Effects of increased prenatal testosterone on anxiety-like behavior and spatial memory of laboratory rats

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Introduction

Testosterone belongs to steroid hormones from the androgen group, which are important not only for sexual differentiation of the fetus, but also for the development of the nervous system and many aspects of behavior and cognitive abilities. Morphologically and physiologically testosterone is a determinant of male sex, it influences the development of reproductive system and secondary sexual characteristics, distribution of fat and hair growth [1]. Testosterone is mainly produced by the Leydig cells of the testes and fewer extent in kidney, liver, adrenal cortex [2] and in other peripheral tissues (fat and muscle tissue), even in some regions of the brain. Testosterone like other gonadal hormones have double effects: organizational and activational. Organizational (prenatal) effects of hormones occur during critical periods of development when exposure to gonadal hormones can cause permanent sex differences. Thus, organizational effects are permanent, irreversible and are responsible for sexual differentiation of the brain [3]. Later in life, testosterone has got activational effect on brain structures, which is dependent on its changing levels. Thus activational effects are acute and reversible that occur throughout life [4]. The aim of our study was to evaluate the organizational effects of testosterone on anxiety and spatial memory in juvenile and adult male and female rats.

Materials and methods

In this study, we used pregnant Lewis rats, which were kept in separate polycarbonate cages and were randomly divided into 4 groups: control (CTRL), testosterone propionate (TST), flutamide (FLU), testosterone propionate with flutamide (TST+FLU). From 14th day of pregnancy until delivery, the rats received either testosterone propionate in dose of 2mg/kg, flutamide in dose of 5mg/kg, both testosterone propionate and flutamide, or olive oil intramuscularly. We housed pups after delivery with their mothers until weaning on postnatal day (PND) 21 and we divided young rats into cages according to sex and birth nest. The housing room was temperature controlled (temperature 22°C, humidity 50%) 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 tested the offsprings in several behavioral tests in pre-adolescent period (PND 25 – 27) and in adulthood (PND 82 – 124). Behavioral testing was conducted during the light phase, although in dark room. In pre-adolescent period, we investigated the behavioral performance of rats in open field test, light/dark box and elevated plus-maze to assess anxiety-like behavior and in simple novelty test to evaluate their memory. We tested rats in period of adulthood in above mentioned tests and in an additional test, in modified Morris water maze to assess their spatial memory.

The **open field apparatus** (5 min) consisted of dark plastic square arena of 100cm x 100cm. The examined behavior included: total distance moved, average velocity of the movement and time spent in the centre zone. The **light/dark apparatus** (5 min) consisted of dark plastic rectangle arena of 45cm x 65cm. It consisted of two equally sized chambers connected by an opening. One chamber was black and covered with a black lid. The second chamber was opened and brightly illuminated. The examined behavior was the amount of time spent in the light chamber. The **plus-maze apparatus** (5 min) consisted of dark plastic arena with two opened arms and

two enclosed arms by walls (30cm high). The arms extended from a central platform. The maze was connected to a metal frame on each end of the arms raising it 60cm above the floor. We recorded the amount of time spent in opened arms. **Simple novelty test** (5 min) we realized in the open field apparatus, but we placed a novel object to the test arena. The examined behaviors included: total distance moved, average velocity of the movement and time spent with investigating a novel object. The **modified Morris water maze** test we realized in a circular pool (diameter 125cm, height 60cm) filled with tap water (24-26°C), which we placed in a brightly illuminated room. The maze was virtually divided into four quadrants and one geometrical figure for orientation as an external cue was placed on the wall of each quadrant. We placed an escape platform (diameter 10cm) into one of four quadrants, hidden 2 cm below the surface of the water (the platform position remained the same during the testing period). The animals were tested in blocks of four trials (a trial duration – 60s) a day, for 4 consecutive days (a same order of animals and starting quadrant at each trial for 4 consecutive days). A trial (60s) consisted of a swim followed by a 30s rest on the platform. If the rat did not find the platform within 60s, the investigator navigated it to the platform. The examined behavior was the time to find the platform. On the day 5 a probe trial occurred. We removed the platform during this trial from the pool. The duration of this trial was 60s and time spent in the platform quadrant was recorded (long – term or reference memory). The starting position for all animals was the quadrant opposite to the platform quadrant.

We measured the behavior of the animals via a computerized animal observation system (EthoVision XT 10, Noldus, Netherlands). We collected blood samples from pregnant females daily, 1 h after injection, from 18th day of pregnancy until the delivery and subsequently measured TST levels in plasma using a commercially available ELISA kit (DRG Diagnostic, Marburg, Germany). We analyzed data using ANOVA with Bonferroni post-hoc test. $P<0.05$ was considered to be statistically significant. We used GraphPad Prism 5.0 for all calculations and tests. Data are presented as mean + standard deviation.

Results

We found significantly higher testosterone levels in plasma of pregnant females in the TST and TST+FLU groups when compared to the CTRL group ($p<0.0001$), while testosterone levels did not differ between CTRL and FLU groups.

Experimental groups differed neither in the analyzed behavioral parameters in any of behavioral tests (open field, elevated plus maze, light/dark box, simple novelty test) nor in preadolescent period and adulthood. In the water maze (period of adulthood), we detected general improvement of animals within acquisition trials. All animals showed improvement from day 1 to day 4, however we observed significant difference between TST and FLU groups on day 4 only ($*p<0,05$). During the probe trial, all groups spent equal time in the quadrant where the platform was previously hidden.

Conclusion

In our experiment prenatal administration of testosterone had no effect on anxiety behavior neither in the preadolescent period nor in period of adulthood. The effects of the testosterone on spatial reference memory remains less clear. In Morris water maze we found significant effect of prenatal testosterone only on 4th day of the test, when TST group was better in finding the platform than FLU group. Studies about the organizational effect of testosterone on behavior are still rare and their conclusions are often contradictory and more research could be aimed on searching strategies during water maze task.

Ethical statement

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

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