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ORIGINAL INVESTIGATION

Sex differences in the potency of κ opioids and mixed-action opioids administered systemically and at the site of inflammation against capsaicin-induced hyperalgesia in rats

Lisa M. Lomas · Andrew C. Barrett · Jolan M. Terner · Donald T. Lysle · Mitchell J. Picker

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Abstract
Rationale Sex differences in the potency of the antinociceptive effects of κ opioids have been reported in various acute pain models with evidence suggesting that these sex differences are mediated by activity in the *N*-methyl-D-aspartate (NMDA) system.
Objectives The purpose of the present study was to evaluate sex differences in the antihyperalgesic actions of selected κ and mixed-action opioids in a persistent pain model and determine if the NMDA system modulates these effects in a sexually dimorphic manner.
Methods Using gonadally intact male and female F344 rats, hyperalgesia was induced by local administration of capsaicin in the tail, after which the tail was immersed in a mildly noxious thermal stimulus (45°C water), and tail-withdrawal latency measured. Opioids were then administered systemically (s.c.) and locally (in the tail) alone, and in selected combinations with the noncompetitive NMDA antagonist dextromethorphan.
Results When administered systemically and locally, the κ opioids spiradoline, U69,593 and U50,488, and the mixed-action opioids butorphanol and nalbuphine, produced dose-dependent antihyperalgesic effects. Whereas the κ opioids were generally more potent in males, sex differences were not observed with the mixed-action opioids. Peripheral receptor activity was confirmed for local administration of κ opioids by the antagonism observed after local, but not intracerebroventricular (i.c.v.), administration of the κ antagonist nor-binaltorphamine (nor-BNI). Dextromethorphan was equally potent in attenuating the antihyperalgesia induced by κ opioids in both males and females.
Conclusions These findings demonstrate sex differences in κ opioid activity in a persistent pain model. Although an NMDA antagonist blocked the effects of κ opioids in this model, these effects were not sexually dimorphic as reported in most acute pain models.

Keywords Sex differences · Capsaicin · Kappa opioid · NMDA · Peripheral opioid receptor · Mixed-action opioid

L. M. Lomas · A. C. Barrett · J. M. Terner · D. T. Lysle · M. J. Picker (✉)
Department of Psychology,
University of North Carolina at Chapel Hill,
Chapel Hill, NC 27599-3270, USA
e-mail: mjpicker@email.unc.edu

Introduction

There is now evidence that male and female rodents differ in their sensitivity to the antinociceptive effects of κ opioids (e.g., Craft and Bernal 2001; Holtman and Wala 2006). In a recent review and analysis, Craft (2003) reported that when collapsed across drugs, studies and species, 39% of the reports indicated that κ opioids were more potent in males, 50% equally potent in males and females, and 11% more potent in females. Both the direction and magnitude of these sex differences appear to be influenced by the dose, type of κ opioid, genotype, and type of pain model (for reviews see Craft 2003; Barrett 2006). In rats, for example, reports of κ opioids being more potent in males have appeared exclusively in studies utilizing acute pain models and are more likely to occur when complete dose–effect functions are determined. Although there are only two reports in which κ opioids were examined in persistent pain models, in these studies, the κ opioids tested were either more potent in females or equally potent in males and females (Binder et al. 2000; Clemente et al. 2004).

Presently, it is unclear if these conflicting findings are a consequence of a sexually dimorphic interaction of opioids with the distinct mechanisms underlying acute vs persistent nociception. For example, persistent nociception requires activation of unmylenated C-fibers and acute nociception activation of large Aδ nociceptive fibers (e.g., Le Bars et al. 2001). Acute and persistent nociception also differ in terms of their duration, neurochemical substrates, and sensitivity to pharmacological manipulation (Le Bars et al. 2001; Richardson and Vasko 2002; Mogil et al. 2003). Thus, the primary goal of the present investigation was to evaluate sex differences in the effects of κ opioids and selected mixed-action opioids with low efficacy at the κ receptor in a persistent pain model. To this end, we conducted tests using the capsaicin-induced hyperalgesia procedure, which is a persistent pain model adapted to the study of sex differences in μ opioid sensitivity (Barrett et al. 2003). One limitation of some persistent pain models is that it is often difficult to control the level of nociception, and baseline levels of nociception are known to be a determinant of sex differences in opioid sensitivity (Cook et al. 2000; Barrett et al. 2002). By altering the dose of capsaicin, however, it is possible to produce comparable levels of nociception in males and females (Barrett et al. 2003). Moreover, inflammation is localized to the tail in the capsaicin model and thus it is possible to examine sex differences in the effects of relatively small doses of κ opioids when their actions are directed at κ opioid receptors located at the site of inflammation. From a clinical perspective, a result of administering small doses locally is that it minimizes potential adverse effects, which is especially critical for κ opioids as adverse central effects limit their clinical utility (Kumor et al. 1986; Rimoy et al. 1991).

Although the mechanisms that mediate the sexual dimorphism in κ opioid sensitivity are unclear, several studies have implicated the NMDA receptor system. Indeed, NMDA antagonists attenuate κ opioid antinociception in male mice and rats but not intact females (Kavaliers and Choleris 1997; Mogil et al. 2003; Holtman and Wala 2006). Ovariectomized female mice (Sternberg et al. 2004) display a similar sensitivity to NMDA antagonists as males, suggesting further that males and females have similar opioid/nociceptive circuitry, but in females, the NMDA component of this circuitry is actively suppressed by the gonadal hormones.

To date, studies of NMDA involvement in sex differences of κ opioid antinociception have been conducted exclusively in models of acute pain and thus its contribution to persistent pain remains to be determined. Experiments of this nature can be complicated by the fact that NMDA antagonists have a direct effect on the development of persistent nociception induced by injections of formalin and Freund's adjuvant, as well as in postsurgical nociception (Eisenberg et al. 1993; Przewlocki and Przewlocka 2001; Nishimura et al. 2004). Such effects are not observed after the administration of capsaicin (Sakurada et al. 1998), and thus this persistent pain model may provide an opportunity to evaluate the contribution of the NMDA system to sex differences with κ opioids. For comparison, tests were conducted in a 50°C warm water tail-withdrawal procedure as in this procedure NMDA antagonists antagonize the effects of κ opioids in a sexually dimorphic manner (Mogil et al. 2003; Holtman and Wala 2006).

Materials and methods

Animals

Gonadally intact male and female F344 rats were obtained from Charles River Laboratories (Raleigh, NC, USA). F344 rats were chosen as previous studies in our lab utilizing this strain have shown large sex differences in opioid antinociception. 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 was used to assess hyperalgesia. Before testing, rats were habituated to handling on one occasion. During testing, each rat was lightly restrained, with the distal 7 cm of the tail immersed in water maintained at 45°C. This temperature was selected as it approximates threshold nociceptive values in adult rats (Falcon et al. 1996). Baseline tail withdrawal latencies were determined in each rat before administration of any compounds, and rats that failed to maintain their tails in the water for a full 15 s were excluded from testing. Subsequently, capsaicin was injected 3.5 cm from the tip of the tail. All injections of capsaicin were made under light halothane anesthesia, with rats recovering from this procedure within 2–3 min. After administration of capsaicin, tail-withdrawal latencies decreased from 15 s to approximately 3.5–4.5 s with this effect being consistent in both males and females. Rats were tested once per week with no more than five tests per animal. Previous studies conducted in our laboratory indicated that this frequency of testing and the number of tests produced reliable and consistent tail-withdrawal latencies (Barrett et al. 2003).

