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Characterization of the antinociceptive effect of oxycodone in male and female rats

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Abstract

A number of investigators have shown that sex plays an important role in the analgesic effects of opioids. Typically, the antinociceptive responsiveness to mu opioid agonists such as morphine is greater in male than in female rats. The effect of sex on kappa opioid analgesia is less known. The present study was conducted to examine sex-related differences in responsiveness to oxycodone (putative kappa/mu opioid agonist). This information is important since oxycodone is widely used clinically for treatment of pain. The present results indicated that oxycodone had a greater antinociceptive response in female rats compared to male rats. This sex specific responsiveness to oxycodone, however, was lost with chronic administration. The greater antinociception in female rats was even more prominent with U50,488H (selective kappa agonist). Further, low (subanalgesic) doses of oxycodone and U50,488H enhanced the sensitivity to pain (hyperalgesia) to a greater extent in male than in female rats. This is in contrast to the previously shown greater hyperalgesic effect of subanalgesic doses of the mu opioid agonist, morphine, in female than in male rats. The present findings suggest that sexual dimorphism in the effect of opioids is related to the opioid receptors on which they predominately act.

Keywords: Oxycodone; U50, 488H; Morphine; Kappa agonist; Mu agonist; Sexual dimorphism; Antinociception; Hyperalgesia; Locomotion

1. Introduction

Sex-related differences in pain perception and pain inhibition have recently received a great deal of attention (see Fillingim and Gear, 2004 for review). A number of studies have determined the responsiveness to mu opioid agonists (such as morphine) in relation to sex in rodents and humans. Typically, male rats have been observed to be more sensitive to morphine than female rats (see Craft, 2003a,b; Kest et al., 2000; Miaskowski et al., 2000 for review). In contrast, the effect of sex on the antinociceptive action of kappa opioid agonists remains equivocal as only a few animal studies have compared the antinociception produced by these opioids with respect to sex. The results of these studies are conflicting (Bartok and Craft, 1997; Craft et al., 1998; Craft and Bernal, 2001; van Haaren et al., 2000; Stoffel et al., 2005; Tershner et al., 2000).

Oxycodone, a commonly used opioid, appears to exert its antinociceptive effect by action at the kappa opioid receptors in male rats (Ross and Smith, 1997; Ross et al., 2000). However, oxycodone has been shown to bind with greater selectivity to mu than kappa receptors in brain homogenates from male mice (Yoburn et al., 1995). Furthermore, there is a line of evidence that oxycodone has mu opioid agonist properties with abuse liability similar to morphine (Beardsley et al., 2004; Zacny and Gutierrez, 2003). Oxycodone has been reported to have an antinociceptive potency comparable to morphine with fewer side effects (Bruera et al., 1998; Kalso et al., 1991; Heiskanen and Kalso, 1997) and it is available in the slow release form for convenient oral dosing. Despite decades of wide clinical use of oxycodone for the treatment of acute and chronic pain, relatively little is known about its pharmacological properties. In particular, we are aware of no data regarding the sex-related differences in oxycodone antinociception and tolerance.

Therefore, the present study was conducted to determine the antinociceptive effect of oxycodone after its acute and chronic administration in male and female rats (tail-flick test). For

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comparative purposes, the antinociceptive effect of the selective kappa agonist, U50,488H was tested. We have previously found that subanalgesic doses of the mu opioid, morphine, produced hyperalgesia and that this effect was blocked by the *N*-methyl-D-aspartate (NMDA) receptor antagonist, ketamine (Holtman and Wala, 2005). In the present study we wanted to see if this phenomenon extended to other opioid receptor subtypes. Therefore, the pain enhancing properties (hyperalgesic) of oxycodone and U50,488H were examined after administration of low (subanalgesic) doses of these opioids alone and in combination with (±)-ketamine. Finally, since motor impairment may affect the response to noxious stimuli, motor activity was also evaluated after administration of oxycodone and U50,488H in male and female rats. The findings of the present rodent study may have implications for the use of oxycodone in the clinical setting.

2. Methods

2.1. Subjects

Age-matched male and female Sprague–Dawley rats (85–90 days old; 350 and 250 g, respectively) were used. Rats were housed in separate cages (with free access to standard laboratory chow and tap water) in a temperature-controlled room with a 12 h light/12 h dark cycle and with lights on at 6:00 a.m. Male and female rats were tested on alternate days. The estrous cycle was not determined. The effect of possible fluctuation in baseline responsiveness to noxious stimuli due to differences in the phase of the estrous cycle was controlled by normalization of responsiveness to the drug for preinjection baseline each day for each rat. Body weights were determined on the day of the experiment. The rats were habituated to handling prior to testing. The rats were handled in accordance with the National Institutes of Health (publication No. 85-23, revised 1985) and the Guide for the Care and Use of Laboratory Animals. The experiments were performed according to a protocol approved by the University of Kentucky Animal Care and Use Committee.

