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Morphine tolerance in male and female rats

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Abstract

Several studies indicate greater sensitivity to morphine (MOR) analgesia in male compared to female rats under the acute dosing condition. The present study investigated whether the same sex difference in sensitivity persists in MOR-tolerant rats. MOR was administered chronically (7 mg/kg twice daily) until tolerance developed in each rat. Tolerant rats were treated randomly with higher graded doses of MOR (10–25 mg/kg). Analgesia (tail-flick test) and spontaneous motor activity (total locomotion) were measured. The present data confirmed previous studies showing a greater sensitivity to acute MOR in male than in female rats. However, the sex differences seen in MOR sensitivity were abolished in tolerant rats. The rate of acquisition of tolerance was similar in male and female rats. The analgesic response was not affected by motor depression.

Keywords: Morphine tolerance; Analgesia; Locomotion; Sex differences

1. Introduction

There is a large volume of data showing a markedly greater analgesic effect of morphine in male rats compared to female rats under the acute dosing condition (see Craft, 2003a,b; Kest et al., 2000b; Miaskowski et al., 2000, for references). Furthermore, it has been suggested that the development of analgesic tolerance to morphine, administered by cumulative dose, infusion, or repeated dosing for a fixed period of time, is related to sex in rats (Craft et al., 1999b; South et al., 2001) and mice (Kest et al., 2000a, 2001). Little is known, however, as to whether sexual dimorphism in the analgesic effect of morphine persists in tolerant subjects. This information is important in that it will show whether the mechanism(s) responsible for the initial sexual dimorphism of morphine undergoes desensitization during repeated treatment.

The purpose of the present study was to determine whether the onset of tolerance development to the analgesic effect of morphine differs between sexes and whether sex differences are sustained in morphine tolerant rats. The design of this study differs from the previous ones in that the chronic administration of morphine was terminated immediately after the development of tolerance. This experimental design allowed the effects of higher doses of morphine to be tested under standardized conditions in tolerant male and female rats. Additionally, since motor depression has been implicated in affecting the analgesic response to morphine, total locomotion was determined.

2. Materials and methods

2.1. Subjects

Male and female (~ 350 and 250 g, respectively), age-matched (90 days old), Sprague–Dawley rats (Harlan) were used in this study. The estrous cycle was not determined in female rats. Each rat was kept separately in a transparent cage with free access to standard laboratory chow and tap water. The rats were housed in a temperature-controlled environment with a 12-h light/12-h dark cycle (lights on at 6:00 a.m.) and were handled in accordance with the NIH *Guide for the Care and Use of Laboratory Animals* (Publication No. 85-23, revised 1985). Experiments were performed according to a protocol approved by the University of Kentucky Institutional Animal Care and Use Committee.

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2.2. Drugs

Morphine sulfate was purchased from Mallinckrodt (St. Louis, MO). On the day of each experiment, a fresh solution of morphine was made with physiological saline. The drug was administered intraperitoneally in a volume equal to 1 ml/kg. The dose of morphine refers to the sulfate salt.

2.3. Analgesia

Antinociception was assessed by the tail-flick test using a standard tail-flick apparatus (EMDIE, Instrument, Roanoke, VA). The heat source focused on a blackened segment of the tail ($\sim$ 1 to 1.5 in. from base and 2 in. in length). The sensitivity of the instrument was adjusted to provide baseline tail-flick latency (TFL) of 2–3 s. A cutoff time of 10 s was used to avoid any injury to the tail. Tail-flick latency was obtained by recording the time from the onset of heat stimulus to withdrawal of the tail from the heat source. All animals were handled and trained in the test situation before the initiation of the tail-flick test. Responses to noxious stimuli were determined on the day of the experiment before (twice) and 30 and 60 min postmorphine injection. The analgesic effect was calculated as percentage of maximum possible effect (%MPE): %MPE=[(postinjection TFL − baseline TFL)/(10 − baseline TFL) × 100].

