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$_{50}$ 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 the 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$_{50}$ 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; $t^{1/2}$, 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) %MPE is labeled 0 baseline (sec) 30 sec (cutoff) − baseline (sec) × 100

For the ED50 determinations, the rats received injections with at least four or five doses of morphine or alfentanil yielding responses that fell between ED10 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 t1/2 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):

\[
t_{1/2} = \frac{\ln(2)}{\text{slope of regression curve of log drug concentration vs time}}
\]

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
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 ED$_{50}$ for alfentanil-induced antinociception; the ED$_{50}$ 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 t$_{1/2}$ for each sex. The t$_{1/2}$ 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. t$_{1/2}$ 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 our previous studies, we were unable to conclude that gender differences exist in antinociception induced by opioid peptides (Kepler et al., 1991) and in antinociception induced by stressors that is thought to be mediated by endogenous opioid peptides (e.g., Romero et al., 1988; Kavaliers and Innes, 1987a, b, 1988). Taken as a whole, our data and earlier work suggest that there are intrinsic sex-related differences in antinociception induced by endogenous and exogenous opioids.

In earlier studies (Cicero et al., 1996), we observed that males were 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 the peak antinociceptive effect, the magnitude of analgesia and the duration of the antinociceptive response; the ED$_{50}$ in males was also approximately half that in females. Our observations extend these previous findings and suggest that the sex differences we have observed in opiate-induced antinociceptive activity are not unique to morphine, but also occur with other mu selective agonists, such as alfentanil.

In our previous studies, we were unable to conclude whether the male-female differences observed in the antinociceptive activity of morphine were due to intrinsic differences in the sensitivity to morphine, presumably at the level of the central nervous system, or rather more simply reflected altered biodistribution of morphine. Our studies provide, to our knowledge, the first evidence that sex-linked differences in the pharmacokinetics of morphine probably cannot explain the large sex differences that have been observed in morphine’s acute pharmacological profile. We found that the peak concentration of morphine in blood and brain and its $t^{1/2}$ were identical in males and females subsequent to the s.c. injection of comparable doses of morphine. Moreover, in as yet unpublished findings, we have found no gender differences in free and bound morphine concentrations in blood. Thus, it seems unlikely that gross differences in the bioavailability of morphine can explain the large sex-related differences that have been found in the magnitude and duration of morphine-induced antinociceptive activity.

It is, of course, possible that morphine concentrations at key loci in brain and/or in the spinal cord that mediate opiate-induced antinociceptive activity might be influenced by sex, but it is difficult to envision any mechanism by which this could occur nor does it appear to be a testable hypothesis. Specifically, the number of sites involved in morphine-induced antinociception, as measured by the hot-plate test in our studies, and by the hot-plate, tail-flick and acetic acid-induced writhing tests in our earlier studies (Cicero et al., 1996), are extensive. Detecting gender-related differences in morphine concentrations in multiple and highly discrete brain regions would present formidable technical difficulties with any technique currently available (e.g., RIA or mass spectrometry). Therefore, at this time it seems most reasonable to hypothesize that the differences we (Cicero et al., 1996) and others (Kavaliers and Innes, 1987a, b; Romero and Bodnar, 1982; Romero et al., 1988) have observed between males and females in terms of morphine’s antinociceptive effects reflect intrinsic gender-related differences in the sensitivity to morphine at its sites of action in the central nervous system and cannot simply be ascribed to differences in the bioavailability or metabolism of morphine.

One interesting set of observations related to our studies of blood and brain morphine concentrations and the disappearance of morphine is that the time to peak concentrations 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 related to dose (fig. 4), with disproportional increases in morphine concentrations at higher doses, but computer analysis failed to show a significant departure from linearity. Although these observations are only tangentially related to the issue of gender differences in opiate-induced antinociception 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 because 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 females 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 suggested in abstract form that the discriminative stimulus properties of morphine may be influenced by gender. Interestingly, these authors speculate in these two abstracts that
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 ovariectomy 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 stimulus 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


