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Gender differences in the cardiovascular responses to morphine and naloxone in spinal rats

Silvia L. Cruz *, Gabriela Rodríguez-Manzo

Departamento de Farmacología y Toxicología, Cinvestav, IPN, Apartado Postal 22026, Mexico City 14000, D.F., Mexico

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Abstract

Putative gender differences in opiate cardiovascular effects were evaluated in spinal rats. After a 4-h exposure to a single dose of morphine (30 mg/kg, i.v.), abstinence was precipitated by naloxone (0.03–3 mg/kg, i.v.). Morphine produced a long-lasting bradycardia and a transient increase in arterial pressure that was similar in both genders. Thereafter, blood pressure decreased both in males and females. Naloxone precipitated a similar dose-dependent heart rate increase in both sexes and a gender-dependent increase in blood pressure. This sex difference appeared in the shape of the response. Prazosin (0.2 mg/kg), prior to naloxone, reduced the pressor response in all animals, suggesting a similar participation of the noradrenergic system in both genders. The present results extend to acute dependence the notion of a sex-dependent differential effect of morphine. The need to consider gender as a factor when studying the effects of opioids is highlighted.

Keywords: Opiate; Dependence, acute; Gender difference; Cardiovascular system; Blood pressure; Heart rate; Rat, spinal

1. Introduction

Gender differences in several acute opiate responses are well documented. Among them, sex-related differences have been established for the effects of morphine on discriminative stimulus (Craft et al., 1999), locomotor activity (Kavaliers and Innes, 1987), temperature (Quock et al., 1985), corticosterone deficit (Nock et al., 1998) and, the most consistent finding, on antinociception. Thus, several authors have found that male rodents are, in general, more sensitive to morphine-induced analgesia than female animals in various models of nociception (Baamonde et al., 1989; Lipa and Kavaliers, 1990; Cicero et al., 1996; Boyer et al., 1998 but see Ali et al., 1995). Although less explored, there are several studies reporting on gender differences in the chronic effects of opiates. For example, Ali et al. (1995), found that females displayed greater abstinence responses than males after a naloxone challenge in morphine-treated rats. Similarly, Katovich and O'Meara (1987) reported that the skin temperature response to naloxone in the morphine dependent rat was more pronounced in females than in males. On the other hand, recent data from Craft et al. (1999) revealed that male rats showed a higher withdrawal score and a greater tolerance to morphine-induced antinociception than females. Regardless of the response analysed and the direction of the changes, a sex-related differential response to opiates is a common finding.

The development of dependence is generally considered as a result of chronic exposure to opiates, however, it is now well established that an antagonist can precipitate withdrawal symptoms after a single administration of an agonist such as morphine (Heishman et al., 1989; Cruz et al., 1993; Easterling and Holtzman, 1997). This state is known as acute dependence and has been demonstrated in several experimental paradigms and in humans. Our group has worked with the spinal rat made acutely dependent to morphine. In this preparation, a significant increase in blood pressure can be elicited by naloxone in animals that received a single injection of 30-mg/kg morphine. This response depends on the dose of the agonist, the dose of the antagonist used for precipitating abstinence and the time of morphine exposure (Cruz and Villarreal, 1993).

Sympathetic hyperactivity has long been recognised as an objective and reliable estimate of morphine withdrawal intensity (see, for instance, Martin and Eades, 1964; Dixon and Chandra, 1987; Delle et al., 1990). Previous data from

* Corresponding author. Tel.: +52-5-483-2853; fax: +52-5-483-2863.
E-mail address: jcmolcruz@compuserve.com.mx (S.L. Cruz).

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our laboratory show that the noradrenergic systems plays an important role in the cardiovascular component of withdrawal in the spinal rat. Thus, the general purpose of the present study was to establish if there are gender differences in the cardiovascular responses to morphine administration and naloxone-precipitated abstinence in spinal rats made acutely dependent to morphine. An additional objective was to investigate if the noradrenergic system plays a role in the mediation of these putative differences.

