Role of Exogenous Testosterone Administration On Biochemical Parameters in Aged Male Rats

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Abstract

The present experiment was performed to observe the effects produced by high dose of a testosterone ester on the reproductive organ and body weight changes in the adult rat, and to correlate these effects with the serum hormone changes. The present study has used the benzoate ester of testosterone (Testosterone benzoate, TB) in the adult male rat (300-350 g). The aim was to co-relate the reproductive organ and body-weight changes with changes in the serum hormone levels following the administration of the ester. TB was injected i.p. for five (5) consecutive days at a dose of 100 mg/kg body-weight. The control rats were injected with vehicle (arachis oil) at the same dose. The rats were killed on the 6th, 12th, 18th, 24th and 36th days. Controls for only the 6th and 36th days were kept. Reproductive organ weight, body-weight and testosterone (T) levels in serum and testis together with serum FSH and serum LH levels were observed. The testes weights remained similar ($p < 0.05$) to those in the control rats until the 18th days and were reduced on the 36th day. The epididymis weights were not changed until the 36th days, while the androgen-dependent seminal vesicle and ventral prostate weights were increased (< 0.05) compared to those in the control rats. The body-weights remained unchanged at the 6th day but were significantly ($p < 0.05$) decreased on the 36th day. The serum testosterone (ST) concentrations were highly raised on the 6th day, came at the control level on the 18th day and were significantly decreased (< 0.05) on the 36th day. The testicular testosterone (TT) content remained significantly lower ($p < 0.05$) from the 6th to the 36th days post-injection. The serum LH and FSH levels also remained significantly lower ($p < 0.05$) throughout the treatment period. It appears that the elevated serum T levels exerted dual effect in the adult rats, namely, enhanced growth of the androgen-dependent organs and an inhibition over the hypothalamo-pituitary-testicular axis. Inhibition of the said axis was evident by the lower levels of the serum LH and FSH; probably due to this, the TT-content remained all through lower, and perhaps this low TT-content for the long period had led to the low testis weights ($p < 0.05$) on the 36th day. This experiment therefore, demonstrates the effects of exogenous androgen administration in the adult male rat physiology.

Keywords: *Androgens, Age, Central Excitation, Cognition, Anxiety, Novelty-Induced Behaviors.*

1. INTRODUCTION

Androgens such as testosterone are ligands that bind and activate nuclear androgen receptors through deoxyribonucleic acid (DNA) genomic mechanisms1 or alter neuron function through nongenomic interactions.2 Testosterone is an endogenous hormone and also a drug of abuse.3 It is a potent sex steroid produced by the testes in males and a precursor to estrogen synthesis from the ovary in females. In common with all androgens, testosterone has androgenic (masculinizing) and anabolic (muscle building) effects; hence, androgens are collectively known as anabolic androgenic steroids (AASs).

There is an increasing prevalence in the use of testosterone in hormone replacement therapy in elderly men who are predisposed to age-dependent testosterone deficiency due to decreasing testosterone levels associated with debility and reductions in total testosterone with normal aging.4 The use and abuse of androgenic steroids in adolescents is also increasing rapidly;5 testosterone in this age group is used largely for enhancement of physical appearance and athletic performance.6 However, with repeated use of AASs, individual psyche and behavior seem to be strongly affected, with noticeable induction of aggressive behaviors and hostility. Mood disturbances such as depression and hypomania are also documented.7 Abuse of AASs is a potential global epidemic. According to Sagoe et al, the

global lifetime prevalence rate of AAS use is 3.3%, with nonmedical use of AASs fast becoming a public health issue of deep concern.8 An emerging increase in use of testosterone in young, gonadally intact men in addition to older gonad-deficient men implies a new area of focus for exogenous testosterone research. In addition, more effort needs to be put into studying age-related differences in response to testosterone administration.

