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Gender-related Differences in the Antinociceptive Properties of Morphine[1]

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ABSTRACT

As part of an effort to examine gender-related differences in the abuse liability of morphine, the present parametric study was undertaken to systematically establish whether there are gender-related differences in the antinociceptive activity of morphine in rats. Our results showed that male rats were uniformly more sensitive to the antinociceptive properties of morphine than were females in three different assays, *i.e.*, the hot-plate, tail-flick and abdominal-constriction tests. This enhanced sensitivity to morphine was reflected in the peak antinociceptive effect, the magnitude of antinociception (*i.e.*, area under the time-action curve), the duration of the antinociceptive response and the 50% effective dose. These differences appear to reflect markedly enhanced central nervous system sensitivity to morphine in males, compared with females, because we observed no gender-linked differences in serum levels of morphine after its injection, at the time when peak antinociceptive effects were observed. Furthermore, these gender-related differences appear to be reflected in antinociception thought to be mediated by both spinal and supraspinal mechanisms. Finally, our results suggest that the acute effects of steroids play little role in the gender-related differences observed, because short-term castration did not alter the gender-related differences we observed. Rather, it appears more probable that the organizational effects of steroids during critical periods in development, which determine gender-related distinctions, may be significant in the male-female differences we have observed. In view of a great deal of largely anecdotal data for humans that suggest that there may be gender-related differences in the abuse liability of psychoactive substances, the model described in this paper may provide a means to examine this important issue.

It has been suggested that there are gender-related differences among humans in the acute pharmacological response to psychoactive drugs, their reinforcing properties and the development of tolerance and physical dependence and perhaps in drug addiction treatment and outcome (Griffin *et al.*, 1989; Lex, 1991; Bailey *et al.*, 1993; Kosten *et al.*, 1995; Rapp *et al.*, 1995). Despite the importance of these issues, it is surprising that there are few systematic studies that have rigorously documented the nature and characteristics of gender-related differences in the acute and chronic effects of psychoactive drugs with substantial abuse potential.

As part of an effort to explore gender-related differences in the acute response to morphine and the subsequent development of tolerance and physical dependence, we have examined whether there are male/female differences in opiate-induced antinociception in rats. A number of previous studies have examined whether there are gender-linked differences in the antinociceptive activity of morphine and, in a more general sense, opioid-induced antinociception. Although no gender-related differences in antinociceptive activity induced by μ-opiate agonists have been found in some experiments (Grossman *et al.*, 1982; Kepler *et al.*, 1991; Islam *et al.*, 1993), in many studies male/female differences have been found in terms of both morphine-induced antinociception and opioid-mediated antinociception induced by stress (*e.g.*, Romero and Bodnar, 1986; Kavaliers and Innes, 1987a,b, 1988; Romero *et al.*, 1988). When gender-related differences have been found, males have generally been observed to be more sensitive than females.

Despite the existing literature related to gender differences in opioid-induced antinociception, a number of questions remain unanswered. For example, we are unaware of a single study that has determined whether serum morphine levels are equivalent in males and females at doses producing maximal gender-related differences in the antinociceptive activity of morphine. Thus, the possibility exists that any gender-related differences observed in the antinociceptive activity of morphine may be related more to gender-derived differences in the bioavailability of morphine than to intrinsic differences in brain sensitivity to the drug. In addition,

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ABBREVIATIONS: CNS, central nervous system; ED_{10}, 10% effective dose; ED_{50}, 50% effective dose; ED_{90}, 90% effective dose; MPE, maximal possible effect.

whereas several investigators have carried out extensive dose- and time-response analyses and used several antinociceptive tests to examine gender-related differences in opioid-mediated analgesia (*e.g.*, Kavaliers and Innes, 1987a,b; Kepler *et al.*, 1989), in many studies only one antinociceptive test was used and limited dose- and time-response information was obtained. Because it is well established that antinociceptive assays in animals assess different aspects of the analgesic activity of morphine (*e.g.*, spinal *vs.* supraspinal), the use of a single assay raises the possible confounding factor of assay variability and, more importantly, limits the generality of the conclusions that can be drawn. Moreover, the pharmacological limitations inherent in restricted dose- and time-response analyses restrict any conclusions that can be drawn about intrinsic gender-related differences in the sensitivity to morphine.