2.4. Motor activity

Total locomotion (LOC) was assessed using the Opto-Varimex infrared photocell-based activity monitor (Columbus Instrument, OH). Beam interruptions were monitored along a single axis and spontaneous motor activities [ambulation and total activity (includes stereotypic activity)] were scored on a front panel counter during two 5-min sessions before morphine injection (baseline) and 30 min after the administration of morphine. Data were normalized for baseline (LOC = postinjection LOC − baseline LOC).

2.5. Procedure

Male and female rats were injected two times a day with morphine (7 mg/kg ip, approximately at 0800 and 1700 h). Analgesia (TFL) was assessed daily after the morning dose of morphine. This procedure was continued until the development of analgesic tolerance. Each rat was considered to be tolerant to morphine when the %MPE was $\leq 5\%$ (30 and 60 min after injection). Rats still received the afternoon dose of morphine even if they were determined to be tolerant following morning testing. The tolerant rats were then randomly challenged within 24 h with a single injection of higher doses of morphine (10, 15, 20, and 25 mg/kg).

2.6. Data analysis

The effective doses (ED_{50}) were calculated from the log dose–response (%MPE) curves (Tallarida and Murray, 1987). Data were analyzed by one-way ANOVA, two-way repeated measures (RM) ANOVA, post hoc Student–Newman–Keuls (SNK) test, Fisher exact test, and *t* test. Differences were considered significant at $P < .05$.

3. Results

Table 1 indicates that neither the baseline response to noxious stimuli (preinjection TFL) or baseline motor activity (preinjection LOC) was affected by repeated morphine treatment in rats. The baseline TFL was not sex related in morphine-naïve rats (before the first injection) or in morphine-tolerant rats. Naïve female rats were more active than their male counterparts. The same trend was observed in morphine-tolerant rats.

Fig. 1 illustrates analgesia (%MPE) [panel A] and motor activity (LOC) [panel B] on the first day, on the next to last day (about 24 h before the development of tolerance) and on the last day (tolerance) of chronic morphine treatment (7 mg/kg ip twice/day). Analysis of the %MPE revealed a significant sex and time effect as well as a time and sex interaction [$F(1,119) = 9.9$, $P < .005$; $F(2,119) = 100.6$, $P < .0001$; $F(2,119) = 8.2$, $P < .001$, respectively, 2-way RM ANOVA]. The present data suggest that the lesser analgesic responsiveness to morphine in female than in male rats on Day 1 ($39.8 \pm 4.3\%$ vs. $69.1 \pm 5.4\%$) corresponds with faster achievement of tolerance (5.4 ± 0.54 days vs. 8.0 ± 0.61 days) in female than in male rats. However, the correlation between the initial %MPE score and the number of days to reach tolerance was not of statistical significance in each individual rat. Analysis of LOC activity showed that the sex effect was below the level of statistical significance, whereas a time effect and interaction between sex and time were statistically significant [$F(2,119) = 5.8$, $P < .005$; $F(2,119) = 4.2$, $P < .025$, respectively, 2-way ANOVA]. The first exposure to morphine produced a greater analgesia that was accompanied by lesser motor activity in male compared to female rats. The repeated administration of morphine resulted in diminished analgesic responsiveness to morphine and enhanced motor activity. On the next to last day of treatment,

Table 1
Baseline (preinjection) responsiveness to noxious stimuli [tail-flick latency (TFL)] and baseline spontaneous motor activity [total locomotion (LOC)] in morphine (MOR)-naïve rats [before the first injection (Day 1)] and in MOR-tolerant rats [before the last injection (tolerance)]

Rats	TFL (s)		LOC (5-min score)	
	Male	Female	Male	Female
MOR naïve	2.47 ± 0.10	2.41 ± 0.08	191.4 ± 10.4	249.4 ± 22.7 *
MOR-tolerant	2.65 ± 0.16	2.46 ± 0.09	189.7 ± 15.0	229.9 ± 18.6

* Significantly different from male rats ($P < .025$, *t* test).

