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FACTORS DETERMINING THE KINETICS OF A SINGLE DOSE OF TESTOSTERONE IN RATS

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Abstract - The results from different authors regarding testosterone and cognitive research show controversial results. One of the reasons may be the form of testosterone used in the experiment. The aim of our study was to evaluate the testosterone levels in plasma and its kinetics after the single application of either a long-acting or a short-acting form of this hormone. Twenty female and twenty male adult Wistar rats were divided into two groups that were either gonadectomized or not. The two groups were divided into 4 subgroups depending on whether the animals received testosterone propionate or testosterone isobutyrate intramuscularly. Samples for analysis were collected before and at 2, 4, 24, 48, 72, 96 and 168 h after injection. The results showed significant differences in the dynamics between rapid and depot forms of testosterone, together with the rebound effect and hormonal negative feedback. These aspects of testosterone kinetics need to be considered when planning experiments on the physiology of testosterone.

*Key words***:** Testosterone, rapid form, depot form, kinetics, rats

INTRODUCTION

Testosterone circulates in blood bound to the sex hormone binding protein (SHBG), and in a smaller amount it is bound to other plasma proteins, for example albumin. SHBG is produced in the liver and the secretion is stimulated by estradiol and thyroxine and reduced by testosterone, growth hormone and glucocorticosteroids. Under normal conditions, 45 to 70% of testosterone is bound to SHBG, 30 to 55% to albumin (but this relationship is very weak) and approximately 2% circulates in the unbound form. The combination of free testosterone and albumin-bound testosterone is called a biologically free or bioavailable fraction (van Rooij et al., 2011).

The results of scientific research into testosterone show that this hormone participates in sexual (O'Connor et al., 2011), social (Gabor et al., 2011) and spatial (Spritzer et al., 2011) behavior. Testosterone levels can be measured in serum, plasma or saliva using a variety of commercially available kits. The most commonly used are ELISA kits, which are already well established. Nevertheless, there are some controversies between the results of individual experiments. For example, Frey and Lacey have found that exogenous administration of testosterone, dihydrotestosterone and 5α-androstane-3α, 17β-diol (3α-Diol) during training in the open field improved the memory of rats (Frye et al., 2001). Similar results have been obtained by Bimonte-Nelson in a study with older

rats (Bimonte-Nelson et al., 2003). In contrast, Naghdi et al. (2005b) in their study indicated that exogenous testosterone has no effect on spatial memory or that this androgen can impair memory (Naghdi et al., 2005a).

Natural testosterone taken orally or injected intramuscularly is rapidly cleared by the liver and is therefore clinically ineffective. For this reason, in our study we used esters of testosterone. Esterification of testosterone at the 17-hydroxyl position produces compounds with increased lipid solubility. These derivates, administered by intramuscular injection in oil, represent the most commonly prescribed androgens. The solubility and rate of absorption of these compounds varies depending on the fatty acid chain. After they leave the depot, the esters are rapidly metabolized to free steroid, which interacts with target tissues. The testosterone released is available also for bioconversion to dihydrotestosterone and estradiol (Lakshman et al., 2010).

Testosterone levels may be affected by the levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). These hormones are secreted by the pituitary gland and their secretion is controlled by the hypothalamus, specifically by the gonadotropin-releasing hormone (GnRH). FSH binds with receptors in Sertoli cells and stimulates spermatogenesis. LH stimulates testosterone production in Leydig cells, which in turn may act on the Sertoli and peritubular cells of the seminiferous tubules and stimulates spermatogenesis. However, the level of testosterone can significantly influence concentrations of LH and FSH by itself. After the administration of exogenous testosterone (testosterone propionate), LH levels in serum were suppressed to undetectable levels and FSH levels were significantly suppressed to 60 - 70% of the baseline values (Capell et al., 1972). Decreased levels of these hormones can also result in a reduced production of endogenous testosterone, because of the feedback mechanisms, which at high levels of testosterone provides for a reduction in the concentration of LH and also GnRH, and hence the amount of testosterone (Kletter et al., 1992).

The aim of our study was to evaluate testosterone levels in the plasma of male and female intact and gonadectomized rats after a single application of testosterone propionate or testosterone isobutyrate.

MATERIALS AND METHODS

Animals

Our study was carried out on 20 female and 20 male Wistar rats, aged 3 months. The animals were divided into two major groups: intact and gonadectomized (either ovariectomized or orchidectomized) with 4 subgroups each: females treated with testosterone propionate (n=5), females treated with testosterone isobutyrate (n=5), males treated with testosterone propionate (n=5), males treated with testosterone isobutyrate (n=5). The rats were housed in groups on a 12 h light:12 h dark cycle (lights on at 06:00 h), at a temperature of 24±1°C and 45±1% humidity. Food and water were available *ad libitum* in their home cages.

