Sex-Related Differences in Morphine’s Antinociceptive Activity: Relationship to Serum and Brain Morphine Concentrations

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ABSTRACT
In earlier studies, it was shown that male rats were considerably more sensitive to the antinociceptive properties of morphine than females in several antinociceptive assays. The purpose of our studies was to examine whether these male-female differences might be due to differences in the blood and brain levels of morphine attained after its s.c. injection rather than to intrinsic differences in the central nervous system sensitivity to the drug. Our results confirmed that males were considerably more sensitive than females to the antinociceptive properties of morphine on the hot-plate test; the ED\textsubscript{50} in males was approximately half that found in females. These sex differences were not unique to morphine because males were also more sensitive to the antinociceptive properties of the potent mu agonist, alfentanil. With respect to the pharmacokinetics of morphine, we found that there was a linear relationship in both males and females between the dose of morphine injected and the blood and brain levels achieved 60 min after the injection when the sex-linked differences in morphine-induced antinociception was greatest; no sex differences were found in the peak levels of morphine attained in blood or brain at any dose of morphine. Furthermore, there were no sex-linked differences in the elimination half-life of morphine from blood and, similarly, there were no differences in the disappearance of morphine from brain. On the basis of these data, it appears that the sex-related differences we have observed between males and females in the response to morphine’s antinociceptive activity cannot be explained by differences in the pharmacokinetics of morphine. Rather, it appears that sex differences in morphine-induced antinociception are related to inherent differences in the sensitivity of the brain to morphine.

We have previously examined whether there are male-female differences in the antinociceptive activity of morphine in the rat (Cicero et al., 1996). Our results and those of several other groups (e.g., Kepler et al., 1989; Baamonde et al., 1988; Islam et al., 1993) demonstrated marked sex-related differences in morphine-induced antinociception. In our earlier studies (Cicero et al., 1996), males were found to be more sensitive to the antinociceptive properties of morphine in three different assays: the hot-plate, tail-flick and writhing tests. This enhanced sensitivity to morphine was reflected in a much higher antinociceptive response, the magnitude of antinociception and the duration of the response in males when compared to females at comparable doses of morphine. Moreover, we found that the ED\textsubscript{50} for morphine-induced antinociception was at least 50% lower in males than in females.

Although there has been relatively little systematic examination of other sex-related differences in the acute or chronic effects of the opiates, Craft and Stratmann (1995) and Craft and Bartok (1996) have reported in abstract form that morphine served as a discriminative stimulus at somewhat lower doses in females than in males. Thus, it may be that differences between males and females can be observed across a spectrum of morphine’s acute pharmacological effects.

Our studies were carried out to assess whether the very large sex-related differences in morphine-induced antinociception that we (Cicero et al., 1996) and others (e.g., Kepler et al., 1989; Baamonde et al., 1988; Islam et al., 1993) have observed, and the suggestion of sex-related differences in the discriminative stimulus properties of morphine (Craft and Stratmann, 1995; Craft and Bartok, 1996), could be explained solely on the basis of differences in blood and brain levels of morphine subsequent to its acute administration. Despite the fact that several groups have documented sex-related differences in the pharmacological response to morphine, we are not aware of a single report that has determined whether these gender differences might be related to differences in the pharmacokinetics of morphine after its s.c. injection. As a result, it is not possible at this time to conclude with any level of certainty that there are fundamental gender-related differences in the sensitivity of the nervous system to opiates. Before such a conclusion can be made, it is

ABBREVIATIONS: %MPE, per cent maximum possible effect; \textsuperscript{1/2}, half elimination rate; RIA, radioimmunoassay.
imperative to demonstrate that male-female differences in the response to morphine, and possibly other mu agonists, can be documented even when blood and brain levels of morphine are equivalent in both sexes after the injection of comparable doses of morphine.

Our studies have examined this important issue. Specifically, we have measured peak blood and brain levels of morphine and the elimination half-life of the drug at doses that produced maximal differences in morphine-induced antinociception in male and female rats. We also examined whether the sex-differences we observed in morphine’s antinociceptive activity was in some way unique to this drug or was a more general effect of mu opiate agonists, such as alfentanil.

**Methods**

**Humane Care of Laboratory Animals**

All of the studies described herein were reviewed and approved by the institutional animal care and use committee, particularly with respect to the ethical standards for research on pain in conscious animals.

