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Gender-linked differences in the expression of physical dependence in the rat

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Abstract

In earlier studies, it was shown that there were gender differences in several aspects of the pharmacological profile of morphine, including its antinociceptive activity, discriminative stimulus properties and its reinforcing effects. The purpose of the present studies was to examine whether there might also be gender-related differences in the development of physical dependence, as reflected in the expression of an opiate withdrawal syndrome upon cessation of chronic morphine administration. We found that a more severe spontaneous withdrawal syndrome was produced by chronic morphine injections or morphine pellet implantation in male rats than in females. The duration of the withdrawal syndrome was also longer. In contrast to our observations with spontaneous withdrawal, we found no gender differences in the naloxone-precipitated withdrawal syndrome induced by chronic morphine administration. These observations suggest that there are gender differences only in the expression of the spontaneous withdrawal syndrome, but not in the neuro-adaptive changes associated with the generation of physical dependence as reflected by naloxone-precipitated withdrawal.

Keywords: Opiate physical dependence; Gender differences; Morphine concentrations in blood and brain; Morphine, physical dependence; Morphine, withdrawal syndrome; Opiate withdrawal, gender differences

1. Introduction

We have previously demonstrated that there are male–female differences in the antinociceptive activity of morphine and other mu agonists (e.g., alfentanil) in the rat (Cicero et al., 1996, 1997). Our results, and those of several other groups (Baamonde et al., 1988; Islam et al., 1993; Kepler et al., 1989), have shown that male rats display a considerably greater degree of antinociception at comparable doses of morphine than do females; the ED_{50} for morphine-induced antinociceptive activity in males was also approximately half that in females on several different antinociceptive tests. These differences appear to reflect intrinsic gender-related differences in the sensitivity of the brain to morphine, since we have found that the levels of morphine in blood and brain were similar in male and female rats at comparable doses (Cicero et al., 1996, 1997).

The scope of gender-related differences in other aspects of opiate pharmacology are largely unknown, but have been the subject of some recent investigations. For example, Craft et al. (1998, 1999) reported that morphine served as a discriminative stimulus at lower doses in females than in males. In a recently published study (Cicero et al., 2000), we have also found that morphine served as a positive reinforcing agent in a place conditioning paradigm in both male and female rats, but that the dose–response curves were markedly different. At low doses, morphine induced a strong preference for the drug-associated chamber in both males and females, but as the dose was increased, the preference for the drug-associated chamber declined sharply in males, whereas in females, a very strong preference was still observed at doses up to near lethal levels.

These data raise the important issue of whether there may be differences in other aspects of the pharmacology of opiates, including their abuse liability. Surprisingly, there is a paucity of preclinical or clinical literature in which potential differences in the abuse potential of opiates have been assessed. The absence of any systematic data related to this important point is somewhat surprising, since it has been reported that, at least for alcohol and cocaine (Bailey

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et al., 1993; Ball et al., 1995; Babor et al., 1992; Griffin et al., 1989; Kosten et al., 1995; Lex, 1991; Rapp et al., 1995), male and female substance abusers may differ in the severity of their abuse, may have different treatment outcomes and/or require different prevention strategies. Whether these data reflect psychosocial or inherent biological differences between the sexes has not been examined.

The present studies were carried out to assess whether the development of physical dependence, as assessed by the expression of the opiate withdrawal syndrome, displays gender differences comparable to what has been observed with some of the acute effects of morphine. The development of physical dependence is a common feature of opiates and may contribute to their abuse potential (Siegel, 1977; Wikler, 1980), although whether it is a necessary or causal factor leading to abuse remains a subject of considerable theoretical debate (Bozarth, 1994; Koob, 1992; Stewart and Wise, 1992). Nevertheless, physical dependence is clearly a prominent feature of chronic opiate abuse and, therefore, we felt it important to examine whether there were gender differences in this important aspect of the pharmacology of the opiates. Specifically, we have assessed whether the magnitude of the withdrawal syndrome, which, in the rat, is characterized by wet-dog shakes, diarrhea and most prominently body weight loss (Blasig et al., 1973; Bozarth, 1994; Cicero and Meyer, 1973; Way et al., 1969; Wei et al., 1973), is quantitatively and qualitatively different in male and female rats after spontaneous and naloxone-precipitated withdrawal from chronic morphine administration.