2.2. Apparatus

A standard tail-flick apparatus (EMDIE, Instrument Co., Roanoke, VA) was used to determine the tail-flick latency (TFL). The heat source was focused on a segment of the rat tail (about 1–1.5 in. from base and 2 in. in length). TFL was measured by recording the time from the onset of the heat stimulus to the tail withdrawal from the heat source. In order to determine the antinociceptive response, the sensitivity of the instrument was adjusted to provide an average baseline TFL of 2–3 s. A cutoff time of 10 s was used to avoid tail damage. In order to more readily observe the effects of subanalgesic doses of opioids, a low intensity radiant heat was used (average baseline TFL of about 10–12 s; cutoff time = 20 s). A previous study demonstrated that the TFL decreased in a linear fashion with increasing thermal stimuli (intensity 0.5–2.5) and that sex-related differences were not significant in rats (Holtman and Wala, 2005). All animals were handled and trained in the test situation before the initiation of the tail-flick test. In order to generate time action curves, responses to noxious stimuli were determined two times (15 min apart and averaged) prior to and one time at 15, 30, 60, 90 and 120 min after injection (acute studies). In the chronic study, the TFL was determined twice (at 15 and 30 min after injection and averaged).

The Opto-Varimex photocell-based activity monitor (Columbus Instruments, Columbus OH) measured total locomotor activity (LOC, 5 min score) prior to (baseline) and 60 min after injection. The beam interruptions were monitored along a single axis and all activities (ambulatory counts plus stereotypic activity) were scored on the front panel counter. Motor activity was determined immediately after the tail-flick test. All testing was conducted between 0900 and 1400 h.

2.3. Procedure

Oxycodone, U50,488H and (±)-ketamine hydrochloride were dissolved in physiological saline immediately prior to administration by the intraperitoneal (IP) route (1 ml/kg). The same rats (10 or 8/drug/sex) were used repeatedly throughout the acute study (with a minimum of 96 h between testing). The order of the doses of oxycodone (0.25, 0.5, 1, 2, 4 mg/kg) or U50,588H (2.5, 5, 10, 15 mg/kg) was balanced by the double block Latin-square design. Male rats were additionally dosed with 20 mg/kg of U50,488H. Control rats (5/sex) were injected with saline. Separate groups of rats (5/sex/treatment) were injected with a low (subanalgesic) dose of oxycodone (10 mcg/kg) or U50,588H (25 mcg/kg) alone and in combination with (±)-ketamine (1.5 mg/kg; 30 min prior to opioid). Control rats were pretreated with saline. In the chronic studies, oxycodone (1 mg/kg) was administered twice daily in male and female rats (6/sex). Each rat was considered to be tolerant to oxycodone when the percent of the maximal possible effect (%MPE) was $\leq 5\%$.

2.4. Drugs

Oxycodone hydrochloride was obtained from Sigma, St. Louis, MO; U50,488H from UpJohn, Kalamazoo MI; and (±)-ketamine hydrochloride from Sigma-Aldrich, St. Louis, MO.

2.5. Data analysis and statistics.

The tail-flick latency (TFL) obtained for each rat at each postinjection time point was converted to %MPE = [(TFL postinjection − TFL preinjection)/(cut off − TFL preinjection)] × 100. The time action curves were generated for %MPE vs time. Areas under the time–action curves ($AUC_{0-120min}$) were calculated by the trapezoidal rule. This appears to be more accurate representation of total drug effect. The $AUC_{0-120min}$ and maximal %MPE were plotted vs. the dose. The dose that produced %MPE = 50 (ED_{50}) along with

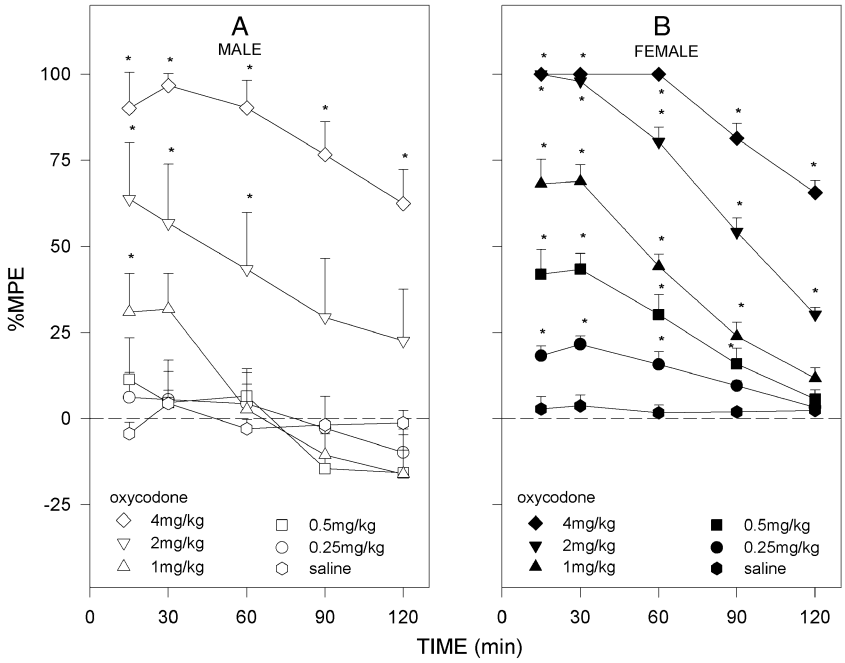

Fig. 1. Time course of the antinociceptive effect produced by graded doses of oxycodone (IP) in male (A) and female (B) rats. Responsiveness on tail-flick test is presented as maximal possible effect (%MPE). Data are mean ± SEM (10 rats/sex). *Significantly different from saline ($P < 0.05$; post-hoc SNK).