**Materials.** Sprague-Dawley rats were purchased from Harlan Sprague Dawley, Indianapolis, IN. They were used at 60 to 80 days of age in all studies. Morphine-sulfate and alfentanil-HCl were generously provided by the National Institute on Drug Abuse (Rockville, MD). The hot-plate apparatus was obtained from Technilab Instruments, Inc. (Pequannock, NJ). The iodinated morphine RIA kit, which was derived from the principles and methods of Yoburn et al. (1985), was purchased from Diagnostic Products Corporation, Los Angeles, CA. The antibody is specific to morphine with less than 0.03% cross reactivity for morphine-3-glucuronide and less than 0.1% cross-reactivity for morphine-6-glucuronide. The lower limit of sensitivity of the assay was 125 pg and the standard curve was linear over the range 125 to 2500 pg (R = 0.97). Interassay variation was 3% and intraassay variation was less than 5% in all studies described herein.

**Hot-plate.** Groups of male and female rats (N = 12 in each group) were tested on the hot-plate that was set at 58°C to yield base-line (i.e., non-drug treated) %MPE is labeled 0.1% cross-reactivity for morphine-3-glucuronide and less than 0.1% cross-reactivity for morphine-6-glucuronide. The lower limit of sensitivity of the assay was 125 pg and the standard curve was linear over the range 125 to 2500 pg (R = 0.97). Interassay variation was 3% and intraassay variation was less than 5% in all studies described herein.

For the ED₅₀ determinations, the rats received injections with at least four or five doses of morphine or alfentanil yielding responses that fell between ED₅₀ and ED₉₀ (see data analysis).

**Serum and brain levels of morphine.** Morphine serum and brain levels were measured in groups (N = 10–12) of male and female rats receiving s.c. injections with doses of morphine ranging from 2.5 to 15.0 mg/kg; this dose range was used because the doses fell between the ED₁₀ and ED₉₀ for morphine-induced antinociception on the hot-plate assay. The rats were killed 60 min after the injection at which time maximal antinociceptive activity and sex-linked differences were found on the hot-plate test. Serum and whole brains were collected. Serum levels of morphine were measured in unextracted samples by the RIA kit described above. The whole brains were homogenized in 0.4 N NaOH and allowed to dissolve overnight at 0 to 4°C. Morphine brain levels were measured directly in these extracts by RIA. Protein levels were measured by the method of Bradford (1976); concentrations in brain were expressed as ng morphine/mg protein. To examine the disappearance rate of morphine from blood and brain, male and female rats received s.c. injections with 5.0, 7.5 or 15 mg/kg morphine and were killed by decapitation at intervals ranging from 15 to 240 min. Serum and brain levels of morphine were measured as described above.

**Statistical analysis.** All differences between males and females were analyzed by analysis of variance followed by post hoc analysis. The tᵢ/₂ of morphine from blood was calculated using the following formula and was carried out using the exponential decay program in PRISM (Graph Pad Inc., San Diego, CA):

\[
t_{i/2} = \frac{\ln 0.692}{slope \ of \ regression \ curve \ of \ log \ drug \ concentration \ vs \ time}
\]

To determine the ED₅₀ values in the hot-plate test, groups of rats received injections with four or five doses of morphine or alfentanil falling between the ED₁₀ and ED₉₀. The rats were tested at the time of the maximal antinociceptive effect in each assay (60 min for morphine and 5 min for alfentanil). Data were expressed in terms of %MPE. The ED₅₀ determinations and the 95% confidence limits (shown in brackets) were determined by nonlinear regression analyses using PRISM.

**Results**

**Morphine-induced antinociception.** In agreement with our earlier studies (Cicero et al., 1996), a single dose of morphine produced marked sex-related differences in morphine’s antinociceptive activity on the hot-plate test. As shown in figure 1, a dose of 10 mg/kg morphine produced significant antinociceptive activity in males reaching a maximum of approximately 75 to 90%MPE at 30 to 60 min after the injection. In contrast to these results, the %MPE in females at this dose was approximately one-third of that in males at the time of peak antinociception. In addition, as we reported previously (Cicero et al., 1996), the magnitude of antinociception, as reflected in the area under the time-action curve, and the duration of antinociception was also significantly greater in males than females (fig. 1). The ED₅₀

![Fig. 1. The antinociceptive response to morphine on the hot-plate test, expressed as %MPE, in male and female rats (N = 12 in each group) at the time intervals shown. Base-line (i.e., non-drug) %MPE is labeled 0 in this figure; morphine (10.0 mg/kg) was injected immediately after time 0 and %MPE was measured at the intervals shown for 3 hr. Values are means (±S.E.M.). Repeated measures analysis of variance demonstrated significant (P < .001) gender and time related differences.](image310x113 to 561x301)
in males was markedly lower than that observed in females [6.3 mg/kg (5.8–7.4) vs. 12.6 mg/kg (10.94–13.69), respectively]. These dose-response curves are not shown because they are virtually identical to those we have previously published (Cicero et al., 1996).

