Sex Differences in Supraspinal Morphine Analgesia Are Dependent on Genotype

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ABSTRACT

Several variables have been reported to affect the expression of sex differences in the analgesic potency of morphine. Although the effect of genetic background on morphine analgesia has been well documented, the relevance of genotype to sex differences in morphine analgesia has rarely been considered. The present study investigated morphine dose-response relationships in male and female mice of 11 inbred mouse strains on the tail-withdrawal test after i.c.v. administration. Large differences in morphine analgesic potency were observed between strains, reflecting the important influence of genotype on this trait. We identified three strains (AKR/J, C57BL/6J, and SWR/J) in which males displayed approximately 3.5- to 7.0-fold greater sensitivities to the analgesic effects of morphine than did their female counterparts. In contrast, in the CBA/J strain, females were found to be approximately 5-fold more sensitive to morphine than were the males. In all other strains, morphine potency estimates between the sexes were not statistically different. These data support the importance of genotype, sex, and their interaction in the mediation of morphine analgesia and suggest that equivocal findings regarding opioid sex differences in the literature may be partially accounted for by the use of different subject populations. The fact that female mice of the AKR/J and CBA/J strains exhibit 35-fold different morphine analgesic potency and that males of these strains are equally sensitive should facilitate the mapping and identification of sex-specific genes of relevance to morphine analgesia.

The analgesic effect of morphine in humans is subject to wide individual differences (Lasagna and Beecher, 1954), and it is becoming increasingly appreciated that sex differences may contribute to this variability (for reviews, see Berkley, 1997; Kest et al., 1998). Our understanding of the contribution of sex differences in morphine analgesia has been largely aided by research in rodent populations. For example, the peak, total, and duration of morphine analgesia after systemic administration have been shown to be greater in males than in females in both rats (Baamonde et al., 1989; Islam et al., 1993; Cicero et al., 1996) and mice (Kavaliers and Innes, 1987; Lipa and Kavaliers, 1990; Candido et al., 1992; Kavaliers and Innes, 1992a, b). A strong case that sex differences in morphine analgesia may be mediated by differential central nervous system mechanisms can be made based on the observations that male rats display greater analgesic effect than females after the administration of morphine into the cerebral ventricles (Kepler et al., 1989) or the rostral ventromedial medulla (Boyer et al., 1998).

However, the effect of sex on morphine analgesia remains equivocal because several studies have failed to observe significant sex differences (Kasson and George, 1984; Islam et al., 1993; Ali et al., 1995). This lack of consistent findings may reflect methodological differences between studies. Indeed, among the variables that have been demonstrated to affect sex differences in opioid analgesia are dose (Kepler et al., 1989; Bartok and Craft, 1997), nociceptive assay used (Baamonde et al., 1989; Islam et al., 1993; Bartok and Craft, 1997), postinjection testing latency (Bartok and Craft, 1997), time of testing (i.e., circadian rhythmicity) (Kavaliers and Innes, 1987), and subject age (Islam et al., 1993). Not usually considered is the well documented fact that the analgesic potency of morphine can vary between subpopulations of a single species (for a review, see Mogil et al., 1996b). For example, large differences in morphine analgesia have been reported between compared selectively bred, inbred, and recombinant inbred mouse strains, reflecting a strong genetic component in the mediation of morphine analgesic sensitivity. However, experiments directly addressing the issue of whether the expression of sex differences in morphine or opioid analgesia varies with genotype are rare. One study reported that the magnitude of sex differences in morphine

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ABBREVIATIONS: AD50, half-maximal analgesic dose; %MPE, maximum possible effect; CI, confidence interval; NMDA, N-methyl-D-aspartate; SW, Swiss-Webster; QTL, quantitative trait locus.
Sex and Genetic Effects on Morphine Analgesia

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analgesia (males > females) differed between Sprague-Dawley and Wistar-Furth rats (Kasson and George, 1984). We observed greater analgesia from the enkephalin-derived μ-opioid receptor agonist [d-Ala²,N-MePhe⁴,Gly-ol⁵]-enkephalin in male than in female mice selectively bred for high-, but not low-, stress-induced analgesia (Kest et al., 1995). In an intriguing finding, Rady and Fujimoto (1997) report that heroin analgesia is mediated alternately by μ- and δ-opioid receptors, respectively, in ICR and Swiss-Webster (SW) strains; this genetic difference displays sex-influenced dominant inheritance, such that male (ICR × SF₁) hybrids display the ICR phenotype, whereas female SF₁ hybrids display the SW phenotype. Finally, endogenous opioid analgesic mechanisms, which are known to represent the substrate on which morphine acts to produce pain inhibition, are activated by forced cold-water swims in inbred male DBA/2 mice but not in female mice of this same strain or in C57BL/6 mice of either sex (Mogil and Belknap, 1997). These studies all suggest that genotype (i.e., allelic differences at relevant genetic loci) may contribute to sex differences in opioid analgesia and that the use of different subject populations may be at least partially responsible for the ambiguous state of the literature regarding these differences.