SEM was then calculated by previously described method (Tallarida et al., 1997). Antinociception and hyperalgesia were defined as any significant increase and decrease from baseline, respectively. Locomotor activity (LOC) was normalized for preinjection baseline (LOC postinjection − LOC preinjection). Statistical analysis was performed with the help of linear regression, one- and two-way repeated measures (RM) ANOVA with post-hoc Student–Newman–Keuls (SNK) method. All values represent mean ± SEM of "n" rats. The level of significance was $P \leq 0.05$.

3. Results

3.1. The antinociceptive effect of oxycodone

The antinociceptive effect of oxycodone (0.25–4 mg/kg, IP) was determined in male and female rats. The onset of antinociception (time of maximal effect ≈ 30 min) was similar in male and female rats (Fig. 1A, B). An enhanced sensitivity to noxious stimuli (hyperalgesia) was observed later in the time course after low doses of oxycodone (0.25–1 mg/kg) in male

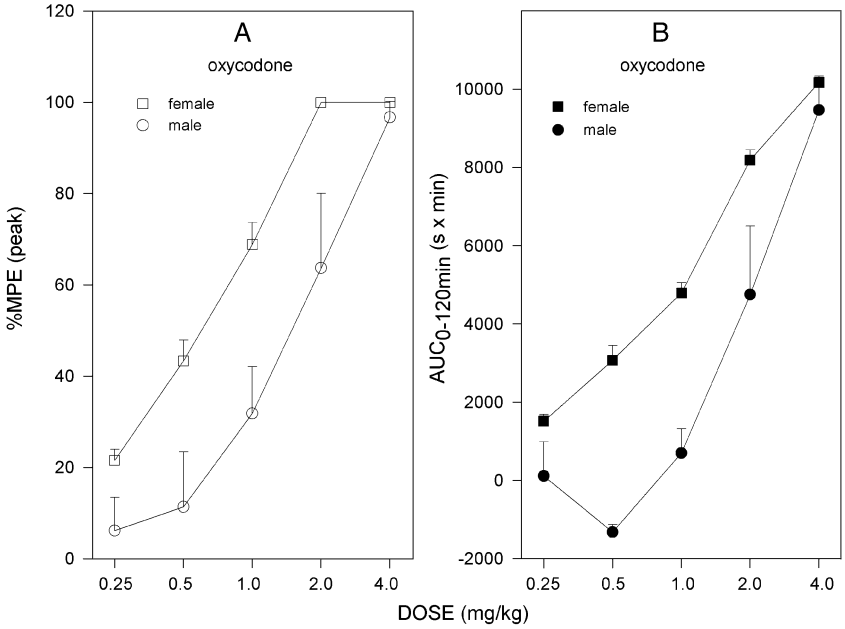

Fig. 2. Dose–response curves for antinociception produced by oxycodone in female and male rats. The antinociceptive effects are presented as: (A) Peak values for percentage of maximal possible effect (%MPE) and (B) Areas under the time action curves ($AUC_{0-120min}$). Data are mean ± SEM (10 rats/sex).