2. Materials and methods

2.1. Animals

Male and female Wistar rats weighing between 200 and 300 g were used. All experimental procedures were performed under approval of our institutional ethics committee. Animals were kept under 12 h light:12 h dark conditions with lights on at 07:00 h, controlled temperature (22–24°C) and free access to food and water. At least six animals were used for each naloxone dose and each rat was used only once. Experiments were performed between 0800 and 1400 h and the series were completed within the same season of the year. Female rats were ovariectomised under pentobarbital anaesthesia (30 mg/kg, i.p.) at least 2 weeks before the experiments to avoid the influence of oestrus cycle fluctuations. After the conclusion of the experimental series, the animals were euthanised.

2.2. Drugs

Morphine hydrochloride was obtained from Merck (Darmstadt, Germany) via the Mexican Ministry of Health. Naloxone hydrochloride, prazosin free base, and gallamine triethiodide were purchased from Sigma (St. Louis, MO). All drugs, except prazosin, were dissolved in saline. Prazosin was initially dissolved in propylene glycol and then in saline to reach a final concentration of 20%. All substances were i.v. injected in a volume of 1 ml/kg.

2.3. General procedure

The detailed procedure has been previously described (Cruz and Villarreal, 1993). Briefly, a tracheostomy was performed under ether anaesthesia to mechanically assist the ventilation with a rodent respirator pump (BioScience, 2 cm^3/100 g, 54 breaths/min). A spinal transection by electrocoagulation at the level of C1 was done and the brain was destroyed with a short steel rod introduced through the foramen magnum. After bilateral vagotomy, indwelling cannulae were placed in the left carotid artery for blood pressure monitoring (Gould Statham P23 1D transducer connected to a Grass polygraph) and in the right jugular vein for treatment administration. Heart rate was assessed by integration of the blood pressure signal by a cardiotachometer (Grass). Treatments were administered after a 70-min resting period following spinal transection.

Acute dependence was induced by a single dose of morphine (30 mg/kg). Animals receiving saline instead of morphine served as controls. Four hours later, naloxone was administered to both control and morphine-treated groups (see below). A 5-min infusion of gallamine (final dose 20 mg/kg) was given to all animals that received morphine, starting 10 min before naloxone administration. This blocking agent, used to avoid muscular movements during withdrawal, does not affect cardiovascular responses when given as an infusion (Cruz and Villarreal, 1993).

Two experiments were conducted. The first one to study the cardiovascular effects of opiates and the second one to determine if the noradrenergic system participates in the mediation of the abstinence response precipitated by naloxone (see below).

2.4. Experiment 1: cardiovascular effects of opiates

For this experiment, a total of 120 animals were used. Four independent groups were established ($n = 30$, each): male and female rats treated with morphine and male and female rats treated with saline. This number of animals was required to make dose–response curves for the abstinence response precipitated by five increasing doses of naloxone ($n = 6$, each). As a result of this design, data from 30 animals of each group were available for the study of acute morphine or saline effects. Such a large number was not necessary to perform statistical analysis, thus, samples of 12 animals per group were randomly chosen.

2.4.1. Acute morphine effects

Heart rate (HR) and mean arterial pressure (MAP) were continuously recorded. The changes observed after morphine/saline administration were determined at several times (from 1 min to 4 h) and compared with control pre-treatment values.

2.4.2. Effects of naloxone in acutely dependent rats

Naloxone was administered to male and female rats 4 h after morphine injection. The abstinence response precipitated by the antagonist was recorded for 30 min following administration and a dose–effect function was determined (naloxone 0.03, 0.1, 0.3, 1, or 3 mg/kg). Saline-treated animals served as controls.

2.5. Experiment 2: participation of the adrenergic system in naloxone-precipitated abstinence

For this experiment, two additional groups were established: morphine-treated male and female rats ($n = 6$ each)

that received 0.2 mg/kg prazosin (an α_1-adrenoceptor antagonist) 5 min before 3 mg/kg naloxone. As in the previous experiment, abstinence was precipitated after 4 h of morphine exposure. Results were compared with the data of morphine-treated animals that received the same naloxone dose in the previous experiment.

