Rapid Behavioral Effects of Sex Hormones in Rats

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Background

Sex hormones have well-described organizational and activational effects on behavior. The mechanism of action of these effects is supposed to involve activation of intracellular steroid receptors acting as transcription factors in the nucleus. Beyond these genomic effects, rapid non-genomic effects have been described for both, testosterone and estradiol [1, 3]. Behavioral effects mediated by these rapid actions are largely unknown, however, it is expected to be within 30 minutes of testosterone or estradiol application. Proposed mechanisms based on Michels G. and Hoppe U. C. [2] can be seen in Figure 1. To our knowledge no studies regarding non-genomic effects of sex hormones and behavior were published so far. The aim of our experiment was therefore to describe the rapid behavioral effects of testosterone and estradiol in male castrated rats.

Methods

Twenty adult male Wistar rats (Anlab, Prague, Czech Republic) were obtained for the experiment. Upon delivery, the animals were allowed two weeks for acclimatization. Animals were kept in separate cages in a controlled environment (temperature 22ºC, humidity 50%) with 12:12 light-dark cycle, light period starting at 8:00 pm, with *ad libitum* access to water and food pellets. The research on animals was approved by the ethical committee of the Faculty of Medicine, Comenius University.

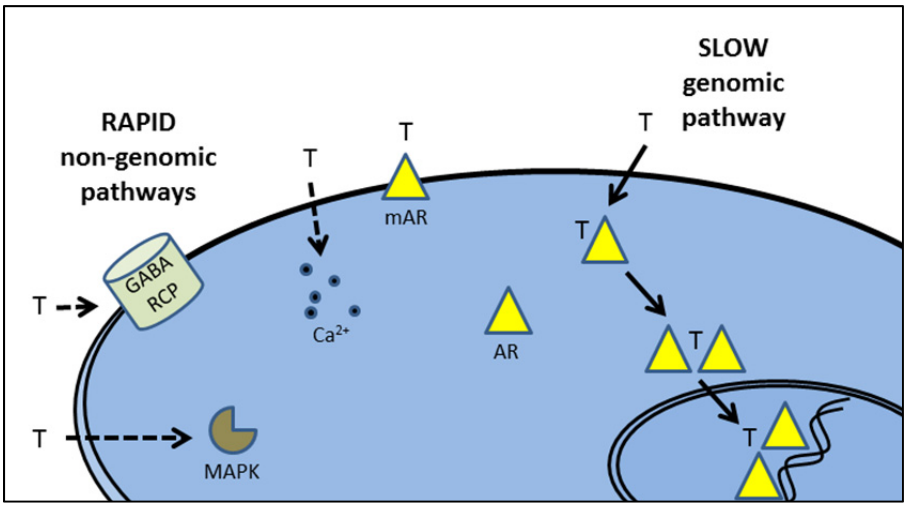

Figure 1. Rapid non-genomic and slow genomic pathway of testosterone (T) action. While in the slow pathway, T binds intracellular receptors and then it enhances the expression of various genes , in rapid pathway, T binds receptors on plasmatic membrane, and the action is mediated through different second messenger cascades (MAPK – Mitogen Activated Protein Kinase; Ca^{2+}- ionized calcium; A R – androgen receptor; GABA RCP – GABA-ergic receptor; mAR – membrane androgen receptor).

Surgery

After two weeks of acclimatization (12-weeks old animals), all rats underwent surgery under general anesthesia (ketamine 100mg/kg + xylazine 10mg/kg in the same syringe, applied intramuscularly). Except of the control sham group, the rest of the animals were castrated prior to experiment. Castration was performed through a small incision in the scrotum, where both testes and epididymis were ligated with absorbable suture and excised. The skin was sutured in two layers with absorbable silk size 4-0. All animals were allowed two weeks for recovery, after the surgery.

Hormonal supplementation

The castrated rats were injected with testosterone 5mg/kg, estradiol 0.5mg/kg or olive oil. Sham-operated group was injected with olive oil.