The role of the sex steroids in the antinociceptive effects of morphine has also been examined. Castration and ovariectomy have been found to increase, decrease or leave unchanged opioid-mediated antinociception (Chatterjee *et al.*, 1982; Romero *et al.*, 1987, 1988; Bodnar *et al.*, 1988; Kepler *et al.*, 1989; Islam *et al.*, 1993). All of the factors outlined above (*i.e.*, antinociceptive assay variability and restricted dose-response analysis) may contribute to these discrepancies, but in addition the time after castration or ovariectomy has been variable. Because steroids exert short-term membrane-mediated effects and long-term organizational effects on the brain (*e.g.*, Young, 1961; Arnold and Breedlove, 1985; Breedlove, 1992, 1994), the time after castration is a critical confounding variable.

The present parametric study was undertaken to systematically establish whether there are gender-related differences in the antinociceptive activity of morphine in male and female rats and to evaluate whether sex steroids may be involved in these differences. To ensure that assay variability did not confound the studies, three different tests were used, *i.e.*, hot-plate, tail-flick and abdominal-constriction tests. These tests were selected because it is known that they assess different aspects of pain. The hot-plate and tail-flick assays both measure the response to thermal pain, but the tail-flick response is thought to be mediated principally by spinal mechanisms, whereas the hot-plate response is believed to be mediated by supraspinal mechanisms (Hayes *et al.*, 1978; Yaksh, 1981; Grossman *et al.*, 1982). The abdominal-constriction test measures visceral pain induced by a chemical toxicant, as opposed to a thermal stimulus, and is believed to be mediated principally by higher brain mechanisms (Giesler and Liebeskind, 1976; Baamonde *et al.*, 1989). Thus, these three tests presumably measure different aspects of pain control and provide a means to independently assess whether there are gender differences in the antinociceptive activity of morphine and at what level of the CNS such effects may occur.

In addition to using a range of antinociceptive assays, we have also carried out extensive dose-response analyses to define the respective ED_{50} values in males and females (as an index of sensitivity to morphine) and have also measured the magnitude and duration of analgesia. Furthermore, we have measured morphine levels in serum to determine whether the gender-related differences in the antinociceptive effects of morphine we observed could be explained by gross differences in circulating drug levels, as opposed to any intrinsic differences in CNS or spinal cord sensitivity to the drug.

Methods

Materials. Sprague Dawley-derived rats were purchased from Harlan Sprague Dawley (Indianapolis, IN). They were used at 60 to 80 days of age in all studies. Morphine sulfate was purchased from Sigma Chemical Co. (St. Louis, MO). The tail-flick apparatus was purchased from EMDIE Instruments Co. (Maidens, VA), whereas the hot-plate apparatus was obtained from Technilab Instruments, Inc. (Pequannock, NJ). The iodinated morphine radioimmunoassay kit, which was derived from the principles and methods of Yoburn *et al.* (1985), was purchased from Diagnostic Products Corp. (Los Angeles, CA). The antibody is specific for morphine, with <0.03% cross-reactivity with morphine-3-glucuronide and <0.1% cross-reactivity with morphine-6-glucuronide. The lower limit of sensitivity of the assay was 125 pg, and the standard curve was linear over the range of 125 to 2500 pg (r = 0.97). Interassay variation was 3% and intraassay variation was <5% in all studies described in the paper.

Hot-plate test. Groups of male and female rats (n = 12 in each group) were tested on a hot-plate that was set at 58°C to yield base-line (*i.e.*, non-drug-treated) reaction times of 3 to 5 sec. Four drug-free trials were used to establish base-line reaction times. The end-point was defined as the rats licking their paws or jumping out of the cylinder (which occurred rarely); a 30-sec cut-off point was used if no response occurred. The animals were then injected s.c. with morphine and were tested every 30 min for 4 hr. For the ED_{50} determinations, the rats were injected with a range of doses of morphine to define the ED_{50}; in all cases, at least four or five doses yielding responses that fell between the ED_{10} and ED_{90} were used to calculate the ED_{50} (see "Data analysis").

Tail-flick test. Groups of male and female rats (n = 12) rats were assessed for antinociceptive activity in the tail-flick apparatus. The intensity of the radiant lamp was adjusted to provide base-line levels of 3.0 to 3.5 sec; the same setting was used for both genders, because we observed no gender-related differences in base-line reaction thresholds. After a single base-line trial, the rats were injected s.c. with morphine (four or five doses ranging from the ED_{10} to the ED_{90}) and differences in reaction time were observed 30 min later. In contrast to the procedure used in the hot-plate assay, the animals were tested only once after morphine treatment, to avoid unnecessary pain associated with radiant heat-induced damage to the tail. The end-point was a tail-flick response sufficient to interrupt the photoelectric beam or a cut-off point of 21 sec. Data were expressed either as reaction time (in seconds) or as percentage MPE, as defined below (see "Data analysis").

