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Relationship between circulating testosterone and emotional behavior in rats

Olanrewaju Akinloye Oyekunle[1], Goke Francis Ibironke[2], and Oluwole Adebayo Opabunmi[1]

1 - Ladoke Akintola University of Technology, Ogbomosho, OY, Nigeria

2 - University of Ibadan, Ibadan, OY, Nigeria

Abstract

The experiment was aimed at investigating the relationship between reduced circulating/endogenous testosterone occasioned by orchiectomy and emotional behavior using the open field test. Eighteen male Wistar rats were randomly selected and classified into two groups: orchiectomized and nonorchiectomized. Orchiectomy was carried out by simple sham surgery. After recovery from orchiectomy, plasma testosterone was determined in both groups after which each animal was observed in the open-field for neurobehavioral activities. The result showed a significant ($p < 0.05$) reduction in plasma testosterone concentration as well as the frequencies of novelty-induced neurobehaviors scored in the open field arena in the orchiectomized group when compared with the nonorchiectomized group. Results indicated that a reduction in circulating testosterone exerts behavioral deficits in orchiectomized animals in the form of fear imposed by exposure to a novel environment resulting in fewer activities. This observation was confirmed by the presence of testosterone receptors in specific brain areas associated with behavioral modulation. We therefore conclude that circulating testosterone could be one of the endogenous mechanisms responsible for coping with fear induced by exposure to a novel environment. **Keywords:** orchiectomy; testosterone; open field; fear; neurobehavior; amygdala.

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Introduction

Testosterone is one of the major sex hormones produced by the body, occurring in both males and females. In males, it is produced mainly by the Leydig cells of the testes, whereas the ovaries and placenta produce it in females. The adrenal cortex also secretes it in both sexes (Mazur & Booth, 1998). Apart from being involved in the development of secondary sexual characteristics in males, it is also of special interest in the study of socioemotional and economic behavior because it influences the brain in archetypical situations such as fight, flight, mating, and struggle for status (Coates, 2010; Eisenegger, Haushofer, & Fehr, 2011). Several studies investigated the association between steroid hormones and neurobehavior in both mammals and other animal species (Brown, 1998; Gahr, 1990). Steroid hormones are widely accepted to modulate animal behavior through indirect actions on neurotransmission in the central nervous system (Hayden-Hixton & Ferris, 1991). This notion of behavioral modulation by steroid hormones is based on the fact that steroid hormone receptors are widely distributed in vital brain areas that modulate emotional behaviors including the hippocampus, amygdala, and prefrontal cortex (Verma & Moghaddam 1996; Zahrt, Taylor, Mathew, & Arnsten, 1997). Testosterone has been implicated in the modulation of some behaviors such as aggression (van Honk et al., 2001; van Honk, Schutter, Hermans, Putman, Tuiten, & Koppeschaar, 2004) and fear (van Honk, Peper, & Schutter, 2005) in both humans and experimental animals. Previous reports have firmly established the fear-reducing properties of testosterone, especially exogenously administered testosterone (Aikey, Nyby, Anmuth, & James, 2002; Aleman, Bronk, Kessels, Koppeschaar, & van Honk, 2004; Berridge, 2003; Boissy & Bouissou, 1994). Fear as an index of emotional behavior is a life-saving emotional state that anticipates and adapts to danger (Gallagher & Holland, 1994). However, through multiple genetic, developmental, and environmental factors, the adaptive

Olanrewaju Akinloye Oyekunle and Oluwole Adebayo Opabunmi, Department of Physiology, College of Health Sciences, Ladoke Akintola University of Technology, P.M.B. 4000, Ogbomoso, Oyo State, Nigeria. Goke Francis Ibironke, Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria. Correspondence regarding this article should be directed to: O.A. Oyekunle, Department of Physiology, College of Health Sciences, Ladoke Akintola University of Technology, P.M.B. 4000, Ogbomoso, Oyo State, Nigeria. Phone: 234 803 074 2928. E-mail: oaoyekunle@lautech.edu.ng