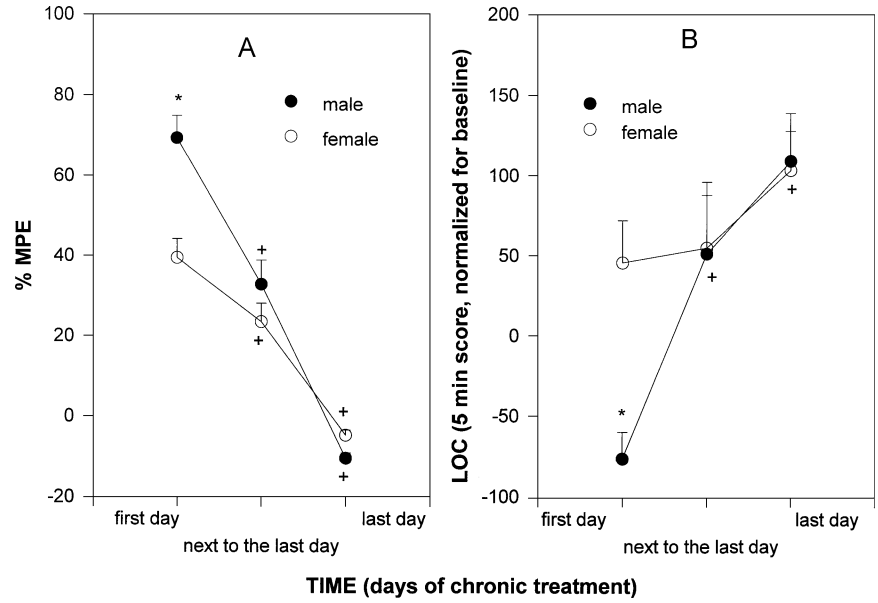

Fig. 1. Analgesia [percentage of maximum possible effect (%MPE)] (panel A) and changes (with respect to baseline) in spontaneous motor activity (LOC) [panel B] after the first exposure to morphine [(MOR) 7 mg/kg ip, Day 1]; during repeated treatment (MOR, 7 mg/kg ip twice daily); about 24 h before the development of tolerance, which required a median time of 7 and 4 days in male and female rats, respectively, and at the last day of repeated treatment [tolerance (%MPE ≤ 5)], which required a median time of 8 and 5 days in male and female rats, respectively. Data are the mean ± S.E.M. (20 rats/sex). * Significantly different from female rats ($P < .05$, post hoc SNK); $^{+}$ Significantly different from Day 1 ($P < .05$, post hoc SNK).

the %MPE was equal to approximately one-half of the initial value (Day 1). On the last day of chronic morphine treatment, the responsiveness to noxious stimuli was enhanced (tolerance-related hyperalgesia). The sex-related differences in motor activity were abolished during chronic morphine treatment and with tolerance.

Fig. 2 compares the number of tolerant (%MPE ≤ 5%) and nontolerant (%MPE>5%) female [panel A] and male [panel B] rats across the time of chronic treatment with morphine. As can be seen, compared to male rats, significantly more female rats had become tolerant to the analgesic effect of morphine on Day 5 (12 vs. 4), Day 6 (15 vs. 6) and Day 7 (17 vs. 6) [$P < .05$, Fisher exact test]. Approximately one-half of the tested female and male rats (10/sex) became tolerant to morphine analgesia by Day 4 and 8, respectively.