Abdominal-constriction test. Groups of male and female rats (n = 15) were injected i.p. with saline or 1.2% (v/v) acetic acid (2 ml/kg). The number of abdominal constrictions were recorded for 30 min after the acetic acid injection. The rats were pretreated with saline or morphine s.c. 15 min before the injection of acetic acid. For purposes of determining the ED_{50} for morphine, at least five doses were used, falling between the ED_{10} and ED_{90}. The groups pretreated with saline before the acetic acid challenge were used to define the MPE.

Serum levels of morphine. To determine whether the gender-related differences observed in the antinociceptive activity of morphine could be explained by differences in serum morphine levels subsequent to its s.c. injection, morphine serum levels were measured in adult male and female rats injected with 5.0, 7.5 or 15.0 mg/kg morphine. The doses were selected to span the range of doses over which maximal gender-linked differences were observed in the three antinociceptive assays used in these studies. Groups of 10 to 15 animals were sacrificed by decapitation and serum was collected 45 min after the morphine injections, the time at which peak analgesic effects were observed in all assays. Serum levels of morphine were determined with the radioimmunoassay kit described above.

Castration and ovariectomy. To determine whether the gender-related differences we observed in morphine-induced antinociception were the result of the acute effects of steroids, groups (n = 12) of male and female rats were sham-operated, castrated or ovariectomized. Two weeks later, ED_{50} values for the antinociceptive activity of morphine in the hot-plate and tail-flick assays were determined precisely as described above. To confirm that the castration and ovariectomy reduced sex steroids to nondetectable levels, serum levels of testosterone, estrogen and progesterone were measured by radioimmunoassay, as described elsewhere (Cicero and Meyer, 1973; Cicero *et al.*, 1986).

Data analysis. To determine the ED_{50} values in the hot-plate, tail-flick and abdominal-constriction assays, groups of rats were injected with four or five doses of morphine falling between the ED_{10} and ED_{90}. The rats were tested at the time of the maximal antinociceptive effect in each assay (60 min for the hot-plate assay, 30 min for the tail-flick assay and 45 min for the abdominal-constriction test). Data were expressed in terms of absolute reaction time or as percentage of MPE, defined as follows:

$$\% \text{ MPE} = \frac{\text{Actual response time (sec)} - \text{base line (sec)}}{\text{Cut-off time (sec)} - \text{base line (sec)}} \times 100$$

For the hot-plate assay, the area under the time-response curve was calculated by methods described elsewhere (Cicero and Meyer, 1973), to assess the magnitude of antinociception (*i.e.*, peak by time). The duration of the antinociceptive response in the hot-plate assay was defined as the time after the injection of morphine when antinociceptive activity returned to pre-morphine base-line levels (*i.e.*, percentage of MPE = 0%) and was calculated by linear regression analysis of the time-action curve for each animal; means ± S.E.M. were then calculated.

All differences between males and females were analyzed by analysis of variance followed by *post hoc* analysis. The ED_{50} determinations and the 95% confidence limits were determined by nonlinear regression analyses using the computer program PRISM (GraphPad Inc., San Diego, CA).

Results

Hot-plate test. A single dose of morphine produced marked gender-related differences in the antinociceptive activity of morphine. As shown in figure 1, a dose of morphine of 12.5 mg/kg produced prolonged reaction times on the hot-plate (*i.e.*, antinociception) in both males and females. However, significantly more antinociceptive activity was produced in males, as reflected in the peak increase in reaction time and the area under the time-action curve. The apparent gender-related difference in the sensitivity to morphine at a single dose was confirmed by evaluating the ED_{50} values 60 min after morphine administration. As shown in figure 2, the dose-response curve for females was shifted markedly to the right, compared with that for males; the ED_{50} was 6.9 mg/kg (95% confidence limits, 6.25–7.28 mg/kg) in males, compared with 11.3 mg/kg (95% confidence limits, 10.84–11.49 mg/kg) in females. The magnitude of the antinociceptive activity of morphine was also considerably greater in males than in females, as determined by the area under the time-response curve (fig. 3). Finally, as shown in figure 4, the duration of analgesia was also significantly greater in males than in females at comparable doses. We observed no differences between the genders in base-line reaction times on the hot-plate.