Antihyperalgesic effects of opioids

For tests examining the antihyperalgesic effects of opioids, a 3.0 and 1.0 μg dose of capsaicin was chosen for males and

females, respectively, as these doses produce a comparable magnitude and duration of hyperalgesia (Barrett et al. 2003). Before initiating testing of opioids, a capsaicin baseline was assessed in each animal. To determine the antihyperalgesic effects of opioids and to determine potency differences in local vs systemic administration, capsaicin was administered in the tail, and one dose of each opioid was injected systemically (s.c.) or locally in the tail. Tail-withdrawal latencies were determined 15 min after the capsaicin/opioid injection, which is the time point corresponding to the peak effect of capsaicin. Additional tests were conducted in which the noncompetitive NMDA antagonist dextromethorphan was administered (s.c. or tail) 15 min before capsaicin injection alone or in combination with U69,593 (s.c. or tail). For all tests, a 15 s cutoff latency was implemented, as this indicated a maximal antihyperalgesic effect (i.e., nociceptive thresholds returned to baseline levels). The doses of dextromethorphan selected for study are similar to those used in previous studies (e.g., Holtman et al. 2003; Craft and Lee 2005), as higher doses are known to alter locomotor activity as well as produce ataxia and stereotypies (Danysz et al. 1994; Plesan et al. 1998; Redwine and Trujillo 2003).

Antihyperalgesic activity of κ opioids at the site of inflammation

To examine the local mediation of locally administered opioids, doses that produced high levels of antihyperalgesia in males and females were administered in the tail along with either local or i.c.v. administration of the selective κ antagonist nor-BNI. For tests with i.c.v. administration of nor-BNI, nor-BNI was administered 24 h before capsaicin. For tests with local (in tail) administration, nor-BNI was administered 15 min before capsaicin. Due to the long duration of nor-BNI, testing for an individual rat was terminated after the completion of this test. To provide a comparison between the results obtained in this persistent pain model with those in an acute pain model, and to identify the dose of nor-BNI to be administered i.c.v. in tests of local mediation of antihyperalgesia, additional tests were conducted using a warm water tail-withdrawal procedure. In this procedure, rats were placed in restraint tubes with the distal 7 cm of the tail immersed in 50°C water and latency to remove the tail measured (see Cook et al. 2000 for details). Tail withdrawal latencies were typically between 10–11 s in both males and females. After baseline latencies were assessed, U69,593 was administered and the latency to remove the tail from the 50°C warm water was determined. A 15-s cut-off to tail-withdrawal was imposed to avoid tissue damage. For these tests, nor-BNI was administered i.c.v. 24 h before administration of U69,593.

Data analysis

For dose–effect curves examining the antihyperalgesic effects of opioids, latencies to tail-withdrawal after administration of the drug were converted to the percentage of the maximum possible effect using the following equation: $\%\ \text{antihyperalgesic effect} = [(\text{observed} - \text{baseline})/(15\ \text{s} - \text{baseline})] \times 100$. 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, an ANOVA was also conducted with sex and dose as between-groups factors. 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 the opioid. For statistical analyses using ANOVA, the alpha level was set at 0.05. Relative potency estimates were also calculated by comparing the potency of s.c. administration of opioids with local administration of opioids within males and within females. For this analysis, dose ratios were calculated in a manner described by (Tallarida and Murray 1987), in which a common slope was determined between linear regression lines representing the two dose–effect curves, and then the distance between the regression lines calculated. Differences in the relative potency of s.c. and tail administration of opioids were considered to be significant if the 95% confidence interval did not overlap 1.0 (by using a *t* test).

To calculate the % maximal antinociceptive effect, tail-withdrawal latencies for the 50°C warm water tail-withdrawal procedure were converted to percent antinociceptive effect using the following equation: $\%\ \text{antinociceptive effect} = [(\text{test latency} - \text{baseline})/(15\,\text{s} - \text{baseline})] \times 100$. The time-course evaluation of a drug's effect was measured in this assay with an ANOVA on area under the curve used for statistical analysis. Area under the curve was estimated by the Trapezoidal Rule using available statistical software (Tallarida and Murray 1987).

Intracerebroventricular injections

Rats were stereotaxically implanted with a single 22-gauge cannula into the left lateral ventricle [anterior–posterior (AP) +0.9, medial–lateral (ML) +1.5, dorsal–ventral (DV) −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 nor-BNI in a 5 μl volume with a 28-gauge injector that protruded 1 mm beyond the tip of the cannula. The injector was connected by a length of tubing to a 10 μl Hamilton microsyringe.

Drugs

The following drugs were used: nor-binaltorphamine, trans-3,4-dichloro-*N*-methyl-*N*[2-(1-pyrolidinyl)cyclohexyl] benzeneaceamide methanesulfonate (U50,488) (all provided by the National Institute on Drug Abuse), dextromethorphan hydrobromide monohydrate, spiradoline mesylate, butorphanol tartrate, nalbuphine HCl, 1-oxaspiro [4,5]dec-8-yl benzeneacetamide (U69,593), and capsaicin (all purchased from Sigma–Aldrich, St. Louis, MO, USA). 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 injection in the tail, capsaicin (0.1 ml volume when administered alone) was mixed in the same syringe as the test drugs in a 0.1 ml volume. For other tests, drugs were administered s.c. in a volume of 0.5–1.0 ml/kg.

Results

κ opioids Figure 1 shows that in both males and females, the κ opioids spiradoline, U50,488 and U69,593 produced dose-dependent increases in antihyperalgesia, with maximal or near maximal effects obtained at the highest doses tested. As shown in Table 1, spiradoline was the most potent of the κ opioids in both males and females when administered locally, whereas U69,593 and U50,488 were approximately equally potent. In contrast, when administered systemically, U50,488 was the most potent, whereas spiradoline and U69,593 were approximately equally potent. Table 2 shows that sex differences were observed in the potency of these κ opioids, with the antihyperalgesic potency of systemic and local administration of spiradoline and U50,488 being greater in males. Analyses based on ANOVA for spiradoline confirmed these observations, indicating a main effect for sex (systemic: $F_{1,12}$=10.3,

