ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Short communication Acta Neurobiol Exp 2015, 75: 457–461

Does the non-genomic effect of testosterone on social anxiety require the presence of a classical steroid receptor?

Barbora Filová[1, 2]*, Emese Domonkos[2], Veronika Borbélyová[2], Janka Bábíčková[2], Ľubomíra Tóthová[2], Daniela Ostatníková[3], Peter Celec[2, 4, 5, 6], and Július Hodosy[2, 3]

[1]Institute of Medical Physics, Biophysics, Informatics and Telemedicine, Comenius University, Bratislava, Slovakia, [2]Institute of Molecular Biomedicine, Comenius University, Bratislava, Slovakia, [3]Institute of Physiology, Comenius University, Bratislava, Slovakia, [4]Institute of Pathophysiology, Comenius University, Bratislava, Slovakia, [5]Department of Molecular biology, Comenius University, Bratislava, Slovakia, [6]Molecular Medicine Center, Slovak Academy of Sciences, Bratislava, Slovakia, * Email: basafilova@gmail.com

Steroid hormones may act through a rapid mechanism that does not require an intracellular steroid receptor and its effects on gene expression. In this study we have analysed this so-called non-genomic effect of testosterone on social anxiety in rats of both sexes using androgen and oestrogen receptor blockers. Male rats were divided into four groups: SHAM-CTRL (a sham operated group treated with oil as vehicle, n=10), SHAM-TST (a sham operated group treated with testosterone at a dose of 1 mg/kg, n=10), GDX-CTRL (a castrated group treated with oil, n=10) and GDX-TST (a castrated group treated with testosterone at a dose of 1 mg/kg, n=10). Female rats were divided into two groups: OVX-CTRL (an ovariectomized group treated with oil, n=10) and OVX-TST (an ovariectomized group treated with testosterone, n=10). The intracellular androgen receptor was blocked with flutamide and both intracellular oestrogen receptors were blocked with tamoxifen (a selective oestrogen receptor modulator). Rats were tested one hour after oil or testosterone administration in the social interaction test. Although the concentration of testosterone was higher in testosterone groups, no significant difference in social interaction was observed between the groups. In summary, in this first study focusing on the non-genomic effects of testosterone on social interaction no rapid effects of testosterone in adult rats were found. Further studies should analyse potential non-genomic effects of testosterone on other forms of social behaviour.

Key words: testosterone, non-genomic effect, social interaction, androgen receptor, oestrogen receptor

Most of studies dealing with the effects of steroids on behaviour examine only the classical genomic pathways. The genomic mechanism requires the binding of a steroid to an intracellular receptor, translocation of this complex into the nucleus and thus inducing protein synthesis and RNA transcription (McEwen 1991). This process is relatively slow and may take several hours or days. In addition to this slow effect, non-genomic effects of steroids were described in 1942, when Hans Salye observed a rapid anaesthetic effect after the application of progesterone. This effect was further used for the development of steroid anaesthesia (Salye 1942).

Correspondence should be addressed to B. Filová
Email: basafilova@gmail.com

Received 24 March 2015, accepted 6 October 2015

In contrast to the genomic effect, the non-genomic effect is fast (within few seconds or minutes) for the activation of DNA transcription (Foradori et al. 2008) and it does not depend on gene transcription or protein synthesis. It rather involves steroid-induced modulation of cytoplasmic or cell membrane-bound regulatory proteins (Simoncini and Genazzani 2003). However, it is still not clear, if this effect is induced directly by the steroid in the absence of a receptor or can be mediated through a classical steroid receptor or a distinct non-classical steroid receptor that is possibly associated with the plasma membrane (Heinlein and Chang 2002).

