Sex differences in NMDA antagonist enhancement of morphine antihyperalgesia in a capsaicin model of persistent pain: Comparisons to two models of acute pain

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Abstract

In acute pain models, N-methyl-D-aspartate (NMDA) antagonists enhance the antinociceptive effects of morphine to a greater extent in males than females. The purpose of this investigation was to extend these findings to a persistent pain model which could be distinguished from acute pain models on the basis of the nociceptive fibers activated, neurochemical substrates, and duration of the nociceptive stimulus. To this end, persistent hyperalgesia was induced by administration of capsaicin in the tail of gonadally intact F344 rats, following which the tail was immersed in a mildly noxious thermal stimulus, and tail-withdrawal latencies measured. For comparison, tests were conducted in two acute pain models, the hotplate and warm water tail-withdrawal procedures. In males, the non-competitive NMDA antagonist dextromethorphan enhanced the antihyperalgesic effect of low to moderate doses of morphine in a dose-and time-dependent manner. Across the doses and pretreatment times examined, enhancement was not observed in females. Enhancement of morphine antinociception by dextromethorphan was seen in both males and females in the acute pain models, with the magnitude of this effect being greater in males. These findings demonstrate a sexually-dimorphic interaction between NMDA antagonists and morphine in a persistent pain model that can be distinguished from those observed in acute pain models.
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1. Introduction

Sex differences in opioid antinociception have been studied extensively in acute pain models (e.g., hotplate), with the majority of reports indicating that male rodents and monkeys are more sensitive to the antinociceptive effects of μ opioids than their female counterparts (Negus and Mello, 1999; Cook et al., 2000; Craft and Bernal, 2001). Several recent lines of physiological, pharmacological and behavioral evidence suggest that this sexual dimorphism may extend to the manner in which NMDA antagonists modulate the actions of μ opioids (D'Souza et al., 1999, 2002; Bryant et al., 2006). Of particular clinical relevance is the finding that NMDA antagonists produce a sexually-dimorphic effect on acute μ opioid antinociception. Indeed, Grisel et al. (2005) reported that dextromethorphan enhanced morphine antinociception in male and ovarectomized female mice, but attenuated morphine antinociception in intact females. Similarly, Craft and Lee (2005) reported that dextromethorphan enhanced morphine antinociception to a greater extent in male rats, with this effect being most evident in a hotplate procedure. Not all reports indicate that NMDA antagonists produce sexually-dimorphic effects on morphine antinociception (e.g., Bryant et al., 2006; Holtman et al., 2003), with these discrepancies most likely explained by differences across studies in type of NMDA antagonist, dose of NMDA antagonist, procedure, and rodent genotype. The dose of morphine tested is also critical, as some NMDA antagonists enhance low levels of antinociception produced by morphine and attenuate high levels (Nemmani et al., 2004; Craft and Lee, 2005).

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In contrast to these findings in acute pain models, there are no investigations evaluating sex differences in NMDA–opioid interactions using persistent pain models. Nociception induced by persistent and acute nociception differ along a number of critical dimensions, including their neurochemical and anatomical substrates, duration, frequency, and type of nociceptive response, as well as the type of nociceptive fibers they activate (LaMotte et al., 1992; McCall et al., 1996; Le Bars et al., 2001; Kayser et al 2007). The distinct mechanisms underlying acute vs persistent pain have been shown to be critical determinants of sex differences in both opioid sensitivity and NMDA–opioid interactions. Indeed, recent studies suggest that the sex differences in μ opioid antinociception apparent in acute pain models may not be apparent in all persistent pain models (Barrett et al., 2003; Cook and Nickerson, 2005). Moreover, NMDA antagonists have been shown to selectively block the antinociceptive effects κ opioids in males when examined in various models of acute pain, but not in models of persistent pain (Mogil et al., 2003; Holtman and Wala, 2006; Lomas et al., 2007). Clearly, while acute nociceptive assays are a beneficial starting point, it is essential to determine if the sexually-dimorphic manner in which NMDA antagonists modulate the effects opioids is apparent in persistent pain models.

The purpose of the present study was to examine sex differences in the extent to which the NMDA antagonist dextromethorphan alters the antihyperalgesic effects of morphine in a model of persistent pain. In the model selected for study, the chemical irritant capsaicin was administered directly into the tail resulting in inflammation, vasodilatation and a hyperalgesic response localized at the site of administration (Caterina et al., 1997; Holzer, 1991; Winter et al., 1995). The hyperalgesia induced by capsaicin was then measured by a decrease in the latency to tail-withdrawal from an acute presentation of a mildly noxious thermal stimulus (45 °C water), and in both males and females this hyperalgesia persists for 60–90 min (Barrett et al., 2003). Unlike some models used to examine persistent pain (e.g., formalin-induced hyperalgesia), in the capsaicin procedure NMDA antagonists do not alter the development or persistence of the hyperalgesic response (Sakurada et al., 1998; Lomas et al., 2007). As such, this model provides a unique opportunity to examine sex differences in NMDA–opioid interactions against a persistent form of nociception. In order to facilitate comparisons across studies, as well as provide a direct comparison across acute and persistent pain models using the same drugs, doses and rodent strain, tests were also conducted using a hotplate (52 °C) and warm water tail-withdrawal (52 °C) procedure.

2. Materials and methods

2.1. Animals

Intact male and female F344 rats were obtained from Charles River Laboratories (Raleigh, NC, USA). 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. Animals used in this study were cared for in accordance with the guidelines of the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill and the "Guide for the Care of and Use of Laboratory Animals" (Institute of Laboratory Animal Resources, National Academy Press, 1996).