properties of fear can go awry (Charney, 2004; Gross & Hen, 2004), leading to aggression or destruction. The destructive value of excess fear has been emphasized in both animal and human models of psychopathology (LeDoux, 1996; Lang, Davis, & Öhman, 2000). This behavior is often exhibited when the fear circuits of the brain become hyperexcitable (Coplan & Lydiard, 1998; Tilfors et al., 2001). The neurobiological mechanism thought to be importantly involved in these fear circuits is the endocrine–neuroendocrine amygdala cascade where testosterone is a key component (Corodimas, LeDoux, Gold, & Schulkin, 1994; Schulkin, Gold, & McEwen, 1998). The present study therefore explored the relationship between reduced circulating/endogenous testosterone induced by orchiectomy and fearful behavior in the open field test.

Methods

Animal handling

Eighteen mature male Wistar rats (200–250 g) were used for the study. Animals were housed in the preclinical animal house of the College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso. Animals were randomly assigned to two groups: orchiectomized and nonorchiectomized (n=9 per group). They were maintained under standard laboratory conditions and a 12 h/12 h light/dark cycle at 23±2ºC with free access to food and water throughout the experiment. All experimental procedures were approved by the institutional animal ethics committee.

Chemicals

Ketamine was obtained from Sigma (St. Louis, MO, USA). The other chemicals, including methylated spirit and penicillin, were analytical-grade and procured locally.

Orchiectomy

Bilateral orchiectomy was performed as described by Svensson, Berntsson, Engel, & Soderpalm (2000). Under ketamine anesthesia (50 mg/kg, i.p.), a small surgical incision was made in the center of the scrotum. Each testicle was exposed through the surgical orifice. The ductus deferens and main arteries and veins were isolated, ligated, and severed, allowing the testicle and epididymis to be removed. The incision was then closed, sutured, and swabbed with povidone–iodine solution. The postoperative procedure was implemented, and the rats were housed in separate cages and allowed free access to food and water for approximately 4 weeks before the experiment.

Determination of plasma testosterone levels

Blood samples (2.5ml) were collected through the saphenous vein with partial restraint in orchiectomized and nonorchiectomized animals 24 h prior to the open field test. The samples were centrifuged at 3000 rpm for 3–5 min using a Uniscope laboratory centrifuge (Model SM800B, Surgifriend Medicals, Essex, UK). Plasma testosterone levels were determined using a standard enzyme-linked immunosorbent assay as described by Tietz (1995) and the Microwell Method (Dialab, Wiener Neudorf, Austria) with parallel measurements in the respective calibrators attached to the kit.

Neurobehavioral study: open field test

The open field apparatus was constructed of square plywood (96 × 96 cm) with 60 cm high walls. One of the walls was made of Plexiglas to facilitate an unobstructed view of the animal in the box. The floor was painted green and divided into 16 squares by parallel and intersecting white lines (Bhattacharya & Satyan, 1997). The rats were individually placed in one corner of the open field, and the following behaviors were visually scored for 5 min in both orchiectomized and nonorchiectomized rats: locomotion, rearing, and grooming. The maze was located in a 1.8 × 4.6 m test room and lit by a 60 W red lamp for background lighting.

The rats were carried to the test room in their home cages and handled by the base of their tails at all times. The rats were placed in the center or one of the four corners of the open field and allowed to explore the apparatus for 5 min. After the 5-min test, the rats were returned to their home cages, and the open field was cleaned with 70% ethyl alcohol and permitted to dry between tests. To assess habituation to the novelty of the arena, the rats were exposed to the apparatus for 5 min on 2 consecutive days. The following behaviors were scored: total locomotor activity (i.e., the frequency with which the animal crossed the grid lines with all four paws and the frequency of rearing were taken as an index of locomotor activity; Walsh & Cummins, 1976), rearing (i.e., the frequency with which the animal stood on its hind legs in the maze; Brown, Corey, & Moore, 1999), and grooming (i.e., the frequency of face washing and paw licking while stationary in the maze; Brown et al., 1999).

Statistical analysis

Data are expressed as the mean ± standard error of the mean and analyzed using Student's t-test. Values of $p<.05$ were considered statistically significant.