2.6. Statistics and data analysis

Naloxone effects on heart rate in morphine-treated rats were expressed as the change in heart rate with respect to pre-naloxone levels. Since the abstinence pressor response exhibited a complex pattern, the following parameters were evaluated: (a) the maximum increase in MAP (given by the highest peak effect observed during the first 10 min after naloxone administration); (b) the total area under the MAP tracing (considering the 30-min recording period); and (c) the number of major pressure peaks. A major peak was considered a phasic activity, lasting more than 2 min and raising MAP at least 50% above the tonic level. A two-way analysis of variance (ANOVA), considering sex, time and the putative interaction between these factors, was used for the study of the acute effects of morphine. This test was also applied for the analysis of the effects of naloxone in morphine or saline-treated rats, considering gender and naloxone dose as factors. Additionally, for each gender, a one-way ANOVA for repeated measures was performed followed by Dunnett's test when needed. Mann–Whitney Rank Sum test was applied for comparisons between median responses of independent groups. The SigmaStat program (version 2.03) was used for statistical analysis.

3. Results

3.1. Experiment 1: cardiovascular effects of morphine

3.1.1. Acute morphine effects

3.1.1.1. Effects on heart rate. A single dose of 30 mg/kg morphine produced an immediate decrease in heart rate (in the first minute) of similar magnitude in males and females (see Table 1). This bradycardic effect remained constant in males for the duration of the experiment, while progressively recovered in females 1 h after morphine administration. Although the trends were clear, the gender × time interaction was not statistically significant (two-way ANOVA, $d.f. = 6$, $F = 0.717$, $P = 0.636$). Control spinal male and female rats that received saline instead of morphine did not show significant changes in heart rate (Table 1; two-way ANOVA gender × time interaction: $d.f. = 6$, $F = 0.049$, $P = 1.0$).

3.1.1.2. Effects on mean arterial pressure. In both genders, morphine produced an immediate and brief increase in blood pressure (of approximately 15 mm Hg) that lasted less than 2 min. Thereafter, MAP values went back to control levels and remained relatively stable for 1 h in females and for less than 30 min in males. After these times, mean arterial pressure slightly, but constantly, decreased until the end of the observation period (Table 1). Saline administration to control rats also produced a brief and mild increase in blood pressure of less than 5 mm Hg that, however, reached statistical significance. Except for this transient change, saline-treated females showed no

Table 1
Time course of acute morphine effects in spinal rats ($n = 12$)
One-way ANOVA for repeated measures for HR in morphine-treated groups: (males, $F = 12.96$, $d.f. = 6$, $P < 0.001$; females, $F = 6.94$, $d.f. = 6$, $P < 0.001$), in saline-treated groups: (males, $F = 1.54$, $d.f. = 6$, n.s.; females, $F = 0.98$, $d.f. = 6$, n.s.). One-way ANOVA for repeated measures for MAP in morphine-treated groups: (males, $F = 41.98$, d.f. = 6, $P < 0.001$; females, $F = 29.24$, $d.f. = 6$, $P < 0.001$), in saline-treated groups: (males, $F = 13.06$, $d.f. = 6$, $P < 0.001$; females, $F = 5.90$, $d.f. = 6$, $P < 0.001$).

	Control	Morphine or saline	Time after treatment administration				
			10 min	30 min	1 h	2 h	4 h
HR (beats per min)							
Morphine-treated groups							
Males	322.5 ± 13	285.4 ± 11[a]	291.3 ± 12[a]	287.5 ± 12[a]	283.8 ± 13[a]	285.4 ± 13[a]	298.0 ± 14[a]
Females	314.6 ± 10	277.5 ± 9[a]	275.4 ± 8[a]	284.6 ± 7[a]	291.7 ± 8	295.0 ± 10	321.3 ± 14
Saline-treated groups							
Males	312.5 ± 9	298.3 ± 9	309.2 ± 11	303.3 ± 11	308.8 ± 13	308.3 ± 12	313.7 ± 8
Females	299.6 ± 10	288.8 ± 10	295.4 ± 11	288.3 ± 11	287.1 ± 12	292.5 ± 15	306.7 ± 9
MAP (mm Hg)							
Morphine-treated groups							
Males	72.7 ± 1.5	87.5 ± 3[a]	69.6 ± 1.8	67.9 ± 1.6[a]	64.6 ± 1.4[a]	62.1 ± 1.2[a]	64.2 ± 1.7[a]
Females	66.5 ± 1.4	80.6 ± 2[a]	65.8 ± 1.2	63.3 ± 1.5	62.3 ± 1.3	61.3 ± 1.8[a]	61.9 ± 1.7[a]
Saline-treated groups							
Males	67.9 ± 2	72.5 ± 2.3[a]	67.3 ± 2	66.3 ± 2	63.9 ± 2[a]	62.7 ± 2.3[a]	61.0 ± 2.9[a]
Females	71.0 ± 1.5	74.6 ± 1.8[a]	70 ± 1.4	69.8 ± 1.5	69.2 ± 1.4	67.9 ± 2.2	68.9 ± 2.1

[a] $P < 0.05$; Dunnett's test.