2.2. Testing

2.2.1. Capsaicin

A warm water tail-withdrawal procedure was used to assess hyperalgesia induced by an injection of capsaicin in the tail. During testing, each rat was lightly restrained, with the distal 7 cm of the tail immersed in water maintained at 45 °C, a relatively innocuous nociceptive stimulus (Lynn and Carpenter, 1982). Baseline tail-withdrawal latencies were determined before administration of any drugs, and rats that failed to maintain their tails in the water for 15 s were excluded from subsequent testing, this occurred in 2 animals during testing for this study. Following determination of baseline latencies, 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 an average of 3.5–4.5 s with this effect being comparable in both males and females.

For tests of the antihyperalgesic effects of morphine and dextromethorphan, 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, a capsaicin baseline was assessed in each rat. Approximately 1 week later, capsaicin was administered in the tail, followed by an intraperitoneal (i.p.) injection of saline, morphine or dextromethorphan alone or in selected combinations. Dextromethorphan was chosen as it is used clinically and has previously been shown to enhance morphine antinociception in a number of acute nociceptive procedures (Grisel et al., 2005; Plesan et al., 1995). Although a number of pretreatment times and doses were examined for both dextromethorphan and morphine, tail-withdrawal latencies were always determined 15 min after the capsaicin tail injection, which is the time point corresponding to the peak effect of capsaicin. 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). Rats were tested approximately once per week with no more than 5 tests per animal, with each group having 6–8 animals. 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).

2.2.2. Warm water tail-withdrawal and hotplate procedures

Prior to testing, rats were habituated to restraint tubes, baseline tests were conducted in both the warm water tail-withdrawal and hotplate procedures, and each rat was administered a 2.5 mg/kg dose of morphine (no data were collected for this test). This habituation protocol was designed, in part, to limit the impact of stress-induced antinociception

which has previously been reported to be influenced by sex but minimized by habituation (Mogil and Belknap, 1997; Dhabhar et al., 1997).

During both habituation and testing in the warm water tail-withdrawal procedure, rats were placed in restraint tubes, the distal 7 cm of the tail immersed in 52 °C water, and the latency to tail-withdrawal recorded. An increase in the latency to remove the tail from the warm water was taken as a measure of nociception, with an upper cutoff limit of 15 s to minimize tissue damage. In this procedure, baseline tail-withdrawal procedures ranged from 7–10 s in males and 6.5–9 s in females. After a baseline tail-withdrawal latency was determined, rats were placed on the hotplate which was set at 52 °C. Latency to hind-paw lick or an escape response was then determined, with an upper cutoff time of 60 s. In this procedure, baseline to hind-paw lick or an escape latencies ranged from 20–35 s in males and 17–35 s in females. In each series of tests, two baseline latencies were determined prior to drug administration. Subsequently, rats were administered an i.p. injection of saline, morphine and dextromethorphan alone or in selected combinations. Data for both the warm water tail-withdrawal and hotplate procedures were collected across a 2 h interval. No rat was exposed to the testing procedures more than 6 times and at least 6 days separated each test.

2.3. Data analysis

2.3.1. Capsaicin

For the antihyperalgesic effects of drugs, latencies to tail-withdrawal following administration of drug were converted to the percentage of the maximum possible effect using the following equation: % antihyperalgesic effect = [(observed-baseline)/(15 s-baseline)] × 100. An ANOVA analysis was then used to determine differences across dose/drug combinations and sex. In instances in which there was a main effect for drug condition and sex, post-hoc tests were conducted using the Fisher's protected least significant difference test to compare dose combinations within sex. For statistical analyses, the alpha level was set at 0.05. The dose of morphine 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.

2.3.2. Warm water tail-withdrawal and hotplate procedures

In order to calculate % maximal antinociceptive effects, tail-withdrawal latencies (warm water) and lick/escape latencies (hotplate) were converted to percent antinociceptive effect using the following equation: % antinociceptive effect = [(test latency-baseline)/(cutoff-baseline)] × 100. The time-course evaluation of a drug effects was measured in these procedures and thus area under the curve was used for statistical analysis. Area under the curve was estimated by the Trapezoidal Rule using available statistical software (Tallarida and Murray 1987®). An ANOVA analysis was then used to determine differences across doses and sexes. Post hoc tests and ED$_{50}$ values were calculated in a manner similar to that

described above. For statistical analyses, the alpha level was set at 0.05.

2.4. Drugs

The following drugs were used: morphine sulfate (provided by the National Institute on Drug Abuse), dextromethorphan hydrobromide monohydrate, and capsaicin (both purchased from Sigma-Aldrich Co., 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. Capsaicin was injected alone in the tail in a 0.1-ml volume. Saline, morphine and dextromethorphan were administered i.p. in a volume of 0.5 to 1.0 ml/kg. Small amounts of lactic acid were added to dextromethorphan to promote solubility. As dextromethorphan occasionally caused lesions and severe adverse effects in both male and female rats at the 30 mg/kg dose, testing at this dose was limited.