It is known, that steroid hormones such as testosterone are able to influence the behaviour of people and animals. Some studies have shown that female mice

when compared to male mice spent less time in the open arms of the elevated plus maze (Frick et al. 2000, Johnston and File 1991), indicating increased anxiety as behaviour of females. In male rats, increased anxiety was observed in the elevated plus maze and open-field test after castration (Edinger and Frye 2004). In contrast, after administration of testosterone to castrated rats, the anxiety was reduced (Fernandez-Guasti and Martinez-Mota 2005). Testosterone also plays an important role in social behaviour, especially in males. It can influence reproductive behaviour and aggressiveness (Sessa et al. 2013). Frye and Seliga observed that castration and also testosterone supplementation can decrease the social interaction in comparison with intact male rats (Frye and Seliga 2001). However, little is known about the non-genomic effect of testosterone on male and female social behaviour. The aim of our work was to describe the non-genomic effect of testosterone on the social behaviour of OVX female and GDX male rats after the administration of both androgen and oestrogen receptor blockers to distinguish it from the genomic mechanism.

Our experiment was carried out on 40 male and 20 female Wistar rats (Anlab, Prague, the Czech Republic). The animals were housed in groups of 5 with food and water *ad libitum* under controlled conditions: temperature 25±2˚C and humidity 55±10%. The light/dark cycle was maintained at 12 hours each of light and dark (light on 08:00). The female rats were divided into 2 groups: a control group (OVX-CTRL, n=10) and a testosterone group (OVX-TST, n=10) and male rats were divided into 4 groups: a sham operated group treated with oil (SHAM-CTRL, n=10), a sham operated group treated with testosterone (SHAM-TST, n=10), a castrated group treated with oil (GDX-CTRL, n=10) and a castrated group treated with testosterone (GDX--TST, n=10). This study was conducted in accordance with the European Communities Council Directive 86/609/EEC.

In the age of 15 weeks, the animals were anaesthetized with ketamine (100 mg/kg, Narkamon inj, Bioveta, Czech Republic) and xylazine (10 mg/kg, Xylariem inj, Riemser, Germany) and gonadectomised or sham operated. Both testes were extracted through a small incision made at the posterior tip of the scrotum and ligated with a silk suture, or both ovaries were extracted through a small incision in the lower abdomen and ligated with the silk suture. Intact male rats underwent sham surgery without gonadectomy.

Two weeks after the surgery, animals received flutamide, an androgen receptor blocker (20 mg/kg; Flutamide, Sigma, St. Louis, USA) and tamoxifen, a selective oestrogen receptor modulator (SERM) with mixed agonist/antagonist properties depending on the target tissue (10 mg/kg; Tamoxifen dihydrogen citrate, PLIVA – Lachema, Brno, Czech Republic). The dosages were determined according to previously published experiments (Frye and Rhodes 2002, Robinson et al. 2012). In this experiment we did not use animals without blocker antagonist administration because our hypothesis was that non-genomic effect of testosterone can be mediated via non-classical steroid receptors associated with the plasma membrane. Flutamide, a classical androgen receptor blocker, does not cross-react with any of the steroid receptors (Gao et al. 2006). Tamoxifen is a non-steroidal oestrogen receptor blocker and acts as an antagonist in the central nervous system. It was proved to block the effect of estradiol on dendritic spines of hippocampal neurons (Cyr et al. 2002). However, experiments have shown that tamoxifen can also influence the expression of a progesterone receptor in some tissues (Mourits et al. 2002). The blockade was administered a day before and subsequently 2 hours before the testing. The control groups received olive oil (Oleum Olivae, VULM, LtD., Slovakia). Testosterone groups received 1 mg/kg of testosterone (testosterone propionate T1875, Sigma, St. Louis, USA) intramuscularly an hour prior to the testing (de Beun et al. 1992). After the behavioural testing, blood samples were collected and concentration of testosterone in the plasma was measured with the commercially available ELISA kit (DRG Diagnostic, Marburg, Germany).