The aim of the present study was to begin a more comprehensive examination of the putative interaction of genotype and sex in the potency of morphine analgesia. Toward this end, morphine half-maximal analgesic dose (AD₅₀) estimates in male and female mice of 11 inbred strains were compiled using the tail-withdrawal test. To minimize the possible influence of sex differences in morphine pharmacokinetic parameters (Candido et al., 1992; Craft et al., 1996; but see Cicero et al., 1996, 1997), we used the i.c.v. route of administration. The data demonstrate that the magnitude and direction of sex differences in morphine analgesia are dependent on genotype.

Materials and Methods

Strains. Naïve, adult mice of both sexes (n = 8/sex/strain) of the following inbred strains were obtained from The Jackson Laboratory (Bar Harbor, ME): 129/J, A/J, AKR/J, BALB/cJ, C3H/HeJ, C57BL/6J, CBA/J, LP/J, SJL/J, and SW/J. For clarity, the J substrain identifier will be omitted. All mice were shipped at 6 weeks of age (except BALB/c mice, which are only available at 6-8 weeks of age, housed four to a cage in a temperature-controlled (22°C) environment, and maintained on a 12:12-h light/dark cycle (lights on at 7:00 AM). Mice were allowed free access to food (Purina chow) and water. Testing occurred at 7 to 8 weeks of age in all cases.

Morphine Administration. Morphine sulfate was generously supplied by the National Institute for Drug Abuse (Rockville, MD) and was dissolved in 0.9% physiological saline. Injections were made into the lateral cerebral ventricle according to the method of Haley and McCormick (1957) with animals under oxygen/isoflurane inhalant anesthesia. Briefly, a small midline incision was made in the scalp of anesthetized mice, and the lambda was located. Drug then was injected directly through the skull at a point 2 mm rostral and lateral to the lambda at a depth of 3 mm using a 10-μl Hamilton microsyringe with a 27-gauge needle. All injections were made in a volume of 5 μl. After each injection, the incision was closed with a stainless steel wound clip.

Tail-Withdrawal Assay. Testing proceeded in two daily sessions near mid-photophase (10:00 AM to 12:00 noon; 2:00-4:00 PM) to reduce circadian effects on nociceptive and analgesic sensitivity. Because of availability restrictions and practical considerations, strains were tested in groups based on their arrival date (group 1: AKR, C3H/He, SJL, SWR; group 2: BALB/c, C57BL/6, CBA; group 3: 129, A, LP). Within each group, however, both strain and sex were completely counterbalanced, so one mouse per strain per sex was tested in each session.

After a 30-min habituation to the testing room, mice were assayed for baseline nociceptive sensitivity on the 49°C tail-withdrawal test. In this assay of acute, thermal nociception, the mouse is gently restrained, and the distal half of the tail is immersed in water maintained at 49.0 ± 0.2°C with an immersion circulator pump (Fisher Isotemp model 71). Latency to reflexive withdrawal of the tail was measured twice by an experimenter to the nearest 0.1 s, with each determination separated by 20 s. The two determinations were then averaged. The tail-withdrawal test was chosen because of its stability even after repeated exposures at this highly noxious water temperature. A cutoff latency of 15 s was used to prevent the possibility of tissue damage.

Dose-Response Studies. Immediately after baseline latency assessment, morphine analgesic potency was determined, using cumulative dose-response curves to reduce the number of mice required. All subjects were injected with increasing doses of morphine (0.1, 0.5, 1.0, 2.0, 3.6, 6.5, and 11.7 μg) in succession. Tail-withdrawal latencies were retested 15 min after each injection, and subsequent doses of morphine were administered immediately after withdrawal latency assessment at each dose. This procedure was repeated until each mouse displayed a cutoff tail-withdrawal latency average of >15 s.