3. Results

Fig. 1 shows that in males and females morphine produced dose-dependent increases in antihyperalgesia, with near maximal effects obtained at the highest dose tested. ANOVA indicated a main effect for dose ($F_{4,64}$=26.9, P<0.05), no main effect for sex, or a dose × sex interaction. ED$_{50}$ values for morphine in females 6.22 mg/kg (95% CL: 3.95–9.79) and males 4.45 mg/kg (95% CL: 2.57–7.86) were also similar. Across the dose range examined, dextromethorphan produced only minimal levels of antihyperalgesia in both males and females, with no dose tested producing greater than an 11% effect. The higher doses of dextromethorphan did, however, produce sedation and disruption of motor performance,
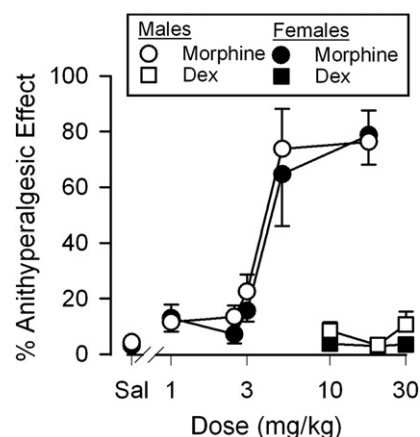

Fig. 1. Antihyperalgesic effects of morphine and dextromethorphan administered systemically in male and female rats (n=6–8). A warm water tail-withdrawal procedure was used for testing in which the distal 7 cm of the tail was immersed in water maintained at 45 °C. Equally effective doses of capsaicin were injected 3.5 cm from the tip of the tail 15 min prior to the test with all drugs administered at the same time as capsaicin. Vertical bars represent the standard error; where not indicated, the standard error fell within the data point. Asterisks (*) indicate significant difference in the antihyperalgesic effects of morphine alone vs in combination with selected doses of dextromethorphan.
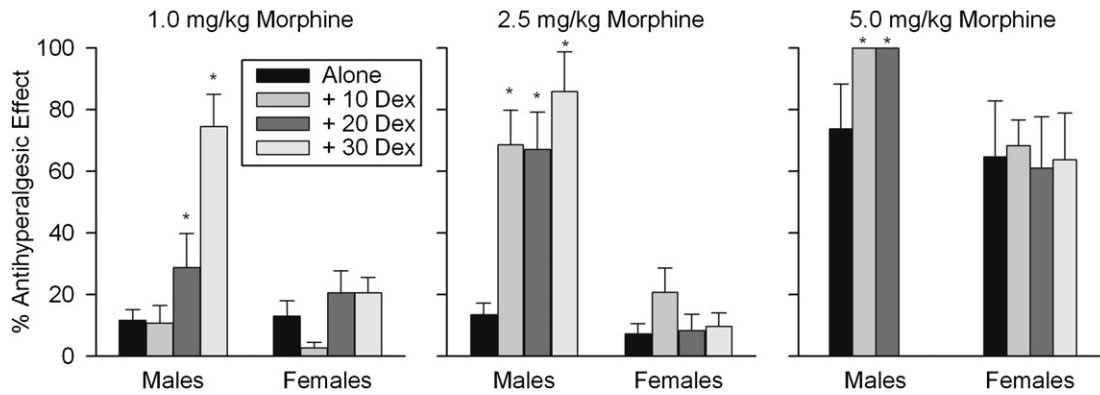
Fig. 2. Antihyperalgesic effects of morphine alone and in combination with dextromethorphan in male and female rats ($n=6$–8) in the capsaicin-induced hyperalgesia procedure. Procedural details are as described in Fig. 1. Vertical bars represent the standard error; when not indicated, the standard error fell within the bar. Asterisks (∗) indicate significant difference in the antihyperalgesic effects of morphine alone vs in combination with selected doses of dextromethorphan.

although these effects were typically short lived and did not interfere with testing.

Fig. 2 shows that in males dextromethorphan enhanced the antihyperalgesic effect of doses of morphine that produced minimal (1.0 and 2.5 mg/kg) to moderate (5.0 mg/kg) antihyperalgesic effects when administered alone. At the two lowest doses of morphine tested, 20 and 30 mg/kg dextromethorphan increased the antihyperalgesic effect of morphine from 11% to 74%, and from 13% to 86%, respectively. At the highest dose of morphine tested, 10 mg/kg dextromethorphan increased levels of antihyperalgesia from 73% to 100%. In females, no dose of dextromethorphan altered the antihyperalgesic effects produced by morphine. For 1.0 and 2.5 mg/kg morphine, ANOVA confirmed a main effect for sex (1.0: $F_{1,48}=12.5$, $P<0.05$; 2.5: $F_{1,41}=64.6$, $P<0.05$), drug (1.0: $F_{3,48}=13.9$, $P<0.05$; 2.5: $F_{3,41}=8.69$, $P<0.05$), and a sex × drug interaction (1.0: $F_{3,48}=6.4$, $P<0.05$; 2.5: $F_{3,41}=6.45$,

$P<0.05$). At 5.0 mg/kg morphine, ANOVA indicated a main effect for sex, ($F_{1,32}=7.57$, $P<0.05$), but no main effect for drug and no sex × drug interaction. Post hoc tests confirmed that, with the exception of 1.0 mg/kg morphine/10 mg/kg dextromethorphan combination, in males all dose combinations enhanced ($P<0.05$) the antihyperalgesic effect of morphine. In contrast, enhancement of the antihyperalgesic effects of morphine was not obtained in females regardless of the dose combination tested.