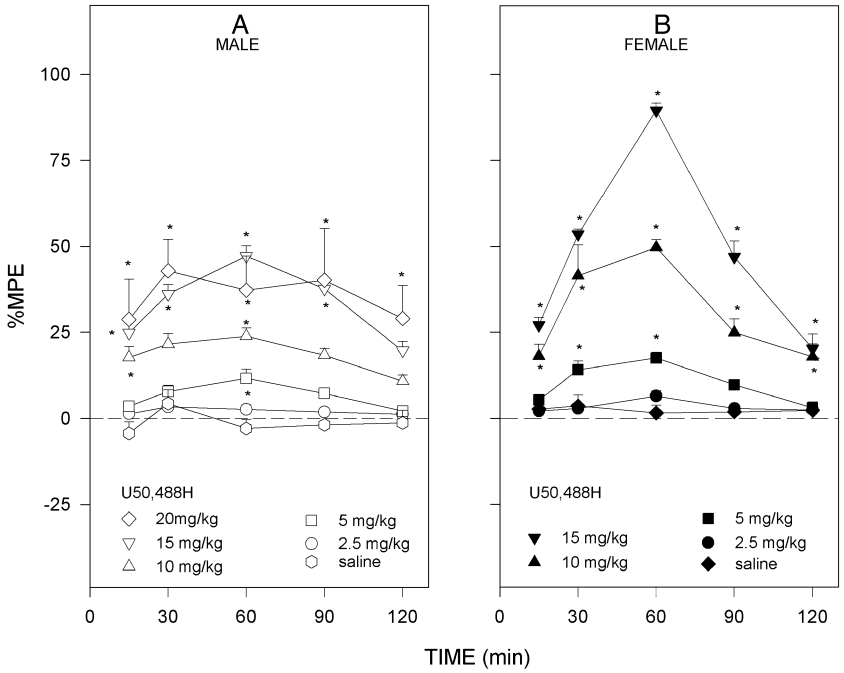

Fig. 3. Time course of the antinociceptive effect produced by graded doses of U50,488H (IP) in male (A) and female (B) rats. Responsiveness on tail-flick test is presented as maximal possible effect (%MPE). Data are mean ± SEM (8 rats/sex). *Significantly different from saline ($P < 0.05$; post-hoc SNK).

rats (high intensity radiant heat). Saline produced no significant effect. The baseline (preinjection) responsiveness to noxious stimuli was similar in male and female rats (TFL = 2.87 ± 0.15 and 2.15 ± 0.05 s, respectively).

The antinociceptive effect of oxycodone was linearly related to dose (peak %MPE, $AUC_{0-120min}$) (Fig. 2A, B). The maximum effect (%MPE = 100) was produced by doses equal to 2 and 4 mg/kg in female and male rats, respectively. Overall, the responses (peak %MPE) were significantly related to dose and were greater in female than in male rats (dose: $P < 0.0001$; sex: $P < 0.0005$; dose × sex: NS; 2-way RM ANOVA). The potency of oxycodone was about two-fold greater in female than in male rats (ED_{50} = 0.63 ± 0.04 vs. 1.46 ± 0.13 mg/kg, respectively).

3.2. The antinociceptive effect of U50,488H

The selective kappa agonist, U50,488H (2.5–15 mg/kg) was administered in male and female rats. Time courses of antinociception were similar in male and female rats (with a

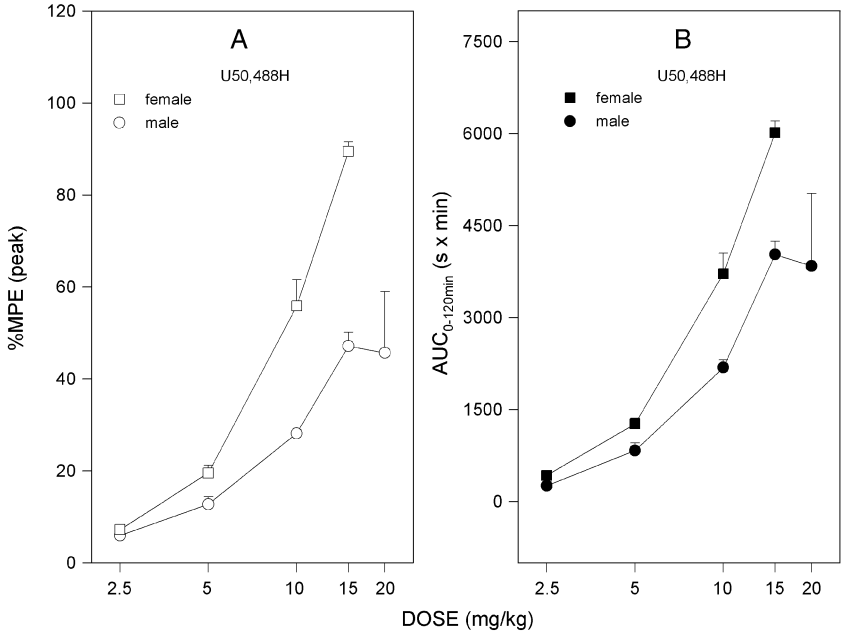

Fig. 4. Dose–response curves for antinociception produced by U50,488H in female and male rats. The analgesic effects are presented as: (A) Peak values for percentage of maximal possible effect (%MPE) and (B) Areas under the time action curves ($AUC_{0-120min}$). Data are mean ± SEM (8 rats/sex).

maximum at about 60 min) (Fig. 3A, B). No delayed hyperalgesia was observed during the duration of testing (120 min).

Antinociceptive responses were linearly related to the dose of U50,488H (peak %MPE and $AUC_{0-120min}$). The dose equal to 15 mg/kg produced approximately maximum effect in female rats while the antinociceptive effect was observed to plateau (%MPE ≈ 50) at a significantly lower level in male rats (Fig. 4A, B). U50,488H-induced antinociception was significantly related to dose, was more pronounced in female than in male rats and the interaction between sex and dose was of statistical significance (dose: $P<0.0001$; sex: $P<0.0001$; dose × sex: $P<0.0001$; 2-way RM ANOVA). The ED_{50} was equal to 9.17 ± 0.37 mg/kg in female rats. Partial agonistic properties of U50,488H were indicated by the 50% ceiling effect at doses equal to 15–20 mg/kg in male rats.