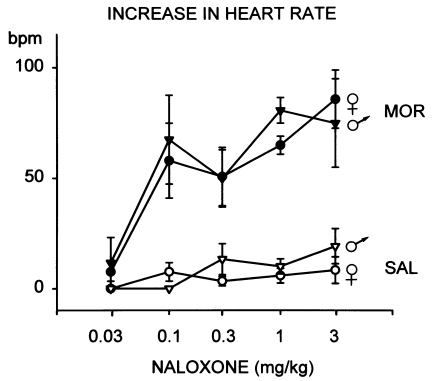

Fig. 1. Dose–response curves for increases in heart rate (beats per minute, bpm) produced by naloxone administered to morphine-treated (filled symbols) and saline-treated (empty symbols) male (triangles) and female (circles) spinal rats. Each point represents the mean ± S.E.M. of at least six animals.

significant variations in blood pressure along the experimental session. In contrast, a significant decrease in blood pressure was observed in male control rats. This effect appeared 1 h after saline and remained for the rest of the experiment. In spite of the apparent differences in the time course of both morphine and saline, the two-way ANOVA revealed no significant gender × time interaction effect in any group (morphine treated animals: $d.f. = 6$, $F = 0.836$, $P = 0.54$; saline-treated animals: $d.f. = 6$, $F = 0.507$, $P = 0.802$). On the other hand, since these differences appeared both in saline and morphine-treated rats, the gender difference cannot be attributed to morphine treatment.

3.1.2. Effects of naloxone in acutely-dependent rats

3.1.2.1. Effects on heart rate. When naloxone was given to rats treated with morphine for 4 h, a biphasic heart rate effect was observed. As previously reported (Cruz and Villarreal, 1993) an initial and brief bradycardia (less than 2 min) was seen in both morphine and saline-treated rats (data not shown). After this, an increasing and long-lasting tachycardia that reached its maximum in the first 10 min was observed in morphine-treated, but not in morphine-free animals. Based on these findings only the tachycardic response was analysed in this work. Fig. 1 shows the dose–response curves for naloxone in all groups. Naloxone elicited a similar dose-dependent increase in heart rate in both morphine-treated (filled symbols) male and female rats (two-way ANOVA for gender: $d.f. = 1$, $F = 0.213$, $P = 0.646$; for naloxone dose: $d.f. = 4$, $F = 11.704$, $P < 0.001$; for gender × naloxone interaction: $d.f. = 4$, $F = 0.288$, $P = 0.885$). By contrast, no effects were seen after naloxone in control saline-treated animals (empty symbols; two-way ANOVA for gender: $d.f. = 1$, $F = 1.520$, $P = 0.223$; for naloxone dose: $d.f. = 4$, $F = 1.921$, $P = 0.121$;