In order to determine if these sex-specific effects of dextromethorphan were a consequence of a sexual dimorphism in the time course of morphine or dextromethorphan actions, tests were conducted in which selected doses of these drugs were administered at different pretreatment times. Fig. 3 shows that in males dextromethorphan enhanced the antihyperalgesic effect of morphine at all pretreatment times tested. In females, varying the pretreatment time of dextromethorphan failed to alter the antihyperalgesic effectiveness of morphine. ANOVA
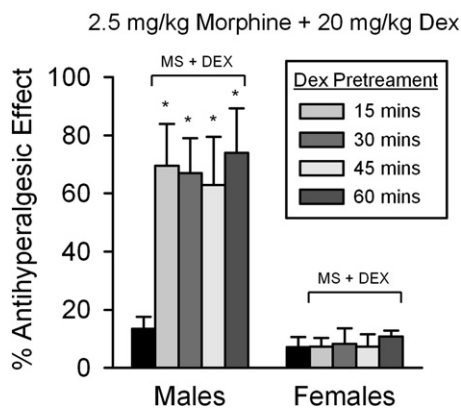

Fig. 3. Antihyperalgesic effects of morphine alone and in combination with a dextromethorphan in male and female rats ($n=6$–8) in the capsaicin-induced hyperalgesia procedure. The dose combination of morphine and dextromethorphan were selected as they produced high levels of antihyperalgesia in males (see Fig. 2). Dextromethorphan was administered at various pretreatment times before testing, while morphine was always administered 30 min before testing. Additional 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 morphine alone vs in combination with dextromethorphan.
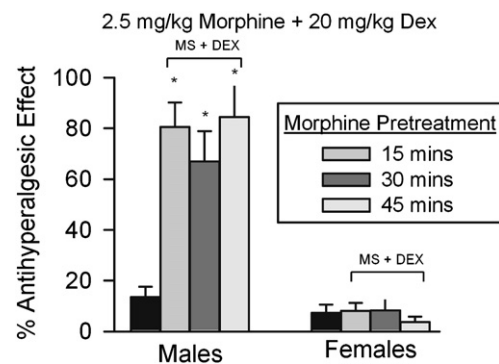

Fig. 4. Antihyperalgesic effects of morphine alone and in combination with dextromethorphan in male and female rats ($n=6$–8) in the capsaicin-induced hyperalgesia procedure. The dose combination of morphine and dextromethorphan were selected as they produced high levels of antihyperalgesia in males (see Fig. 2). Morphine was administered at various pretreatment times before testing, while dextromethorphan was always administered 30 min before testing. Additional 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 morphine alone vs in combination with dextromethorphan.
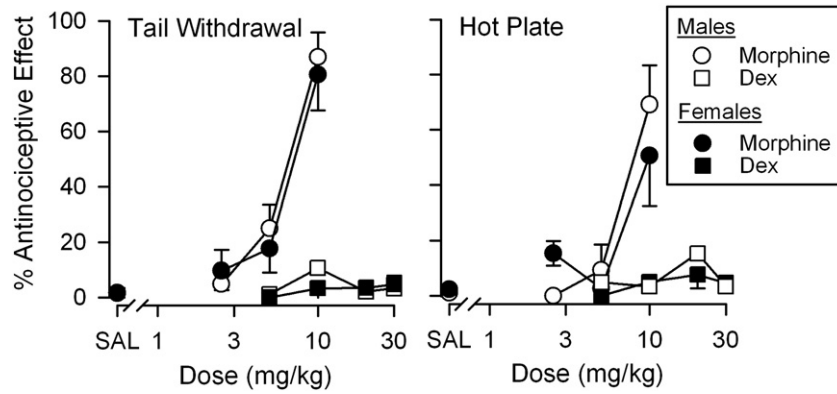
Fig. 5. Antinociceptive effects of morphine and dextromethorphan administered systemically in male and female rats ($n=6$–8) in a warm water tail-withdrawal procedure (left panel) and a hotplate procedure (right panel). All data reflect the effects of these drugs at a 30 min pre-session injection time. In the warm water tail-withdrawal procedure, the distal 7 cm of the tail was immersed in water maintained at 52 °C and latency to withdrawal the tail from warm water was recorded. In the hotplate procedure rats were placed on the 52 °C hotplate and latency to hind-paw withdrawal/escape was recorded. Control data (SAL) indicate the effects of saline administration. Vertical bars represent the standard error; where not indicated, the standard error fell within the data point.

confirmed a main effect for sex ($F_{1,48}=97.3$, $P<0.05$), pretreatment time ($F_{3,48}=9.106$, $P<0.05$), and a sex × pretreatment time interaction ($F_{4,48}=9.75$, $P<0.05$). Post hoc tests also confirmed enhancement ($P<0.05$) of the antihyperalgesic effect of morphine at all dextromethorphan pretreatment times in males, but at no pretreatment time in females.

Fig. 4 shows the antihyperalgesic effect of morphine when administered at various pretreatment times before dextromethorphan. For males, all pretreatment times produced an enhancement of morphine antihyperalgesia, whereas in females enhancement was not observed at any pretreatment time. ANOVA confirmed a main effect for sex ($F_{1,66}=55.6$, $P<0.05$),