2. Methods

2.1. IUCAC approval

All of the experiments and protocols employed in these studies were approved by the Institutional Animal Care and Use Committee.

2.2. Materials

Sprague–Dawley rats were purchased from Harlan Sprague Dawley, Indianapolis, IN. They were used at 60–80 days of age. They were housed individually on a 12-h light/dark cycle and given food and water ad libitum. Morphine sulfate and morphine pellets (75 mg free base) were generously provided by the National Institute on Drug Abuse (Rockville, MD).

2.3. Spontaneous withdrawal after morphine administration

In the first study, groups of male and female rats ($n=15$ in each independent group) were injected twice daily with morphine. The regimen began with a dose of 10 mg/kg injected subcutaneously twice a day and escalated over a 14-day period to a final dose of 40 mg/kg twice daily. In the second series of studies, separate groups of male and female rats ($n=15$ each) were implanted subcutaneously, under light brevital anesthesia, with a single 75-mg morphine pellet. The pellets were encased in nylon mesh to provide for the easy removal of the pellets after implantation. In the double injection study, injections stopped on the 14th day, and withdrawal was monitored and scored as described below; in the pellet implantation studies, the pellet was removed (under light brevital anesthesia) 3 days after implantation and the withdrawal syndrome was scored.

Beginning the day after the last injection or pellet removal, and continuing at daily intervals for 7 days, two independent raters assessed the severity of the withdrawal syndrome in male and female rats according to a slightly modified rating scale described elsewhere (Gellert and Holtzman, 1978). This rating scale includes all of the signs of withdrawal, which are weighted according to their prominence. The signs and symptoms monitored and their weighting factors are shown in Table 1. These observations took place for a 60-min period in the morning on each test day.

These two studies (i.e., pellet implantation and double injection) were replicated three times, and all of the data presented in this paper represent the pooled results of three experiments.

2.4. Precipitated withdrawal after injections of morphine or morphine pellet implantation

Separate groups of male and female rats ($n=15$) were injected with morphine twice daily as described above or were implanted with a single 75-mg morphine pellet. In the double-injection study, 1 h after the last injection on Day 14, the rats were injected with naloxone (0.25 mg/kg) and the severity of the withdrawal syndrome was rated for a 60-min period. In the pellet implantation studies, the pellet

Table 1
Graded and checked signs of withdrawal

Graded signs	Factor
Body weight loss	1 for each 1% body weight loss
Wet-dog shakes	
1–2	2
3–10	4
10+	6
Number of escape attempts	
2–4	1
5–9	2
10+	3
Checked signs[a]	
Diarrhea	2
Teeth chattering	2
Profuse salivation	7
Chromodacryorrhia	5
Ejaculation	3

[a] Scored by the factor shown if it is present or not—not graded as to severity.

was not removed; 3 days after pellet implantation, the rats were injected with doses of naloxone ranging from 0.005 to 1.0 mg/kg. In both studies, the rats were observed for 60 min for withdrawal symptoms using the rating scale shown in Table 1.

2.5. Data analysis

All differences between males and females were analyzed by repeated-measures analysis of variance followed by post hoc analysis. For the ratings, complex chi-square analyses were performed.

3. Results

3.1. Spontaneous withdrawal signs after twice-daily injections of morphine

The results of the spontaneous withdrawal studies after twice-daily injections of morphine are shown in Figs. 1 and 2. In Fig. 1, the weighted withdrawal factor is presented and in Fig. 2, body weight loss (as a % of initial weight) after spontaneous withdrawal. Body weight loss was selected for illustrative purposes, in this and other figures, since it most clearly parallels the fully rated withdrawal syndrome, and can be used as a highly predictive, single factor of withdrawal (Cicero and Meyer, 1973; Gellert and Holtzman, 1978). As can be seen, male rats had more severe withdrawal than females for up to 4 days postwithdrawal. The duration of the withdrawal response also was more protracted in males than in females (Figs. 1 and 2).

