent when less effective opioids are tested (Kavaliers and Innes, 1987; Craft et al., 1998; Negus and Mello, 1999; Patrick et al., 1999; Craft and Bernal, 2001; Barrett et al., 2002). For example, in the warm water tail-withdrawal procedure, little or no sex differences are observed with spiradoline, U69,593 or U50,488 (Craft et al., 1998; Negus and Mello, 1999; Patrick et al., 1999; Craft and Bernal, 2001; Barrett et al., 2002), opioids that produce high levels of antinociception under almost all experimental conditions. In contrast, large sex differences have been reported in rats with enadoline, bremazocine and nalorphine under conditions in which these opioids produce low to intermediate levels of antinociception (Craft and Bernal, 2001; Barrett et al., 2002; but see Bartok and Craft, 1997). Although enadoline and nalorphine were also tested in the present investigation, enadoline produced a maximal effect in both males and females, whereas nalorphine was ineffective in both sexes. Thus, the effectiveness of the opioids under the present experimental conditions may not have been optimal to detect underlying sex differences in κ-opioid receptor pharmacology.

In summary, these findings indicate marked sex differences in mechanical nociception, but a similar involvement of the endogenous opioid system in males and females. Additionally, marked sex differences were apparent in the antinociceptive effects of μ-, but not κ-, opioid receptor agonists, and these effects were apparent even under conditions in which baseline nociceptive latencies were equated. Comparing modalities in which sex differences are apparent to modalities in which sex differences are lacking may facilitate strategies for identifying the underlying mechanism(s) of sex differences in opioid-induced antinociception.

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