Over the years however, extensive research has been conducted on the behavioral consequences of testosterone administration9,10 in rodents,11 nonrodents,12–15 and gonadally intact16,17 and gonadectomized animals.17,18 Numerous researchers have also studied the effects of either endogenous or exogenous testosterone on various behavioral paradigms such as16,18–24 sexual behaviors,25–27 obesity or lipid profile,28,29 and gender differences.30–32 With regard to age-related difference in testosterone effects, Chambers et al27 studied the effects of brain androgen binding/metabolism, testosterone, and sexual behavior in intact and gonadectomized old and young male Fischer 344 rats and reported significant differences in sexual behaviors but no significant difference in testosterone effects on aromatase activity. Laroche et al33 studied how shipping during the prepubertal period or adulthood influenced steroid hormone response in male and female mice and concluded that a reduction in behavioral response to exogenous testosterone administration is seen in prepubertal mice compared to adult mice, irrespective of gender.

Most of the studies conducted dealt with effects of either prenatal or prepubertal testosterone administration on adult behaviors; however, a dearth of literature exists in relation to the effect of exogenous testosterone administration (in two separate age groups) on novelty-induced behaviors, and emotionality in mice. The rationale for this study is the need for a better understanding of the behavioral changes that accompany testosterone supplementation at physiologic (<3 mg/kg/day) and supraphysiologic doses (>3 mg/kg/day)34 in gonadally intact animals. We believe that gonadally intact animals model a significant subset of testosterone users in real life. Over the years, research had concentrated on the effects of exogenous testosterone post-gonadectomy; however, many testosterone users are gonadally intact. In this study, we investigated the effect of age, testosterone administration, and their interactions in gonadally intact prepubertal and aged male mice.

2. METHODS

Drugs

Olive oil (Goya) and testosterone propionate (TP; Hubei-Datong Biochemical Company Limited) were given as i.p. injections of specific doses of testosterone.

Subjects

Inbred Swiss albino male mice were used in this study. Mice were housed in plastic cages measuring 41 cm × 31 cm × 26 cm. Housing is a temperature-controlled quarters (22°C–25°C) with 12 hours of light daily. Mice had free access to food and water, except during the behavioral tests. All procedures were conducted in accordance with the approved institutional protocols and within the provisions for animal care and use prescribed in the scientific procedures on living animals.

3. EXPERIMENTAL METHOD

The Swiss albino male mice were divided into two main age-related (prepubertal [postnatal day (PND) 29] and aged [18 months old]) groups. Mice in each age-related group were randomly divided into three groups for the open-field test, four groups for the cognition tests, and four groups for the anxiety-related test. Control groups received i.p. injection of vehicle (olive oil), test groups received doses of TP (2.5 and 5.0 mg/kg/day) daily for a period of 21 days, while animals in the fourth group received a standard drug (diazepam at 0.5 mg/kg for the anxiety test and scopolamine at 1 mg/kg for cognition test). Animals were weighed weekly. Behavioral testing after acute dose was carried out 30 minutes after administration of first dose of vehicle, testosterone, or standard drug, while behavioral testing after sub-chronic dose was carried out 30 minutes after last dose of vehicle, testosterone, or standard drug. Blood samples were collected from mice (animals used in the open-field test) one day after completion of behavioral tests to assay circulating total testosterone levels. Testosterone or vehicle was administered in the morning, and blood samples were collected two to three hours after completion of behavioral tests. Animals were euthanized using diethyl-ether, and blood was collected via a cardiac puncture.

Behavioral testing

The behavioral models used were the open-field, Y-maze, radial-arm, and elevated plus maze (EPM). Behavioral tests were conducted in a quiet room between the hours of 8 a.m. and 2 p.m. On each of the test days, mice were transported in their home cages to the testing room and allowed 30 minutes to acclimatize before testing; for all behavioral tests, at least 5 minutes was allowed between testing each individual animal to ensure the maze was completely dry and residual odor of ethanol had been dispersed. At the beginning of the behavioral tests, each animal was placed in the apparatus, and its behavior was videotaped for subsequent analysis. After testing, each mouse was removed from the maze and returned to its home cage, and all interior surfaces were cleaned thoroughly with 70% ethanol and then wiped dry to remove any trace of odor.

Open field

Novelty-induced behaviors were recorded in the open field (horizontal locomotion [line crossing], rearing, and grooming) over a 20-minute period.35 The open-field box is a rectangular arena comprised of a hard floor measuring 36 cm × 36 cm × 26 cm and made of white-painted wood with its floor divided by permanent red markings into 16 equal squares. The mice were placed in the center of the field and covered by a small dome that was then removed at the beginning of video recording of their activities.