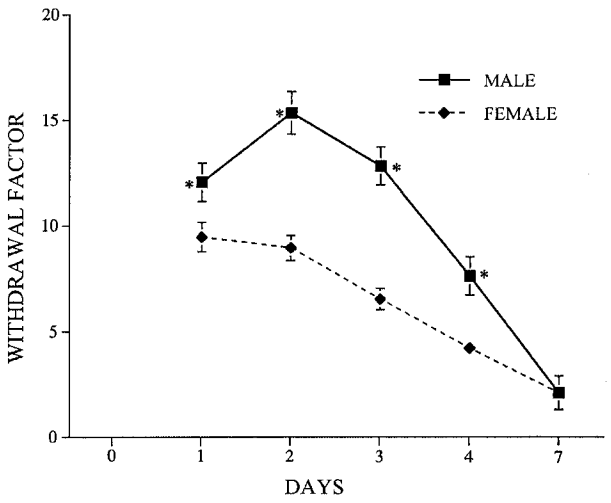

Fig. 1. Withdrawal scores in male and female rats ($n=15$ in each of three replications) following cessation of twice daily injections of morphine. Values are means (±S.E.M.) of three experiments. * Significantly ($P<.05$) higher at comparable time points than in females.

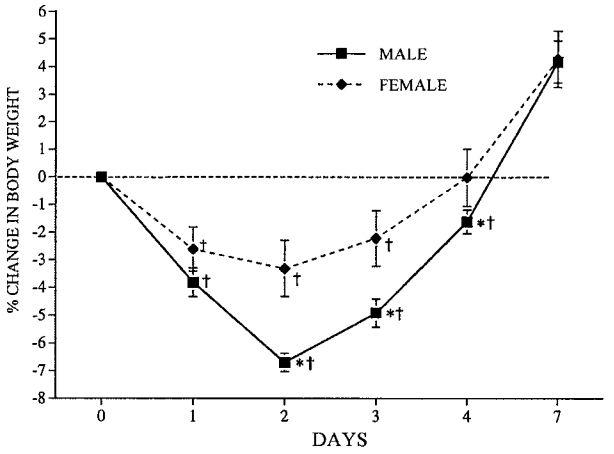

Fig. 2. Mean (±S.E.M.) body weight loss, expressed as % loss in body weight, subsequent to cessation of chronic morphine injections twice daily ($n=15$). The dotted line represents the prewithdrawal baseline body weight. Values are mean (±S.E.M.) of three experiments. Repeated-measures ANOVA demonstrated significant ($P<.01$) gender and time-related differences. * Significantly ($P<.05$) greater at comparable time points in females; †significantly ($P<.05$) lower than baseline.

3.2. Spontaneous withdrawal signs after morphine pellet implantation

Since the total withdrawal ratings were relatively modest after spontaneous withdrawal following twice-daily injections of morphine, separate groups of male and female rats were implanted with morphine pellets as described in the Methods. Figs. 3 and 4 show the withdrawal factor and body weight loss, respectively, in male and female rats implanted with a single morphine pellet for 3 days, which was then removed, and the withdrawal syndrome rated for 60 min at the daily intervals shown. As can be seen, males had much more severe withdrawal scores than females. Notably, as

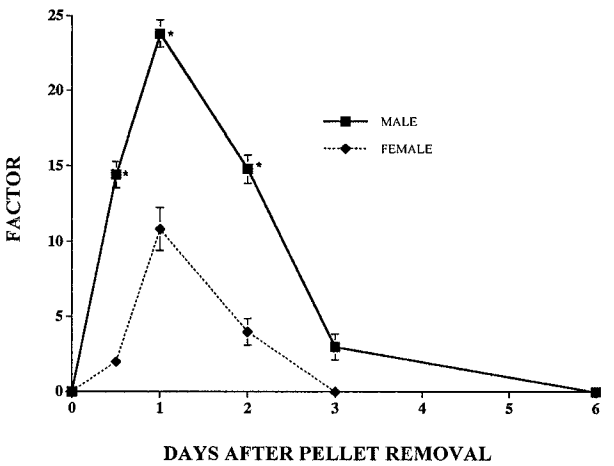

Fig. 3. Graded withdrawal factor in male and female rats ($n=15$) implanted with a single morphine pellet for 3 days, which was then removed. Values are means (±S.E.M.). * Significantly ($P<.01$) different than in females.