3.3. The hyperalgesic effects of oxycodone and U50,488H

The hyperalgesic properties of oxycodone and U50,488H were tested after administration of very low (subanalgesic) doses in male and female rats. The baseline responsiveness to noxious stimuli (preinjection TFL) was equal to 12.5 ± 0.49 s and 11.1 ± 0.35 s in male and female rats, respectively (low intensity radiant heat). Both oxycodone (10 mcg/kg) and U50,488H (25 mcg/kg) decreased TFL (indicative of hyperalgesia) in male rats. The same low dose of U50,488H (25 mcg/kg) enhanced TFL in female rats. In order to determine if the hyperalgesia involved a NMDA receptor mechanism, low doses of oxycodone and U50,488H were coadministered with (±)-ketamine (1.5 mg/kg). The hyperalgesic effect of oxycodone and U50,488H was blocked by the NMDA receptor antagonist, (±)-ketamine (1.5 mg/kg) (Fig. 5A, B, C, D). This dose of (±)-ketamine alone does not have antinociceptive activity in the low intensity tail-flick test (Holtman and Wala, 2005).

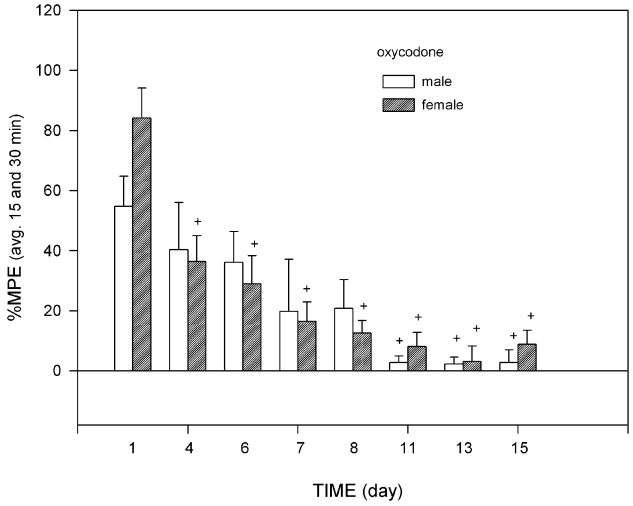

Fig. 6. Time courses for oxycodone-induced antinociception after repeated administration (1 mg/kg, 2×/day) in male and female rats. Antinociception is presented as percentage of maximal possible effect (%MPE). Data are mean ± SEM (6 rats/sex). +Significantly different from Day 1 ($P<0.05$; post-hoc SNK).

3.4. The locomotor effects of oxycodone and U50,488H

The effect of oxycodone and U50,488H on locomotor activity was determined in male and female rats. Spontaneous

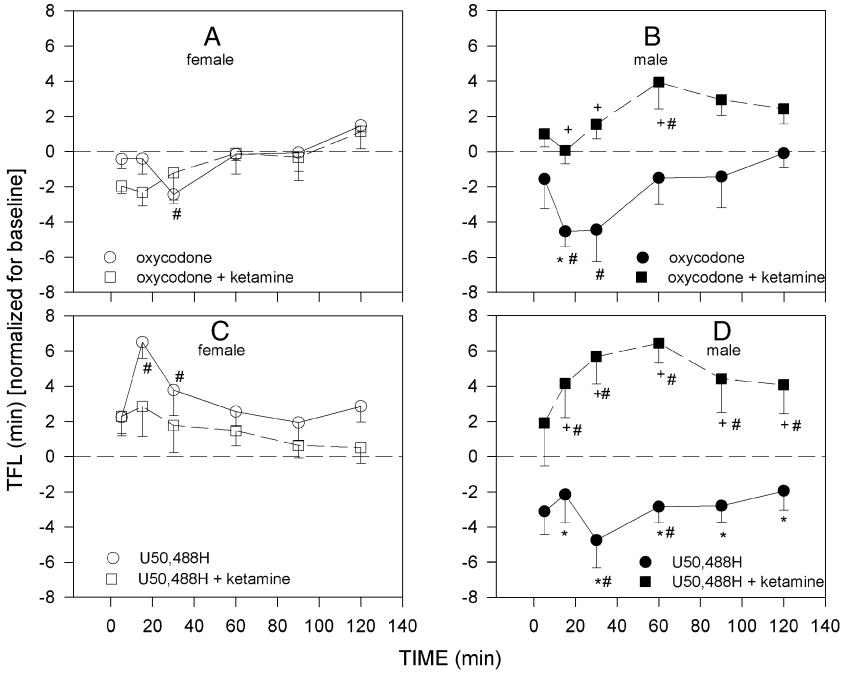

Fig. 5. Time courses for the effect produced by low (subanalgesic) IP doses of oxycodone alone (10 mcg/kg) and oxycodone (10 mcg/kg) combined with (±)-ketamine (1.5 mg/kg) in female rats (A) and male rats (B); U50, 488H alone (25 mcg/kg) and U50,488H (25 mcg/kg) combined with (±)-ketamine (1.5 mg/kg) in female rats (C) and male rats (D). Data are mean ± SEM (5 rats/sex/treatment). #Significantly different from baseline value (preinjection); *Significantly different from identically treated female rats; +Significantly different from the oxycodone- or U50,488H-treated rats of the same sex; ($P<0.05$; post-hoc SNK).