Fig. 6. Time-course assessment of the antinociceptive effects of selected doses of morphine administered alone (dotted lines) and in combination with dextromethorphan in female and male rats ($n=6$–8) in a warm water tail-withdrawal (52 °C) procedure. Vertical bars represent the standard error; where not indicated, the standard error fell within the data point. Data in the right most panels reflect area under the curve (AUC) analyses for administration of morphine alone and in combination with dextromethorphan over 2 h time course. Asterisks (*) indicate a significant sex difference in AUC.
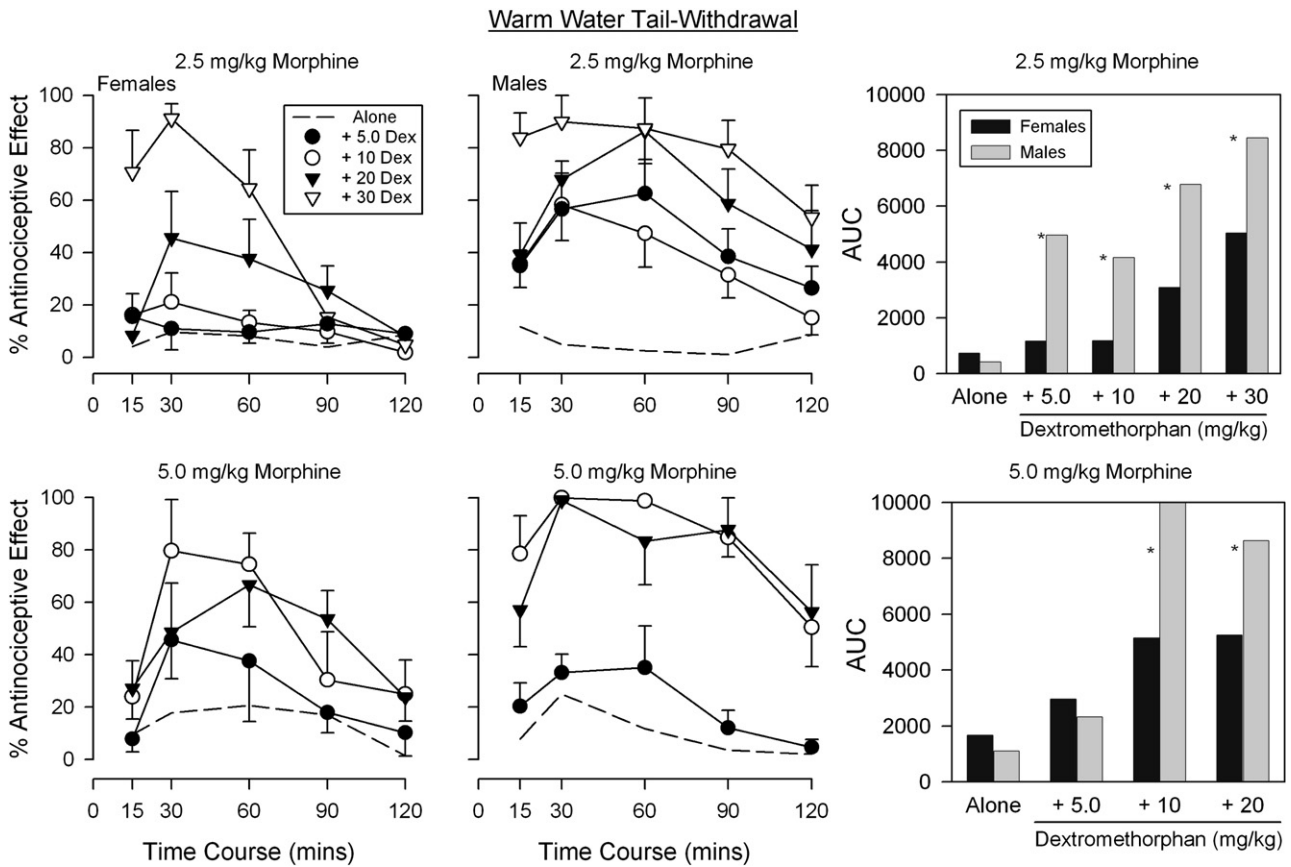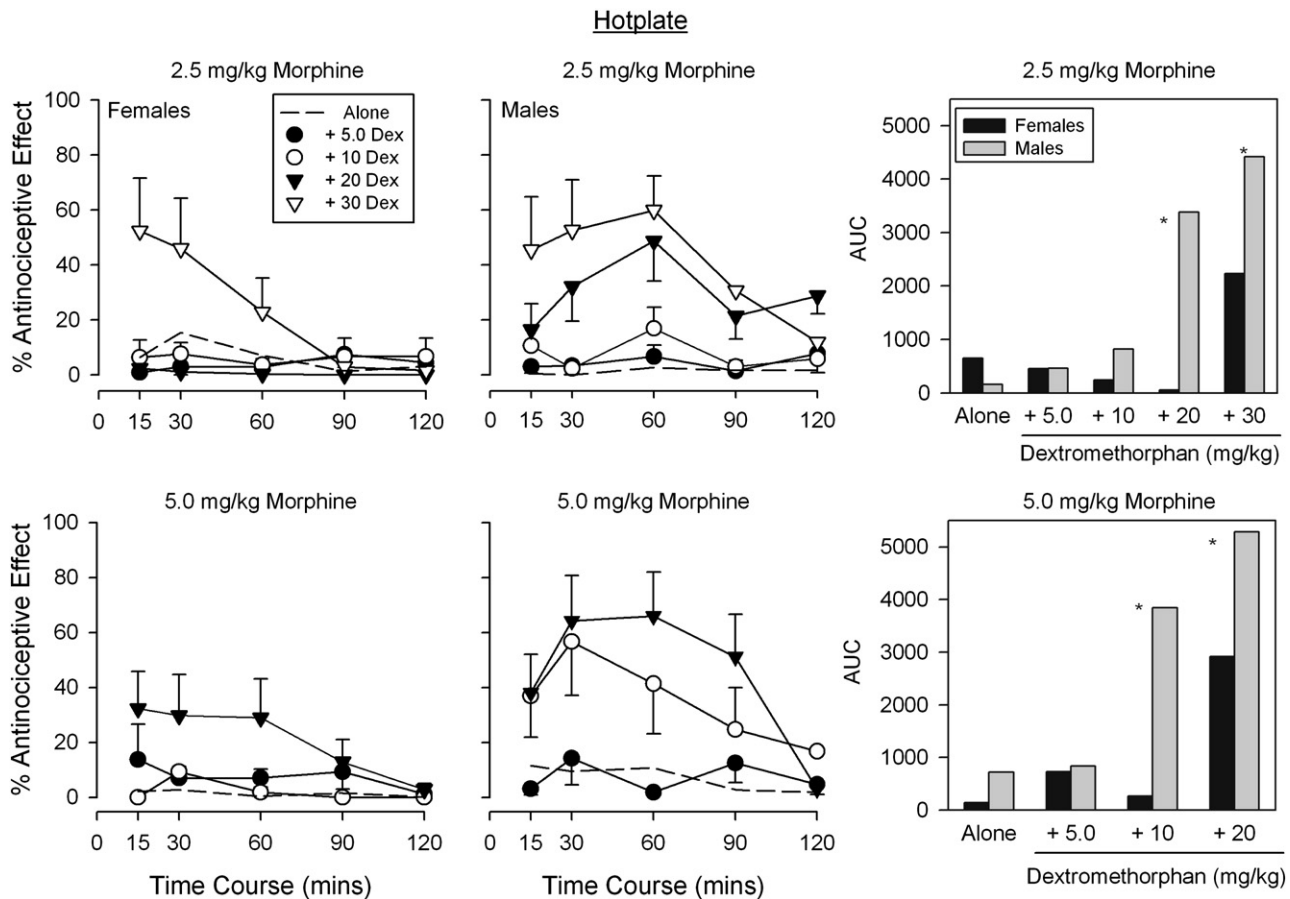
Hotplate

