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·*Original Article*·

Effect of testosterone on morphine withdrawal syndrome in rats

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Abstract

Aim: To determine whether testosterone is involved in morphine withdrawal syndrome (WS). **Methods:** In order to induce dependency, rats were treated with subcutaneous injection of morphine (days 1–2, 5 mg/kg; days 3–5, 7.5 mg/kg; days 6–8, 10 mg/kg), and after the last dose of morphine (day 8) WS was induced by intraperitoneal injection of naloxone (1 mg/kg). Wet dog shake (WDS), abdomen writhing (AW), and jumps (J) were recorded as indicators of WS. **Results:** The severity of WDS, AW, and J in male rats was greater than that in females. Accordingly, in 4-week castrated and flutamide-treated (10 mg/kg/day for 8 days, i.p.) male rats, WDS, AW, and J were significantly decreased compared to male control rats. Testosterone replacement therapy (10 mg/kg/day for 8 days, i.m.) in 4-week castrated rats restored the severity of WDS, AW, and J behaviors to the level of non-castrated male rats, whereas testosterone potentiated the WDS behavior in non-castrated male rats. **Conclusion:** It can be concluded that testosterone might be effectively involved in morphine WS. *(Asian J Androl 2008 Sep; 10: 765–769)*

Keywords: testosterone; castration; flutamide; morphine; withdrawal syndrome

1 Introduction

Several studies have shown sex-related differences in many pharmacological properties of morphine such as antinociception [1–4], tolerance to analgesia [5], and stimulant effects [6]. For most abused drugs, there has been a long-standing "gender gap" in frequency of use and addiction; that is, men are more likely than women to use and become dependent on drugs [7]. Cicero *et al*. [8] reported that severity of spontaneous morphine withdrawal syndrome (WS) in male rats is greater than that in female rats. These differences appear to reflect intrinsic gender-related differences in the sensitivity of the brain to morphine, as it has been shown that the levels of morphine in blood and brain are similar in male and female rats at comparable doses [2, 9].

It has been reported that the development of tolerance and dependence on morphine can be inhibited by concomitant chronic treatment with neurosteroids such as allopregnanolone, pregnenolone sulfate, or progesterone [10]. Furthermore, dependency on morphine markedly decreases the brain concentrations of neurosteroids pregnenolone, progesterone, pregnenolone sulfate, and

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testosterone [11, 12], suggesting that changes in the concentration of endogenous neurosteroids might be related to the development of morphine dependence and withdrawal. It has been shown that finastride, as a 5α-reductase inhibitor, could attenuate the development and expression of naloxone-precipated WS [13]. According to other studies, morphine-induced antinociception might be altered by ovarioectomy, pregnancy, and/or exogenous hormones [14, 15], whereas the effect of male gonadal hormones on withdrawal syndrome has not been well studied. In the present study we showed that testosterone plays an effective role in severity of naloxone-precipitated WS of morphine.

2 Materials and methods

2.1 Ethics

Allprocedures were carried out under the ethical guidelines of the Tabriz University of Medical Sciences (Tabriz, Iran) and the studies received approval by the Ethics Committee of the Tabriz University of Medical Sciences, according to the guide for the care and use of laboratory animals [National Institutes of Health (USA) Publication No. 85-23, revised 1985].

2.2 Drugs

All drugs were prepared fresh on the days of experimentation. Testosterone enanthate (Darupakhsh, Tehran, Iran) and flutamide (Sigma, Taufkirchen, Germany) were dissolved in sterile caster oil and ethanol–water (2:1, v/v), respectively. Other drugs such as morphine (Temad, Tehran, Iran) and naloxone (Darupakhsh) were dissolved in 0.9% saline. The dosage of testosterone (10 mg/kg/day, i.p.) and flutamide (10 mg/kg/day, i.m.) was prepared according to Nayebi and Rezazadeh [16].

2.3 Animals

Male and female Wistar rats, weighing 225–250 g, were obtained from the central animal house of the Tabriz University of Medical Sciences. Animals were housed in standard polypropylene cages, four per cage, under a 12 h: 12 h light:dark schedule at an ambient temperature of 25 ± 2ºC and were allowed free food and water. Rats were divided randomly into 13 experimental groups, each comprising eight animals.