Surgery

Castrated animals were anesthetized with ketamine and xylazine (100 mg/kg and 25 mg/kg respectively) and 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 an incision in the lower abdomen and ligated with silk suture. Intact animals underwent sham surgery, without gonadectomy. Testosterone was applied intramuscularly in a single dose 2 weeks after surgery.

Treatment

Testosterone propionate and testosterone isobutyrate were applied in a single 5 mg/kg dose intramuscularly. Blood samples for testosterone analysis were obtained before testosterone application, 2 and 4 h after application and for the next 4 consecutive days. The last blood sample was obtained at day 7 after testosterone application. All blood samples were taken between 8:00 and 10:00 from the tail vein into col-

lection tubes with K_3EDTA and then centrifuged (3 min at 5000 x g). Plasma was stored frozen (-20°C) until analysis. Testosterone levels were determined by the commercially available ELISA Kits (DRG International, New Jersey, USA).

Statistical analyses

All statistical analyses were performed by XLStatistics version 09.09.24. ANOVA was used for comparison of differences between the groups applied with testosterone propionate and testosterone isobutyrate, and within all the groups. P values less than 0.05 were considered to be statistically significant.

RESULTS

In the intact female rats the testosterone levels in both groups reached their maximum levels in the second hour after testosterone administration (62.05±4.3 nmol.l^{-1} and 36.65±16.01 nmol.l^{-1} for the testosterone-propionate and testosterone-isobutyrate groups, respectively). Subsequent cleavage of testosterone was observed sooner in the testosterone isobutyrate group ($p<0.05$), within 2 h from the peak values, while in the testosterone propionate group this was observed within next 22 h (Fig. 1).

In intact male rats, the maximum concentration was reached in both subgroups in the fourth hour after testosterone administration (59.45±12.54 nmol.l^{-1} and 40.88±11.20 nmol.l^{-1} for the testosterone propionate and testosterone isobutyrate groups, respectively). A significant decrease towards the baseline values was observed within 48 h after injection for the testosterone isobutyrate group and within 24 h after injection for the testosterone propionate group ($p<0.05$, Fig. 2).

Maximal concentrations for both groups of castrated male rats were reached within two hours after testosterone application (61.15±5.54 nmol.l^{-1} and 34.88±11.29 nmol.l^{-1} for the testosterone propionate and testosterone isobutyrate groups, respectively). A significant decrease for both groups was observed within the next 48 h ($p<0.05$; Fig. 3).

In the ovariectomized (OVX) females, both groups reached their maximal testosterone concentration within 4 h (79.71±4.55 nmol.l^{-1} and 32.48±8.42 nmol.l^{-1} for the testosterone propionate and testosterone isobutyrate groups, respectively). A significant decrease of testosterone levels was found within the next 24 h for the female OVX testosterone propionate group and within 48 h for the female OVX testosterone isobutyrate group ($p<0.05$; Fig. 4).

DISCUSSION

The evaluation of testosterone levels is useful in many studies dealing with the influence of testosterone on cognitive function and brain development, because many investigators have published different results indicating that the administration of testosterone either improves (Roof, 1993, Spritzer et al., 2011) or impairs spatial memory and learning (Harooni et al., 2008, Naghdi et al., 2001). One of the reasons for this can be the form of testosterone which has been applied, the time of behavioral measurement or the number of applied doses. For example, Naghdi and Harooni used intra CA1 administration of testosterone enanthate in several doses, and both authors showed impairment in spatial abilities. On the contrary, the research groups of Roof and Spritzer pointed out that the application of testosterone propionate actually decreases the number of errors made in spatial tasks, thus improving orientation in space. Apart from the form and method of application of testosterone, all four works also differ in the amount of testosterone used and the number of overall applied doses.

The aim of our study was to investigate the differences between two forms of testosterone, the fast-acting testosterone propionate and long-acting testosterone isobutyrate. These two forms differ in the number of carbon atoms; testosterone propionate has three and testosterone isobutyrate has four carbon atoms (Miescher et al., 1936). Our results show a significant difference in kinetics after a single dose of these two forms of testosterone, as well as in the maximal concentration reached, where the more rapid testosterone form increased the levels of tes-

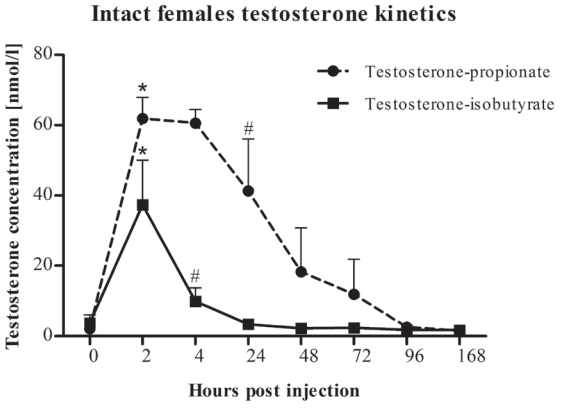

Fig. 1. Comparison of intact female group. Maximal levels of testosterone were reached for both subgroups at 2 h post-injection, and a subsequent decrease was observed within 24 and 4 h after injection for the testosterone propionate and testosterone isobutyrate subgroups, respectively. * denotes significant change in comparison to baseline values; # denotes significant change in comparison to maximal reached values ($p<0.05$ for * and #).