Fig. 7. Time-course assessment of the antinociceptive effects of selected doses of morphine administered alone (dotted lines) and in combination with dextromethorphan in female and male rats ($n = 6$–8) in a hotplate (52 °C) procedure. Vertical bars represent the standard error; where not indicated, the standard error fell within the data point. Data in the right most panels reflect area under the curve (AUC) analyses for administration of morphine alone and in combination with dextromethorphan over 2 h time course. Asterisks (*) indicate a significant sex difference in AUC.

pretreatment time ($F_{4,66} = 2.8$, $P < 0.05$), and a sex × pretreatment time interaction ($F_{4,66} = 9.7$, $P < 0.05$). Post hoc tests confirmed an enhancement ($P < 0.05$) of the antihyperalgesic effect of morphine at all dextromethorphan pretreatment times in males, whereas in females enhancement was not observed at any pretreatment time.

Fig. 5 shows the effects of morphine and dextromethorphan alone in males and females in two acute pain models, the warm water tail-withdrawal and hotplate procedures. Across the dose range examined, dextromethorphan failed to produce an antinociceptive effect. Alone, morphine produced dose-dependent increases in antinociception in both males and females. Based on morphine ED_{50} values in females (6.47 mg/kg, 95% CL: 4.87–8.60) and males (6.03 mg/kg, 95% CL: 5.08–7.17) there were no sex differences in the potency of morphine in the warm water tail-withdrawal procedure. Time-course analyses of morphine (2.5–10 mg/kg morphine) in males and females (data not shown) indicated that the peak effects of morphine were typically seen at the 30 min test interval, and thereafter the magnitude of morphine antinociception gradually decreased, returning to baseline levels between 90 and 120 min. ANOVA of these time-course data indicated no main effect for sex, a main effect for dose ($F_{3,43} = 41.2$, $P < 0.05$) with no dose × sex

interaction. Similarly, in the hotplate procedure sex differences were not observed in the potency of morphine, as comparable ED_{50} values were obtained in females (12.36 mg/kg, 95% CL: 5.69–26.9) and males (8.06 mg/kg, 95% CL: 5.74–11.3). Peak effect and the duration of morphine (2.5–10 mg/kg) antinociception in this procedure were similar to that observed in the warm water tail-withdrawal procedure (data not shown). ANOVA of these time-course data indicated no main effect for sex, a main effect for dose ($F_{3,44} = 13.5$, $P < 0.05$) with no dose × sex interaction.

Fig. 6 shows the antinociceptive effect of selected doses of morphine when combined with dextromethorphan in the warm water tail-withdrawal procedure. Alone, in both males and females 2.5 and 5.0 mg/kg morphine produced only low levels of antinociception (less than 24%). When administered in combination, dextromethorphan produced a dose-and time-dependent enhancement of morphine antinociception, with this effect observed in both males and females. At the higher doses of dextromethorphan, morphine produced near maximal levels of antinociception with a peak effect and duration of action that was consistently larger in males. When enhancement was observed in males, low to moderate levels of antinociception were generally apparent even at the 120 min test interval. In

contrast, in females antinociception was not typically observed beyond the 60 or 90 min test intervals. ANOVA of area under the curve data for 2.5 and 5.0 mg/kg morphine indicated a main effect for sex (2.5: $F_{1,65} = 39.4$, $P < 0.05$; 5.0: $F_{1,49} = 6.78$, $P < 0.05$), drug (2.5: $F_{4,65} = 23.3$, $P < 0.05$; 5.0: $F_{3,49} = 22.3$, $P < 0.05$), and a sex × drug interaction (2.5: $F_{4,65} = 3.13$, $P < 0.05$; 5.0: $F_{3,49} = 4.12$, $P < 0.05$).