Data Analysis. Analgesia at each dose was expressed as a percentage of the maximum possible effect (%MPE) as calculated by the formula: %MPE = [(postmorphine latency - baseline latency)/cutoff latency - baseline latency] × 100. The use of %MPE takes into account the cutoff latency and individual baseline latencies, so these will not bias the quantification of analgesia.

Two-way ANOVA was used to examine the main effects of strain and sex, and their interaction, on tail-withdrawal baseline latencies. Student’s t test was used for pairwise comparisons between sex within each strain. The morphine dose-response data (%MPE values) were analyzed using the BLISS-21 computer program. This program maximizes the log-likelihood function to fit a parallel set of gaussian sigmoid curves to the dose-response data and provides AD₅₀ values, 95% confidence intervals (CIs), and estimates of relative potency (Uman and Inturrisi, 1981). To evaluate the genetic codetermination between thermal nociceptive sensitivity and morphine analgesia, baseline withdrawal latencies and morphine AD₅₀ strain means were correlated using Pearson’s r statistic. Variance of strain mean AD₅₀ values between sex were also compared using a two-tailed F test. In all statistical tests, a criterion level of .05 was used.

Results

Baseline Sensitivity on Tail-Withdrawal Test. Baseline tail-withdrawal latencies for all strains, between and across sex, are presented in Table 1. There was a highly significant main effect of strain (P < .001) and of sex (P < .001), and the interaction between strain and sex approached significance (P = .084). Indeed, pairwise comparisons of sex for each strain indicate that in three strains, AKR, C3H/He, and C57BL/6, males exhibited significantly higher baseline tail-withdrawal latencies than their female counterparts, reflecting lower nociceptive sensitivity. Thus, sex differences in baseline sensitivity on the tail-withdrawal test are genotype dependent. One-way ANOVAs performed on baseline withdrawal latencies for each sex indicate a significant effect of strain in both males (F₁₀,₇₃ = 5.90, P < .001) and females (F₁₀,₇₂ = 12.02, P < .001), although strain rank order sensitivity was not identical.
Nociceptive sensitivity and morphine analgesia in male and female mice of 11 inbred mouse strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sex</th>
<th>Baseline Latency (s)</th>
<th>AD50 b (μg)</th>
<th>Potency Ratio c</th>
</tr>
</thead>
<tbody>
<tr>
<td>129</td>
<td>♂ + ♀</td>
<td>5.3 ± 0.4</td>
<td>0.33 (0.19–0.55)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td>5.6 ± 0.7</td>
<td>0.35 (0.16–0.73)</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>5.1 ± 0.4</td>
<td>0.34 (0.18–0.66)</td>
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<tr>
<td>A</td>
<td>♂ + ♀</td>
<td>5.9 ± 0.2</td>
<td>0.54 (0.33–0.85)</td>
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</tr>
<tr>
<td></td>
<td>♀</td>
<td>5.4 ± 0.2</td>
<td>0.42 (0.21–0.82)</td>
<td>1.7</td>
</tr>
<tr>
<td>AKR</td>
<td>♂ + ♀</td>
<td>4.0 ± 0.2</td>
<td>1.64 (1.08–2.47)</td>
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<tr>
<td></td>
<td>♀</td>
<td>4.7 ± 0.3</td>
<td>0.60 (0.31–1.11)</td>
<td>6.9 d</td>
</tr>
<tr>
<td>BALB/c</td>
<td>♂ + ♀</td>
<td>5.2 ± 0.2</td>
<td>1.03 (0.66–1.57)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>5.5 ± 0.2</td>
<td>1.23 (0.67–2.19)</td>
<td>0.8</td>
</tr>
<tr>
<td>C3H/He</td>
<td>♂ + ♀</td>
<td>3.8 ± 0.2</td>
<td>0.18 (0.09–0.33)</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>4.2 ± 0.2</td>
<td>0.11 (0.04–0.25)</td>
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<tr>
<td></td>
<td></td>
<td>3.2 ± 0.4 ±**</td>
<td>0.42 (0.19–0.89)</td>
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<tr>
<td>C57BL/6</td>
<td>♂ + ♀</td>
<td>3.1 ± 0.2</td>
<td>2.02 (1.33–3.02)</td>
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<tr>
<td></td>
<td>♀</td>
<td>3.7 ± 0.2</td>
<td>1.02 (0.55–1.83)</td>
<td>3.6 e</td>
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<tr>
<td>CBA</td>
<td>♂ + ♀</td>
<td>4.0 ± 0.2</td>
<td>0.31 (0.18–0.51)</td>
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<tr>
<td></td>
<td>♀</td>
<td>3.8 ± 0.4</td>
<td>0.68 (0.35–1.25)</td>
<td>0.2 f</td>
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<tr>
<td>DBA/2</td>
<td>♂ + ♀</td>
<td>5.1 ± 0.2</td>
<td>0.36 (0.21–0.50)</td>
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<tr>
<td></td>
<td>♀</td>
<td>5.0 ± 0.3</td>
<td>0.42 (0.21–0.82)</td>
<td>0.8</td>
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<tr>
<td></td>
<td></td>
<td>5.3 ± 0.4 ±**</td>
<td>0.33 (0.16–0.67)</td>
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<tr>
<td>LP</td>
<td>♂ + ♀</td>
<td>4.9 ± 0.2</td>
<td>1.33 (0.87–2.03)</td>
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<tr>
<td></td>
<td>♀</td>
<td>4.9 ± 0.3</td>
<td>1.10 (0.66–1.81)</td>
<td>1.6</td>
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<tr>
<td></td>
<td></td>
<td>4.9 ± 0.4</td>
<td>1.71 (1.00–2.88)</td>
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<tr>
<td>SJL</td>
<td>♂ + ♀</td>
<td>3.7 ± 0.2</td>
<td>1.73 (1.10–2.67)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>3.9 ± 0.2</td>
<td>1.64 (0.88–3.00)</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.6 ± 0.2 ±</td>
<td>1.97 (1.03–3.29)</td>
<td></td>
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<tr>
<td>SWR</td>
<td>♂ + ♀</td>
<td>3.4 ± 0.1</td>
<td>2.77 (1.84–4.14)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>3.5 ± 0.2 ±</td>
<td>1.43 (0.79–2.52)</td>
<td>3.5 g</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.2 ± 0.2</td>
<td>4.95 (3.99–6.90)</td>
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</table>