Tail-flick test. At single doses of morphine, males displayed at least twice as much antinociceptive activity as females in the tail-flick assay. As shown in figure 5, this difference was reflected in the respective ED_{50} values; the ED_{50} in males was 4.59 mg/kg (95% confidence limits, 4.16–5.03 mg/kg), compared with 8.17 mg/kg (95% confidence limits, 7.61–8.71 mg/kg) in females. As was true in the hot-plate test, we found no gender-related differences in base-line reaction times in the tail-flick assay.

Abdominal-constriction test. The dose-response curves for morphine-induced inhibition of abdominal constriction produced by acetic acid are shown in figure 6. As was the case with the tail-flick and hot-plate assays, morphine produced markedly more antinociceptive activity in males, compared with females; the ED_{50} in males was significantly lower than that observed in females [ED_{50} = 0.14 mg/kg (95% confidence limits, 0.11–0.18 mg/kg) in males *vs.* 0.53 mg/kg (95% confidence limits, 0.52–0.54) in females].

Serum levels of morphine. Figure 7 shows the serum levels of morphine in males and females 45 min after the injection of 5.0, 7.5 or 15.0 mg/kg morphine. As can be seen,

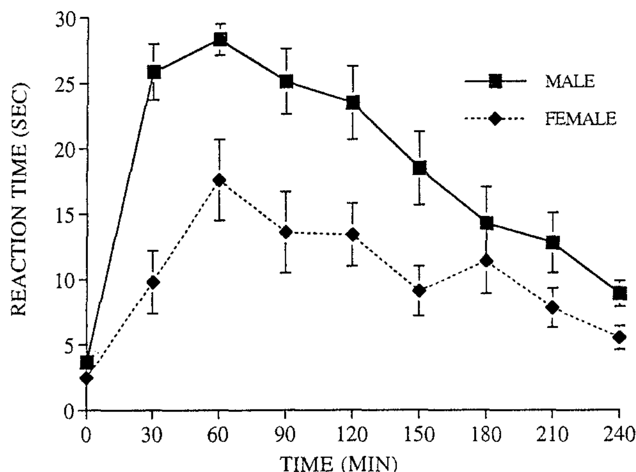

Fig. 1. Reaction times in the hot-plate test for male and female rats (n = 12 in each group) at the time intervals shown. Base-line (*i.e.*, non-drug) reaction times were recorded (time 0), morphine (12.5 mg/kg) was injected immediately after time 0 and reaction time was measured at the intervals shown, for 4 hr. Values are means ± S.E.M. Repeated-measures analysis of variance demonstrated significant ($P < .001$) gender- and time-related differences.

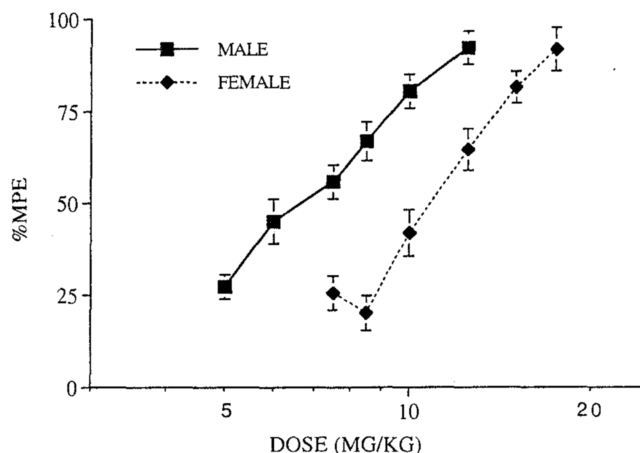

Fig. 2. Dose-response curves for male and female rats in the hot-plate test. Data are expressed as percentage of MPE, as defined in the text. The data represent means ± S.E.M. of three to five replications for each dose (n = 10–12/replicate). Analysis of variance demonstrated significant ($P < .001$) differences between males and females.