Post hoc tests conducted on AUC data revealed that in males 5.0 and 10 mg/kg dextromethorphan were the lowest doses that enhanced ($P > 0.05$) the effects produced by 2.5 and 5.0 mg/kg morphine, respectively. In females, enhancement of morphine antinociception was observed with the 20 mg/kg and 10 mg/kg dextromethorphan doses for the 2.5 mg/kg and 5.0 mg/kg morphine dose, respectively. These analyses also confirmed a significantly ($P < 0.05$) larger antinociceptive effect in males for all doses of dextromethorphan in combination with 2.5 mg/kg morphine and at the two highest doses of dextromethorphan in combination with 5.0 mg/kg morphine.

Similar effects were obtained in the hotplate procedure. Alone, both doses of morphine produced only low levels of antinociception, less than 11% in males and 15% in females. As shown in Fig. 7, the antinociceptive effects of morphine were enhanced by the two higher doses of dextromethorphan in males, and only at the highest dose in females. This effect was evident both in terms of the peak effect and duration of morphine antinociception. For both doses of morphine, ANOVA of area under the curve data indicated a main effect for sex (2.5: $F_{1,65} = 13.9$, $P < 0.05$; 5.0: $F_{1,51} = 7.45$, $P < 0.05$) and drug (2.5: $F_{4,65} = 14.8$, $P < 0.05$; 5.0: $F_{3,51} = 7.7$, $P < 0.05$). A sex × drug condition interaction was evident only at 2.5 mg/kg morphine ($F_{4,65} = 5.98$, $P < 0.05$). Post hoc tests confirmed that the two highest doses of dextromethorphan enhanced the antinociceptive effect of both 2.5 and 5.0 mg/kg morphine to a greater extent ($P < 0.05$) in males. For both doses of morphine, enhancement of morphine antinociception was observed at the two highest doses of dextromethorphan in males, and only at the highest of dextromethorphan in females.

4. Discussion

4.1. Models of acute pain

One purpose of the present investigation was to examine the effects of the non-competitive NMDA antagonist dextromethorphan on morphine antinociception in two acute pain models, the hotplate (52 °C) and warm water tail-withdrawal (52 °C) procedures. In these procedures, morphine was approximately equally potent in males and females. The lack of sex differences in opioid antinociception has similarly been reported, and is most apparent when only a limited number of doses or opioids are tested (Craft, 2003). The failure to observe sex differences in morphine antinociception in these procedures provided an opportunity to evaluate potential interactions with NMDA antagonists under conditions in which baseline levels of morphine antinociception were comparable in both males and females. Under these conditions, dextromethorphan produced a dose-and time-dependent enhancement of morphine antinoci-

ception, with this effect observed in both males and females. In a number of instances, the combination of dextromethorphan and low doses of morphine produced maximal antinociceptive effects. The magnitude of these dextromethorphan-induced enhancements of morphine antinociception, however, was consistently larger in males and observed at lower doses of dextromethorphan. Such finding are consistent with previous studies indicating that in acute pain models NMDA antagonists enhance the peak effect and duration of morphine antinociception in both rats and mice (Grisel et al 2005, Nemmani et al., 2004, Craft and Lee, 2005). These findings extend those reports to a strain of rats (F344) known to display large sex differences in μ opioid antinociception (Terner et al., 2003), and confirm findings indicating that dextromethorphan can produce relatively large increases in morphine antinociception (e.g., Plesan et al., 1999).

In the hotplate procedure, the enhancement observed in male and female rats was smaller than that observed in the warm water tail-withdrawal procedure. These findings extend previous reports of differences across acute nociceptive assays in the extent to which NMDA antagonists enhance morphine antinociception. For example, Craft and Lee (2005) reported greater enhancement in male Sprague–Dawley rats in a hotplate procedure, yet failed to observe enhancement in a warm water tail-withdrawal procedure. Although greater effects were observed in the present investigation in the warm water tail-withdrawal procedure with F344 rats, this discrepancy could reflect a rodent strain-dependency, and there is evidence that rat strain and substrain are critical determinants of sensitivity to μ opioid antinociception (Terner et al., 2003, Kest et al., 1999), sex differences in μ opioid antinociception (Terner et al., 2003, Kest et al., 1999), and the extent to which dextromethorphan enhances the antinociceptive effects of μ opioids in males (Bulka et al., 2002, Plesan et al., 1999).

4.2. Persistent pain model

The major purpose of the current investigation was to evaluate NMDA–opioid interactions in a model of persistent pain. In the procedure selected for study, administration of the chemical irritant capsaicin in the tail produces a 60–90 min hyperalgesic response to mildly noxious warm water (45 °C). In this procedure, systemically administered morphine was equally potent at reducing capsaicin-induced antihyperalgesia in males and females (Barrett et al., 2003). Dextromethorphan, which had no antihyperalgesic effect when administered alone, produced a dose-dependent enhancement of morphine antihyperalgesia in males. The higher doses of dextromethorphan tested increased morphine antihyperalgesia by 63–73%. In contrast, in females dextromethorphan failed to enhance the antihyperalgesic effect of morphine. This sexually-dimorphic effect was observed across doses of morphine that produced both low and moderate levels of morphine antihyperalgesia. Such findings markedly contrast with those reported in acute pain models, where enhancement is typically observed in both males and females (Craft and Lee, 2005; Grisel et al 2005; present investigation).