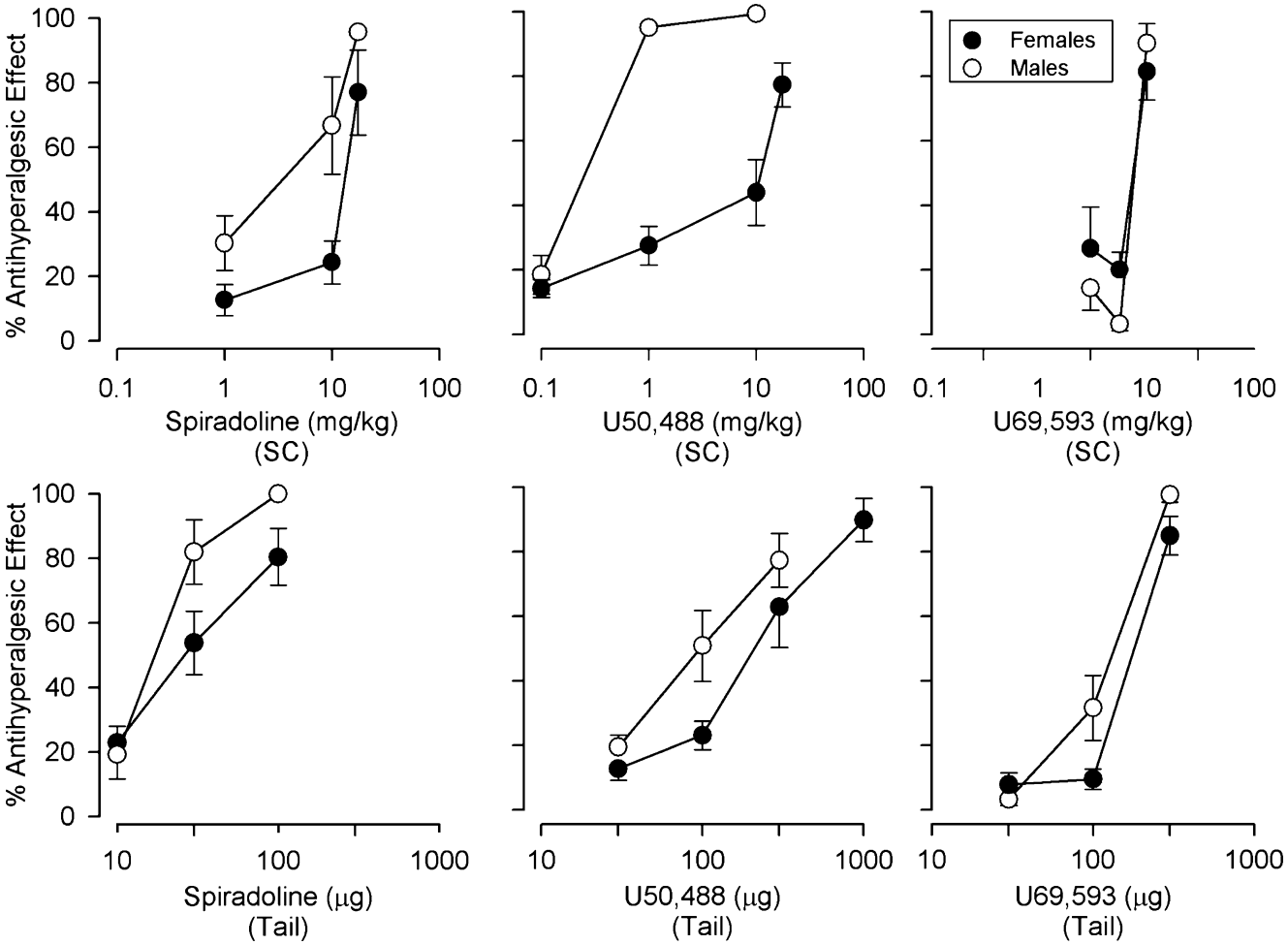

Fig. 1 Antihyperalgesic effects of spiradoline, U50,488 and U69,593 administered systemically (s.c.) and locally (tail) in male and female rats (n=6–8). Data for systemically administered drugs are expressed in milligram per kilogram and for locally administered drugs in micrograms. A warm water tail-withdrawal procedure was used for testing in which the distal 7 cm of the tail was immersed in water maintained at 45°C. Equally effective doses of capsaicin were injected 3.5 cm from the tip of the tail 15 min before the test with all opioids administered at the same time as capsaicin. *Vertical bars* represent the standard error; where not indicated, the standard error fell within the data point

Table 1 ED50 values (95% confidence limits) and relative potency ratios (95% confidence limit) for male and female rats (n=6–8) in tests conducted with spiradoline, U50,488, U69,593, butorphanol, and nalbuphine when administered systemically (s.c.; mg/kg) and locally (tail; μg)

Drug	Males	Females	Potency ratio
Spiradoline			
Tail	0.019 (0.013–0.266)	0.028 (0.019–0.45)	1.84 (1.23–2.92)[a]
SC	2.82 (1.41–5.64)	11.2 (4.58–27.2)	3.19 (1.57–14.85)[a]
U69,593			
Tail	0.11 (0.09–0.14)	0.16 (0.11–0.22)	1.32 (0.94–1.88)
SC	6.92 (5.49–8.74)	6.5 (4.7–8.98)	0.88 (.609–1.24)
U50,488			
Tail	0.099 (0.068–0.15)	0.19 (0.14–0.28)	1.98 (1.11–4.05)[a]
SC	0.30 (0.157–0.58)	4.36 (1.95–9.75)	18.6 (6.67–69.3)[a]
Butorphanol			
Tail	0.031 (0.022–0.444)	0.05 (0.37–0.07)	1.51 (0.88–2.76)
SC	0.203 (0.087–0.486)	0.113 (.004–0.36)	1.48 (0.40–5.76)
Nalbuphine			
Tail	0.35 (0.28–0.44)	0.38 (0.31–0.45)	1.09 (0.82–1.49)
SC	7.86 (4.54–13.6)	3.05 (1.21–7.75)	1.91 (0.89–4.72)

[a] More potent (P<.05) in males than females as determined by relative potency ratio.

P<0.05; local: $F_{1,12}$=4.7, P<0.05) and dose (systemic: $F_{1,24}$ =23.9, P<0.05; local: $F_{1,24}$=39.5, P<0.05), but no dose × sex interaction. Similarly, a main effect for dose (systemic: $F_{2,24}$=61.7, P<0.05; local: $F_{2,26}$=27.2, P<0.05) and sex (systemic: $F_{1,12}$=55.8, P<0.05; local: $F_{1,13}$=4.5, P<0.05) was observed for U50,488. A significant dose × sex interaction was observed for systemic ($F_{2,24}$=20.2, P<0.05) but not local, administration of U50,488. In contrast to spiradoline and U50,488, sex differences with U69,593 were observed only after local administration ($F_{1,13}$=5.2, P<0.05). Analyses also revealed a main effect of dose ($F_{2,26}$=156.4, P<0.05) and a dose × sex interaction ($F_{2,26}$=3.4, P<0.05). Although there was no main effect for sex with systemic administration of U69,593 or a dose × sex interaction, there was a main effect for dose ($F_{2,12}$=45.5, P<0.05).

Antihyperalgesic activity of κ opioids at the site of inflammation To determine the role of κ opioid receptors at the site of inflammation in mediating the antihyperalgesic effect of κ opioids, U69,593 was administered locally in combination with both local and i.c.v. administration of the κ antagonist nor-BNI. As shown in panels "a" and "b" of Fig. 2, the effects of U69,593 were attenuated by local but not i.c.v. administration of nor-BNI, suggesting that the effects of U69,593 were mediated by κ opioid receptors at the site of inflammation. ANOVAs indicated a main effect for treatment ($F_{2,29}$=61.6, P<0.05) with post hoc analyses confirming attenuation by local (P<0.05) but not by i.c.v. administration. There was, however, no main effect for sex or a sex × condition interaction.

Table 2 Relative potency ratios (95% confidence limit) for spiradoline, U50,488, U69,593, butorphanol and nalbuphine when administered systemically (s.c.) and locally (tail) in male and female rats (n=6–8)

Drug	Males	Females
Spiradoline	192.2 (102.8–364.8)[a]	102.0 (42.1–205.9)[a]
U50,488	3.30 (1.17–6.61)[a]	16.38 (7.032–74.1)[a]
U69,593	69.23 (49.2–98.8)[a]	44.02 (26.1–71.5)[a]
Butorphanol	9.83 (4.01–27.9)[a]	3.15 (0.579–12.2)
Nalbuphine	19.82 (11.7–34.0)[a]	11.54 (7.34–17.4)[a]

[a] More potent (P<.05) when administered locally than systemically as determined by relative potency ratio.

For comparison, and to determine if the dose of nor-BNI selected for i.c.v. administration was appropriate, similar tests were conducted in a 50°C warm water tail-withdrawal procedure. In this procedure, systemic administration of opioids is known to produce antinociception via central and/or spinal sites. To equate the antinociceptive effects produced by U69,593, a higher dose was used in females (5.6 mg/kg) than males (3.0 mg/kg). In this procedure, systemic administration of U69,593 produced a time-dependent antinociceptive response, with a peak effect observed 30 to 45 min after administration (Fig. 2, panels "c" and "d"). Although complete dose–response testing was not conducted, with this higher dose the peak effect of U69,593 was slightly smaller in the females. In this procedure, local administration (i.e., in the tail) of U69,593 failed to produce an antinociceptive effect in either males or females. Administration of nor-BNI via the i.c.v. route completely attenuated the effects of U69,593, suggesting that in this procedure the effects of U69,593 were mediated by spinal or supraspinal κ opioids receptors. ANOVA indicated a main effect for treatment ($F_{2,26}$=27.1, P<0.05) with post hoc analyses confirming attenuation (P<0.05) by i.c.v. administration of nor-BNI. Post hoc analyses also confirmed a difference (P<0.05) in the antinociceptive effects of systemic and local administration of U69,593 in the tail withdrawal procedure. No main effect for sex or a sex × condition interaction was found.