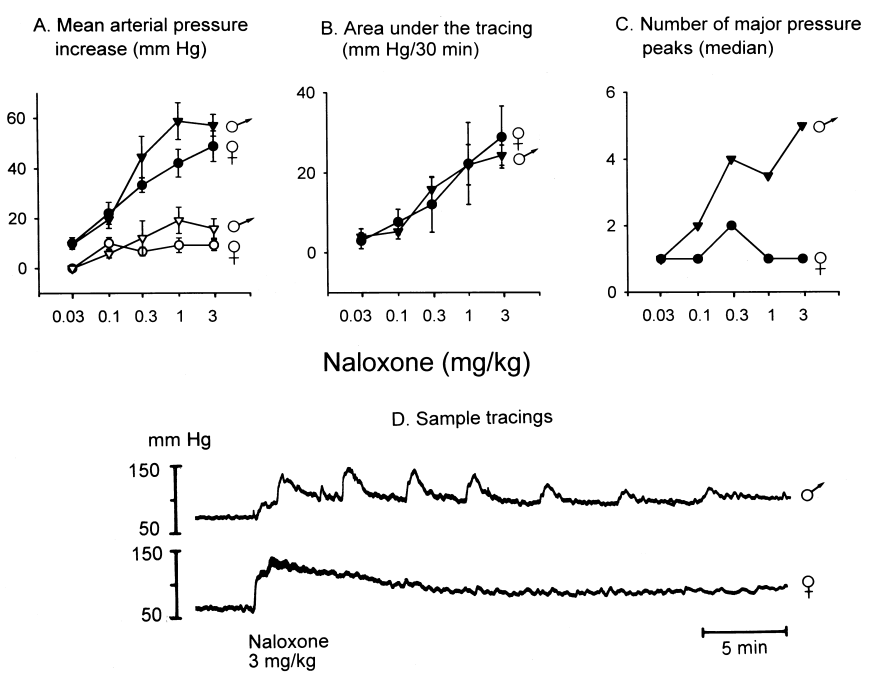

Fig. 2. (A) Dose–response curves for increases in mean arterial pressure (MAP) precipitated by naloxone in male (triangles) and female (circles) spinal rats treated with morphine or saline (filled and empty symbols, respectively). (B) Dose–response curves for the area under the MAP tracing considering the 30-min recording period in morphine-treated rats. (C) Dose–response curves showing the median number of major pressure peaks as a function of naloxone in morphine-treated animals. In all curves, each point represents the mean ± S.E.M. of at least six animals. D) Sample tracings of the pressor responses observed after the administration of 3 mg/kg naloxone in male and female rats previously exposed to 30 mg/kg morphine for 4 h. Several major pressure peaks (seven in this example) are superimposed to basal oscillations, (variations of approximately 5 mm Hg). In females, only one major pressure peak that slowly returns to basal levels is observed.

for gender × naloxone interaction: $d.f. = 4$; $F = 1.666$; $P = 0.173$).

3.1.2.2. Effects on mean arterial pressure. Fig. 2 shows the naloxone dose–response curves for mean arterial pressure in morphine-treated males and females (filled symbols) and in saline-treated animals (empty symbols). Changes in MAP after naloxone administration were evaluated by the maximum increase in blood pressure (A), the area under the MAP tracing (B) and the number of major pressure peaks (C, see Section 2). The increase in MAP (panel A) showed a naloxone dose-dependent relationship in both morphine-treated males and females. In control rats, the opiate antagonist lacked an effect. The two-way ANOVA revealed that there was a gender-related difference in morphine-treated animals. No significant gender × naloxone interaction was found (for morphine-treated animals: $d.f. = 1$, $F = 4.6$, $P = 0.037$ for gender; $d.f. = 4$, $F = 28.759$, $P < 0.001$ for naloxone dose; $d.f. = 4$; $F = 1.273$; $P = 0.239$ for gender × naloxone interaction. For saline-treated rats: $d.f. = 1$, $F = 2.354$, $P = 0.131$ for gender; $d.f. = 4$, $F = 2.011$, $P = 0.107$ for naloxone dose; $d.f. = 4$, $F = 1.453$, $P = 0.231$ for gender × naloxone interaction).

As can be seen in sample tracings of Fig. 2 (panel D), a striking difference between genders was observed in the shape of the naloxone-precipitated responses in morphine-treated animals. It is worth mentioning that blood pressure in the spinal rat showed several minor peaks (oscillations of pressure of approximately 5 mm Hg) along the recording session. When naloxone was administered, a significant and long-lasting increase in MAP was seen superimposed to these basal oscillations in both sexes. However, a gender difference appeared in the pattern of this increase. Thus, males consistently exhibited several major pressor peaks in contrast to females that showed only one major peak that slowly returned to basal levels. Due to this complex pattern, an analysis of the area under the MAP tracing and of the number of major pressure peaks of morphine-treated animals was performed. Panel B shows that there were no differences in the area under the MAP tracing between genders (two-way ANOVA: $d.f. = 1$, $F = 0.102$, $P = 0.751$ for gender; $d.f. = 4$, $F = 30.261$, $P < 0.001$ for naloxone dose; $d.f. = 4$; $F = 0.775$; $P = 0.547$ for gender × naloxone interaction). The median number of major pressure peaks in morphine treated rats are shown in panel C. Statistical analysis of this parameter revealed significant differences for gender ($d.f. = 1$, $F = 29.823$, $P < 0.001$), naloxone dose ($d.f. = 4$, $F = 4.025$, $P = 0.007$) and gender × naloxone interaction ($d.f. = 4$; $F = 3.227$; $P = 0.02$; two-way ANOVA).