**Alfentanil-induced antinociception.** The time and dose-response curves for alfentanil-induced antinociception in male and female rats are shown in figures 2 and 3, respectively. As shown in figure 2, although alfentanil produced a substantial degree of antinociceptive activity in males and females at a dose of 0.20 mg/kg, there were, as was the case with morphine, significant differences between the sexes. Males achieved much greater levels of antinociceptive activity than females from 5 to 30 min after the injection of this single dose of alfentanil (fig. 2). Figure 3 shows the dose-response curves for alfentanil-induced antinociception 5 min after its s.c. administration in males and females. There were marked sex-related differences in the dose-response functions and the calculated ED50 for alfentanil-induced antinociception; the ED50 in males was less than half that observed in females [0.146 mg/kg (0.142–0.148) compared to 0.203 (1.96–2.10)].

**Serum and brain levels of morphine.** Figures 4 and 5 show the morphine serum and brain levels, respectively, in male and female rats 60 min after doses ranging between 2.5 to 15.0 mg/kg. The 60-min survival interval was selected because peak morphine–induced antinociceptive activity, and pronounced sex-related differences, were observed at this time. There were no differences observed between males and females at any dose in the serum or brain morphine levels reached at this time. Figures 6 and 7 show the time-course for serum and brain morphine concentrations, respectively, in male and female rats subsequent to the injection of 5.0, 7.5 or 15.0 mg/kg morphine. There were no statistically significant differences between males and females either in the peak morphine levels attained in serum nor in morphine’s elimination from blood. The data shown in Figure 6 were analyzed using the exponential decay program in PRISM. No statistically significant differences were found in the slopes of the morphine elimination curves from blood (plotting log dose vs. time) for sex or dose and thus all of the data were combined to estimate the t1/2 for each sex. The t1/2 for morphine in blood was 43.83 min (±3.83) for males compared to 42.6 min (±7.62) for females.

As shown in figure 7, there were also no statistically significant differences in peak morphine concentrations in brain or in its apparent disappearance. t1/2 rates from brain were not calculated because the slopes of the disappearance curves were multiphasic, suggesting a multicompartment model, and there were too few data points to permit a calculation of disappearance rates as determined by computer analysis (PRISM). However, at the time points used in these studies when maximal differences were observed in morphine’s antinociceptive activity, there were no sex-related differences in brain morphine concentrations at 5, 7.5 or 15.0 mg/kg morphine from 15 to 180 min after its s.c. injection.

**Discussion**

The results of these studies confirm the marked sex-related differences in the antinociceptive activity of morphine that we (Cicero et al., 1996) and others (e.g., Kepler et al., 1989; Baamonde et al., 1988; Islam et al., 1993) have previ-
In the bioavailability of morphine can explain the large sex-
tensions in blood. Thus, it seems unlikely that gross differences
inherent differences in free and bound morphine concentra-
tions consequent to the s.c. injection of comparable doses of morphine.

We cannot explain the large sex differences that have been ob-
served in morphine’s acute pharmacological profile. We
assume that the peak concentration of morphine in blood and
brain seems to be dependent on dose in both sexes (figs. 6 and 7). In addition, there appears to be a suggestion that blood morphine concentrations may not be linearly re-
lated to dose (fig. 4), with disproportional increases in mor-
ephine concentrations at higher doses, but computer analysis
failed to show a significant departure from linearity. Al-
though these observations are only tangentially related to the
issue of gender differences in opiate-induced antinocicep-
tion and do not alter the conclusion that pharmacokinetic
differences cannot explain the gender differences we have
observed, we believe it important to raise these issues be-
cause they may be relevant to a more general examination or
reexamination of the pharmacokinetics of morphine in the
rat.

Our observations document rather clearly that there are
marked sex-related differences in one key component of mu
opiate agonist’s pharmacological properties: antinociception.
Whether there are other differences between males and fe-
males in terms of their response to other actions of mu
agonists, particularly the very important issue of their abuse
liability, has not been fully explored. In fact, these issues
seem to have received very little rigorous attention. Although
there has been some speculation in the clinical literature that
gender may play a role in the abuse liability of cocaine and
alcohol, and perhaps in prevention strategies and treatment
outcome (Bailey et al., 1993; Griffin et al., 1989; Lex, 1991;
Kosten et al., 1995; Rapp, 1995), we have been unable to find
any clinical studies that have addressed whether there are
gender differences in the abuse liability of opiates.