Behavioral testing

Five minutes after an injection, animals were tested in the open field test (5 min), simple novelty test (5 min), light-dark box (5 min) and forced swim test (3 min). The whole battery of tests was conducted within 30 minutes after injection, since 30 minutes is believed to be the non-genomic effect. The order of tests was chosen from the least to the most stressful. EthoVision XT version 8.5 obtained from Noldus was used as tracking software. Open field test consisted of a square arena of 100cm x 100cm. Animals were placed in the center of the arena, and subsequently were left to explore the maze for 5 minutes. Time in central zone, which was lit, speed, track and distance were recorded. Simple novelty test was performed in the same arena as the open field test; however, new object was inserted into the north-west quadrant of the open field arena. Light/dark box consisted of a box 80cm x 40cm that was divided in the half by a wall with entrance. Half of the box was covered and thus floor inside was in dark. Animals were inserted in the dark box and their behavior was tracked for five minutes. Time in light zone was recorded and evaluated. Forced swim test arena was obtained from Noldus. It consists of a cylindric water tank of 45cm height and 30cm in diameter. The arena was backlit by infrared light. Animals were allowed to swim in the water tank for 3 minutes, during which 3 types of behavior were recorded: A. Immobility - when the animal was not moving, B. Mobility – when the animal was partially moving, and C. Complete mobility – when the animal was swimming around, or was trying to get out of cylinder. Immobility time as a marker of depression-like behavior was calculated and further analyzed.

Statistical analysis was performed by XLStatistics 12.02.10 and GraphpadPrism v. 5.0. ANOVA with Bonferroni post hoc test were used. $P<0.05$ was considered to be statistically significant. Data presented as mean + standard deviation.

Results

Testosterone ($p<0.05$) and also estradiol ($p<0.05$) decreased the time spent in the light part of the light-dark box (see Figure 2). In the open field estradiol increased time spent in the central square ($p<0.05$) compared with castrated and testosterone group but not with control group (Figure 3). No differences in depressive behavior in the forced swim test measured as immobility time was observed between groups (Figure 4). No differences between the groups were found in the simple novelty test (data not shown).

Conclusion

Rapid behavioral effects of testosterone seem to include an anxiogenic effect. The rapid action of estradiol on anxiety differed according to the test used. As far as we know, we are first to describe such rapid testing by battery of tests, in order to evaluate non-genomic effects of sex hormones on behavior. Nevertheless, further studies using larger groups of animals to conquer inter-individual variability, which is high, should reproduce the results and analyze these effects in females as well as further validate the battery of tests.

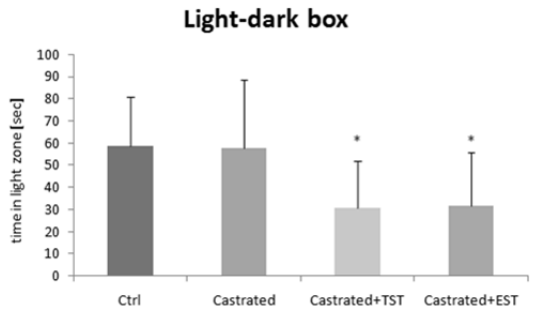

Figure 2. Light/Dark box results. Testosterone as well as estradiol decreased the time spent in the light part of the light-dark box. * denotes p<0.05 vs. control, data presented as mean + SD.TST – testosterone, EST – estradiol, Ctrl – control group.

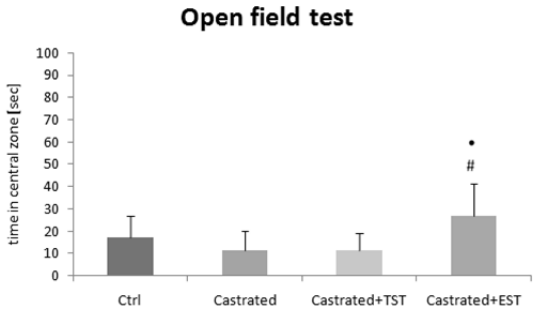

Figure 3. Open field results. Estradiol proved to have anxiolytic effect since, time in central lit zone was in estradiol group increased. # denotes p<0.05 in comparison to castrated group, • denotes p<0.05 in comparison to castrated+TST group. TST – testosterone, EST – estradiol, CTRL – control group.

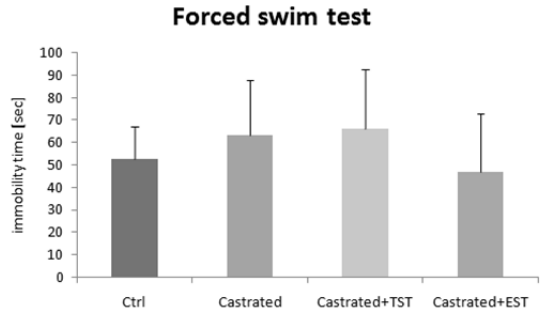

Figure 4. Results of forced swim test. No significant changes were found between groups. TST – testosterone, EST – estradiol, Ctrl – control group.

References

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