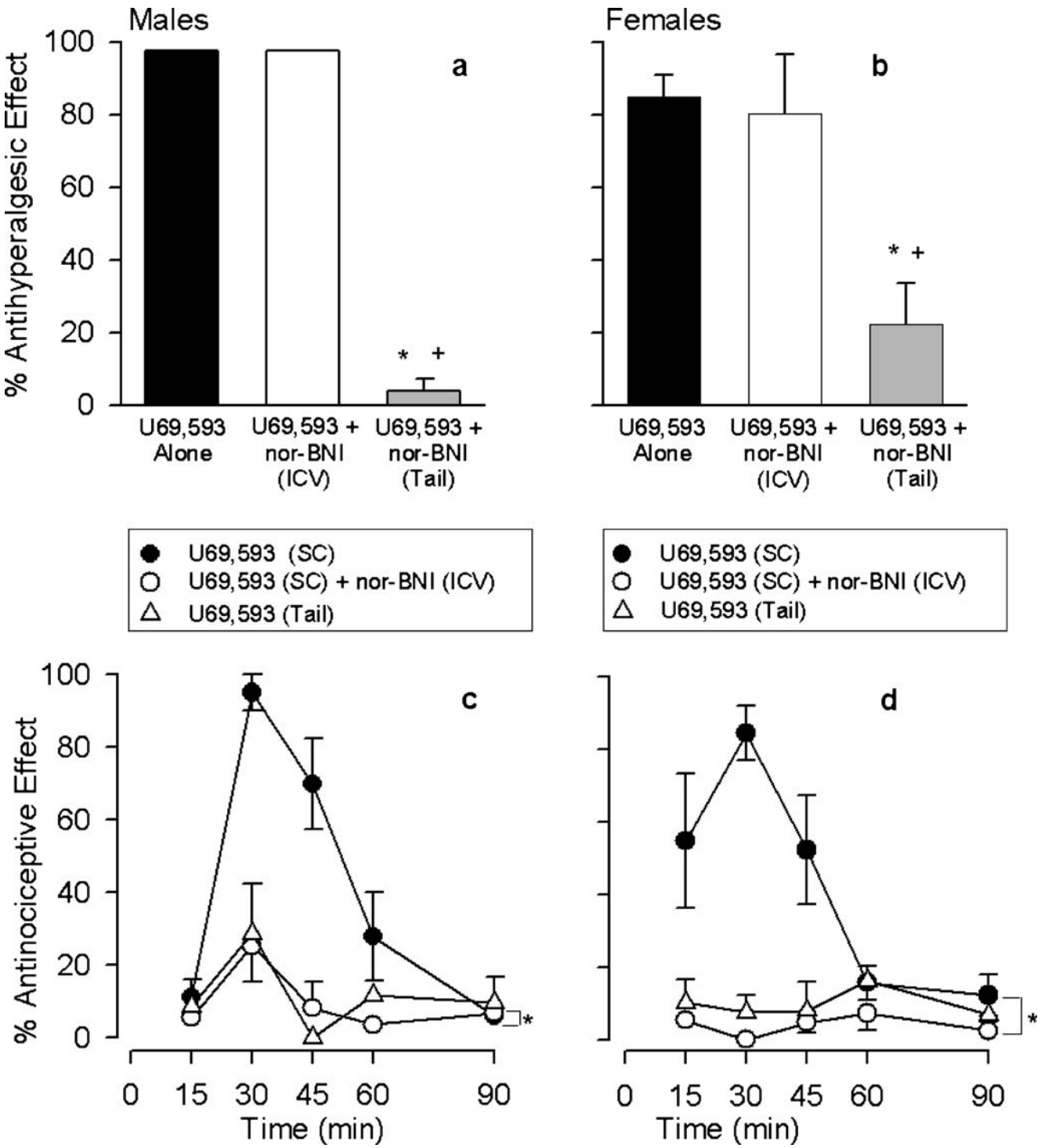

Figure 3 shows that in the capsaicin preparation, local administration of nor-BNI attenuated the antihyperalgesic effects of spiradoline in both males and females. In contrast, nor-BNI failed to attenuate the effects produced by U50,488. ANOVA indicated a main effect of drug condition for spiradoline ($F_{1,23}$=22.5, P<0.05) but no main effect for sex or a sex × condition interaction.

Mixed-action opioids Figure 4 shows that the mixed-action opioids butorphanol and nalbuphine produced dose-depen-

◀ **Fig. 2** **a** and **b** Antihyperalgesic effects of local (tail) administration of U69,593 (300 μg) alone and in combination with central (i.c.v.) or local (tail) administration of nor-BNI (10 μg) in male and female rats (n=5) in the capsaicin preparation. Additional details are as described in Fig. 1. *Asterisks* (*) indicate a significant difference between the antihyperalgesic effects of U69,593 alone (tail) vs in combination with nor-BNI (tail). *Crosses* (+) indicate a significant difference in the antihyperalgesic effect of the combination of U69,593 administered in the tail and nor-BNI administered i.c.v. vs to the combination of U69,593 and nor-BNI administered in the tail. **c** and **d** Antinociceptive effects of U69,593 alone when administered systemically (3.0 mg/kg males; 5.6 mg/kg females: these doses were determined to be equally effective in males and females) and in combination with central (i.c.v.) administration of nor-BNI (10 μg) in the warm water tail-withdrawal procedure in males and females. In this procedure, the distal 7 cm of the tail was immersed in water maintained at 50°C and latency to withdrawal the tail from warm water was recorded. Antinociceptive effects of U69,593 administered locally (tail; 300 μg) are also shown. All tests were conducted in male and female rats (n=5). For all panels, *vertical bars* represent the standard error; where not indicated, the standard error fell within the bar or the data point. *Asterisks* (*) indicate a significant difference in the antinociceptive effects of U69,593 alone (s.c.) vs in combination with nor-BNI (i.c.v.)

dent increases in antihyperalgesia, with maximal or near maximal effects obtained at the highest dose tested. In both males and females, nalbuphine was more potent when administered locally, whereas butorphanol was more potent when administered locally in males but not in females (Table 2). ANOVA analyses indicated a main effect of dose for both drugs (butorphanol: systemic: $F_{3,33}$= 22.8, P<0.05, local: $F_{3,39}$=42.8, P<0.05; nalbuphine: systemic: $F_{2,24}$=27.3, P<0.05, local: $F_{2,26}$=212, P<0.05), but no effect of sex or dose × sex interaction. Thus, in contrast to the κ opioids tested, there were no sex differences in the antihyperalgesic potency of either nalbuphine or butorphanol (see Table 1).

Figure 5 shows that local administration of nor-BNI attenuated the antihyperalgesic effects of nalbuphine in both males and females, but failed to attenuate the effects produced by butorphanol. ANOVA indicated a main effect for drug condition only for nalbuphine ($F_{1,23}$=660, P< 0.05), and no main effect for sex or a sex × condition interaction. Although nor-BNI failed to attenuate the effects of butorphanol there was a sex × condition interaction ($F_{1,36}$=5.37, P<0.05).