3.1.3. Experiment 2: participation of the adrenergic system in naloxone-precipitated abstinence

This experiment further analysed the pressor responses in morphine-treated rats of both genders. The effects of

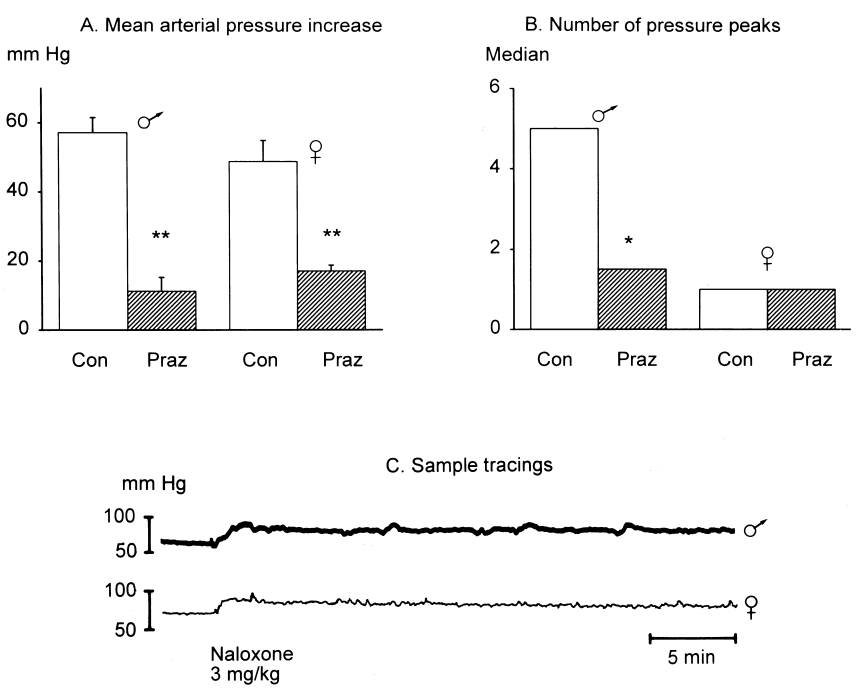

Fig. 3. Effects of prazosin (0.2 mg/kg) pretreatment (−5 min) on naloxone-precipitated abstinence responses in spinal rats treated with 30 mg/kg morphine for 4 h. (A) Increases in mean arterial pressure produced by 3 mg/kg naloxone in the presence of prazosin (Praz, dashed bars) and in control rats that only received morphine (Con, empty bars). Each bar represents the mean ± S.E.M. of at least 6 determinations. (B) Median number of pressure peaks in prazosin-pretreated (Praz) and control (Con) rats in response to naloxone (3 mg/kg). (C) Sample tracings of naloxone-precipitated abstinence responses in male and female rats pretreated with prazosin. $^{*}P < 0.05$; $^{**}P < 0.01$, Mann–Whitney's Rank Sum test.

administering 0.2 mg/kg prazosin 5 min prior to naloxone are shown in Fig. 3. Control responses were increases in mean arterial pressure observed in animals exclusively treated with morphine (empty bars). As shown in panel A, naloxone produced a pressor response in both sexes that was reduced by prazosin pre-treatment (dashed bars). This reduction affected also the magnitude of the pressure peaks. Although these were still identifiable, their magnitude did not meet the definition of a major pressure peak any longer. Thus, considering the overall reduction of the response elicited by prazosin pre-treatment, pressure peaks, in contrast to major pressure peaks, were defined as phasic activity, lasting more than 1 min and raising MAP at least 15% above the tonic level. Using these criteria, it was found that the number of pressure peaks (panel B) was also clearly reduced by prazosin, but only in males (from a median value of approximately 5 peaks to a value close to 2). Only one peak of pressure was seen in the vast majority of morphine-treated female rats and prazosin pre-treatment did not abolish the peak. Sample tracings are included to illustrate the nature of the responses observed. It is worth mentioning that, although the reduction in the number of peaks was clear, an important individual variability was observed, since some males showed only one peak, while others exhibited up to 5.

