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Research report

The effect of opiodergic system and testosterone on anxiety behavior in gonadectomized rats

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HIGHLIGHTS

- Gonadectomy decreased %OAT and %OAE.
- I.p. injection of testosterone and morphine in GDX male rats increased %OAT and %OAE.
- I.p. injection of naloxone in GDX male rats decreased %OAT and %OAE.
- I.p. injection of testosterone 1 h before infusion of morphine in GDX male rats did not significantly alter %OAT and %OAE.
- I.p. injection of testosterone 1 h before administration of naloxone in GDX male rats decreased %OAT and %OAE.

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ABSTRACT

Background and aim: Removal of the testes (gonadectomy; GDX), the primary source of androgens, increases anxiety behavior in several tasks. Opioids are known to play a role in mediating the effects of androgen. In the present study, the effect of testosterone and opioidergic system on anxiety behavior was investigated.

Methods: Adult male Wistar rats were bilaterally castrated. The elevated plus maze which is a useful test to investigate the effects of anxiogenic or anxiolytic drugs in rodents was used.

Results: The data indicated that there is a decrease, 10 days after castration, in the percentage of OAT (the ratio of time spent in the open arms to total times spent in any arms × 100) and OAE (the ratio of entries into open arms to total entries × 100) but not locomotor activity, showing anxiogenic-like effects of gonadectomy. Intraperitoneal injection of testosterone (200, 300 and 450 mg/kg) and morphine (2.5, 5 and 7.5 mg/kg), before testing 10 days after castration, showed an increase in OAT and OAE. Furthermore, injection of naloxone (5 and 7.5 mg/kg, i.p.), 5 min before testing 10 days after castration, decreased OAT and OAE. Also, injection of a significant dose of testosterone (300 mg/kg, i.p.), 1 h before the injection of different doses of morphine (1, 2.5, 5 and 7.5 mg/kg, i.p.), 10 days after castration, did not significantly alter OAT, OAE and locomotor activity. While, administration of a significant dose of testosterone (300 mg/kg, i.p.), 1 h before the infusion of different doses of naloxone (1, 2.5, 5 and 7.5 mg/kg, i.p.), 10 days after castration, decreased OAT and OAE.

Conclusion: The results show the involvement of testosterone and opioidergic system in anxiogenic-like behaviors induced by gonadectomy.

1. Introduction

Removing the testes (gonadectomy, GDX), the prime source of testosterone, results in higher levels of behavior indicative of anxiety in a variety of tasks, in male rats [1–4]. For example, GDX male rats show decreased activity in the central area of the open field and in the open arms of the elevated plus maze [1,4–7], reduced drinking in Vogel's punished drinking test, increased freezing behavior [1], operant tests of spatial and non-spatial working memory and behavioral flexibility, as well as performance on a progressive reward ratio task [5]. Testosterone administration can reverse some of the effects of GDX [1,3,4,7,8]. Testosterone is a main circulating androgen [9]. In humans and in rodents, testosterone are known to be involved in the anxiolytic-like behaviors [10–15], aggressive behavior [15,16], motivation [17], circadian activity rhythms [18], regulation of neural structure [19], mood and cognition [20]. Endogenous testosterone levels have been revealed to be inversely associated with anxiety and depression severity [21]. Further-

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more, metabolites of testosterone may mediate some of androgens' influence on anxiety. Separate reports indicate that replacement with testosterone or its non-aromatizable, 5α-reduced metabolite dihydrotestosterone (DHT) to GDX rats similarly decline anxiety behavior. DHT may be further reduced by the 3α-hydroxysteroid dehydrogenase enzyme (3α-HSD) to 3α-androstanediol (3α-diol). 3α-Diol administration can decrease anxiety behavior of ovariectomized female rats or intact male rats [3,4,7].

Moreover, morphine is involved in anxiety behavior [22]. Several studies have reported an anxiolytic function for morphine and μ-opioid receptor agonists when injected peripherally, whereas μ-opiate receptor antagonists tend to be anxiogenic [23–27]. Similarly, κ-opioid receptor agonists have strong anxiolytic effects in the elevated plus maze, which are also antagonized by naloxone [24]. Also, δ-opioid receptors are involved in the control of emotional responses, including anxiety levels and depressive-like behaviors [28]. Some study indicated that the endogenous opioid system could influence testosterone level via effects on the hypothalamic–pituitary–gonadal axis and the testes [29]. Thus, functional interactions between testosterone and opioid system in behavior control seem possible.

The elevated plus maze (EPM), which is composed of two open and two closed arms, has been used to assess behavioral parameters indicating anxiety in laboratory animals [23,27,30,31]. The evaluation of anxiety is based on the percent preference for open arms. Considering that the animals actively avoid the open arms of an elevated platform, lower percent preference for the open arm confirms intense anxiety. This model is very suitable for the study of anxiolytic or anxiogenic drugs [23,24,30,32]. In this test, the percentage of time in the open arms of the elevated plus maze provides the best measure of anxiety, whereas the number of the closed arms entries provides the best measure of motor activity [33].