NMDA modulation of κ opioid antihyperalgesia To evaluate the sexually dimorphic role of the NMDA system in modulating κ opioid antihyperalgesia, the NMDA antagonist dextromethorphan was administered in combination with both local and systemic administration of U69,593. Alone, dextromethorphan (3.0–30 mg/kg) had no antihyperalgesic effects (data not shown). However, at the higher does tested, there was a clear disruption of motor coordination and sedation. As shown in Fig. 6, in both sexes systemic administration of dextromethorphan produced a dose-dependent attenuation of the antihyperalgesic effect of systemic administration of U69,593, with almost complete attenuation observed at the highest dose of dextromethorphan tested. ANOVA confirmed a main effect for dose ($F_{3,46}$=39.33, P<0.05), but no main effect for sex or a sex × dose interaction. Post hoc analysis indicated a significant (P<0.05) difference between U69,593 alone and the combination of U69,593 with 3.0 and 30 mg/kg

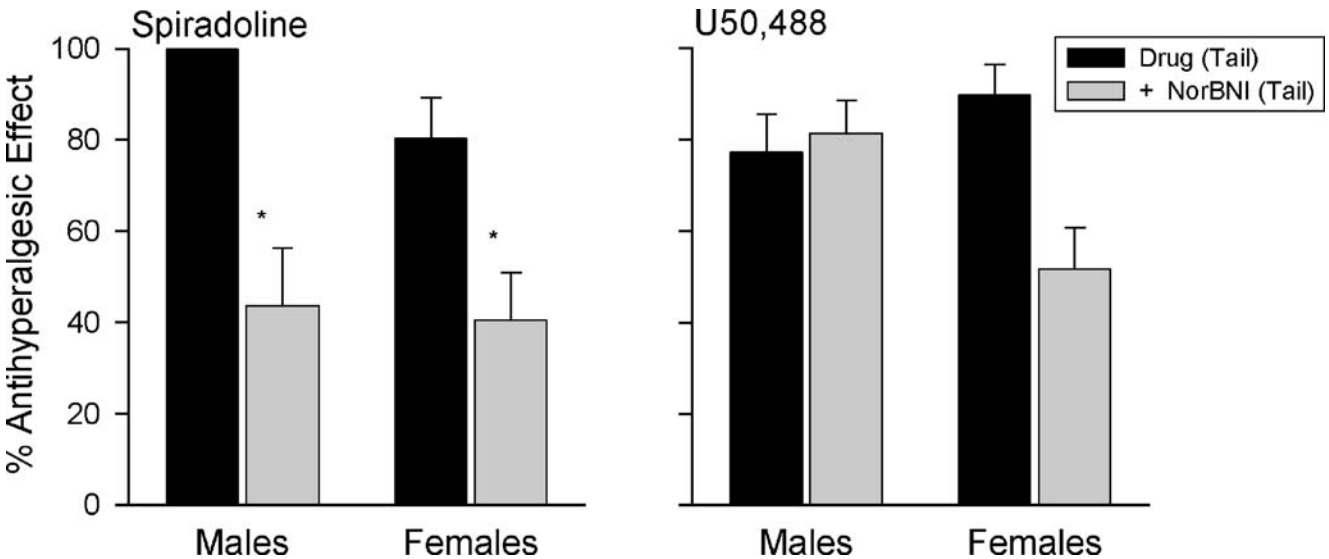

Fig. 3 Antihyperalgesic effects of local (tail) injections of spiradoline (100 μg), U50,488 (300 μg males, 1,000 μg females: these doses were determined to be equally effective in males and females), alone and in combination with local injections of nor-BNI (10 μg) in male and female rats (n=6–8) in the capsaicin preparation. Procedural details are as described in Fig. 1. *Vertical bars* represent the standard error; where not indicated, the standard error fell within the bar. *Asterisks* (*) indicate a significant difference between the antihyperalgesic effects of opioids alone vs in combination with nor-BNI

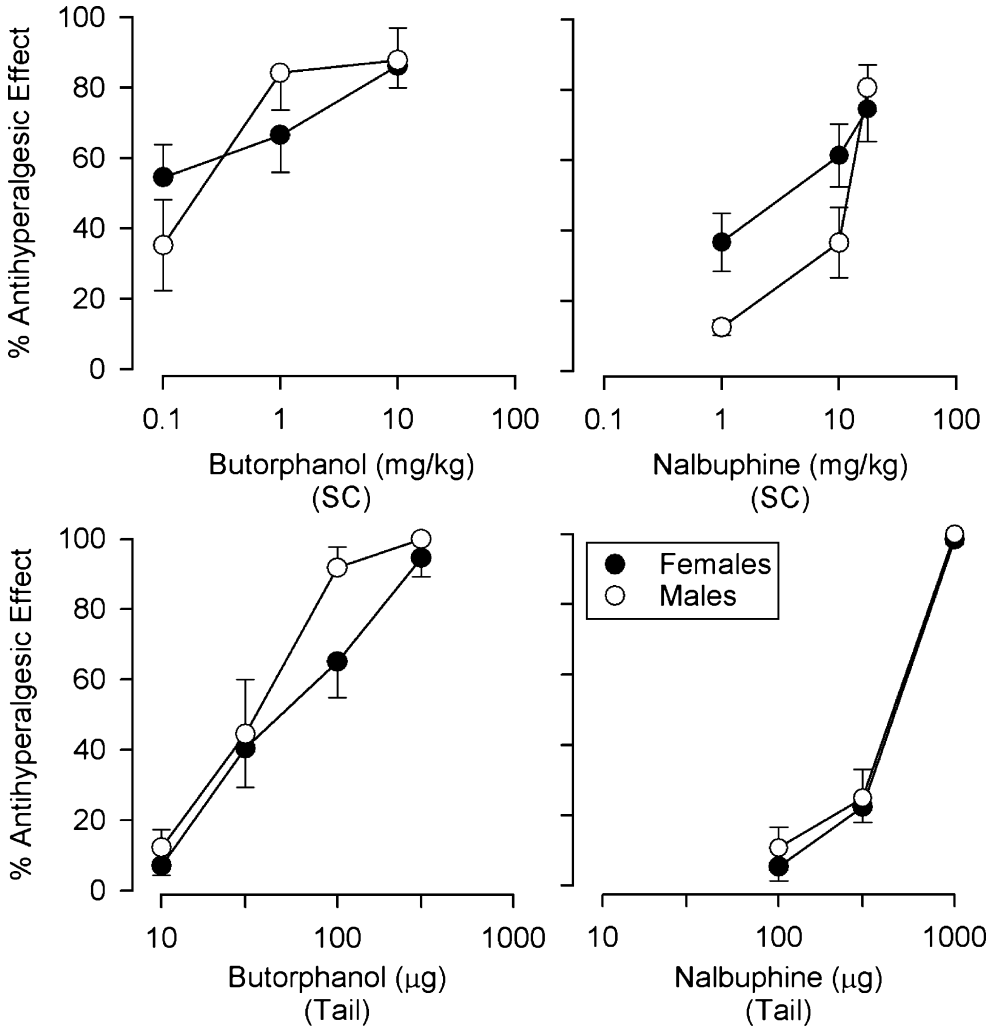

Fig. 4 Antihyperalgesic effects of the mixed-action opioids butorphanol and nalbuphine administered both systemically (s.c.) and locally (tail) in male and female rats (n=6–8) in the capsaicin preparation. Data for systemically administered drugs are expressed in milligram per kilogram and for locally administered drugs in micrograms. Other procedural details are as described in Fig. 1. *Vertical bars* represent the standard error; when not indicated, the standard error fell within the bar

dextromethorphan. In comparison, as shown in Fig. 7, dextromethorphan administered systemically produced a small attenuation (less than 30%) of the antihyperalgesia produced by local administration of U69,593 in both sexes. Local administration of dextromethorphan also failed to attenuate the antihyperalgesia produced by local U69,593 administration in both males and females. ANOVA analysis indicated a main effect for treatment ($F_{2,34}$=3.29, P<0.05), but no main effect for sex or a sex × treatment interaction. Post hoc analysis confirmed the combination of dextromethorphan administered systemically and U69,393 locally was different (P<0.05) than U69,593 alone.