In the preclinical literature, there also appears to be a
paucity of data with respect to sex-differences in the abuse
liability of opiates, but Craft et al. (1995, 1996) have sug-
gested in abstract form that the discriminative stimulus
properties of morphine may be influenced by gender. Inter-
estingly, these authors speculate in these two abstracts that

![Fig. 5. Mean (±S.E.M.) brain levels of morphine (ng/mg protein) in male
and female rats receiving injections (N = 12–15) with morphine (2.5–15
mg/kg morphine) and killed at the intervals shown (plotted as a log
function of dose). Analysis of variance revealed no significant gender
differences.](image-url)
females can discriminate morphine at lower doses than males which they interpret to suggest that female rats are more sensitive to morphine’s effects than are males. If these differences are confirmed in more extensive studies, they apparently would be exactly in the opposite direction of what we have observed with antinociception; i.e., that males appear to be much more sensitive than females to morphine’s antinociceptive properties. It is clearly premature at this point to speculate on the significance of the nature and direction of sex differences in the acute pharmacological profile of morphine, but it appears that, at least in male and female rats, there may be gender differences in at least two important aspects of the pharmacology of morphine: antinociception and its discriminative stimulus properties. It is equally apparent, however, that very few rigorous and systematic studies have addressed this important issue in animals or humans and that more comprehensive experiments are needed.

If one makes the logical assumption that the sex-related differences in morphine-induced antinociception observed in the present and earlier studies are in some manner due to differences in the central nervous system sensitivity to morphine, one reasonable hypothesis is that there are differences between males and females in the number or affinity of those opiate receptors involved in mediating antinociception. In this connection, Hammer (1984, 1985, 1990, 1994) has reported sex-linked differences in the number and regional distribution of opioid receptors in sexually dimorphic brain regions in males and females (e.g., Hammer, 1984, 1985, 1990; Hammer et al., 1982). It is noteworthy, however, that Hammer (1984, 1985, 1990, 1994) found differences in opiate receptors only in one or two highly discrete brain loci; in most important respects the overall opiate receptor profiles in brain were remarkably similar in males and females. Given that we have found differences in morphine-induced antinociception thought to be mediated by multiple sites in the nervous system and, if it is true that there are gender differences in other aspects of opiate pharmacology such as its discriminative stimulus properties (Craft et al., 1995, 1996), the number of potential areas in brain which should reflect dimorphism in opiate receptor profiles should be very large. The fact that Hammer (e.g., 1984, 1985, 1990; Hammer et al., 1994) failed to detect wide-spread male-female differences in opiate receptor densities in brain suggests either that there are no gender-related differences in the affinity or density of opiate receptors, suggesting that these differences are mediated by postreceptor events; or that any differences that do exist cannot be detected using any technique currently available.

One of the more reasonable explanations of sex-related differences in the response to opiates is, of course, that sex steroids may mediate these effects. Although in some prior studies, removal of steroids by castration and/or ovarietomy influenced the antinociceptive response to morphine in adult rats (e.g., Islam et al., 1993; Romero and Bodnar, 1982; Bodnar et al., 1988; Romero et al., 1988; Baamonde et al., 1988), other studies (Cicero et al., 1996; Kepler et al., 1989) have failed to find any effects at all. These data suggest that sex-related differences in morphine-induced antinociceptive activity may not be dependent on the acute membrane-mediated effects of sex steroids. Rather, it may be more reasonable to postulate that the sex-related differences we have
observed are determined in large part by the organizational effects of steroids mediating sexual differentiation of brain morphology and neurobiology that occur in the very late prenatal or early postnatal period rather than in adulthood (Arnold and Breedlove, 1985; Goy et al., 1964; Breedlove, 1992, 1994). We are currently examining 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.

It should be clear from this discussion that we and others have documented robust differences between males and females in the antinociceptive and perhaps discriminative stimulatory properties of morphine. However, it seems equally clear that, although we may be able to eliminate some obvious mechanisms that could be involved (e.g., pharmacokinetic variables) in these gender differences, we are not yet in a position to speculate on the mechanisms involved. As an initial step in this direction, a particularly useful set of experiments will be to determine whether sex differences can be observed in other aspects of morphine’s pharmacology, such as its reinforcing properties or the development of tolerance and physical dependence. These studies would either tend to better focus mechanistically oriented studies if the differences are found to be highly specific, or suggest that a more broadly based series of studies need to be contemplated if sex differences are observed in many or all aspects of opiate pharmacology. Nevertheless, our studies would seem to provide a foundation on which to begin an examination of whether other sex-related differences may be found in the acute and chronic action of opiates and the mechanisms which might be involved.

In conclusion, our experiments conclusively demonstrate pronounced sex-related differences in the antinociceptive properties of morphine and perhaps other mu opiate agonists (e.g., alfentanil). Most importantly, these gender-differences appear to reflect markedly enhanced central nervous system sensitivity to morphine in males when compared to females as opposed to any differences in the bioavailability or metabolism of morphine. The mechanisms underlying these striking sex-related differences are unknown.

References