Anxiety test

Anxiety-related behaviors (time spent in the open) were measured in the EPM.37 The EPM is plus shaped, with two open arms measuring 25 cm × 5 cm × 5 cm lying across from each other and perpendicular to two closed arms measuring 25 cm × 5 cm × 16 cm with a center platform (5 cm × 5 cm × 0.5 cm). The closed arms are enclosed by two high walls (16 cm), while the open arms have no side walls. Animals are placed in the central platform facing the closed arm and behaviors recorded for five minutes. The observer retired behind a screen to be obscured from the mouse's view. The criterion for arm visit was considered only when the animal decisively moved all its four limbs into an arm.

Testosterone assay

Plasma was separated by centrifugation and stored at −20° until assayed. Total serum testosterone was measured using commercially available radioimmunoassay kits , following the manufacturer's instructions. The kits had a lower limit of detection of 0.04 ng/mL, with intra- and interassay coefficients of variation of less than 5%. Mice in the vehicle-administered prepubertal group showed total testosterone levels that were not detectable (below the detection limit for the assay, 0.04 ng/mL); for this group, serum testosterone levels were recorded as 0.04 ng/mL to allow for statistical comparisons.

Statistical analysis

Data were analyzed using Chris Rorden's ezANOVA for windows, version 0.98. Hypothesis testing was performed using analysis of variance (ANOVA). All data were first tested for normality and variance homogeneity, prior to statistical analysis. Having been found to be normally distributed and variances homogeneous, we tested the hypothesis that patterns of behaviors exhibited by gonadally intact male mice following administration of testosterone are dependent on age using ANOVA. Multifactorial ANOVA was used to test the effects of three main factors: dose, age (prepubertal vs aged), and duration of administration (acute vs subchronic) on behaviors in the open-field, Y-maze, radial-arm maze, and EPM, while two-factor ANOVA was used to analyze the main effect of dose and age on markers of blood testosterone levels. Tukey's honest significant difference test was used for within- and between-group comparisons. Results are expressed as mean ± standard errors of mean (SEM), P values less than 0.05 were considered statistically significant.

4. RESULTS

Effect of TP on body weight

Figure 1 shows the effect of TP on body weight. Effect was measured as percentage change in body weight and was defined as the difference between the final and initial body weights divided by initial weight multiplied by 100. Two-factor ANOVA revealed a significant main effect of testosterone dose ($F(2,54) = 286$, $P < 0.001$), age of mice ($F(1,54) = 4503$, $P < 0.001$), and very strong interactions between testosterone dose × age ($F(2,54) = 489$, $P < 0.001$). Pairwise comparisons of the effects of age (prepubertal vs aged) on weight revealed that prepubertal mice gained weight significantly compared to aged mice, following administration of either vehicle or testosterone.