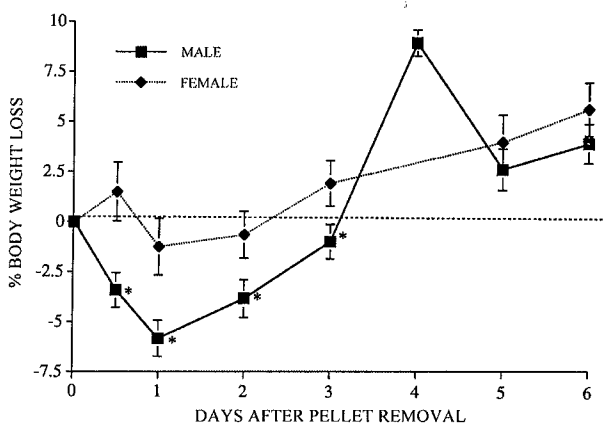

Fig. 4. Mean (±S.E.M.) body weight loss, expressed as % loss in body weight in male and female rats ($n=15$ in each group) implanted with a single morphine pellet for 3 days, which was then withdrawn. * Significantly ($P<.01$) greater than in females.

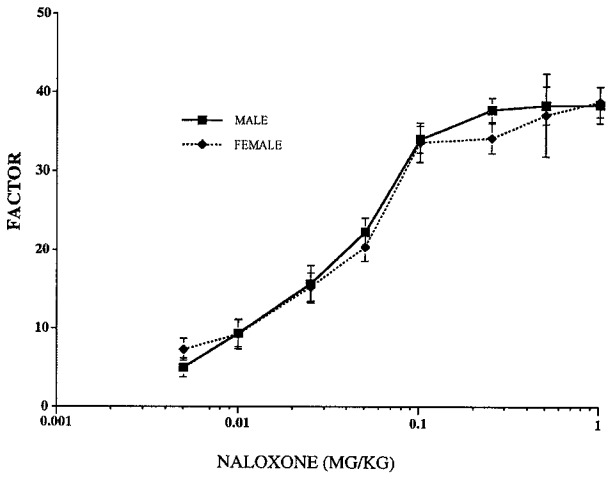

Fig. 6. Mean (±S.E.M.) withdrawal scores observed in male and female rats ($n=15$) implanted with a single morphine pellet for 3 days and then injected with the doses of naloxone shown (log scale). No significant gender differences were found by ANOVA.

shown in Fig. 4, males lost approximately 6% of their body weight 24 h after pellet withdrawal, whereas females lost less than 1%, which was not significantly different from the prewithdrawal baseline. The withdrawal syndrome was also considerably more prolonged in males than in females (Figs. 3 and 4).

3.3. Gender differences in precipitated withdrawal

To examine whether gender-related differences also existed in naloxone-precipitated withdrawal, as was found with spontaneous withdrawal, male and female rats were injected with morphine for 14 days as discussed above. One hour after the last morphine injection on Day 14, the rats were injected with naloxone (0.25 mg/kg) to precipitate withdrawal. The results for the weighted factor and body weight loss are shown in Fig. 5. No differences were observed in the withdrawal syndrome in either sex. We also examined naloxone-precipitated withdrawal subsequent to pellet implantation, which produces substantively more withdrawal behavior than the double injections (Cicero and Meyer, 1973). The rats were injected with naloxone (doses from 0.005 to 1 mg/kg) 3 days after pellet implantation, and the withdrawal syndrome was scored for 60 min. The results of this study are shown in Figs. 6 and 7 for the withdrawal factor and body weight loss, respectively. As shown in Figs. 6 and 7, in marked contrast to our results