Table 1
Onset of tolerance to antinociception following repeated administration of oxycodone (1 mg/kg, twice daily) in male and female rats ($n=6$ rats/sex)

Tolerance development with chronic oxycodone treatment									
Day	1	4	6	7	8	11	13	15	16
Male	0/6	0/6	0/6	2/6	2/6	5/6	5/6	6/6	6/6
Female	0/6	0/6	0/6	2/6	2/6	4/6	5/6	5/6	6/6

Data are presented as number of oxycodone-tolerant rats (%MPE $\leq$ 5) across time of repeated treatment.

motor activity was significantly related to dose ($P<0.00025$ and $P<0.001$ for oxycodone and U50,488H, respectively; 2-way RM ANOVA) but not to sex. Higher doses of oxycodone (2 and 4 mg/kg) caused a reduction in locomotor activity in male and female rats. Locomotor activity decreased linearly with dose of U50,488H in female rats while the dose–response curve had a U-shape in male rats (data not shown).

3.5. Tolerance to oxycodone

Repeated testing (in 96 h intervals; acute study) did not alter the antinociceptive effects produced by oxycodone and U50,488H (days: NS; 1-way RM ANOVA). On the other hand, the responsiveness to oxycodone progressively decreased with time of twice-daily (1 mg/kg) chronic treatment (tolerance) (Fig. 6). The antinociceptive effect of oxycodone (%MPE) was significantly related to time of repeated dosing ($P<0.0001$; 2-way RM ANOVA) but not sex. A similar number of male and female rats became tolerant (%MPE equals to $\leq$5%) across the time of chronic oxycodone treatment (Table 1). However, the initial antinociceptive responsiveness to oxycodone was considerably higher in female rats and thus a proportionally longer period of time would be expected for the %MPE to decline towards the baseline. Furthermore, a loss of about half of the initial antinociceptive potency of oxycodone was achieved faster in female rats (4 and 2 rats on day 4 and 6, respectively) compared to male rats (3, 2 and 1 rats on day 6, 7 and 8, respectively). Taken together, the present findings suggest that the rate of tolerance development (a loss of antinociceptive potency over time) was greater in female than in male rats. Baseline TFL (2.3 ± 0.11 s and 2.1 ± 0.11 s in male and female rats, respectively) did not systematically increase or decrease across time of oxycodone repeated dosing (TFL fluctuation: 3.0 ± 0.20–1.9 ± 0.14 s and 2.7 ± 0.19–1.8 ± 0.20 s in male and female rats, respectively). Body weights did not significantly change during chronic oxazepam treatment (female rats: 244.3 ± 4.37 vs. 246.7 ± 5.02 g; male rats: 380.8 ± 2.34 vs. 377.5 ± 6.0 g for day 1 vs. day 14).

4. Discussion

The present study demonstrated that oxycodone, had a greater antinociceptive response in female rats compared to male rats. This greater antinociception in female rats was even more prominent with the selective kappa agonist, U50,488H. In striking contrast, previous data from our laboratory (an identical protocol) showed that the antinociceptive effect of morphine, a predominantly mu opioid agonist, was lesser in female than in male rats (Holtman et al., 2003a, b). A greater antinociceptive effect of morphine in male rats has also been shown by numerous laboratories for several assays including tail-flick (Cicero et al., 1996; Kepler et al., 1989; Krzanowska and Bodnar, 1999), hot-plate (Cicero et al., 1996; 1997), tail withdrawal (Boyer et al., 1998; Craft et al., 1999) and electric shock jump (Kepler et al., 1989; Krzanowska and Bodnar, 1999) in rats. Therefore, the present data seem to support the idea that the antinociceptive effect of oxycodone is mediated (at least in part) by kappa opioid receptors (Ross and Smith, 1997; Nielsen et al., 2000; Ross et al., 2000). Together, these findings suggest that sex-related differences in the antinociceptive effects of opioids are dependent on the receptor at which they act.

The present data on oxycodone in intact rats (tail-flick test) are in contrast with a recent study indicating that this opioid was more potent in male than in female Freund's adjuvant-treated arthritic Lewis rats (paw pressure test) (Cook and Nickerson, 2005). The experimental design (strain, pain model, noxious stimuli) may be the reason for the discrepancy. Sex-related differences in baseline pain thresholds were found in arthritic rats (females exhibited greater hyperalgesia than males) but not in intact rats. Alteration of the endogenous opioid system in arthritic rats may play a role (Millan et al., 1986). We are not aware of any other rodent studies that directly compare antinociceptive responsiveness to oxycodone with regard to sex. However, available data on the relative potencies of oxycodone and morphine in male and female rats suggest that sex could play a role (Cleary et al., 1994; Leow and Smith, 1994; Nielsen et al., 2000; Poyhia and Kalso, 1992; Ross et al., 2000). The route of administration also appears to be important as oxycodone is metabolized to the mu-opioid agonist, oxymorphone (Poyhia and Kalso, 1992). Sex-related differences in first pass metabolism (if any) will be more prominent after PO and IP routes of administration.