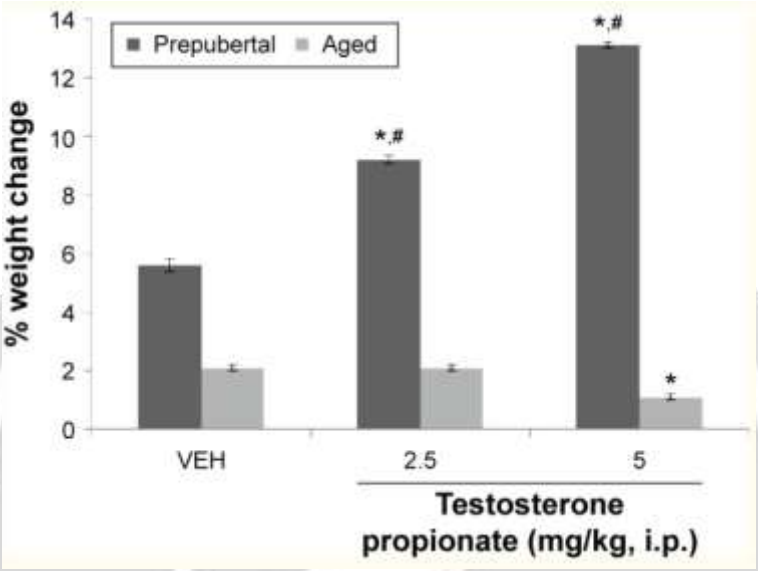

Figure 1 Effect of TP on body weighs

Pairwise comparisons of testosterone dose against vehicle revealed a significant increase in weight in prepubertal mice at 2.5 mg/kg of TP ($t(18) = 13.94$, $P < 0.001$) and 5 mg/kg of TP ($t(18) = 29.43$, $P < 0.001$), while in aged mice, increase in body weight was significantly lesser at 5 mg/kg ($t(18) = 7.07$, $P < 0.001$) compared to corresponding vehicle-treated groups. At 2.5 mg/kg, no significant difference was seen.

Effect of TP on novelty-induced behaviors

Figures 2–4 show the effects of TP on horizontal locomotion, rearing, and grooming, respectively. MANOVA with TP dose, age, and duration of administration (acute behavioral test vs subchronic behavioral tests) as main factors revealed a significant main effect of TP dose ($F(2,108) = 211$, $P < 0.001$), duration of administration ($F(1,108) = 1418$, $P < 0.001$), and age ($F(1,108) = 760$, $P < 0.001$), with strong interactions between testosterone dose × duration of administration ($F(2,108) = 292$, $P < 0.001$), TP dose × age ($F(2,108) = 55.8$, $P < 0.001$), duration of administration × age ($F(1,108) = 2832$, $P < 0.001$), and TP × duration of administration × age ($F(2,108) = 966$, $P < 0.001$).

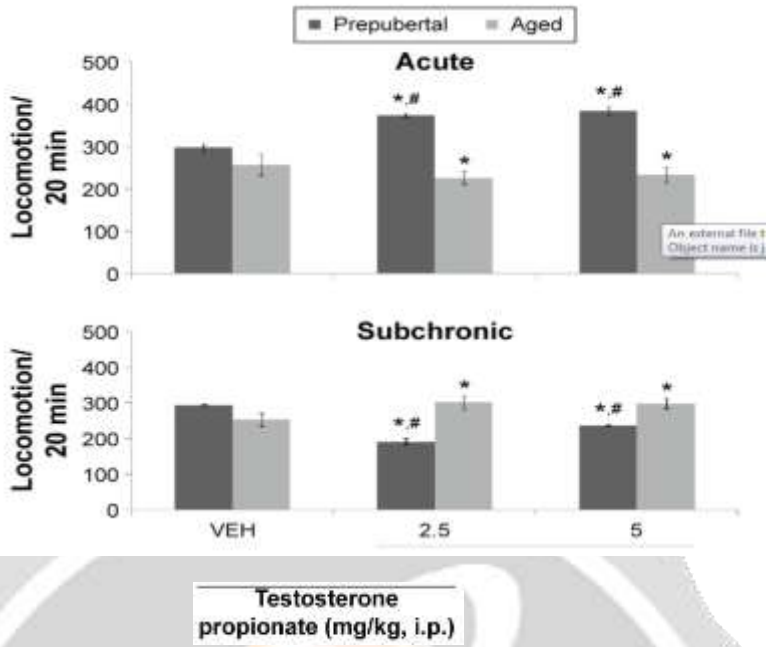

Figure 2 Effects of TP on open-field locomotor activity.