Fig. 3 shows the dose–response curves for the analgesic effect (%MPE) [panel A] and LOC effect of morphine (10–25 mg/kg) [panel B] in male and female rats rendered

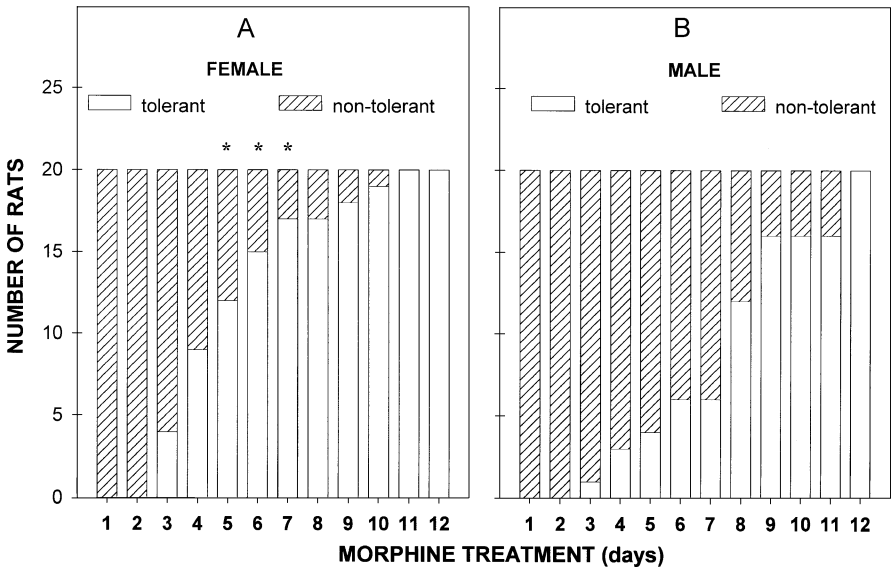

Fig. 2. Onset of tolerance to analgesia following repeated administration of MOR 7 mg/kg ip twice daily in female and male rats (20/sex). Data are presented as the number of MOR-tolerant (%MPE ≤ 5) and nontolerant (%MPE > 5) rats across time of repeated treatment. * Significantly different from male rats ($P < .05$, Fisher exact test).

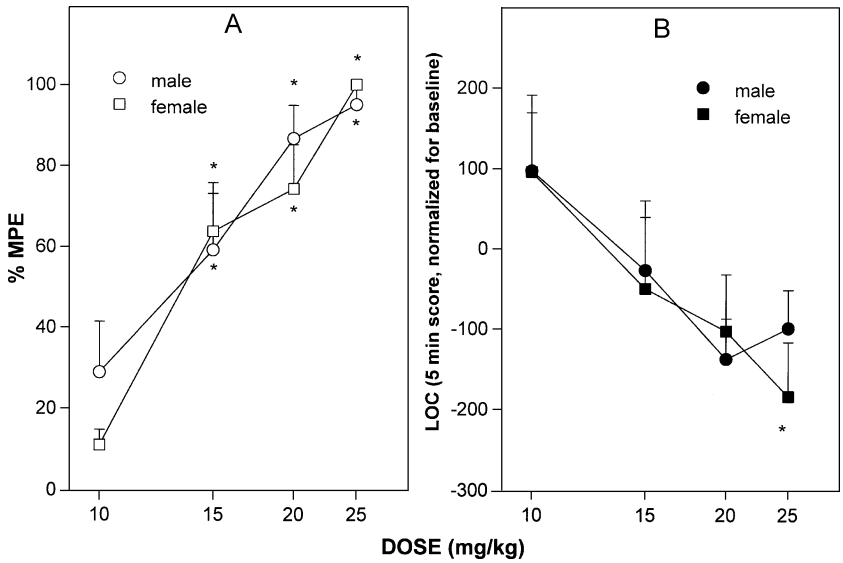

Fig. 3. Dose–response curves for antinociception (%MPE) (panel A) and LOC (panel B) after the acute administration of MOR in MOR-tolerant male and female rats. Tolerance was induced by repeated administration of MOR (7 mg/kg ip twice daily). A single dose of MOR (10–25 mg/kg ip) was administered within 24 h after the development of tolerance in each rat. Data are mean ± S.E.M. (five rats/sex/dose). * Significantly different from MOR 10 mg/kg ($P < .05$, post hoc SNK).