4. Discussion

4.1. General

The purpose of this work was to characterise the acute effects of morphine and the changes elicited by naloxone after morphine exposure on the cardiovascular system of male and female spinal rats.

The results of this study can be summarised as follows: (1) An acute morphine administration produced a similar decrease in heart rate in both genders. Although this bradycardic effect lasted longer in males than in females, the difference did not reach statistical significance. (2) Morphine produced an initial brief increase in mean arterial pressure that was similar in both sexes. After this transient change, a mild but constant decrease in blood pressure was seen. (3) In morphine-treated rats, naloxone produced a dose-dependent increase in heart rate that was similar in both sexes. (4) Naloxone produced a dose-dependent increase in blood pressure that was gender dependent. Although both sexes exhibited an important and long-lasting increase in this parameter, a phasic response with several major pressure peaks was seen in males, but not in females. (5) Prazosin reduced the abstinence pressor response precipitated by naloxone with similar efficacy in males and females. Besides, a reduction in the number of pressure peaks was seen in males.

4.2. Acute morphine effects

There are several studies on the acute cardiovascular effects of opiates (Buccafusco, 1983; Chan et al., 1999) but, to our knowledge, the issue of putative gender differences has not been addressed. Present results show that the previously described morphine-induced bradycardia (Randich et al., 1993) and transient increase in blood pressure (Cruz and Villarreal, 1993; Chan et al., 1999) are not influenced by gender. Although a sex-related variation is seen in the time course of both acute morphine and saline effects on blood pressure, this variation seems to reflect basal pre-existing conditions and not a morphine-specific effect. Hence, morphine naive female rats do not show pressor changes during 4.5 h, while males exhibit a significant reduction in this parameter towards the end of the recording period. These data suggest that a different physiological response to spinal transection could occur in males and females.

In a previous work, we reported that mean arterial pressure values were relatively stable in spinal male rats along a similar recording period than the one used in this study (Cruz and Villarreal, 1993). In that work, a trend in blood pressure to decrease along time was noticed, but it did not reach statistical significance. Present results in males and females were obtained analysing a bigger sample of animals (12 instead of 7) and now the trends previously observed are proved to be statistically significant.

4.3. Effects of naloxone in acutely dependent rats

The main finding of this paper is that there are gender differences in the shape of the pressor responses precipitated by naloxone in morphine-dependent spinal animals. Male rats exhibit several major pressure peaks while female animals show only one. Moreover, in males, the number of major pressure peaks was a function of the dose of the antagonist. In morphine-naive animals of both genders, naloxone produces a mild effect that is not significant from the statistical point of view. It is worth mentioning that the doses of naloxone selected for these studies do not produce disturbances in blood pressure per se (Cruz and Villarreal, 1993). As to the heart rate, naloxone elicits a long-lasting tachycardia in morphine-dependent animals that is absent in saline-treated controls and independent of the animal's gender.

Sex differences in the pressor abstinence response elicited by naloxone could reflect, at least, two different phenomena: (a) variations, among sexes, in the state of dependence itself, or (b) a differential responsiveness of the cardiovascular system inherent to genders. From our results, no conclusion can be drawn as to the basis underlying the differences here observed. Nevertheless, it is inter-

esting to mention that there are data in the literature showing gender differences both in the intensity of naloxone-precipitated withdrawal (see below) as well as in cardiovascular responsiveness (see, for instance, Freedman et al., 1987; Luzier et al., 1998).

A few experimental studies indicate that there are sex differences in morphine-induced dependence and withdrawal, however, the results are not consistent. Depending on the experimental paradigm used, females display greater abstinence responses than males (Katovich and O'Meara, 1987; Ali et al., 1995) or show lower withdrawal scores and less tolerance to morphine-induced antinociception (Craft et al., 1999). Interestingly, although sex differences have been addressed for both acute and chronic effects of morphine, to our knowledge, no gender-related differences have been described for acute dependence.