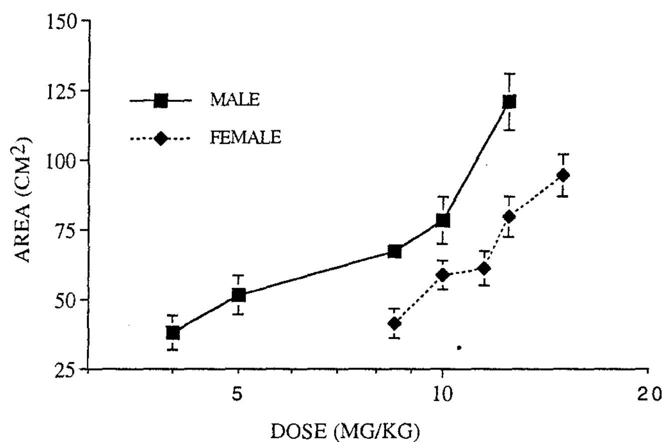

Fig. 3. Area under the analgesic curve for the data shown in figure 2 (mean ± S.E.M.) as a function of dose. The data were expressed as reaction time (in seconds) after morphine administration minus the base-line reaction time for the 4 hr after the injection of morphine. The area, which was determined by methods described elsewhere (Cicero and Meyer, 1973), was determined separately for each rat used in the studies shown in figure 2. Analysis of variance demonstrated significant (P < .001) differences between males and females.

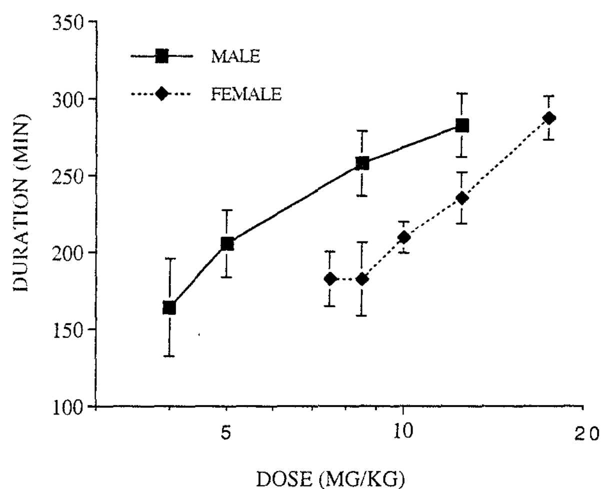

Fig. 4. Duration of analgesia for the rats depicted in figures 2 and 3, plotted as a function of dose. Duration was defined as the time when the percentage of MPE reached zero, as determined by nonlinear regression (with the computer software program PRISM) of the decay curve for the antinociceptive response. The *y*-intercept was determined for each animal used in the experiments described in figures 3 and 4, and the means ± S.E.M. were determined. Analysis of variance demonstrated significant (P < .001) differences between males and females.

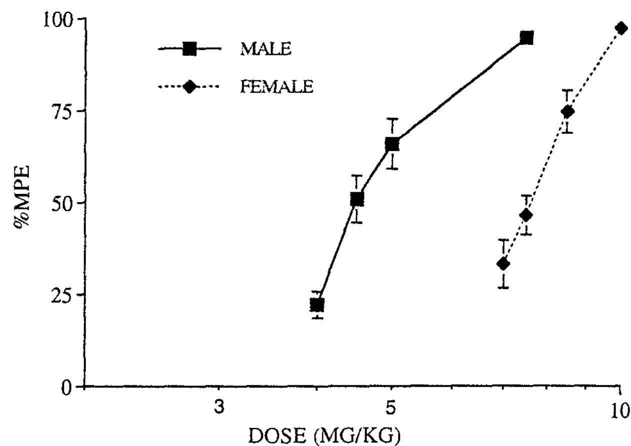

Fig. 5. Dose-response curves for male and female rats in the tail-flick test. Data are expressed as percentage of MPE, as defined in the text. The data represent means ± S.E.M. of three to five replications for each dose (n = 10–12/replicate). Analysis of variance demonstrated significant (P < .001) differences between males and females.

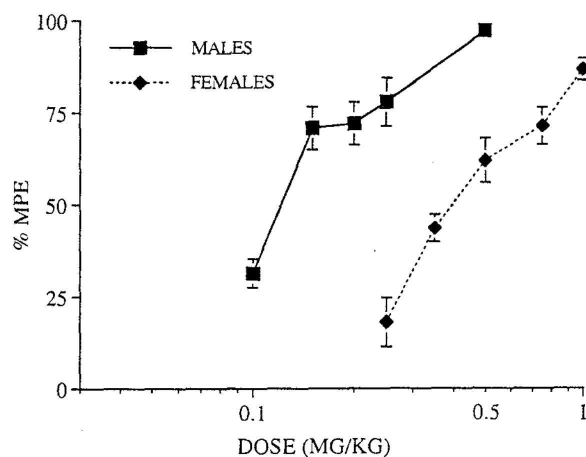

Fig. 6. Dose-response curves for male and female rats in the abdominal-constriction test. Data are expressed as percentage of MPE, as defined in the text. The data represent means ± S.E.M. of two replications for each dose (n = 15/replicate). Analysis of variance demonstrated significant (P < .001) differences between males and females.

there were no differences between males and females in the serum morphine levels attained at the time maximal differences in the antinociceptive activity of morphine were observed (figs. 1–6).

