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...
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-precipitated WS [13]. According to other studies, morphine-induced incoercibility 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

All procedures 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 castor 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 Rezaazadeh [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 ± 2°C 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
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 withdrawal syndrome (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 $P < 0.01$, respectively) by injection of testosterone (Cas + VT); $P < 0.01$ compared with male and VT-treated male 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-
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