To investigate the possible non-genomic effect of testosterone on rat social behaviour, the social interaction test was used. This test can be utilized to assess anxiety and exploratory behaviour (File and Hyde 1978). The design of the test was similar as was described by Frye (Frye 2001). The familiar social test apparatus consisted of a rectangular 100×100 cm box placed in a dark room. The middle of the maze was well lit. In contrast to the test used by Frye (2001), we placed the strange animal of the same gender in the corner of the box in a cage to avoid attacks between animals. Tested rats were positioned in the middle of the maze and allowed to freely explore the maze for 10 minutes. Behavioural data were analysed by the tracking software EthoVision XT 10 (Noldus, Netherlands). The total distance moved (cm),

mean velocity of the movement (cm/s) and time spent in social interaction with a strange animal (moving in the zone of strange animal and sniffing) were calculated. Measurements from social interaction test and the concentration of testosterone of males were statistically evaluated by using the two-way analysis of variance ANOVA with Bonferroni post-hoc correction. Social behaviour and the plasma testosterone concentration of females were statistically evaluated by using unpaired non-parametric Mann-Whitney test. $P<0.05$ was considered to be statistically significant. Results are presented as mean plus standard error of the mean.

The two-way ANOVA for male testosterone concentrations have showed significant differences due to surgery ($F_{1,34}=15.54$, $P<0.001$), treatment ($F_{1,34}=26.75$, $P<0.0001$) and the interaction surgery x treatment ($F_{1,34}=8.717$, $P<0.01$). *Post hoc* comparison has showed significant difference ($P<0.0001$) between GDX-CTRL and SHAM-CTRL groups that were treated with oil. After comparison of both testosterone groups we have found no significant difference ($P=0.9539$; Fig. 1A). The OVX-TST group of females had a significantly higher concentration of testosterone ($P<0.0001$) when compared with the OVX-CTRL group (Fig. 1B).

While the concentration of testosterone was significantly increased, two-way ANOVA for male social interaction have not revealed significant influence of surgery ($F_{1,35}=0.6796$, $P=0.4153$), treatment ($F_{1,35}=0.2076$, $P=0.6514$) and a surgery by treatment interaction ($F_{1,35}=0.0029$, $P=0.9575$). In addition, *post hoc* comparison has shown no significance differences in interaction time of tested male rats ($P=0.9999$; Fig. 2A). The unpaired non-parametric Mann-Whitney test has also showed no significant differences in social interaction between OVX-CTRL and OVX-TST female rats (P=0.999; Fig. 2B). Similarly, no significant difference was found in velocity and distance moved (data not shown).

In this study we have hypothesized that a rapid effect of testosterone can be mediated via a non-classical androgen receptor associated with the plasma membrane. For this reason we used classical steroid receptor blockers – flutamide and tamoxifen. Non-genomic mechanisms of steroid hormones can occur within few seconds or minutes after increasing their concentration and are considered to be independent of receptors acting in the nucleus. Aikey et al. observed that effect of testosterone (within 30 minutes) on anxiety after a single application of testosterone propionate in male mice (Aikey et al. 2002). There are also some studies, which suggest, that testosterone can quickly affect variety of other behaviour e.g.: coupling behaviour (James and Nyby 2002) or affective states in males (Alexander et al. 1994).