Effects of castration on morphine-induced antinociceptive activity. Castration and ovariectomy 2 weeks before testing produced no discernible effects on the antinociceptive activity of morphine in males or females in the hot-plate test (fig. 8) or tail-flick assay (fig. 9). The dose-response curves were overlapping, with no observed differences in the ED_{50} values as a result of changes in gonadal status. In addition, there were no differences found in base-line reaction times between castrated or sham-operated males and females in either the hot-plate or tail-flick assays (data not shown). Sex steroid levels were nondetectable at the 2-week postcastration interval used.

Discussion

The results of these studies demonstrate marked gender-related differences in the antinociceptive activity of morphine. Males were uniformly more sensitive to the antinociceptive properties of morphine in three different assays, *i.e.*, the hot-plate, tail-flick and abdominal-constriction tests. This enhanced sensitivity to morphine was reflected in the peak analgesic effects and the ED_{50} determinations for all three assays and, in the case of the hot-plate test, the total magnitude of analgesia (*i.e.*, the area under the time-action curve) and the duration of the antinociceptive response. These differences in the antinociceptive response to morphine do not seem to be related to differences in the availability of morphine after its acute s.c. injection, because equivalent serum levels were found in male and female rats. Thus, the differences observed apparently reflect intrinsic

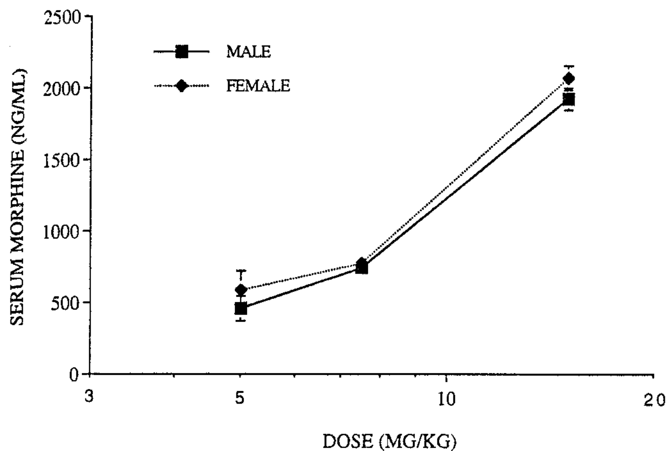

Fig. 7. Serum levels of morphine (mean ± S.E.M.) in male and female rats (n = 15) injected with 5.0, 7.5 or 15.0 mg of morphine and killed 45 min later. Analysis of variance revealed no significant gender differences.

gender-related differences in sensitivity to the effects of morphine at its site of action.

Our results are in general agreement with a number of previous studies that have demonstrated gender-related differences in the antinociceptive activity of morphine and opioid-mediated antinociception induced by stressors (Romero and Bodnar, 1986; Kavaliers and Innes, 1987a,b, 1988; Romero *et al.*, 1988; Baamonde *et al.*, 1989; Kepler *et al.*, 1991). However, our studies have extended these observations and, more importantly, addressed a number of important issues that previously had not been systematically examined. For example, although several groups have used full dose- and time-response analyses and multiple assays to assess the generality of gender-linked differences in opioid-mediated analgesia (*e.g.*, Kavaliers and Innes, 1987a,b; Kepler *et al.*, 1989), in most studies only single antinociceptive tests and/or single doses of opiate at single postinjection time intervals were used. Thus, conclusions regarding the generality of gender-related differences in opiate-mediated antinociception and the magnitude of these differences have been difficult to make.

In the studies described in this paper, we have attempted to avoid all of these potentially confounding variables and extend previous studies in this important area. Specifically, to ensure that assay variability did not contribute to any gender-related differences in the antinociceptive activity of morphine, three tests were used, *i.e.*, hot-plate, tail-flick and abdominal-constriction tests. The hot-plate and tail-flick assays both measure the response to thermal pain, but the tail-flick response is thought to be mediated principally by spinal mechanisms, whereas the hot-plate response is believed to be mediated by supraspinal mechanisms (Hayes *et al.*, 1978; Yaksh, 1981; Grossman *et al.*, 1982). The abdominal-constriction test measures visceral pain induced by a chronic toxicant, as opposed to a thermal stimulus, and is believed to be mediated principally by higher brain mechanisms (Giesler and Liebeskind, 1976; Baamonde *et al.*, 1989). By using these three tests, which clearly measure different aspects of pain control, we have provided the most comprehensive data obtained thus far to independently assess whether there are gender-related differences in the antinociceptive activity of morphine and at what site such effects may be mediated. Our data clearly indicate that, first, the gender-related differences are not assay specific and, second, these differences appear to occur at both the spinal and supraspinal levels, at least to the extent that these assays reflect these mechanisms. Clearly, however, because our studies involved only systemic injections, more definitive data must be obtained using intraspinal and intracranial injections before conclusions regarding site-specificity can be considered definitive.