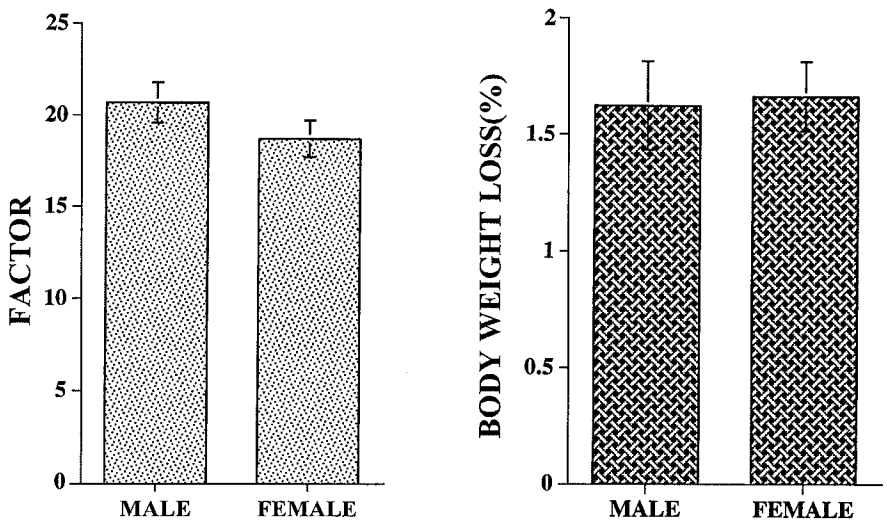

Fig. 5. Mean (±S.E.M.) body weight loss, expressed as % loss in body weight and the graded withdrawal factor in male and female rats ($n=15$ in each group) injected twice daily as described in the Methods. Naloxone (0.25 mg/kg) was injected 1 h after the last morphine injection, and the withdrawal syndrome was rated for 60 min. There were no significant differences.

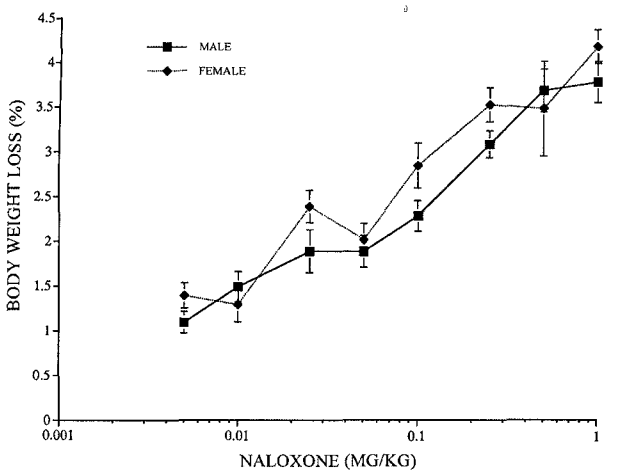

Fig. 7. Body weight loss, expressed as % of body weight reduction, in male and female rats ($n = 15$) implanted with a single morphine pellet for 3 days and then injected with the doses of naloxone shown (log scale). Values are means (±S.E.M.) of three experiments. ANOVA revealed no significant gender effects.

with spontaneous withdrawal, there were no differences observed between males and females in the withdrawal scores and the naloxone dose–response curves were identical.

4. Discussion

The results of these studies establish that the expression of physical dependence on morphine is more severe in male than in female rats during spontaneous withdrawal after chronic morphine administration. These observations suggest that, at least with respect to spontaneous withdrawal, the severity of the withdrawal response to opiates is considerably greater in males than in females. It appears that these differences may be associated with gender-related distinctions in the sensitivity of the central nervous system to the dependence-producing properties of morphine, since it has been observed that pharmacokinetic factors (e.g., blood and brain levels of morphine) are no different in male and female rats administered morphine either acutely or chronically (Cicero et al., 1996, 1997). In contrast to our results with spontaneous withdrawal, it is important to note that we found no gender differences in naloxone-precipitated withdrawal, which, as discussed further below, suggests that equivalent degrees of physical dependence were generated in males and females during chronic morphine administration, but that, for reasons that are currently unclear, the severity of the spontaneous withdrawal syndrome is markedly gender dependent.