tolerant to morphine (7 mg/kg). Morphine produced a dose-related increase in analgesia [$F(3,19) = 10.1$ and 25.1, for male and female rats, respectively, $P < .0001$, one-way ANOVA] that was accompanied by a decrease in LOC in female rats [$F(3,19) = 3.7$, $P < .05$, one-way ANOVA], but not in male rats. The %MPE and LOC were significantly related to dose [$F(3,39) = 31.2$, $P < .0001$, and $F(3,39) = 5.8$, $P < .005$, respectively; two-way RM ANOVA] but not to sex. The effective analgesic doses (ED_{50}) of morphine were equal to 13.1 ± 0.85 and 13.9 ± 0.39 mg/kg for tolerant male and female rats, respectively.

In general, the total dose of morphine that was administered before the subsequent higher dose testing tended to be less in female than in male rats [51.8 ± 8.2; 93.8 ± 17.9; 60.2 ± 10.3; 71.4 ± 16.9 mg/kg (female) vs. 105 ± 10.8; 116.2 ± 23.2; 77.0 ± 12.5; 121.8 ± 16.8 mg/kg (male) in rats randomized to 10, 15, 20, and 25 mg/kg, respectively]. However, the between-sex differences in total dose of morphine (chronic) were of statistical significance for rats challenged with 10 mg/kg ($P < .005$, t test) only, but not for the 15–25 mg/kg doses of morphine (acute). The within-sex between-group differences in the total dose of morphine were not of statistical significance. Furthermore, there was no correlation between the duration of treatment and/or total morphine dose (during tolerance development) and analgesia score for acute morphine in individual rats.

4. Discussion

The present study confirmed that the chronic administration of morphine (7 mg/kg ip twice/day) resulted in the development of tolerance to the analgesic effect in male and female rats. Decreased responsiveness to morphine was not due to adaptation to the repeated application of noxious stimuli since sensitivity to radiant heat (baseline reaction time in the tail-flick assay) was similar in naïve and morphine-treated rats. It is worth noting that the responsiveness to saline (1 ml/kg ip) in the tail-flick test was similar in male and female rats (Holtman et al., 2003a). In addition, male and female rats demonstrated similar responses to saline (administered subcutaneously) in the hot-plate and tail-withdrawal tests (Bartok and Craft, 1997). The present data revealed that the decrease in analgesia was accompanied by an enhancement in motor activity. This is in accord with the observation that behavioral sensitization occurs as opposed to the development of tolerance to the analgesic effect of morphine (Powell and Holtzman, 2001). The present data on acute morphine (Day 1) replicate the literature by demonstrating greater antinociception (tail-flick test) (Cicero et al., 1996; Kepler et al., 1989; Krzanowska and Bodnar, 1999) and less motor activity (Boyer et al., 1998; Wala et al., 2001) in male rats in comparison to female rats. Similarly, in most reported studies where other nociceptive tests (hot plate, tail withdrawal, abdominal constriction, paw pressure, shock jump) were used, morphine also produced more potent analgesic effects in male than in female rats. However, there are some studies where no sex differences in analgesia were observed, or where more analgesia was produced in female than in male rodents. There is a line of evidence suggesting that several factors such as the pain assay procedure, hormonal status, species, and strain play a role in sexual dimorphism (see Craft, 2003b; Fillingim and Ness, 2000; Mogil et al., 2000, for reviews). Our data

revealed that the initial sex-related differences in the analgesic and motor effects of morphine were abolished during chronic (twice/day) treatment. This was also observed after intermittent (one dose/week) morphine administration (Craft et al., 1999b). Current and previous data indicate that sexually dimorphic modulation of antinociception seems to be related exclusively to the acute administration of morphine in rats.