The mechanisms underlying the gender-related differences observed in the present studies are unknown. It seems doubtful, however, that differences in the bioavailability of morphine after its s.c. injection are involved. As discussed above, we found that males and females attained the same peak morphine levels in serum at doses of morphine that produced maximal gender-related differences in the antinociceptive activity of morphine (fig. 7). Thus, it seems unlikely that the bioavailability of morphine can explain the extremely large differences observed between males and females in the present studies at the time at which peak analgesic responses were found. However, these conclusions must be tempered by the fact that we measured morphine levels only after administration of these representative doses of morphine, and it is possible that more extensive dose-response analyses and determinations of morphine elimination rates may reveal that pharmacokinetic differences could be involved in the gender-related differences that we and others

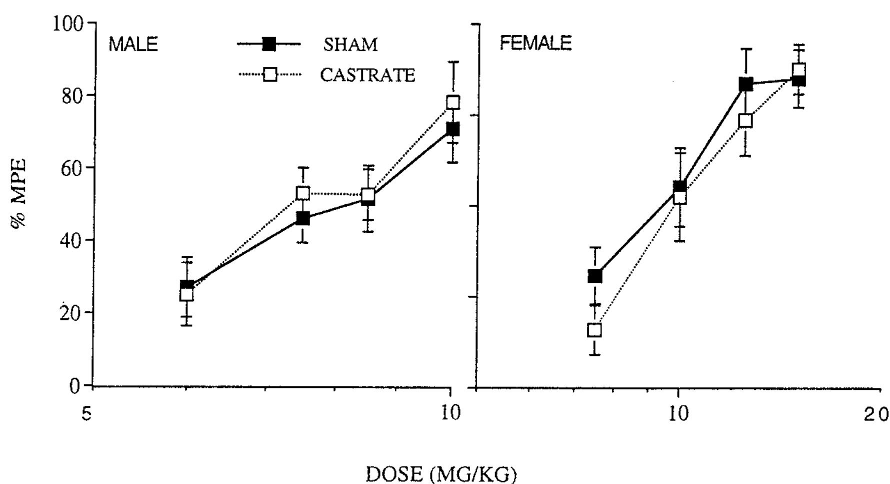

Fig. 8. Dose-response curves for antinociceptive activity in male and female rats sham-operated (SHAM) or castrated (CASTRATE) 2 weeks before testing in the hot-plate assay. Data are means ± S.E.M. of three replications (n = 12 in each study); the studies were carried out identically to those shown in figures 1 to 4. Analysis of variance revealed no significant effects of gonadal status in either males or females.

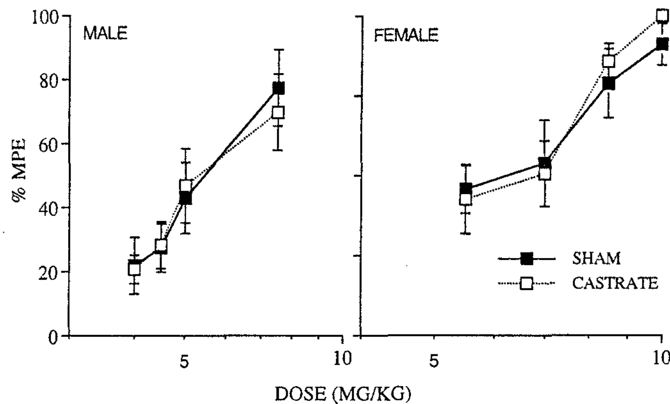

Fig. 9. Dose-response curves for antinociceptive activity in male and female rats sham-operated (SHAM) or castrated (CASTRATE) 2 weeks before testing in the tail-flick assay. Data are means ± S.E.M. of three replications (*n* = 12 in each study); the studies were carried out identically to those shown in figure 5. Analysis of variance revealed no significant effects of gonadal status in either males or females.

have observed. Furthermore, it is possible that brain levels of morphine, particularly in regions mediating the antinociceptive activity of morphine, were much lower in females than males, but it is difficult to envision any mechanism by which this could occur. Nevertheless, this possibility is being explored, as well as more extensive pharmacokinetic analyses.