While studies suggest that sex differences in μ opioid antinociception are not due to opioid pharmacokinetics, binding affinity, receptor density or μ receptor-stimulated [^{35}S]GTPγS binding (Kepler et al., 1991; Candido et al., 1992; Cicero et al., 1996; Selley et al., 2003), some studies suggest a longer onset and shorter offset of morphine antinociception in females (Sarton et al., 2000). Moreover, some NMDA antagonists and their major metabolites have a longer plasma half-life in female rats (e.g., Ramachander et al., 1977; Shelnutt et al., 1999). Consequently, it was possible that the failure to observe enhancement in females was a consequence of the time course of the drugs examined. To evaluate this possibility, pretreatment times ranging from 15–60 min for both morphine and dextromethorphan were examined. Across the pretreatment times examined, dextromethorphan-induced enhancement of morphine antihyperalgesia was observed only in males. These findings suggest that the sexual dimorphism in NMDA–opioid interactions observed in the present investigation was not a consequence of sex differences in the pharmacokinetics of dextromethorphan or morphine, but rather reflects sex differences in the extent to which the NMDA system modulates the effects produced by μ opioids.

The mechanism underlying this sexual dimorphism in NMDA–opioid interactions and why differences are more pronounced in models of persistent than acute pain have not been determined. Previous studies have shown a sex-dependency in NMDA-κ opioid interactions that is apparent in acute but not persistent pain models (Holtman and Wala, 2006; Lomas et al., 2007). Moreover, a sex-dependency in NMDA-μ opioid interactions have been observed in stress-induced analgesia (e.g., Mogil et al 1993), the development of morphine tolerance (Bryant et al., 2006) and morphine-induced c-Fos expression (D'Souza et al., 1999, 2002). Some evidence also suggests that these sex-dependent effects may be mediated by estrogen, as estrogen can modulate NMDA receptors in various brain regions (Cyr et al., 2001).

4.3. Estrous cycle

Although the present investigation represents a preliminary investigation of NMDA–opioids interactions in a persistent pain model, a weakness in our approach was the failure to determine estrous cycle or the potential influence of gonadal hormones. Indeed, the recent finding that the level of dextromethorphan-induced enhancement of morphine antinociception in ovarectomized female mice was comparable to that of male mice suggests that gonadal hormones may play a role in mediating NMDA–opioid interactions (Grisel et al., 2005). This finding is supported by evidence that female rats in diestrous show a greater enhancement of morphine antinociception by the NMDA antagonist LY235959 than in other estrous phases (Craft and Lee, 2005). Moreover, estrous cycle has been shown to influence the development of nociception in a variety of pain models (Aloisi and Ceccarelli, 2000; Ren et al., 2000), including the capsaicin procedure (Barrett et al., 2003). Given the pervasive nature of the influence of gonadal hormones on both nociception and opioid activity, it would not be surprising

if gonadal hormones influenced NMDA–opioid interactions in persistent pain models. In the present investigation, however, it was unlikely that estrous cycle played a significant role in determining the extent to which dextromethorphan altered morphine-induced hyperalgesia. In particular, female rats consistently displayed less individual variability than in their male counterparts, an effect that would not be expected had different phases of the estrous cycle selectively influenced responsiveness to dextromethorphan and morphine.

4.4. Implication for other persistent pain models

It is well established that categorization of pain as being either acute or persistent represents an oversimplified construct. Numerous types of persistent pain have been well characterized (e.g., inflammatory, neuropathic), with each type mediated by distinct neurochemical substrates. For example, whereas activation of non-NMDA excitatory amino acid receptors modulate the development and persistence of nociception in a plantar incision model of pain (Zahn et al., 1998), NMDA receptors are involved in the hyperalgesia induced by Freund's adjuvant and formalin (e.g., Ren and Duhbar, 1993) as well as that produced by following neuropathic injuries (Mao et al., 1992). In contrast, capsaicin-induced hyperalgesia involves activation of neuorokinin receptors, but not NDMA receptors (Lao et al., 2003). As such, it is possible that the sexually-dimorphic manner in which NMDA antagonists enhance μ opioid antihyperalgesia may be specific to certain pain models, and thus to certain types of pain. While it is important to examine the effects of the combination of NMDA antagonists and morphine in multiple persistent pain models, such experiments may prove difficult as NMDA antagonists can have a direct effect on the development of nociception. The lack of NMDA involvement in the development of the hyperalgesic response in the capsaicin model made this an effective model in which to initiate study of the effects of NMDA antagonist on μ opioid antihyperalgesia.

4.5. Potential clinical relevance

While acute pain models represent a beneficial starting point to examine the effects of NMDA antagonists on μ opioid antinociception, ultimately in the clinical population these drugs will be used in conditions of persistent or chronic pain. In clinical studies, the extent to which NMDA antagonists enhance the analgesic effects of opioids has produced mixed results (e.g., Dudgeon et al., 2007). For example, in cancer pain patients titrating doses of morphine or morphine/dextromethorphan combinations, the combination therapy provided satisfactory pain control at half the morphine dose (Katz, 2000). In contrast, in patients with chronic, non-neuropathic pain (e.g., low back pain, osteoarthritis), enhancement was not observed (Galer et al., 2005). The current findings may help explain some of these discrepant findings as they demonstrate that NMDA–opioid interactions are dependent upon the type of nociception as well as the sex of the subject. Although sex has not been systematically examined in clinical studies, a recent set of case reports

indicated that the NMDA antagonist ketamine was more effective in enhancing opioid analgesia in 2 male chronic pain patients then in a female patient (Bell, 1999). Determining the mechanism by which sex and the type of persistent pain mediate NMDA–opioid interactions should shed light on the potential clinical utility of combining NMDA antagonists with μ opioids.

Acknowledgements

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