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Hormonal responses to different sexually related conditions in male rats

H. Bonilla-Jaime *, G. Vázquez-Palacios, M. Arteaga-Silva, S. Retana-Márquez

Department of Reproductive Biology, Universidad Autonoma Metropolitana-Iztapalapa, Mexico City, C.P. 09340, Mexico

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Abstract

Plasma levels of corticosterone (C) and testosterone (T) increase after sexual activity in males of several species. However, the physiological significance of these increases has not been elucidated. In the present study, hormonal response to different conditions linked to sexual activity was assessed. In the first experiment, plasma levels of C and T were assessed both in sexually experienced and naive male rats after the following conditions: (A) control group, without sexual stimulation; (B) males exposed to ovariectomized females; (C) males exposed to intact, non-receptive females; (D) males exposed to receptive females with the vagina obstructed, to avoid intromission; (E) males exposed to receptive females: but separated by a grid that prevents physical contact; (F) males exposed to receptive females during 30 min. In a second experiment, experienced male rats were allowed to repeatedly copulate until reaching the criteria for sexual exhaustion, and 24 h later, they were allowed to copulate. Once sexually related conditions ended, males were killed and their blood was obtained. C and T plasma levels were assessed by HPLC with ultraviolet (UV) detection. Results indicate that T did not increase significantly in naive male in any sexual condition, while in the experienced males, significant increases were observed with the mere presence of a receptive female and also after ejaculation. These increases were significantly larger in experienced males. On the other hand, C also increased in all sexual conditions, both in experienced and naive rats; however, the increase observed was larger in experienced males. Regarding sexual satiety, both C and T increased after copulating ad libitum to satiety. T increased almost three-fold compared to control, while C increased two-fold. No significant changes were observed in either one of the steroids 24 h after sexual exhaustion, even though males remained with a receptive female during an hour. These results show that sexual experience has an important influence on the hormonal response to sexual activity. C rises could be directly related to sexual arousal involved in the different sexual conditions, while T rises seem to have a direct relationship with both the motivation and execution aspects of masculine sexual behavior.
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Introduction

The hormonal influence on the regulation of sexual behavior is well known. However, the importance of mating behavior in triggering an endocrine response has been poorly analyzed. Several reports show that plasma levels of corticosterone (C) and testosterone (T) increase after sexual activity in males of many species. In male rats, increases in luteinizing hormone (LH), prolactin (Prl) and T have been reported when subjects reach ejaculation (Kamel and Frankel, 1978; Kamel et al., 1977). A similar hormonal response has been observed in pigs (Borg et al., 1991) and rabbits (Agmo, 1976). In contrast, in stallions and bulls, the increase of T after sexual behavior was not observed after sexual stimulation followed by ejaculation (Borg et al., 1991; Rabb et al., 1989). In rats, the increase of T has been observed only in sexually experienced males, but not in naive males (Kamel et al., 1975). Furthermore, the only presence of a receptive female rat increases the levels of luteinizing hormone and T (Graham and Desjardins, 1980).

On the other hand, it has been reported that, in sexual satiety, which consists of a prolonged sexual inhibition in response to copula ad libitum (Beach, 1956; Larsson, 1956), the motivational component of reproductive behavior is diminished (Rodriguez-Manzo and Fernández-Guasti, 1995). It has been suggested that T is involved in motivational aspects of sexual behavior (Alexander et al., 1994; Stoleru et al., 1993), hence, the modifications in plasma levels of this steroid after sexual satiety were also analyzed in this study.

* Corresponding author. Departamento de Biología de la Reproducción, Universidad Autónoma Metropolitana-Iztapalapa, C.P. 09340, Iztapalapa, D.F. Mexico. Fax: +1 5255 5804 4930.
E-mail address: bjh@xanum.uam.mx (H. Bonilla-Jaime).

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Concerning adrenal steroids, it has been observed that completion of one or more services (intromission plus ejaculation) results in a significant elevation of cortisol concentration in stallions, bulls and pigs (Borg et al., 1991; Rabb et al., 1989). In sexually experienced male rats (Retana-Marquez et al., 1998) and mice (Bronson and Desjardins, 1982), as well as in naive males (Szechtman et al., 1974), plasma levels of C rise after copulatory activity. To our knowledge, an evaluation of the response of C to different sexual situations has not been made.

Despite the number of species that have been investigated in this regard, the significance of these hormonal increases due to sexual stimulation is still far from clear. In this study, the response of T and C to different intensities of sexual stimulation was assessed, both in sexually experienced and inexperienced male rats.

Materials and methods

Adult Wistar rats (weight: 300–350 g) from our own vivarium were used in this study. Animals were kept (5 per cage) in a room under constant temperature and humidity and with a reversed 12/12 light cycle (lights on: 21 h). Food and water were available ad lib. Sexual behavior tests were done during the dark phase of the cycle (three h after light off) and under a dim red light. Male rats were placed in a circular Plexiglas arena (45 cm diameter) during 5 min for habituation before introducing a stimulus female artificially brought into estrus with a sequential treatment of 10 μg of estradiol benzoate (SC, 0.1 ml corn oil, 48 h before testing) plus 1 mg of progesterone 44 h later. Males were randomly assigned to the experienced or inexperienced group. Males in the experienced group were tested for sexual behavior in three test. Only those subjects that displayed 3 ejaculations in the last test were included. Sexual behavior tests were performed with a 3-day period between them. Experimental procedures were performed following the NIH guidelines for handling and care of animals.

