Non-genomic effects of testosterone on social behavior in laboratory rats

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Introduction

Testosterone is a steroid hormone, which has a crucial role for development of the brain and its functions, including behavior and cognitive abilities. It influences the development of reproductive system and secondary sexual characteristics, distribution of fat and hair growth [1]. In men, testosterone is mainly produced by Leydig cells of the testes [2], while in females it is mainly generated by the ovaries and adrenal cortex. Molecular mechanism of testosterone action can vary. Testosterone can act through slower genomic pathway or through rapid non-genomic pathway [3]. Studies that examine the non-genomic effect of testosterone on male and female social behavior are still rare. The aim of our work was to find out the non-genomic effect of testosterone on the social behavior of OVX (ovariectomized) female and GDX (gonadoectomized) male rats after the administration of androgen and estrogen receptor blocker to exclude the genomic pathway.

Experimental procedures and surgery

In our experiment, we used 20 female and 40 male Wistar rats (Anlab, Prague, Czech Republic). We housed the animals in separate polycarbonate cages in a controlled environment (temperature 22±2°C, humidity 50%±10%) with a 12:12 light – dark cycle, light phase from 08:00 a.m. to 8:00 p.m. The animals had *ad libitum* access to a standard diet and tap water. We randomly divided the female rats into 2 groups: control group (OVX-CTRL, n = 10) and testosterone group (OVX-TST, n = 10) and male rats into 4 groups: sham operated group treated with oil (SHAM-CTRL, n = 10), sham operated group with testosterone (SHAM-TST, n = 10), castrated group treated with oil (GDX-CTRL, n = 10) and castrated group with testosterone (GDX-TST, n = 10).

Male rats on postnatal day (PND) 47 underwent a castration (removal of testes) or sham surgery (without removal of the testes) under general anesthesia (intraperitoneal injection of ketamine (100mg/kg) and xylazine (10 mg/kg)). All female rats at age of 15 weeks underwent ovariectomy, when we extracted both ovaries. All animals were allowed two weeks for recovery after the surgery.

Hormonal supplementation and behavioral testing

We tested the rats in period of adulthood (PND100 – 125) in the test of social interaction. The apparatus consisted of dark plastic square arena 100cm x 100cm. In a corner of test arena we placed a cage with novel stranger rat of the same gender as testing animal. We put the tested animal in the middle of the maze and allowed to freely explore the maze for 10 minutes. The examined behaviors included: total distance moved, average velocity of the movement and time spent with investigating the stranger rat. The time spent with a novel stranger rat expressed the capability of social interaction and reflected the degree of exploratory and anxiety-like behavior. Rats received hormonal supplementation 1 hour before behavioral testing and group assignment was as follows: OVX-CTRL (olive oil – 1 μl/g); OVX-TST (testosterone propionate – 1 mg/kg); SHAM-CTRL (olive oil – 1 μl/g); SHAM-TST (testosterone propionate – 1 mg/kg), GDX-CTRL (olive oil – 1 μl/g); GDX-TST (testosterone propionate – 1 mg/kg). Animals received flutamide, an androgen receptor blocker (20 mg/kg) and tamoxifen, nonselective

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estrogen receptor blocker (10 mg/kg) one day before testing, and subsequently 1 hour before it. We collected blood samples from tail vein after behavioral testing and subsequently measured TST levels in plasma using a commercially available ELISA kit (DRG Diagnostic, Marburg, Germany). We analyzed the behavior of animals by a computerized animal observation system (EthoVision XT 10, Noldus, Netherlands).

Statistical analysis

We analyzed data from social interaction testing and the concentration of testosterone of males using the one-way analysis of variance (ANOVA) with the four groups being the tested factor. For comparison between pairs of groups we used Bonferroni post-hoc test. We analyzed data from behavioral testing and concentration of testosterone of females using unpaired nonparametric Mann-Whitney test. P-values less than 0.05 were considered significant. We used GraphPad Prism 5 for all calculations and tests. Data presented as mean + standard deviation.

Results

We found significantly increased concentration of testosterone in males in SHAM-CTRL ($F(3.36) = 12.11$, $p<0.01$), SHAM-TST ($p<0.001$) and GDX-TST group ($p<0.001$) in comparison with GDX-CTRL group. Testosterone levels did not differ between SHAM-CTRL, SHAM-TST and GDX-TST groups. OVX-TST group of females had significantly higher concentration of testosterone ($p<0.001$) when compared with OVX-CTRL group. In test of social interaction, we did not observe significant differences in time spent in interaction with the stranger rat between tested groups of males ($F(3.35) = 0.2300$) and females. Similarly, we found no significant differences in distance moved and velocity of the movement.

Conclusion

Our experiment did not confirm the rapid non-genomic effect of testosterone on social behavior after blockade of androgen and both estrogen receptors neither in male nor in female rats. Although, the concentration of testosterone in plasma was higher in groups received testosterone propionate, we did not find significant differences in social behavior of tested animals. Further studies are needed to uncover the molecular mechanism of non-genomic effect of testosterone on social behavior.

Ethical statement

All animal procedures were approved by the ethical committee of the Faculty of Medicine, Comenius University.

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