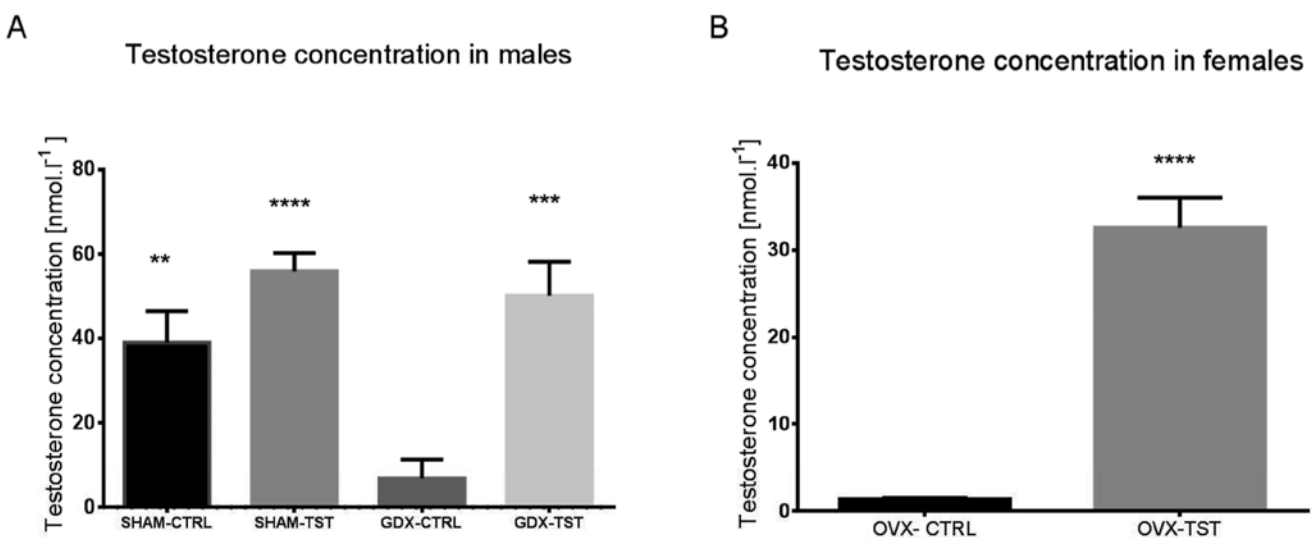

Fig. 1. Concentration of testosterone in plasma after behavioral testing (A) – concentration of testosterone in males, **** denotes $P<0.0001$ in comparison between SHAM-CTRL and GDX-CTRL groups;. (B) – concentration of testosterone in females, **** denotes $P<0.0001$ in comparison with OVX-CTRL group. Data are presented as mean plus standard error of the mean. SHAM-CTRL – sham operated group of males with oil, SHAM-TST – sham operated group of males with testosterone, GDX-CTRL – castrated group of males with oil, GDX-TST – castrated group of males with testosterone, OVX--CTRL – ovariectomized group of females with oil, OVX-TST – ovariectomized group of females with testosterone.

The non-genomic effect of testosterone can be also mediated via local aromatization of testosterone into estradiol in the brain (Nyby 2008). There is some evidence, that estradiol may act rapidly to increase male mating behaviour in rats and quail or aggression in mice (Steinman and Trainor 2010). In order to distinguish the non-genomic effect of testosterone from that of estradiol we administered a selective oestrogen receptor modulator – tamoxifen to all groups of experimental animals. However, our study confirmed no rapid effect of testosterone on social behaviour after blockade of both androgen and oestrogen receptors. Although the testosterone concentration in the plasma was higher in groups which received testosterone propionate, we have found no significant difference in social behaviour of tested male and female rats. Frye and Seliga (2001) observed similar effect of testosterone administration to gonadectomised male rats in the social interaction test. They found no significant difference in comparison with gonadectomised rats treated with oil. However, in contrast to our study, the concentration of testosterone was supra-physiological. In addition, Frye also found significant difference in social interaction of gonadectomised rats and gonadectomised rats treated with testosterone compared to the intact male group.

Results from this study suggest, that the non-genomic effect of steroids can be in some cases induced through non-transcriptional effect of the classical steroid receptors, because we have observed no effect of testosterone on social behaviour after androgen and oestrogen receptor blockade. However, there are some limitations of our study. The effect of flutamide on membrane associated androgen receptors is still not very clear. Some experiments suggest that flutamide may block both intracellular and membrane-associated androgen receptors and not only intracellular receptors (Sato et al. 2010). We did not use animals without steroid receptor blockade and also intact rats. Probably the use of another type of social interaction test, for example the Three-chamber social interaction test, would be more useful for this experiment. Moreover, the castrated status of animal in this study is questionable as have suggested Cross and co-author (Cross and Rosseli 1999). Instead of androgen and oestrogen blockade, the next experiment can be focused on the use of testosterone albumin conjugate (testosterone-BSA) that can acts only on membrane receptors and the application of steroid antagonist is not needed (Naghdi and Asadollahi 2004). Further studies are needed to understand the molecular mechanism of the non-genomic effect of testosterone on social behaviour.