Overall, a greater percentage of female rats than male rats exhibited tolerance to the analgesic effect of morphine at Days 3–11 of repeated treatment. However, it is important to notice that the initial analgesic responsiveness to morphine was considerably lower in female than in male rats (%MPE ≈ 40% vs. 70%, Day 1). Accordingly, a proportionally shorter period would be expected for the %MPE to decline towards the preinjection baseline in female rats compared to male rats. Thus, it is likely that after administration of equipotent doses of morphine, tolerance to analgesia would be achieved at the same time in male and female rats. The observation that one-half of the morphine-treated female rats showed tolerance (%MPE < 5) about twice as fast as one-half of morphine-treated male rats suggests that the rate of development of tolerance (defined as a loss of analgesic potency over time) was similar in both sexes. Although the present data seem to be contradictory to previous reports on sex differences in the development of tolerance in rats (Craft et al., 1999b; South et al., 2001) and mice (Kest et al., 2000a), the interpretation of the results as well as the experimental design may be the reason for the discrepancy. In the first study, where morphine was administered for one or two weeks (10 and 20 mg/kg twice daily, hotplate and tail withdrawal tests), the differences in the rates of tolerance development were determined by the magnitude of potency ratios after cumulative doses of morphine (on Days 8 and 15 of chronic administration and on Days 22 and 43 at withdrawal). The data revealed that the decrease in potency of morphine and the rate of recovery was greater in male than in female rats (Craft et al., 1999b). In the second study, where morphine was infused intravenously, short-term (5 or 10 mg/day; 48 h), the surprisingly lower initial analgesic effect of morphine (hot plate) in male than in female rats (20 vs. 40%MPE) corresponded well with the shorter time to reach baseline (approximately 1 vs. 3 h in male and female rats, respectively) (South et al., 2001). In contrast, the initial response to morphine was similar in male and female mice, whereas a greater reduction in morphine analgesia was observed in female relative to male mice following the chronic administration of morphine (escalating dose schedule: 10–40, 20–80, 40–120 mg/kg, three times a day; 3 and 7 days; tail-flick test) (Kest et al., 2000a). It is worthy to note that chronic administration of the same absolute dose of morphine (5, 10, 20 mg/kg twice daily, 7 and 14 days) produced greater tolerance in male than in female F344 and Lewis rats. However, when the data were reanalyzed for the adjusted functional chronic morphine dose (chronic morphine dose/potency of acute morphine under prechronic condition) morphine tolerance was not related to sex (decrease in morphine potency on Day 7 and/or 15 of chronic treatment) (Barrett et al., 2001).

Sex-related differences have also been observed in other aspects of morphine pharmacology, such as discriminative stimulus (Craft et al., 1996, 1999a) and reinforcing properties (Cicero et al., 1999, 2003) of the drug. In addition, sex-related differences in the development of physical dependence have been characterized. With regard to the latter, the score for naloxone-induced withdrawal was greater in male than in female rats repeatedly exposed to morphine (10 mg/kg sc twice daily; 5 days) (Craft et al., 1999b). In contrast, no sex difference in naloxone-precipitated withdrawal was found in rats chronically treated with either daily injections of morphine (escalating dose 10–40 mg/kg sc, three times a day; 14 days) or continuous exposure to morphine pellets, 75 mg; 3 days) (Cicero et al., 2002a). However, the spontaneous withdrawal syndrome was more severe and lasted longer in male than in female rats. Sex-related differences in naloxone-precipitated abstinence (male>female) were observed in mice following an acute administration of morphine (25–100 mg/kg) but not after chronic administration of morphine via daily injection (escalating dose schedule: 10–40, 20–80, 40–120 mg/kg, three times a day; 3 or 7 days) or continuous infusion (3.4 mg/kg/24 h; 7 days; osmotic pump) (Kest et al., 2001). Finally, chronic morphine exposure has been shown to produce sex differences in regard to corticosteroid-binding globulin and corticosterone levels (Nock et al., 1998).