Results

Testosterone

The statistical analysis of plasma testosterone levels revealed a significant reduction of testosterone levels in orchiectomized animals ($p<.05$) compared with the nonorchiectomized group. Orchiectomy does not completely abolish the secretion of androgens because they can be secreted from sources other than the testes, such the adrenal organ. This explains the slight amount of the hormone detected in the orchiectomized group.

Table1. Effect of orchiectomy on plasma testosterone, total locomotion, rearing, and grooming

Group	Plasma testosterone (nm/L)	Total locomotion	Rearing frequency/5 min	Grooming frequency/5 min
Nonorchiectomized (control)	21.55±.2	82±1.8	39±2.4	44.4±2.9
Orchiectomized (experimental)	3.25±.2*	75.5±1.3*	32.2±2.1*	20.4±2.3*

Data are expressed as mean± SEM (n=9 per group).
*p<.05 compared with nonorchiectomized group.

Locomotion, rearing, and grooming

The open field results showed a significant reduction of the number of grid lines crossed and frequency with which the animals stood on their hindlimbs during the 5-mintest in the orchiectomized group compared with the nonorchiectomized group (p<.05). Paw licking and face washing (i.e., grooming) also significantly decreased (p<.05) in the orchiectomized group compared with the nonorchiectomized group.

Discussion

The open field test (Hall, 1934; Hall & Ballenchey, 1932) provides simultaneous measures of locomotion, exploration, and anxiety. The number of lines crossed and frequency of rearing are usually used as measures of locomotor activity but also reflect exploration and anxiety. A high frequency of these behaviors indicates increased locomotion and exploration and/or lower levels of anxiety (Walsh & Cummins, 1976). The number of central square entries and duration of time spent in the central square are measures of exploratory behavior and anxiety. A high frequency or duration of these behaviors indicates high exploratory behavior and low anxiety levels. However, the number of grid lines crossed and frequency of rearing and grooming were adopted as indices of anxiety/fear in the present study and have been documented as reliable and valid measures of emotional behavior (Ivinskis, 1968; Prescott, 1970).

Table 1 shows that orchiectomized animals exhibited a significant reduction of exploratory behavior, indicating elevated fear or increased anxiety, and this observation was consistent with previous open field studies (Hall, 1934; Archer, 1973; Blanchard, Griebel,& Blanchard, 2001). Several studies have shown that elevated testosterone level reduce anxiety-like or fearful behavior in rodents in several behavioral paradigms (Fernandez-Guasti & Martinez-Mota, 2005; Frye & Seliga 2001; Eisenegger et al., 2011). Therefore, an inverse relationship appears to exist between testosterone levels and exhibition of anxiety-like or fearful behavior. However, the neural mechanism remains unknown. The fear-reducing properties of elevated testosterone have been shown to be γ-aminobutyric acid A ($GABA_A$) receptor-dependent in the amygdala (Hermans, Ramsey, Tuiten, & van Honk, 2004; Hermans, Putman, Baas, Koppeschaar, & Honk, 2006),whereas the opposite relationship is unclear but may also be integrated via various dopaminergic systems in the hippocampus, amygdala, and other parts of the mesocortical system. The presence of androgen receptors in the hippocampus, amygdala, and parts of the mesocortical system in mammals lends credence to this notion (Choate & Resko, 1996; Greco, Edwards, Michael, & Clancy, 1998; Resko, Connolly, Roselli, Abdelgadir, & Choate, 1993; Clancy, Bonsall, & Michael, 1992). These parts of the brain are known to influence emotional aspects of behavior. Lesions and inactivation of these areas have been associated with some symptoms of depression and an inability to cope with fear (Krishnan & Nestler, 2010; Tamminga, 2010; Kritzer & Creutz, 2008). In the present study there was a reduction of circulating testosterone-induced behavioral deficits in orchiectomized animals, resulting in less activity in the form of fear imposed by exposure to a novel environment. Therefore, not only perturbation of the prefrontal dopaminergic system induces behavioral deficits, but reduced circulating testosterone can also induce such deficits as shown by this study. Therefore, we conclude that circulating testosterone may be one endogenous mechanism responsible for coping with fear induced by exposure to a novel environment.

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