* Baseline latency (± S.E.M.) to reflexive withdrawal of the distal half of the tail from 49°C water.
* See text for i.c.v. morphine AD50 calculation. Values in parentheses represent 95% CI endpoints.
* Calculated as female AD50/male AD50 for that strain.
* Females significantly more sensitive than males, p < .05.

Supraspinal Morphine Analgesia. Cumulative dose-response curves for all strains collapsed across sex are presented in Fig. 1, with corresponding AD50 estimates (micrograms per mouse) and 95% CIs presented in Table 1. We observed a wide range of potency estimates between strains, confirming the relevance of genetic background on morphine analgesic sensitivity. Figure 1 also shows cumulative dose-response curves for all strains separately by sex. The larger strain variability apparent for female mice is reflected in the corresponding AD50 estimates for each sex in Table 1. For example, although the largest difference in morphine analgesic potency in males (approximately 14-fold) is observed between the C3H/He and SJL strains, a 41-fold difference in morphine potency distinguishes CBA and SWR females. Subjecting the ratio of the variances to an F test confirmed that this variability between strain AD50 estimates in females was significantly greater than that in males (F10,10 = 3D 12.5, P < .001).

Figure 2 displays pairwise comparisons of morphine analgesic potency for each strain, with derived AD50 estimates presented in Table 1. In three strains (AKR, C57BL/6, and SWR), males exhibited significantly lower morphine AD50 values relative to their female counterparts. Calculated potency ratios (female AD50/male AD50) reveal an approximately 6.9-, 3.6-, and 3.5-fold increase in morphine analgesic sensitivity for male AKR, C57BL/6, and SWR males relative to females, respectively. Conversely, female CBA mice displayed an approximately 5.5-fold increase in analgesic sensitivity to morphine relative to males of this strain. Thus, in the majority of strains tested, there were no significant sex differences in morphine analgesic potency, nor were males exclusively more sensitive than females when sex differences were observed.

We observed negative correlations between baseline tail-withdrawal latencies and morphine AD50 values for all strains across sex (r = −0.61) and for males (r = −0.40) and females (r = −0.68) alone, confirming in females the inverse relationship between initial nociceptive sensitivity and morphine analgesia previously reported for males (Mogil et al., 1996a; Elmer et al., 1997).