Figure 8 shows attenuation of the antihyperalgesic effects of both spiradoline and U50,488 in males and females by dextromethorphan. In these tests, the most effective dose of each opioid was combined with 30 mg/kg of dextromethorphan, with all drugs being administered systemically. ANOVAs indicated a main effect for treatment with spiradoline alone and in combination with dextromethorphan, ($F_{1,23}$=6.87, P<0.05), but no main effect for sex or a sex × dose interaction. ANOVA also indicated a main effect for treatment for U50,488 alone or in combination with dextromethorphan ($F_{1,23}$=1.02, P< 0.05), but no main effect for sex. There was a sex × treatment interaction ($F_{1,23}$=14.737, P<0.05).

For comparison, tests were conducted in the 50°C warm water tail-withdrawal procedure with all drugs being administered systemically. As shown in Fig. 9, equally effective doses of U69,593 were combined with a dose of dextromethorphan that produced the largest reduction in antihyperalgesia in the capsaicin preparation. In these tests, U69,593 produced near maximal effects in both males and females. Dextromethorphan attenuated the antinociceptive effect produced by U69,593 only in males ($F_{1,12}$=36.2, P< 0.05). Comparison of difference scores in the area under the curve analysis confirmed this sexually dimorphic action. Thus, dextromethorphan attenuated the effects of U69,593 in both males and females in the capsaicin preparation, but in the 50°C warm water tail-withdrawal procedure attenuation was observed only in males.

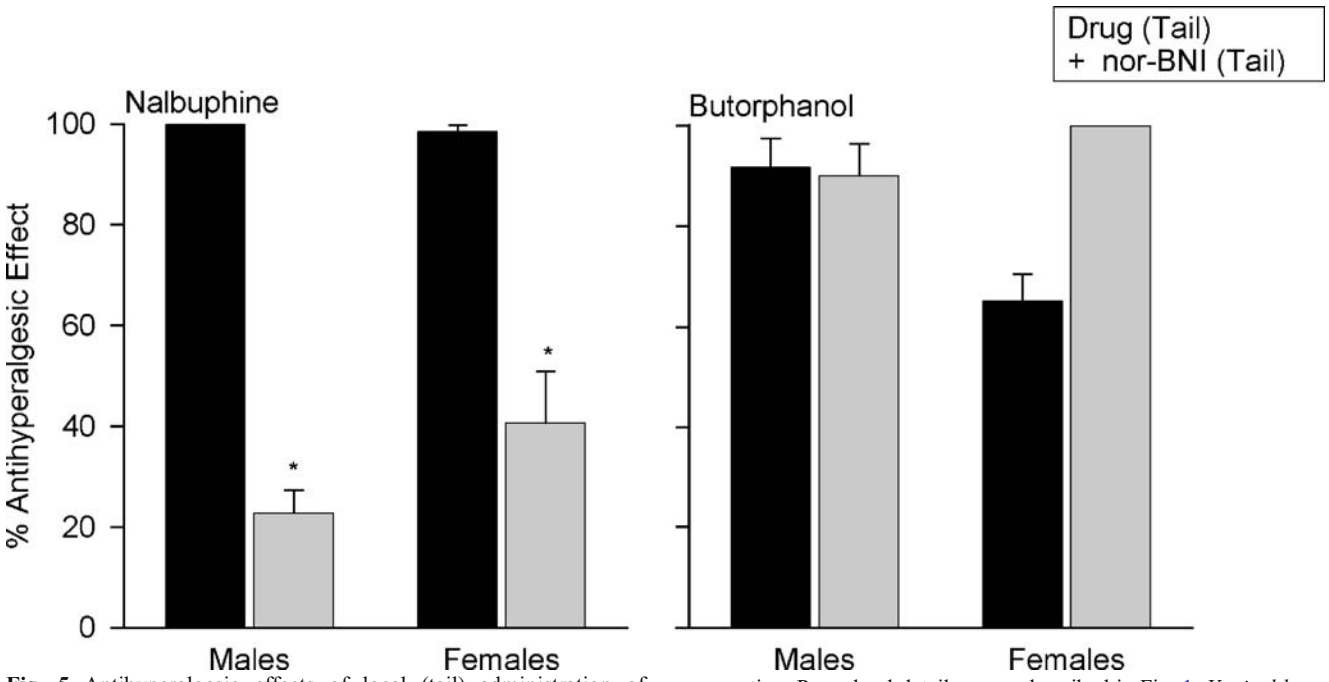

Fig. 5 Antihyperalgesic effects of local (tail) administration of nalbuphine (1,000 μg), butorphanol (100 μg males, 300 μg females: these doses were determined to be equally effective in males and females) alone and in combination with local (tail) administration of nor-BNI (10 μg) in male and female rats (n=6–8) in the capsaicin preparation. Procedural details are as described in Fig. 1. *Vertical bars* represent the standard error, where not indicated, the standard error fell within the bar. *Asterisks* (*) indicate a significant differences in the antihyperalgesic effect when the opioid was administered alone vs in combination with nor-BNI

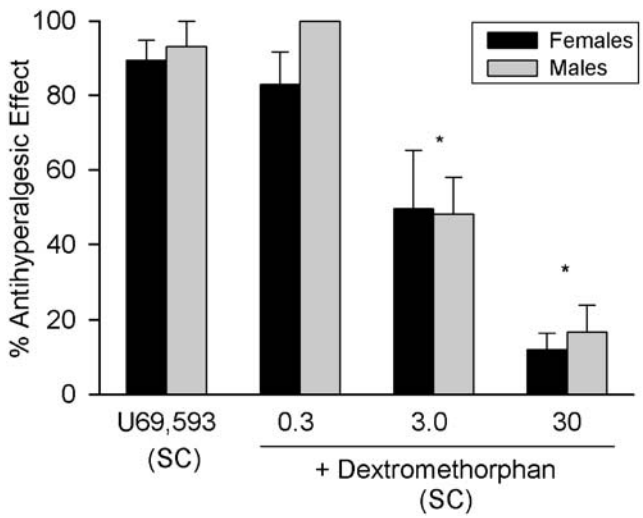

Fig. 6 Antihyperalgesic effects of systemically (s.c.) administered U69,593 (10 mg/kg) alone and in combination with systemically (s.c.) administered dextromethorphan in male and female rats (n=6–8) in the capsaicin preparation. Procedural details are as described in Fig. 1. Alone, dextromethorphan (3.0–30 mg/kg) had no antihyperalgesic effects (data not shown). *Vertical bars* represent the standard error; when not indicated, the standard error fell within the bar. *Asterisks* (*) indicate significant difference in the antihyperalgesic effects of U69,593 alone vs in combination with dextromethorphan

Discussion

Previous studies indicate that κ opioids generally produce greater antinociception in males compared to females, and this effect has been reported in rats, mice, and monkeys (Craft 2003). Unfortunately, the available animal studies have almost exclusively utilized acute nociceptive models, and thus little is known about sex differences in the effectiveness of κ opioids in persistent pain models. Consequently, one purpose of the present investigation was to examine the antihyperalgesic effects of κ opioids after administration of capsaicin, which produces a short-term (30–90 min) hyperalgesic response to mildly noxious thermal stimuli. Across the range of doses tested, spiradoline and U50,488 were more potent in males than females, and this effect was observed after both systemic and local administration at the site of inflammation. U69,593 was also more potent in males, although this effect was observed only after local administration. To date, only two published studies have examined sex differences in κ opioids using persistent pain models. Clemente et al. (2004) reported that local administration of U50,488 was more effective at reducing formalin-induced hyperalgesia in female rats, with the magnitude of this effect being greater during diestrous than proestrous. Similarly, Binder et al. (2000) reported that the peripherally active κ-opioid asimadoline was more effective in female rats at reducing Freunds-induced hyperalgesia when tested against a ther-