The present results (tail-flick test) on U50,488H are consistent with those obtained with the hot plate test (52 °C) showing approximately twofold greater antinociception ($ED_{50}=4.94$ vs. 8.62 mg/kg, respectively) in female than in male Sprague Dawley rats (Craft and Bernal, 2001) and greater antihyperalgesic effectiveness (paw pressure test) in female than in male adjuvant-treated arthritic Dark Agouti rats (Binder et al., 2000). The ceiling effect for U50,488H observed for male rats in the present study has been also reported by previous investigators (D'Anci et al., 2000). However, the present data contradict the studies showing a greater antinociceptive response to U50,488H (tail withdrawal test) in male rats (Craft and Bernal, 2001; Stoffel et al., 2005), male monkeys (Negus and Mello, 1999) and male mice (Kavaliers and Innes, 1987). Overall, the sex-related differences in antinociceptive responsiveness to kappa and mixed action kappa/mu opioid agonists appear to be related to species, strain, pain model and intensity of noxious stimuli (Barrett et al., 2002; Binder et al., 2000; Craft and Bernal, 2001; van Haaren et al., 2000). Clearly, additional testing of oxycodone using different pain model(s) is in order. It is worthy to note, that our

findings regarding the greater sensitivity to oxycodone and U50,488H in female than in male rats are in agreement with the observation of greater analgesic responses to oxycodone (Kaiko et al., 1996) as well as to butorphanol, nalbuphine and pentazocine (clinically used kappa opioid analgesic drugs) in women than in men (Gear et al., 1996a,b, 1999, 2000).

In addition, the locomotor effects of oxycodone and U50,488H have been examined in male and female rats. Overall, the opioid-produced antinociceptive effects were accompanied by a motor deficit. Contrary to sex-related antinociception, changes in locomotor activity were not sex-related for either oxycodone or U50,488H. Therefore, it is unlikely that locomotor activity contributes to the antinociceptive responsiveness in male and female rats.

We have also demonstrated that repeated testing (96 h intervals) did not result in sensitization and/or tolerance to oxycodone and U50,488H. In contrast, twice-daily chronic treatment caused a decrease in the antinociceptive effectiveness of oxycodone (tolerance). The same was observed for morphine (Cicero et al., 1996, Craft et al., 1999; Holtman et al., 2004). Further, as for morphine (Holtman et al., 2004), the sex-specific responsiveness to oxycodone was sustained during intermittent injections but was lost with long-term chronic use. Therefore, sex-related responsiveness to opioid analgesics seems to be abolished with repeated dosing. Loss in the initial potency of oxycodone was faster in female than in male rats. The opposite was demonstrated for morphine (Barrett et al., 2001; Craft et al., 1999). In the current study, complete tolerance to oxycodone (1 mg/kg 2× day; IP) was achieved within 13 days in both male and female rats. Tolerance was seen within 48 and 84 h following chronic IV infusion of oxycodone, (2.5 and 5 mg/24 h, respectively) in Dark Agouti male rats (Nielsen et al., 2000). Tolerance was also demonstrated after cumulative doses of oxycodone (3 days; SC) in male mice (Duttaroy and Yoburn, 1995). Several factors such as species, dose and dosing schedules, route of administration and the nociceptive assay are likely to affect opioid tolerance. Therefore, from this rodent study it is not possible to predict whether responses to acutely and/or chronically administered oxycodone are related to gender in humans. This will require further research.

The mechanism underlying sexual dimorphism in opioid antinociception is not clear. Hormonal, pharmacokinetic, physiological, and behavioral factors have been extensively studied without conclusive results (see Craft, 2003a,b; Cicero et al., 2002, Cook et al., 2000, Fillingim and Gear, 2004; Fillingim and Ness, 2000, Kest et al., 2000, Miaskowski et al., 2000, Selley et al., 2003, for references). The present data also confirm that sex-related responses to oxycodone and U50,488H are not due to the differences in the intrinsic responsiveness to noxious stimuli (baseline TFL), time action curves and locomotor activity. It can be argued that using high intensity radiant heat may obfuscate sex differences in baseline and subsequent antinociception. The literature is not consistent as to whether the pain threshold is related to the sex of the rats. The present data showed that TFL is not related to sex using both high (2–3 s) and low (10–12 s) intensity radiant heat; however, the responsiveness to oxycodone and U50,488H was different in male and female rats. The same was reported for morphine (high intensity radiant heat) by our (Holtman et al., 2003a,b; Holtman and Wala, 2005) and other laboratories (Cicero et al., 1996; Kepler et al., 1991; Krzanowska and Bodnar, 2000). Additionally, we demonstrated that despite similar baseline values sexual dimorphism was observed after intermittent but not chronic administration of oxycodone. It should be noted that the phase of the estrous cycle was not determined in the present study. Overall, it would be difficult to demonstrate its effect on antinociception using a crossover experimental design as variance in antinociception that could be accounted for by the estrous stage is usually low as compared to variance accounted for by the dose. There is a volume of conflicting data indicating that the effect of the estrous cycle on opioid antinociception is complex and not well-defined in rats (see Kest et al., 2000; Miaskowski et al., 2000, for review); however, this factor may contribute to the antinociceptive responsiveness in female rats. Importantly, the present study demonstrated significant sex-related differences in responsiveness to oxycodone and U50,488H using a sample of randomly cycling female rats.