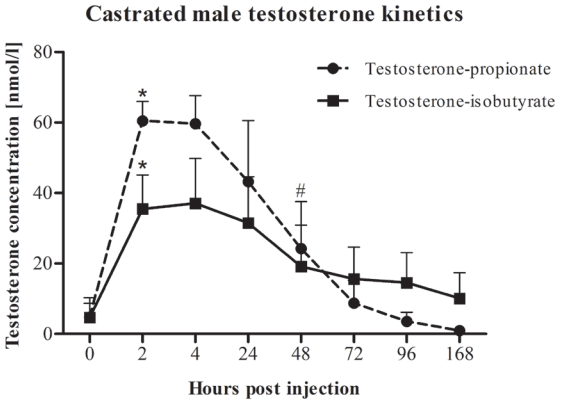

Fig. 3. Comparison of castrated male group. Maximal levels of testosterone were reached for both subgroups at 2 h post-injection, and a subsequent decrease was observed within 48 h after injection for both subgroups. * denotes significant change in comparison to baseline values, # denotes significant change in comparison to maximal reached values ($p<0.05$ for * and #).

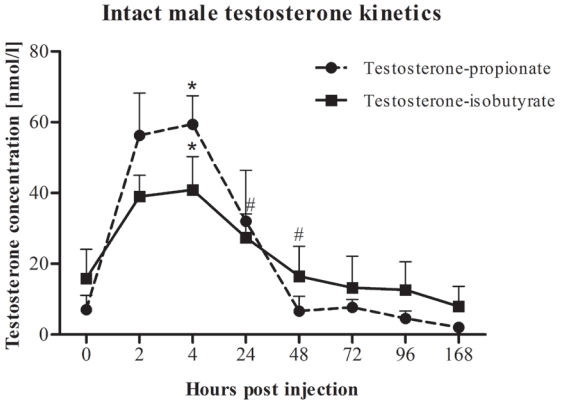

Fig. 2. Comparison of intact male group. Maximal levels of testosterone were reached for both subgroups at 4 h post-injection, and a subsequent decrease was observed within 24 and 48 h after injection for the testosterone propionate and testosterone isobutyrate subgroups, respectively. * denotes significant change in comparison to baseline values, # denotes significant change in comparison to maximal reached values ($p<0.05$ for * and #).

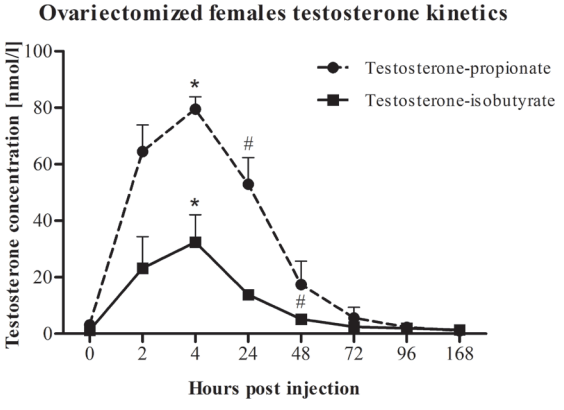

Fig. 4. Comparison of ovariectomized female group. Maximal levels of testosterone were reached for both subgroups at 4 h post-injection, and a subsequent decrease was observed within 24 and 48 h after injection for the testosterone propionate and testosterone isobutyrate subgroups, respectively. * denotes significant change in comparison to baseline values, # denotes significant change in comparison to maximal reached values ($p<0.05$ for * and #).

tosterone supraphysiologically in the first few hours after administration. This effect was seen in both groups, i.e. the intact and gonadectomized animals. Testosterone propionate reached maximum levels two hours, and testosterone isobutyrate four hours, after application. A subsequent decrease of testosterone levels was observed within 24 h after administration for the testosterone propionate group and

within 48 h for the testosterone isobutyrate group. While these results were not surprising, the decrease of testosterone below the initial baseline values on the 7th day after a single dose of testosterone in the intact male rats is rather interesting. This is probably due to the negative feedback of exogenous testosterone to gonadotropin secretion.

In conclusion, both testosterone forms were able to increase the levels of testosterone in the blood of treated animals with quite a predictable pharmacokinetic profile. However, these data suggest that such a pharmacokinetic profile is of particular importance. Before planning experiments on the physiology of testosterone, factors responsible for the actual testosterone level and possible hormonal feedback in the regulatory mechanisms need to be considered.

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