2.4 Surgical procedures

The male rats were fully anesthetized with an i.p. injection of sodium pentobarbital (50 mg/kg). Castration was carried out as follows: the ventral scrotum was shaved and scrubbed with Betadine (Behvazan Co., Rasht, Iran); a 1.5-cm transverse incision was made at midline scrotum; the testes were exteriorized through the incision; the tubules were tied with 0.4 silk suture; the testes, epididymis, and associated fat pad were removed; and the incision was closed with wound clips. A sham operation was carried out by making the scrotal incision, gently manipulating the testes, and closing the incision with wound clips.

2.5 Behavioral study

In order to induce dependency, morphine was injected subcutaneously in a schedule of: days 1–2, 5 mg/kg; days 3–5, 7.5 mg/kg; and days 6–8, 10 mg/kg. Fifteen minutes after the last dose of morphine (on day 8), WS was induced by intraperitoneal injection of naloxone (1 mg/kg). After 15 min, the numbers of wet dog shakes (WDS), abdomen writhing (AW), and jumps (J) were recorded as indicators of WS for a period of 40 min by an observer blind to treatment.

2.6 Expression of data and statistics

Descriptive statistics and comparisons of differences between each data set were calculated using SigmaStat software (version 3.1, obtained from Central Library of Tabriz University of Medical Sciences, Tabriz, Iran). The data were expressed as mean ± SEM and were analyzed by one-way ANOVA in each experiment. In the case of significant variation, the values were compared by Tukey's test. Statistical significance was accepted at the level of $P < 0.05$.

3 Results

3.1 Morphine WS in male and female rats

Figure 1 summarizes the number of withdrawal behaviors in male and female rats. As it has been shown, the number of WDS, AW, and J in male rats was greater than that in females ($P < 0.001$, $P < 0.05$ and $P < 0.01$, respectively). Male rats also showed more severe naloxone-induced WS than females.

3.2 Effect of castration and flutamide on morphine WS

The results of morphine WS in 4-week castrated and flutamide-treated (10 mg/kg/day for 8 days, i.p.) male

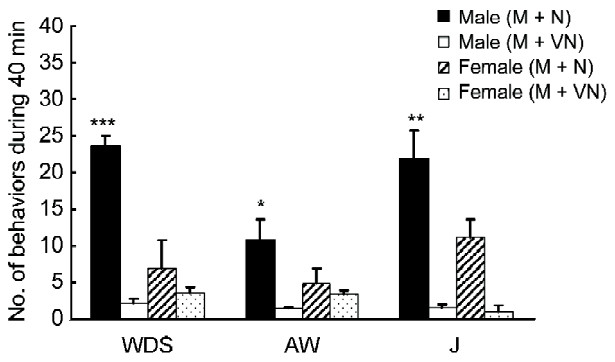

Figure 1. Sex-related differences in morphine withdrawal syndrome (WS), as indicated by wet dog shake (WDS), abdomen writhing (AW), and jumps (J). Data represent the values (mean ± SEM) obtained in male and female experimental groups, expressed as the number of behaviors during 40 min (n = 8 per group). One-way ANOVA was applied followed by Tukey's test. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ compared with female (M + N) rats. M, morphine; N, naloxone; VN, vehicle of naloxone.

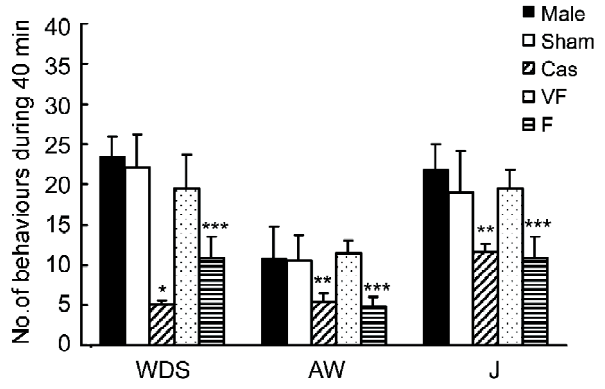

Figure 2. Effect of castration (Cas) and flutamide (F) (10 mg/kg, i.p.) on morphine withdrawal syndrome (WS) in rats, as indicated by wet dog shake (WDS), abdomen writhing (AW), and jumps (J). Each bar represents the number of behaviors during 40 min in experimental groups, expressed as mean ± SEM (n = 8 per group). One-way ANOVA was applied followed by Tukey's test. $^{*}P < 0.001$ and $^{**}P < 0.05$, compared with morphine WS in male and sham-operated rats respectively; $^{***}P < 0.05$ compared with flutamide vehicle (VF)-treated rats and male rats.

rats are shown in Figure 2. Castration caused a decrease in the number of WDS, AW, and J behaviors in comparison with male (non-castrated) and sham-operated rats ($P < 0.001$ and $P < 0.05$, respectively). The number of WDS, AW, and J was decreased ($P < 0.05$) by daily injection of flutamide, as a testosterone receptor antagonist.