We further address a potential mechanism involved with sex differences in opioid-induced antinociception. There is a line of evidence regarding dual antinociceptive/pronociceptive properties of acutely administered mu-opioid analgesics (see Mao, 2002; Simonnet and Rivat, 2003, for references). The overall effect (analgesia, hyperalgesia) appears to depend on the dose. Typically, the enhancement in pain sensitivity (hyperalgesia) is observed as a delayed response after antinociception; however, morphine, at low (subanalgesic) doses also has been shown to produce hyperalgesia in rats (Crain and Shen, 2001; Hamann and Martin, 1992; Holtman and Wala, 2005; Parvini et al., 1993). Thus, hyperalgesia can be demonstrated independently from antinociception. In addition, data from our lab demonstrated that sex differences in the antinociceptive (male>female) and hyperalgesic (female>male) effects of morphine were opposite in rats (Holtman and Wala, 2005) and that delayed hyperalgesia was evident after the administration of an antinociceptive dose of morphine in female but not in male rats (Holtman and Wala, 2005). The present data extended these finding by showing that kappa opioids such as U50,488H (subanalgesic dose), produced hyperalgesia in male rats. The hyperalgesic effect of oxycodone was also more prominent in male than in female rats. Sexual dimorphism in the antinociceptive (female>male) and hyperalgesic (male>female) effects of oxycodone and U50,488H were inverse. We also found delayed hyperalgesia later in the time course after higher dose of oxycodone in male rats (when the concentration is expected to be low). The time courses were followed for only 120 min postinjection; therefore, it is possible that hyperalgesia would have been observed at a later time in female rats. Interestingly, hyperalgesia was also demonstrated after central (ICV) administration of oxycodone (40 nmol) in male Dark Agouti rats (Ross et al., 2000). The present data are in agreement

with previous findings that the analgesic and the pain-enhancing effects (delayed hyperalgesia) of nalbuphine (clinically used kappa opioid analgesic) are more pronounced in women and men, respectively (Gear et al., 1996a,b, 1999). Together, these findings suggest that the pain facilitatory process triggered by opioid(s) is sexually dimorphic. Assuming that the overall effect of the opioid is equal to the sum of antinociceptive and pronociceptive processes, suggests that hyperalgesia counteracts antinociception to a different extent depending upon the selectivity of the opioid drug for mu and kappa opioid receptors as well as sex. This may explain (at least in part) the direction of sex differences in the antinociceptive effects of opioid analgesics in rats.

There is no certain explanation as to why sex differences for opioid analgesics acting predominantly on kappa and mu opioid receptors are opposite in rats (at least in the tail-flick test). Pharmacokinetics/metabolism is not a reason since opposite sexual dimorphism was also apparent after central injection (rostral ventral medulla, RVM) of the selective kappa agonist, U69,593, (Tershner et al., 2000) and morphine (Boyer et al., 1998; Kepler et al., 1991). Further, brain/plasma levels of both U69,593 (Craft et al., 1998) and morphine (Cicero et al., 1997) were comparable in male and female rats. One possible explanation is that the distribution and localization of kappa and mu opioid receptors on pain modulatory neurons differ in male and female rats (Fields and Basbaum, 1994; Hammer, 1990; Pan et al., 1997; Tershner et al., 2000). Moreover, in the RVM the mu and kappa opioid receptors have been found on a physiologically different class of neurons that may play a contradictory role in the transmission of nociception (Fields et al., 1991; Pan et al., 1997). These speculations can be extended to the agonist/antagonist properties of opioids with mixed action on kappa and mu receptors (see Pan, 1998, for review) and sex-related interactions between kappa- and mu-selective opioids (Cook et al., 2000; Tershner et al., 2000). In addition, it is of interest to note that the NMDA receptor antagonist, (±)-ketamine, was able to block the hyperalgesic effects of oxycodone and U50,488H in male rats (present study) as well as morphine-induced hyperalgesia in female rats (Holtman and Wala, 2005). Further, the sex-related modulation of antinociception by (±)-ketamine has been shown to be opposite for morphine (Holtman et al., 2003a) and U69,593 (Kavaliers and Choleris, 1997). This suggests that the NMDA receptor-mediated mechanism may be involved.

In summary, the present study demonstrated sexual dimorphism in the antinociceptive effect of oxycodone in rats. In humans, little is known about oxycodone analgetic sensitivity in males and females. This is important as gender may impact the clinical efficacy of oxycodone in the treatment of pain.

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