This study was funded by VEGA grant No. 1/0406/13 granted by the Ministry of Education of the Slovak Republic, grant APVV-0753-10, grant UK/18/2014 and the project implementation: Comenius University in Bratislava, Science Park supported by the Research and Development Operational Programme funded by the ERDF. Grant number: ITMS 26240220086.

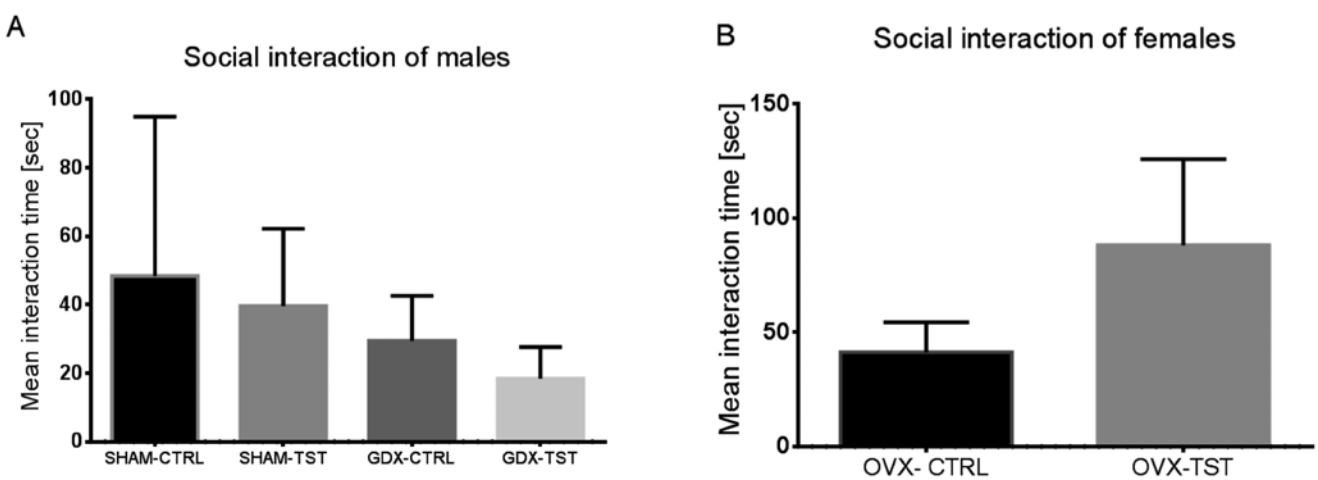

Fig. 2. Test of social interaction (A) – social interaction of males. (B) – social interaction of females. Data are presented as mean plus standard error of the mean. SHAM-CTRL – sham operated group of males with oil, SHAM-TST – sham operated group of males with testosterone, GDX-CTRL – castrated group of males with oil, GDX-TST – castrated group of males with testosterone, OVX-CTRL – ovariectomized group of females with oil, OVX-TST – ovariectomized group of females with testosterone.

REFERENCES

Aikey JL, Nyby JG, Anmuth DM, James PJ (2002) Testosterone rapidly reduces anxiety in male house mice (Mus musculus). Horm Behav 42: 448–460.

Alexander GM., Packard MG, Hines M (1994) Testosterone has rewarding affective properties in male rats: implications for the biological basis of sexual motivation. Behav Neurosci 108: 424–428.

de Beun R, Jansen E, Slangen JL, Van de Poll NE (1992) Testosterone as appetitive and discriminative stimulus in rats: sex- and dose-dependent effects. Physiol Behav 52: 629–634.

Cross E, Roselli CE (1999) 17beta-estradiol rapidly facilitates chemoinvestigation and mounting in castrated male rats. Am J Physiol 276: R1346–R1350.