Plasma levels of C and T were determined in both sexually experienced and inexperienced males after different sexually related conditions. In the first experiment, males were randomly assigned to the following groups and conditions (n = 10):

A) Control group, without sexual stimulation (CON).
B) In the presence of an ovariectomized (OVX) females during 20 min.
C) In the presence of a non-receptive intact female (NREC), during 20 min. Vaginal smears were taken to determine the stage of the estrus cycle. All the females were in diestrus stage at the time of testing.
D) In the presence of a receptive female with an obstruction in the vagina (OBST), during 20 min.
E) In the presence of a receptive female during 20 min but with a grid between them to prevent physical contact (GRID).
F) In the presence of a receptive female during 30 min (EJAC).

Blood was collected immediately after the end of the stimulus, approximately 4 h after the beginning of the dark period.

In a second experiment, sexually experienced males were randomly assigned to one of the following conditions (n = 10):

A) Control group without sexual activity (CON).
B) Copulatory activity until reaching the criteria of sexual satiety (SAT).
C) Sexually satiated males allowed to copulate 24 h after sexual satiety.

The criterion to determine sexual satiety was: 30 min in the presence of a receptive female without displaying any sexual activity (Beach, 1956; Larsson, 1956). Blood was collected after males reached the criteria of sexual satiety approximately 8 h after the onset of darkness.

Hormone assay

Males were decapitated and trunk blood was collected immediately after exposure to the appropriate stimulus. Blood samples were collected in the dark phase, and the time difference between the first and the last subject was approximately 10 min. Corticosterone and testosterone were extracted from plasma and quantified by HPLC with ultraviolet (UV) detection using a modification of the method reported by Woodward and Emery (1987). Blood samples were centrifuged and plasma (1 ml) was mixed with 100 μl 19-nor-testosterone solution (5 mg/ml in methanol) as an internal standard. Steroids were extracted into 5 ml diethyl ether-dichloromethane (60/40 v/v) mixed by vortex and immediately centrifuged for 5 min. The organic phase was vortex mixed with 1 ml HPLC-grade water. After centrifugation, the organic phase (3 ml) was evaporated at room temperature under nitrogen. The residue was redissolved in 100 μl of methanol–water (60:40 v/v). The guard column and the column were equilibrated using HPLC-grade methanol–water (60:40 v/v) at a flow rate of 0.4 ml/min. Separations were made at a temperature of 40°C, in a Waters Symmetry C18 column. A Waters 600-MS system controller was used to flush the mobile phase and the steroids were assessed with a 486 Waters UV absorbance detector (fitted at 250 nm). Results were analyzed using the Maxima 820 Chromatography Workstation, obtained from Waters Co. (Milford, Massachusetts). Inter-assay and intra-assay coefficients of variation (C.V.) were determined using four plasma pools in the 1.5–50 μg/dl (corticosterone) and 0.2–8 ng/ml (testosterone) ranges, covering the normal ranges in rat plasma. The averages C.V.s for intra-day and inter-day precision for testosterone were 2.93% and 7.12%, respectively. The detection limit of the assay for corticosterone was 0.05 μg/dl, and the detection limit of the assay for testosterone was 0.05 ng/ml at a signal to noise ratio of 2:1. The average recovery of the steroids after extraction from blood was about 85%.

Statistical analysis

Mount latencies were analyzed with a non-parametric two-way ANOVA (2 × 4), with experience and experimental condition as factors; post hoc comparisons were made using a Dunn test (Zar, 1984). Hormonal levels were analyzed using a parametric two-way ANOVA (2 × 6), with experience and experimental condition as factors. When significant, post hoc comparisons were made using a Newman–Keuls test. Ejaculation latencies and frequencies were analyzed by Mann Whitney U test. The level of significance was fixed at 0.05.

Results

In naive male rats exposed to OVX females, only two rats displayed mounts; mean mount latency was 384.5 s, and none ejaculated. In males with non-receptive females, all the males displayed mounts (mean = 264 s) and none ejaculated. Males with a grid sniffed females actively. In males exposed to OBST females, only two mounted (mean = 251 s) and none ejaculated. In males allowed to copulate with receptive females, mean mount latency was 43 s, mean ejaculation latency was 642 s and mean ejaculation frequency was 1.9 (Table 1).

In experienced males exposed to OVX females, all of them mounted (mean = 53.6 sec) but none ejaculated. Males exposed to non-receptive females mounted (mean = 32.1 s) but none ejaculated. Males separated from females by a grid actively sniffed females during the 20 min. Males exposed to OBST females displayed mounts (mean = 8.5 s). When experienced males were allowed to copulate with receptive females, their mean mount latency was 15.5 s, mean ejaculation latency was 259.6 s and the mean number of ejaculations was 3.2 (Table 1). Mount latencies observed in experienced males were significantly lower than those of naive males [$H(1,64) = 60.13$; $P < 0.001$], and the sexual condition also influenced this

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