If one makes the logical assumption that the gender-related differences observed in the present studies are in some manner the result of differences in CNS sensitivity to morphine, one reasonable hypothesis is that there are differences between males and females in the number or affinity of the opiate receptors involved in mediating antinociception or the biochemical reactions triggered by receptor occupancy. Although we are unaware of any data suggesting gender-related differences in opioid receptor profiles in those areas mediating analgesia, a number of earlier studies have suggested relatively large gender-linked differences in the number and regional distribution of opioid receptors, particularly in so-called sexually dimorphic regions in males and females (*e.g.*, Hammer, 1984, 1985, 1990; Hammer *et al.*, 1994). Whether such differences can be found with respect to the opioid receptors mediating antinociception remains to be determined.

One prominent explanation of gender-related differences in the response to opiates is, of course, that sex steroids may mediate these effects. In some previous studies, castration and ovariectomy influenced the antinociceptive response to morphine in the tail-flick assay. For example, in some experiments castration was reported to enhance the sensitivity of males to morphine and in others ovariectomy was reported to decrease the sensitivity of females to opioids, in effect equalizing the two genders (*e.g.*, Romero and Bodnar, 1986; Bodnar *et al.*, 1988; Romero *et al.*, 1988; Baamonde *et al.*, 1989; Islam *et al.*, 1993). However, in at least one study no effects of castration or ovariectomy on morphine-induced antinociception were observed (Kepler *et al.*, 1989). In the present studies, we used two assays, the hot-plate and tail-flick assays, which presumably reflect supraspinal and spinal antinociception, respectively. In both of these assays, we observed no shift in the dose-response curves for castrated or ovariectomized male or female animals. These data suggest that the gender-related differences we have observed in morphine-induced antinociceptive activity are not dependent upon the acute membrane- mediated effects of the steroids. We cannot explain the discrepancy between the present results and those reported previously in some (*e.g.*, Romero *et al.*, 1987, 1988; Islam *et al.*, 1993) but not all (*e.g.*, Kepler *et al.*, 1989) studies in which it was claimed that castration produced changes in the antinociceptive activity of morphine. However, this disparity may be the result of the time after castration when antinociceptive activity was assessed, the antinociceptive test used or pharmacological variables, such as the use of single doses of morphine at one time point.

There are, of course, two mechanisms by which sex steroids could mediate gender-based differences in the response to morphine, *i.e.*, acute "activational" effects (Young, 1961) and, perhaps more importantly, long-term organizational effects (Goy *et al.*, 1964; Arnold and Breedlove, 1985; Breedlove, 1992, 1994). Our data suggest that the acute effects of steroids do not play a major role in mediating the antinociceptive effects of the opiates. By inference, our data suggest that the gender-related differences we have observed are the result of intrinsic gender-related differences generated by the organizational effects of steroids, which have been shown to mediate sexual differentiation of brain morphology and neurobiology (Breedlove, 1992, 1994). These effects generally occur at the very late prenatal or early postnatal period, rather than in adulthood. Studies are underway to determine whether alterations in steroid levels during the critical periods in which sexual differentiation occurs can alter the antinociceptive activity of morphine in adult male and female animals.

In conclusion, our experiments conclusively demonstrate pronounced gender-related differences in the antinociceptive effects of morphine. These differences appear to reflect markedly enhanced CNS sensitivity to morphine in males, compared with females, as opposed to any intrinsic differences in the bioavailability of morphine. Furthermore, these gender-related differences appear to exist at both the spinal and supraspinal levels. Finally, our results suggest that the acute effects of steroids play little role in the gender-related differences observed; rather, it appears more probable that the organizational effects of steroids, which occur in the late prenatal and early postnatal stages and in large part determine gender-related distinctions in males and females, may be more significant. The mechanisms underlying these striking gender-related differences are unknown, as is their clinical significance at this point. However, in view of a great deal of largely anecdotal data for humans, which suggest that there may be gender-related differences in the acute effects of psychoactive substances and their abuse liability (Griffin *et al.*, 1989; Lex, 1991; Bailey *et al.*, 1993; Kosten *et al.*, 1995; Rapp *et al.*, 1995), our data may provide a means to begin examining this important issue.

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