Cyr M, Calon F, Morissette M, Di Paolo T (2002) Estrogenic modulation of brain activity: implication for schizophrenia and Parkinson's disease. J Psychiatry Neurosci 27(1): 12–27.

Edinger KL, Frye CA (2004) Testosterone's analgesic, anxiolytic, and cognitive-enhancing effects may be due in part to actions of its 5alpha-reduced metabolites in the hippocampus. Behav Neurosci 118: 1352–1364.

Fernandez-Guasti A, Martinez-Mota L (2005) Anxiolytic-like actions of testosterone in the burying behavior test: role of androgen and GABA-benzodiazepine receptors. Psychoneuroendocrinology 30: 762–770.

File SE, Hyde JR (1978) Can social interaction be used to measure anxiety? Br J Pharmacol 62: 19–24.

Foradori CD, Weiser MJ, Handa RJ (2008) Non-genomic actions of androgens. Front Neuroendocrinol 29: 169–181.

Frick KM, Burlingame LA, Arters JA, Berger-Sweeney J (2000) Reference memory, anxiety and estrous cyclicity in C57BL/6NIA mice are affected by age and sex. Neuroscience 95: 293–307.

Frye CA, Rhodes ME (2002) Enhancing effects of estrogen on inhibitory avoidance performance may be in part independent of intracellular estrogen receptors in the hippocampus. Brain Res 956: 285–293.

Frye CA, Seliga AM (2001) Testosterone increases analgesia, anxiolysis, and cognitive performance of male rats. Cogn Affect Behav Neurosci 1: 371–381.

Gao W, Kim J, Dalton JT (2006) Pharmacokinetics and pharmacodynamics of nonsteroidal androgen receptor ligands. Pharm Res 23(8): 1641–1658.

Heinlein CA, Chang C (2002) The roles of androgen receptors and androgen-binding proteins in nongenomic androgen actions. Mol Endocrinol 16: 2181–2187.

James PJ, Nyby JG (2002) Testosterone rapidly affects the expression of copulatory behavior in house mice (Mus musculus). Physiol Behav 75: 287–294.

Johnston AL, File SE (1991) Sex differences in animal tests of anxiety. Physiol Behav 49: 245–250.

McEwen BS (1991) Non-genomic and genomic effects of steroids on neural activity. Trends Pharmacol Sci 12: 141–147.

Mourits MJE, Ten Hoor KA, van der Zee, Willemse PHB, de Vries, Hollema H (2002) The effects of tamoxifen on proliferation and steroid receptor expression in postmenopausal endometrium. J Clin Pathol 55: 514–519.

Naghdi N, Asadollahi A (2004) Genomic and nongenomic effects of intrahippocampal microinjection of testosterone on long-term memory in male adult rats. Behav Brain Res 153: 1–6.

Nyby JG (2008) Reflexive testosterone release: a model system for studying the nongenomic effects of testosterone upon male behavior. Front Neuroendocrinol 29: 199–210.

Robinson S, Penatti CA, Clark AS (2012) The role of the androgen receptor in anabolic androgenic steroid-induced aggressive behavior in C57BL/6J and Tfm mice. Horm Behav 61: 67–75.

Salye H (1942) Correlation between the chemical structure and the pharmacological actions of the steroids. Endocrinology 30: 437–453.

Sato SM, Johansen JA, Jordan CL, Wood RI (2010) Membrane androgen receptors may mediate androgen reinforcement. Psychoneuroendocrinology 35(7): 1063–1073.

Sessa AK, Harris RM, Hofmann HA (2013) Sex steroid hormones modulate responses to social challenge and opportunity in males of the monogamous convict cichlid, Amatitliana nigrofasciata. Gen Comp Endocrinol 189: 59–65.

Simoncini T, Genazzani AR (2003) Non-genomic actions of sex steroid hormones. Eur J Endocrinol 148: 281–292.

Steinman MQ, Trainor BC (2010) Rapid Effects of Steroid Hormones on Animal Behavior. Nature Education Knowledge 3(10): 1.