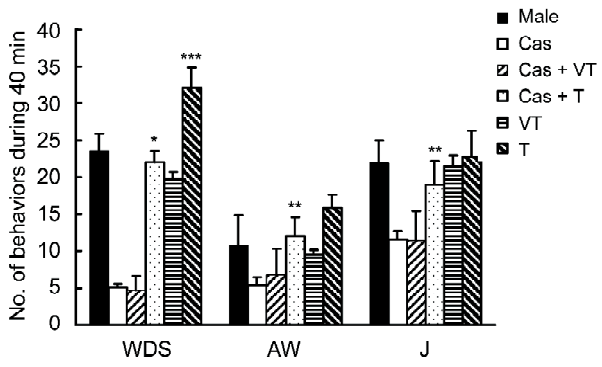

Figure 3. Effect of testosterone (T) (10 mg/kg/day for 8 days, i.m.) replacement therapy and castration (Cas) on morphine withdrawal syndrome (WS), as indicated by wet dog shake (WDS), abdomen writhing (AW), and jumps (J), in male and male castrated rats. Each bar represents the number of behaviors during 40 min in experimental groups, expressed as mean ± SEM (n = 8 per group). One-way ANOVA was applied followed by Tukey's test. $^{*}P < 0.001$ compared with Cas rats and Cas rats treated with vehicle of testosterone (Cas + VT); $^{**}P < 0.05$ compared with Cas rats; $^{***}P < 0.01$ compared with morphine withdrawal syndrome in Male and VT-treated male rats.

3.3 Effect of testosterone replacement therapy on morphine WS

The effect of testosterone replacement therapy (10 mg/kg/day for 8 days, i.m.) on morphine WS was investigated in 4-week castrated rats. As shown in Figure 3, the number of WDS, AW, and J increased to the male rats level ($P < 0.001$ and $P < 0.05$) by injection of testosterone in castrated rats. Accordingly, we observed an increase ($P < 0.01$) in WDS behavior in testosterone-treated male rats in comparison with male (non-castrated) rats.

4 Discussion

The results of this study establish that the expression of physical dependence on morphine is more severe in male rats than in females during naloxone-induced withdrawal after chronic morphine treatment. It appears that these differences might be associated with gender-related distinctions in the sensitivity of the central nervous system to the dependence-producing properties of morphine, as it has been observed that pharmacokinetic factors are the same in male and female rats [2]. Our results are in agreement with the report showing that males have more severe naloxone-induced WS than fe-

males [17]. In contrast, it has been reported that naloxone-precipitated WS appears to be equivalent in most aspects between male and female rats [18]. However, it should be noted that this controversy could be due to differences in doses, duration of treatment, and withdrawal assay methods.

It has been reported that plasma concentration of testosterone and dihydrotestosterone decreases markedly 4 weeks after castration of male rats [19–21]. Therefore, we studied morphine WS in 4-week castrated rats as a model of testosterone-depleted rats. According to our present study, we observed that withdrawal behaviors in male rats are significantly decreased by castration and daily injection of flutamide (a testosterone receptor antagonist). Thus, we might suggest a possible role for testosterone in the behavioral responses to chronic morphine treatment that appeared after naloxone injection. Testosterone has also been found to modify endogenous opioid peptides levels in various sites of brain [22]. Therefore, the roles of endogenous opioid peptides in this regard should not be neglected.

In this study, we showed that the decrease in morphine withdrawal behaviors in male castrated rats was reversed by testosterone replacement therapy. Also, testosterone injections in non-castrated male rats potentiated the severity of behaviors. Recently it has been shown that a higher testosterone level in male rats is one of the most obvious reasons for the development of a clear gender difference in locomotion activity [23], a further indication of the possible role of male androgenic hormone. Another study showed that castration reduces aromatase activity in the hypothalamus–preoptic area of adult rats [24]. As testosterone is converted to estradiol by aromatase enzyme, so attention should be paid to the possible role of estrogens in testosterone-related effects. In conclusion, our data suggest that testosterone has an effective role in severity of morphine withdrawal behaviors. In addition, we suggest that investigation of a possible clinical application of flutamide, as a testosterone receptor antagonist, should be carried out to test its usefulness in diminishing morphine dependency. Further studies are needed to elucidate the exact mechanism of testosterone on brain neuronal systems that are responsible to development of dependency.

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