It should be emphasized that in the present study, morphine was terminated upon the development of tolerance to analgesia in each rat; whereas in previous experiments reported by others, rats or mice of both sexes were exposed to morphine for a fixed period. Under these circumstances, it is likely that either morphine was terminated before the development of tolerance in some rats, or that tolerant rats continued to receive morphine. The latter may be important in that an exaggerated nociceptive response to noxious stimuli (thermal hyperalgesia) occurs in association with tolerance (see Mao, 1999; Vanderah et al., 2001, for reviews). Data from our laboratory revealed that opioid-induced hyperalgesia differs in male and female rats (Holtman et al., 2002; Wala et al., 2002).

The present study indicates that a dose-related response to morphine persists in tolerant rats. In contrast to sex-related differences in morphine analgesia in nontolerant rats ($ED_{50} = 2.92 \pm 0.38$ and 6.17 ± 1.27 mg/kg for male and female rats, respectively, tail-flick test) (Holtman et al., 2003b), the analgesic effectiveness of morphine was similar in tolerant male and female rats ($ED_{50} \approx 13$ mg/kg). Consequently, the potency of morphine was reduced to a greater extent in tolerant male than in tolerant female rats (ED_{50}

increased approximately 3.5- and 1.2-fold, respectively). The same trend was reported for a cumulative dosing procedure and hot-plate test (6.9- and 3.7-fold increase in ED_{50} in male and female rats, respectively) (Craft et al., 1999b) as well as for daily injections and tail-flick test (3.3- vs. 2.4-fold in male and female rats, respectively) (Kasson and George, 1984). Taken together, the accumulated data demonstrate that the initial greater responsiveness to morphine in male rats is abolished at tolerance.

The possible mechanisms underlying sexual dimorphism in the analgesic effect of acute morphine have been extensively studied. The contributions of pain threshold, pharmacokinetics, neuronal circuitry, population and distribution of receptors, and adaptation to aversive events have been considered, but the results are not conclusive (see Craft, 2003a,b; Kest et al., 2000b; Miaskowski et al., 2000, for reviews). Furthermore, data on the effect of gonadal steroid hormones on acute morphine analgesia also are conflicting (see Fillingim and Ness, 2000, for a review). Recent data suggest that the sex differences in morphine analgesia are related to organizational effects of gonadal steroids during development of the brain but not to their acute activational effect in the adult rat (Cicero et al., 2002b). Some investigators report that the effect of the estrous cycle should also be taken under consideration since the baseline responsiveness appears to be lower during estrus in rats (Kepler et al., 1989; Martinez-Gomez et al., 1994; Stoffel et al., 2003). There are conflicting data, however, which show a similar potency of morphine at proestrus/diestrus and lower analgesia at estrous (Stoffel et al., 2003), no differences between estrus and diestrus (Kepler et al., 1989), and greater sensitivity to morphine at diestrus than in proestrus (Berglund and Simpkins, 1988). These studies indicate that the affect of the estrous cycle on morphine analgesia is complex and not well defined in rats. It should be emphasized that although the estrous cycle was not evaluated in the present study the antinociceptive response to morphine was normalized for preinjection baseline each day in each rat. This approach was taken to eliminate a possible artifact due to fluctuation of basal nociception in different phases of the estrous cycle. The fact that (1) female rats at diestrus showed morphine analgesia (acute) approximately equal to their male counterparts (Stoffel et al., 2003) and (2) that chronic morphine decreased proestrus and estrus days and increased diestrus days (Craft et al., 1999b) may suggest that similar morphine potency in tolerant female and male rats is at least partially related to inhibition of the estrus cycle during chronic morphine treatment.

The present data suggest that the mechanisms responsible for the sex-specific intrinsic reactivity of morphine become desensitized during the development of tolerance. There is a line of evidence that tolerance to opioids is linked to the NMDA receptors (see Mao, 1999; Price et al., 2000, for reviews). Recent data from our laboratory revealed that the addition of NMDA receptor antagonists diminished sex differences in acute morphine analgesia in rats (Holtman et al., 2003a). Whether an interaction between the NMDA and opioid receptors applies to the abolishment of sexual dimorphism of morphine as tolerance develops warrants further testing.

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