Our observations are to our knowledge the first in the literature to demonstrate gender-related differences in the magnitude of the spontaneous withdrawal syndrome after chronic opiate administration. However, naloxone-precipitated withdrawal has been examined in two previous studies. In agreement with the present results, Ali et al. (1995) reported that the naloxone-precipitated withdrawal syndrome appeared to be equivalent in most respects in male and female rats treated for a brief time (6 days) with injections of morphine and then injected with naloxone. In contrast to these results, and the present experiments, Craft et al. (1999) found that males had slightly more, but significant, withdrawal syndromes than females after naloxone-precipitated withdrawal in rats injected chronically with morphine. We have no explanation for the differences in our results and others (Ali et al., 1995) and those of Craft et al. (1999), but the very slight degree of withdrawal engendered by a brief period of chronic injections in the latter studies, may have exaggerated a rather small difference in the rated withdrawal syndrome. In the two previous studies just described (Ali et al., 1995; Craft et al., 1999), spontaneous withdrawal unfortunately was not examined, so it appears that the present results may be unique.

The most striking aspect of the data described in this paper is the magnitude of the gender-related differences in the expression of opiate withdrawal. Following cessation of chronic morphine administration or withdrawal of a single 75-mg morphine pellet, male rats had significantly greater scored withdrawal symptoms than females. For example, male rats lost 6–7% of their prewithdrawal body weight 24 h postwithdrawal, whereas females suffered very mild body weight losses. This gender difference is all the more striking in the pellet implantation studies when it is considered that females weigh approximately 20% less than males and, thus, a 75-mg pellet may have been expected to produce greater levels of physical dependence in females than in males, which is precisely the opposite of what we observed. These data clearly suggest that there are intrinsic gender-related differences in the dependence-producing properties of the morphine.

The most interesting observations in this set of studies, of course, is that we found significant gender differences only in spontaneous opiate withdrawal. The naloxone-precipitated withdrawal syndrome was equivalent in both sexes. Moreover, the naloxone dose–response curves also were not different between the two sexes. The relevance of these observations to the issue of changes in opiate receptor profiles during chronic morphine administration will be discussed below, but these data indicate, most significantly, that chronic morphine administration apparently produced equivalent levels of physical dependence in males and females as reflected in naloxone-precipitated withdrawal. Since naloxone-precipitated withdrawal is broadly assumed to accurately reflect the full magnitude of morphine's dependence-producing properties, it appears that males display significantly more spontaneous withdrawal symptoms than females, even though the same degree of physical dependence is produced during chronic morphine treatment. At this point, we can offer no reasonable explanation for the differences we have observed in spontaneous opiate withdrawal if, in fact, the same degree of physical dependence

was produced by chronic morphine administration. However, given the fact that spontaneous withdrawal is the normal event, as opposed to precipitated withdrawal, our observations could have important implications for both preclinical and clinical examinations of morphine's physical dependence-producing properties.