4.4. Participation of the adrenergic system in naloxone-precipitated abstinence

A particular objective of this work was to investigate whether the noradrenergic system played a similar role in the precipitation of the abstinence pressor response in males and females. In a previous study, we found that prazosin administered prior to naloxone clearly reduced the increase in mean arterial pressure characteristic of morphine-dependent male spinal rats (Cruz and Villarreal, 1993). In the same sense, other groups have reported that catecholamines (adrenaline, noradrenaline and dopamine) are released during naloxone-induced withdrawal in rats (Rabadán et al., 1997, 1998). Present results reveal a similar efficacy of prazosin as a blocking agent in males and females, confirming that adrenaline and/or noradrenaline play an important role in the sympathetic manifestations of the abstinence response (Dixon and Chandra, 1987; Chang and Dixon, 1990; Rabadán et al., 1997; Chan et al., 1999). Prazosin pre-treatment also provoked a statistically significant reduction in the number of pressure peaks in males. However, an important individual variation, ranging from 1 to 5 peaks, is observed. The only pressure peak of females does not disappear with prazosin treatment. Yet, in both genders, the magnitude of these responses is reduced by the α_1-adrenoceptor antagonist. The residual pressor response to naloxone after prazosin suggests that other neurotransmitter systems (such as dopamine) are involved. From our results it is not possible to determine the actual nature of the peaks but one possibility is that the lowered pressor response (an increase of about 20 mm Hg instead of approximately 50) is unable to reach the threshold to activate the physiological mechanisms underlying the phasic response. Nevertheless, it appears that the gender difference observed after prazosin in the number of pressure peaks is basal and not attributable to a sex-related differential participation of the α_1 adrenoceptors in this response. Further experiments, specifically designed to establish the pharmacological nature of this component are needed.

The sex-differences in the cardiovascular component of precipitated abstinence here presented could be due to several factors. These include variations among sexes in: (a) opioid pharmacokinetics (Cicero et al. 1997), (b) opioid receptor binding (Zubieta et al., 1999), and density (Weiland and Wise, 1990), (c) gonadal hormone levels (Islam et al., 1993; Kepler et al., 1989), (d) environmental factors, e.g. circadian (Kavaliers and Innes, 1987) and circannual changes (Rodríguez et al., 1980) and, (e) genetic background (Kest et al., 1999). In our study, neither genetic variability, nor differential breeding conditions could have played a role, since animals were all bred in our institution under the same conditions. Circannual and circadian variables were also controlled. Finally, females were ovariectomised to avoid the influence of hormonal fluctuations due to oestrous cycle.

Several of the gender-related differences in morphine effects have been described to depend on steroid hormones. However, the data are inconsistent since reductions (Kepler et al., 1989), increases (Kasson and George, 1984) and no alterations (Cicero et al., 1996) have all been reported, for the same nociception test, as a result of gonadectomy. Besides, other authors have postulated that these variations might be due to the organisational effects of these hormones during the critical brain differentiation period (see, for instance, Cicero et al., 1997). Needless to mention, further experiments are needed to delineate the role played by gonadal hormones in our results.

As to the pharmacokinetic factors, Cicero et al. (1997) reported that similar peak morphine levels were attained in blood and brain of male and female rats during the 60-min period immediately after s.c. injection. In relation to opioid receptors, it has been found that there is an increased binding to μ receptors in women as compared to men in several brain regions (Zubieta et al., 1999). Moreover, denser opiate receptor binding sites have also been described in female rats as compared to males in the sexually dimorphic preoptic area (Hammer, 1984). Present results do not allow any conclusion as to the possible role played by these factors in the responses observed. However, since the main gender difference turned out to be the shape of the naloxone-precipitated pressor response, it could be thought that the difference in the cardiovascular response relies on physiological variables other than opiate availability.

Regardless of the response analysed and the direction of the changes, it appears that gender differences can be observed across a spectrum of morphine's pharmacological effects. In conclusion, although no clear consensus has emerged as to the mechanisms underlying sex-differences in morphine effects, our results, together with previously published observations, highlight the need to consider gender as a factor when studying the effects of opioids.

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