A separate experiment performed on AKR and CBA mice of both strains, in which seven repeated i.c.v. injections of saline were made, revealed an expected but modest (1–2 s) anesthesia/injection-related analgesia (data not shown). Importantly, this analgesia did not increase in magnitude with injection number and was statistically indistinguishable in both sexes and strains. Thus, it is very unlikely that our conclusions are confounded by sex or genotype differences in stress analgesia.
The present findings clearly demonstrate that sex differences in morphine analgesia are genotype dependent. Although in the majority of the strains examined, there was no significant sex difference in the AD50 estimate for morphine analgesia, significantly greater analgesic potency was observed in male AKR, C57BL/6, and SWR mice relative to their female counterparts. The lack of sex differences in most strains may truly reflect the absence of such differences in these genotypes or our lack of statistical power to detect differences of modest magnitude. The present finding that female CBA mice were approximately 5.5-fold more sensitive to the analgesic effects of morphine than were males of this strain highlights the importance of genetic background on not only the magnitude but also the direction of sex differences.

That genotype should have an impact on sex differences in morphine analgesia are genotype dependent. Although in the majority of the strains examined, there was no significant sex difference in the AD50 estimate for morphine analgesia, significantly greater analgesic potency was observed in male AKR, C57BL/6, and SWR mice relative to their female counterparts. The lack of sex differences in most strains may truly reflect the absence of such differences in these genotypes or our lack of statistical power to detect differences of modest magnitude. The present finding that female CBA mice were approximately 5.5-fold more sensitive to the analgesic effects of morphine than were males of this strain highlights the importance of genetic background on not only the magnitude but also the direction of sex differences.

That genotype should have an impact on sex differences in morphine analgesia is consistent with previous reports that have considered sex as a variable in genetic models of exogenous μ receptor-mediated analgesia in rats (Kasson and George, 1984) and mice (Kest et al., 1995). To our knowledge, sex differences in morphine analgesia have without exception been examined in commercially available outbred strains of rats and mice. Large phenotypic differences of relevance to analgesia have been documented between different outbred strains and even between populations of the same outbred strain maintained in different breeding institutions (i.e., vendor effects) (Mogil et al., 1996b). We suggest that variability in genetic background, perhaps acting in concert with other variables (see the introductory paragraphs), may have contributed to some of the conflicting findings in the literature on sex differences in both basal nociceptive sensitivity and opioid analgesia. To directly investigate this possibility, the testing of common outbred strains would be required. We instead chose to investigate only inbred mouse strains, the rodent populations of greatest use for gene-mapping efforts that may shed light on the responsible mechanisms (see below).

It is difficult to assess the accuracy of our AD50 estimates because there are virtually no instances in the literature in which the inbred mouse strains tested here were administered morphine by the i.c.v. route. In the one experiment, to our knowledge, in which more than one of these strains were used, Frigeni et al. (1981) obtained morphine AD50 estimates in male DBA/2 and C57BL/6 mice of 0.21 and 0.82 μg, respectively. These values cannot be easily compared with the values obtained presently (0.36 and 2.02 μg, respectively) because these investigators used a different nociceptive assay, the 51.5°C hot-plate test. It is notable, however, that the potency ratio between these strains from their and our studies are comparable (4.0 and 5.6, respectively). Also engendering confidence in the accuracy of the presently obtained data is the highly significant correlation (r = 0.87, P < .005, eight common strains) between baseline latencies of male mice in the present study and in a separate study conducted 1 year earlier in a different laboratory (Mogil et al., 1998).

**Proposed Mechanisms Underlying Sex Differences in Morphine Analgesia.** One obvious possibility is that morphine bioavailability might differ between the sexes. Higher concentrations of morphine in the brains of male rodents relative to females have been observed after systemic injection in some (Candido et al., 1992; Craft et al., 1996) but not other (Cicero et al., 1996, 1997) studies. The present demonstration of sex differences in morphine potency after i.c.v. administration in mice, as previously reported in rats (Kepler et al., 1989), argues against an exclusive role for pharmacokinetics in accounting for the differential analgesic response of males and females to morphine.

Other investigations have attempted to relate sex differences in opioid analgesia to opioid pharmacodynamic factors.
For example, sex differences in the immunoreactive density of the endogenous opioid peptides leu- and met-enkephalin (and their proteolytic enzymes) (Kavaliers and Innes, 1993), which can affect morphine potency (Vaught and Takemori, 1979), have been demonstrated in the rat (Watson et al., 1986). With respect to opioid receptors, no consistent sex differences in μ or δ opioid receptor populations are observed in rats (Kepler et al., 1991). In mice, both increased levels in males (Mogil et al., 1994) and no differences (Candido et al., 1992) in whole-brain opioid binding between sexes have been reported. The disconnection between analgesia and receptor density is well evidenced by the fact that male mice exhibit a significantly greater morphine analgesic supersensitivity than females after chronic treatment with the opioid antagonist naltrexone despite the absence of sex differences in the magnitude of opioid receptor up-regulation (Candido et al., 1992).