One reasonable hypothesis to explain the gender-related differences in the expression of the opiate withdrawal syndrome is that there are differences in the density or affinity of those opiate receptors, or in the post-receptor processes (i.e., signal transduction), which might be involved in mediating the dependence-inducing properties of morphine. If this were true, one might expect that naloxone-precipitated withdrawal would also display gender-related differences analogous to those observed with spontaneous withdrawal. Specifically, although it has been extraordinarily difficult to establish that chronic opiate administration influences the number or affinity of opiate receptors (for reviews, see Fleming and Taylor, 1995; Johnson and Fleming, 1989; Loh et al., 1988), it is well established that the dose of naloxone required to precipitate withdrawal decreases markedly as the degree of physical dependence increases (Fleming and Taylor, 1995; Loh et al., 1988; Takemori et al., 1973; Way et al., 1969). This shift in the naloxone dose–response curve has been interpreted to suggest that physical dependence induces differences in the affinity of opiate receptors for agonists and antagonists or, more likely, in post-receptor signal transduction processes (for reviews, see Fleming and Taylor, 1995; Loh et al., 1988). If this interpretation is correct, our finding that there were no gender-related differences in the naloxone dose–response curves for precipitated withdrawal would suggest that there are apparently no discernible differences in opiate receptor profiles or in signal transduction processes in males and females subsequent to chronic opiate administration. This conclusion may not be surprising, since although Hammer (Hammer, 1984, 1985, 1990; Hammer et al., 1994) has reported gender-linked differences in the density of opioid receptors in discrete sexually dimorphic brain regions in males and females, in most important respects, the overall opiate receptor profiles in brain were remarkably similar. The present results could provide additional, albeit indirect, evidence that there may also be no readily detectable gender-related differences in chronic opiate-induced changes in the receptors or signal transduction processes, which mediate the development of physical dependence. Based upon these observations, it seems reasonable to conclude that neither changes in opiate receptor profiles nor in post-receptor processes induced by chronic morphine administration can readily explain the gender differences we have observed in spontaneous opiate withdrawal.

One of the more reasonable explanations of gender-related differences in the response to opiates is that sex steroids may mediate these effects. We are unaware of any studies that have examined whether either the acute "activational" effects of the steroids or their organizational effects, which mediate sexual differentiation of brain morphology and neurobiology (Arnold and Breedlove, 1985; Breedlove, 1994; Goy et al., 1964), can explain any of the gender differences observed in morphine's pharmacology. Studies are currently underway examining this possibility.

The results of these studies extend earlier observations from our laboratory (Cicero et al., 1996, 1997) and others (Baamonde et al., 1988; Islam et al., 1993; Kepler et al., 1989) in which it was found that males were more sensitive to the antinociceptive properties of morphine and other mu selective agonists. The generality of gender-related differences in the pharmacology of the opiates has also been explored. In a series of studies, Craft et al. (1996, 1998) have reported that the discriminative stimulus properties of morphine may be influenced by gender with females more sensitive than males. The present observations that there are differences in the dependence-producing properties of the opiates extend these earlier studies and add further evidence that there may be several key gender-linked differences in the acute and chronic pharmacological profile of the opiates: antinociception; their discriminative stimulus properties; and the expression of the withdrawal syndrome. Whether there are gender differences in the abuse potential of the opiates has not yet been directly addressed in any study known to us, which is surprising since it has been speculated that gender may play a role in the abuse liability of cocaine and alcohol (Bailey et al., 1993; Ball et al., 1995; Babor et al., 1992; Griffin et al., 1989; Kosten et al., 1995; Lex, 1991; Rapp et al., 1995). However, in a recent study (Cicero et al., 2000), we observed strong gender-related differences in the ability of morphine to serve as a positive reinforcing agent in a place preference paradigm. Morphine served as a positive reinforcement in both males and females, but as the dose increased, morphine lost its positive reinforcing properties in males but not in females; females continued to show a preference for morphine even at doses that produced significant adverse events, including death (Cicero et al., 2000). The present data would seem to reinforce the later observation and both sets of observations would seem to provide, at the very least, a strong foundation to explore whether gender differences may exist in the abuse potential of opiates.

In conclusion, we have documented robust differences between males and females in the expression of spontaneous opiate withdrawal. These data add further evidence that gender differences can be observed in several aspects of opiate pharmacology, including antinociception, their positive reinforcing properties and discriminative stimulus properties. However, it seems equally clear that, although we may be able to eliminate some obvious factors that could mediate these gender differences (e.g., pharmacokinetic variables and perhaps opiate receptor/signal transduction processes), we are not yet in a position to speculate on the mechanisms involved. These gender differences do, however, appear to reflect markedly enhanced central nervous system sensitivity to the dependence-producing properties of morphine in males

when compared to females, as opposed to any differences in the pharmacokinetics of morphine. The mechanisms underlying these effects, and the generality of our results to other aspects of opiate pharmacology, clearly need to be assessed with particular emphasis on possible gender differences in the abuse potential of the opiates.

Acknowledgments

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