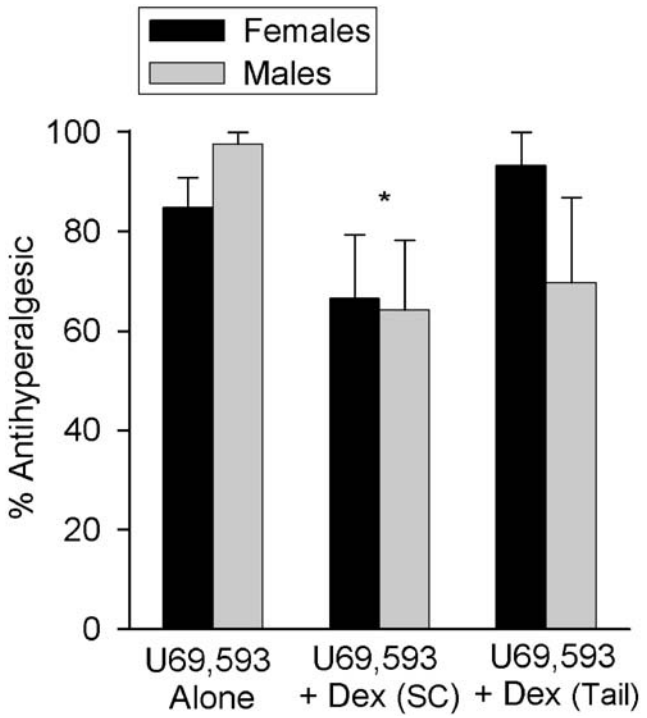

Fig. 7 Antihyperalgesic effects of U69,593 (300 μg) administered locally (tail), and in combination with dextromethorphan (30 mg/kg) administered systemically (s.c.) and locally (tail) in the capsaicin preparation in male and female rats (n=6–8). Procedural details are as described in Fig. 1. *Vertical bars* represent the standard error; when not indicated, the standard error fell within the bar. *Asterisks* (*) indicate significant difference in the antihyperalgesic effects of local administration of U69,593 alone vs in combination with dextromethorphan administered systemically

mal stimulus. Sex differences were not observed in this study when asimadoline was tested against a mechanical stimulus or with PNU50488H. Given that distinct mechanisms contribute to the persistent hyperalgesia induced by injections of formalin, Freunds and capsaicin (McCall et al. 1996; Holzer 1991; Le Bars et al. 2001), identifying the sexually dimorphic action of κ opioids in these persistent pain models will require extensive investigation. However, the present study provides preliminary evidence to suggest marked sex differences in responsiveness to κ opioids and that the direction of these sex differences may be dependent upon the mechanisms underlying the different types of persistent nociception.

The present finding that spiradoline, U50,488 and U69,593 were on average 147, 9.9- and 57-fold more potent when administered locally than systemically, respectively, suggests that in both males and females, the antihyperalgesia induced by local administration of κ opioids was mediated predominantly by activity in the site of inflammation (Ko et al. 1999). Local κ opioid activity was confirmed by the demonstration that in males and females local, but not i.c.v., administration of the κ-opioid antagonist nor-BNI attenuated the effects of local administration of both U69,593 and spiradoline. These data suggest that when administered locally, U69,593 and spiradoline attenuated capsaicin-induced hyperalgesia actions via activity at peripheral κ opioid receptors. These findings extend previous studies by demonstrating sex differences in κ opioid antihyperalgesia mediated by opioid activity at the site of inflammation.

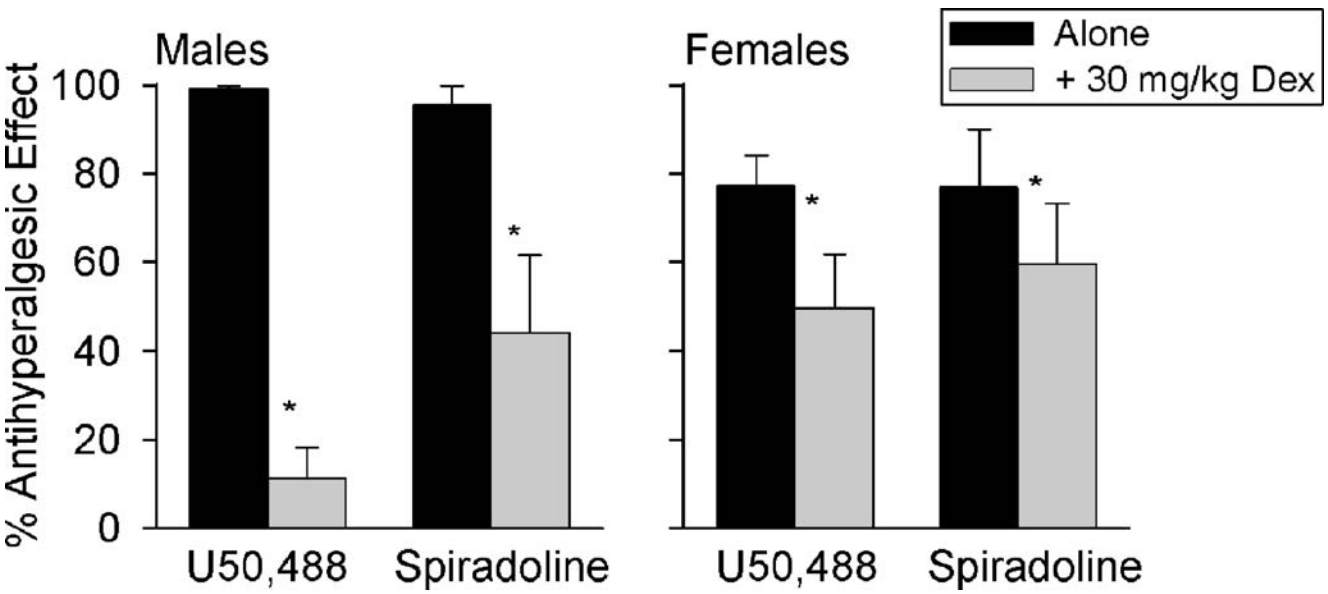

Fig. 8 Antihyperalgesic effects of spiradoline (17.5 mg/kg) and U50,488 (10 mg/kg, males; 17.5 mg/kg, females: these doses were determined to be equally effective in males and females) alone and in combination with dextromethorphan (30 mg/kg) in male and female rats (n=6–8) in the capsaicin preparation. Procedural details are as described in Fig. 1. All drugs were administered systemically (s.c.). *Vertical bars* represent the standard error; where not indicated, the standard error fell within the bar. *Asterisks* (*) indicate significant difference in the antihyperalgesic effects of opioid alone vs in combination with dextromethorphan