Considering the involvement of testosterone and opioidergic system on some behaviors induced by gonadectomy and also the involvement of testosterone and opioidergic system in the modulation of anxiety like behaviors, the purpose of this paper is to report the effects of pharmacological manipulations of testosterone and opiates in the modulation of anxiety processes induced by gonadectomy.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (Pasteur Institute, Tehran, Iran), weighing 250–270 g at the time of surgery, were used. The animals were housed four per cage (50 cm × 50 cm × 40 cm high), in a room under a 12 h light:12 h dark cycle (lights on 08:00 h) and controlled temperature (23 ± 1 °C) with free access to food and water. Male rats were allowed to adapt to the laboratory conditions for at least 1 week before surgery. Animals were handled about 3 min each day prior to behavioral testing. All experiments were performed between 9:00 and 13:00 h and each rat was tested only once. Eight animals were used in each group and each animal was used once only. In this study, all procedures are in accordance with the guide for the Care and Use of Laboratory Animals as adopted by the Ethics Committee of Faculty of Science, Tehran University (357: November 2000).

2.2. Surgery

Male Wistar rats were randomly assigned to one of three groups: normal (intact), castrated, or sham-operated. For surgery, the animals were anesthetized with ketamine hydrochloride 10% (50 mg/kg) plus xylazine 2% (4 mg/kg) in a mixture of 2:1, which was injected in a volume of 2 ml/kg (IP). A 2 cm ventral incision was then made in the scrotum and the skin was cut to expose tunica. The tunica was pierced, and gap was stretched with blunt forceps. The testes were exposed by applying mild pressure to the pelvic region. The spermatic artery was clamped and cauterized, and then the testes were removed. The epididymis, vas deferens and ducts were displaced into the tunica. Sham-operated animals were exposed to the entire procedure noted above with the exception of the removal of the testes (with the aim of measuring possible stress induced by surgery) [6,34–37].

2.3. Elevated plus maze

The elevated plus maze was used as a measure of both anxiogenic and anxiolytic agents in rodents [30]. All experiments were made in a silent and dimly lighted room, separated from the colony room. Male rats were adapted to the testing room for 1 h prior to testing. The elevated plus maze consisted of four arms two of which had no side or end walls (open arms; 50 cm × 10 cm). The other two had side and end walls, but were open on the top (closed arms; 50 cm × 10 cm × 40 cm). Where the four arms intersected, there was a square platform of 10 cm × 10 cm. 10 days after the surgery, the effects of intraperitoneal administration of drugs were tested in the elevated plus maze. Male rats were randomly assigned to treatment conditions and tested in a counter-balanced order. The animals from different experimental conditions were tested sequentially. Rats were individually placed in the center of the elevated plus maze facing a closed arm and allowed 5 min of free exploration. The number of entries into open arms, the number of entries into closed arms, the total time spent in the open arms, the total time spent in the closed arms, rearing, grooming and defecation were measured. The test room was illuminated by two 60-W bulbs located 1.5 m above the apparatus. Raw data were used to manually calculate the anxiogenic and anxiolytic behaviors. Entry was defined as putting all four paws in the maze arms and measured by a hand counter. The percentage of open arm entries and open arm time as standard anxiety indices were measured as follows: (a) OAT (the ratio of time spent in the open arms to total times spent in any arms × 100); (b) OAE (the ratio of entries into open arms to total entries × 100). (c) Total closed and open arm entries were calculated as a relative pure index of locomotor activity [23,26]. Drugs which act on anxiety-like behavior may either increase or decrease OAT and OAE inferring anxiolytic-like or anxiogenic-like response respectively.

In this study, numbers of rearing (the rat maintains an erect posture which is usually associated with sniffing), grooming (the rat rubs its face, ears, mouth, vibrissae and eyes with rapid circular movements of its forepaws) and defecation index (the number of defection boli) were measured as the conventional indices for anxiety-like behaviors [23].

2.4. Drugs

Drugs used in this study were ketamine, xylazine (For surgical procedure; Alfasan Chemical Co., Woerden, and Holland), testosterone (Aboraihan-Daru, Tehran, Iran), morphine sulfate (Temad, Tehran, Iran) and naloxone hydrochloride (Tolid-Daru, Tehran, Iran). Testosterone was dissolved in sesame oil 1 h before the experiment. Testosterone was administered i.p. 1 h prior to testing to ensure that testosterone had time to reach the brain [7]. Morphine and naloxone were dissolved in sterile 0.9% saline just before the experiment. Control animals received saline or sesame. The protocol has been summarized in Table 1