Attempts have also been made to relate sex differences in opioid analgesia to gonadal hormone levels of male and female rodents. Although female rats are reported to display greater analgesic sensitivity to systemic morphine on the mornings of diestrus (Banarjee et al., 1983) and proestrus (Banarjee et al., 1983; Berglund and Simpkins, 1988), Kepler et al. (1989) reported a relative decrease in sensitivity during met/diestrus. Studies with gonadectomized subjects are even more conflicting. For example, although it appears that the effect of adult ovarioectomy on morphine analgesia may be nociceptive modality specific (Baamonde et al., 1989; Ali et al., 1995), reductions (Banarjee et al., 1983; Kepler et al., 1989), increases (Kasson and George, 1984), and no alterations (Cicero et al., 1996) in morphine analgesia have all been reported just for the tail-withdrawal test. Like females, the analgesic potency of systemic morphine in castrated adult rats (Chatterjee et al., 1982; Kasson and George, 1984; Ali et al., 1995; Cicero et al., 1996) and mice (Candido et al., 1992; Ali et al., 1995) is dependent on the nociceptive modality tested, and contradictory findings are reported within the same assay (tail-withdrawal test) for rats (Chatterjee et al., 1982; Kasson and George, 1984; Cicero et al., 1996).

Finally, sex differences have been noted in other neurochemical systems that have an impact opioid analgesia. For example, there is evidence indicating that N-methyl-D-aspartate-sensitive excitatory amino acid receptors mediate morphine analgesia but that selective N-methyl-D-aspartate receptor blockade significantly reduces morphine analgesia in males only (Lipa and Kavaliers, 1990). Sex differences in the antiopioid effects of the endogenous peptides Tyr-MIF-1 and neuropeptide FF on morphine analgesia in mice have also been noted (Kavaliers and Innes, 1992a,b). However, the relevance of these findings remains highly speculative. Overall, no clear consensus has emerged regarding the mechanisms underlying sex differences in morphine analgesia.

**Application of Gene Mapping Strategies.** We believe that further investigation of sex differences in opioid analgesia requires the application of new strategies. We are attempting to delineate the physiological mechanisms underlying differential opioid analgesic sensitivity between the sexes by mapping the relevant genetic loci by linkage analysis (Lander and Botstein, 1989). This approach, called quantitative trait locus (QTL) mapping, can reveal chromosomal regions (and, ultimately, genes) associated with continuously distributed traits such as opioid sensitivity. QTL mapping has been successfully used to identify loci associated with systemic morphine analgesic magnitude on the hot-plate test using C57BL/6 and DBA/2 progenitors (Belknap et al., 1995). However, several important issues remain unexamined. First, these experiments examined morphine analgesia on the hot-plate test, and we and others have determined that the genetic mediation of this trait is at least partially specific to the noiceptive assay used (Mogil et al., 1996a; Elmer et al., 1997). Furthermore, the QTLs identified so far are limited to those polymorphic between DBA/2 and C57BL/6 mice. Finally, this mapping study considered only male mice, and we have provided evidence in two other experiments for the existence of sex-specific, pain-relevant QTLs: a locus containing the δ-opioid receptor gene Oprd1 statistically associated with basal nociception on the hot-plate test in male mice only (Mogil et al., 1997a) and a locus on chromosome 8 mediating nonopioid stress-induced analgesia in female mice only (Mogil et al., 1997b).

Therefore, based on the present findings, we have begun a QTL mapping study using AKR and CBA strains as progenitors. The 35-fold difference in morphine AD50 values between females of these strains should facilitate the identification of QTLs mediating analgesic magnitude in this sex (Lander and Botstein, 1989). In addition, the virtually identical AD50 values between males of these strains should decrease the probability that the QTLs identified will be highly relevant to males. Given the previous success in identifying sex-specific QTLs of relevance to pain processing, it seems reasonable to expect that sex differences in morphine analgesia might also be due at least partially to the action of separate genes in each sex. The identification of such genes and their protein products may shed further light on inconsistencies in the existing literature and, more importantly, on mechanisms underlying the variable actions of this clinically useful drug.

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