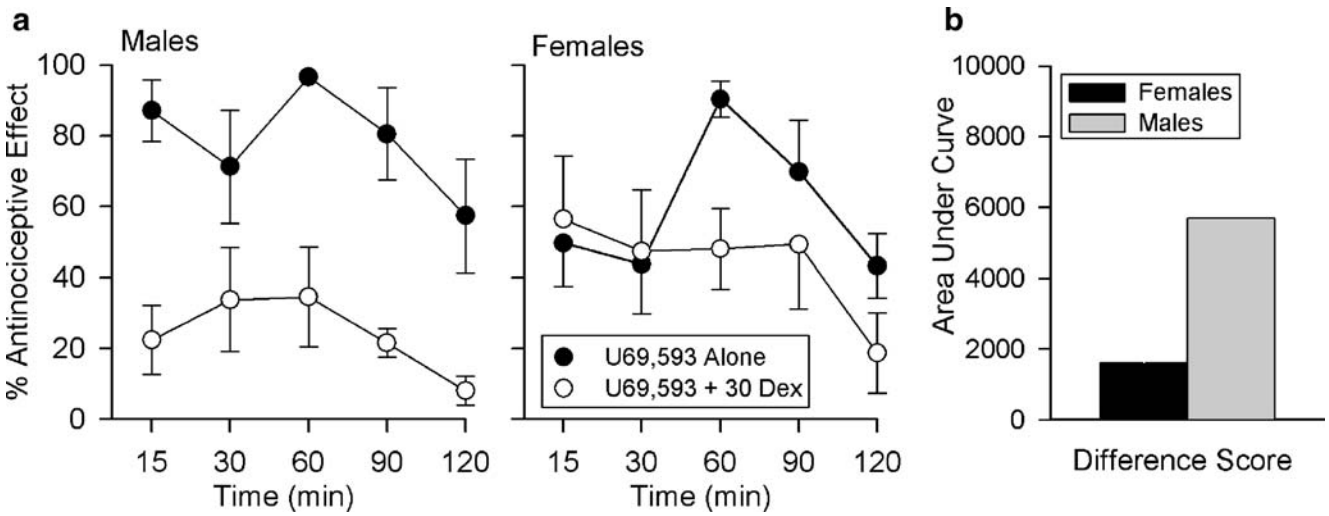

Fig. 9 **a** Antinociceptive effects of U69,593 (3.0 mg/kg males; 5.6 mg/kg females: these doses were determined to be equally effective in males and females) alone and in combination with dextromethorphan (30 mg/kg) when administered systemically (s.c.) in both male and female rats (n=6–8) in the warm water tail-withdrawal procedure. *Vertical bars* represent the standard error; where not indicated, the standard error fell within the data point. **b** Area under the curve (AUC) difference score for systemic administration of U69,593 in combination with dextromethorphan. Data represent the difference in AUC for U69,593 alone—AUC for U69,593 in combination with dextromethorphan in both males and females. *Asterisks* (*) indicate a significant sex difference in AUC difference score

Several possible mechanisms have been demonstrated to account for the greater effectiveness of local administration of opioids in persistent pain models. For example, inflammation is associated with an increased expression of opioid receptors in the dorsal root ganglia and upregulation of opioid receptors in small primary afferent neurons (Ji et al. 1995; Zollner et al. 2003). Inflammatory conditions can also disrupt the perineurial barrier, allowing for greater access of peripherally administered opioids to the opioid receptor population (Antonijevic et al. 1995). Although the present investigation did not directly compare these processes in males and females, the observation that κ opioids were effective when administered locally suggests that these peripheral processes are active in both males and females. Such findings are consistent with previous reports indicating that similar opioid/pain circuitry exists in males and females, but the characteristics of this circuitry can be altered by the gonadal hormones (e.g., Kavaliers and Galea 1995; Sternberg et al. 2004).

As with U69,593 and spiradoline, sex differences were observed with U50,488. In contrast to these κ opioids, local administration of nor-BNI failed to attenuate the effects of U50,488. It is unlikely that the effects produced by local injections of U50,488 were mediated by activity at the μ receptor, as in this preparation, μ opioids are slightly more potent in females (Barrett et al. 2003). Although considered a selective κ agonist, U50,488 can produce antinociception by activating nonopioid receptor sites. For example, the antinociception produced by local injections of the (+)-enantiomer of U50,488 as well as high doses of the (−)-enantiomer are not reversed by nor-BNI (Joshi and Gebhart 2003). This antihyperalgesic effect is believed to be mediated by a direct blockade of sodium channels and not activation of κ opioid receptors. The current findings contrast with those reported previously in capsaicin preparations. In rhesus monkeys, local administration of nor-BNI antagonized the antihyperalgesic effects produced by U50,488 (Ko et al. 1999), and in rats the effects of U50,488 were reversed by doses of the opioid antagonist quadazocine considerably larger than those required to attenuate the effects of the μ agonist fentanyl (Ko et al. 2000). Although it is difficult to reconcile these findings, it does confirm that under some conditions, the local antihyperalgesic actions of U50,488 are not mediated directly by activity at the κ opioid receptor.

Recent studies suggest that sex differences in κ opioid antinociception may result from differential NMDA receptor activity. Indeed, NMDA antagonists selectively attenuate κ opioid antinociception in male and ovariectomized female mice and rats (e.g., Mogil et al. 2003; Sternberg et al. 2004; Holtman and Wala 2006). Whether these sex-dependent effects are apparent in models of persistent pain have yet to be determined. In the present investigation, the NMDA antagonist dextromethorphan attenuated the antihyperalgesic effect of U69,593, spiradoline and U50,488, and this effect was observed in both males and females. In contrast, dextromethorphan attenuated the effects of U69,593 in the 50°C warm water tail-withdrawal procedure only in males, a finding congruent with other studies using acute pain models (Kavaliers and Choleris 1997). Indeed, NMDA antagonists have been shown to selectively antagonize the effects of various κ opioids (e.g., U69,693,

U50,488) in rats and mice using the warm water tail-withdrawal and tail-flick procedures (e.g., Mogil et al. 2003; Holtman and Wala 2006). Collectively, these findings provide evidence to suggest that the NMDA receptor system plays a critical role in mediating sex differences in the effects produced by κ opioids in acute but not persistent pain models.

Direct comparisons between the effects produced by κ opioids in acute and persistent pain models should be interpreted with caution. Hyperalgesia associated with persistent nociception can be distinguished from acute nociception in both the transmission of nociceptive information, presence vs absence of inflammation, type of nociceptive response, as well as the underlying neurochemical substrates (Le Bars et al. 2001; McCall et al. 1996). Even though in the present investigation the same type of nociceptive response (i.e., tail withdrawal from warm water) was utilized with capsaicin in the 50°C warm water tail-withdrawal procedure, there were still numerous procedural and parametric differences between these procedures. Moreover, in these procedures, different doses of U69,593 were required to obtain maximal effects.

With models of inflammation, like capsaicin, opioids can produce their effects via both local at the site of inflammation and central sites, whereas in acute models of pain, the activity of opioids appears to be restricted to central sites (Yaksh 1997; Le Bars et al. 2001). As such, it was possible that the interaction between the NMDA and opioid systems could be apparent at both the central and local level. In the present investigation, systemic administration of dextromethorphan produced minimal attenuation of the antihyperalgesia induced by local administration on U69,593, which contrasts with the almost complete reversal observed after systemic administration of U69,593. A relatively high dose of dextromethorphan administered locally also failed to attenuate the antihyperalgesic effects of local administration of U69,593. These findings suggest that in some pain models, attenuation of κ opioid antihyperalgesia can only be obtained when both the NMDA antagonist and κ opioid are acting centrally.

An additional purpose of the present investigation was to examine the effects of mixed-action opioids with relatively low efficacy at κ receptors (e.g., Dykstra 1990; Butelman et al. 1998). Both butorphanol and nalbuphine were more potent when administered locally at the site of inflammation than systemically. The antihyperalgesia produced by nalbuphine was reversed by local administration of nor-BNI, establishing the contribution of κ opioid receptors at the site of inflammation. In contrast, the finding that nor-BNI failed to antagonize the antihyperalgesia produced by butorphanol suggests receptors other than κ receptors are involved in its antihyperalgesic effects. Despite the differential action of butorphanol and nalbuphine, sex differences were not observed in their antihyperalgesic effects. To some extent, these findings contrast with previous studies indicating that sex differences with butorphanol and nalbuphine are typically larger that those observed with more efficacious opioids in both acute and persistent pain models (Cook et al. 2000; Craft and Bernal 2001; Cook and Nickerson 2005). Although the effects of selective κ opioids have not been examined in humans, some studies do suggest that mixed-action opioids with κ opioids activity have greater antihyperalgesic effects in females (Craft 2003). The discrepancies across these studies may be a consequence of the type of pain model employed, suggesting further that the relative potency of opioids in males and females is specific to certain inflammatory pain models. The factors that make these models different may be critical in understanding sex differences in opioid antinociception.

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