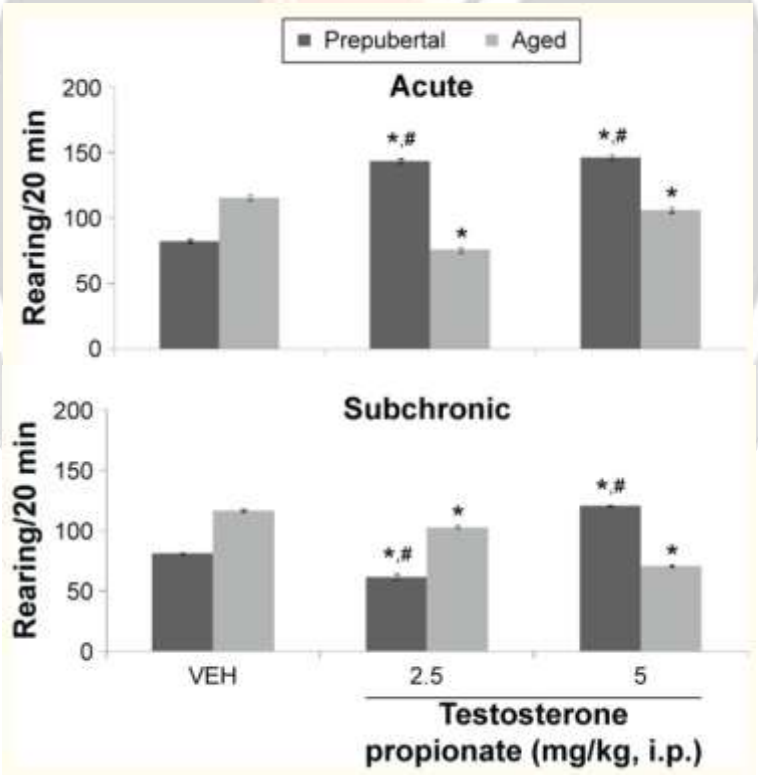

Figure 3 Effects of TP on rearing activity

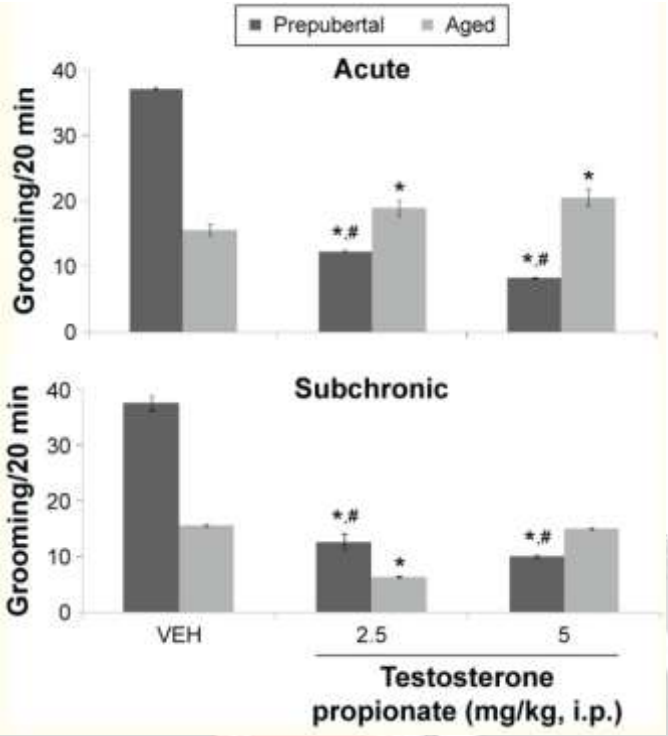

Figure 4 Effects of TP on grooming

Pairwise comparisons of testosterone dose against vehicle following behavioral tests conducted after first dose (hereafter referred to as acute behavioral tests) of either vehicle or TP revealed a significant increase in horizontal locomotion at 2.5 mg/kg of TP ($t(18) = 43.92$, $P < 0.001$) and 5 mg/kg of TP ($t(18) = 36.54$, $P < 0.001$) in prepubertal mice and a significant reduction at 2.5 mg/kg of TP ($t(18) = 6.88$, $P < 0.001$) and 5 mg/kg of TP ($t(18) = 9.07$, $P < 0.001$) in aged mice compared to corresponding vehicle groups. Tests conducted after last dose of vehicle or testosterone (hereafter referred to as subchronic behavioral tests) revealed a significant decrease in locomotor activity at 2.5 mg/kg of TP ($t(18) = 43.66$, $P < 0.001$) and 5 mg/kg of TP ($t(18) = 8.67$, $P < 0.001$) in prepubertal mice and a significant increase at 2.5 mg/kg of TP ($t(18) = 34.23$, $P < 0.001$) and 5 mg/kg of TP ($t(18) = 10.15$, $P < 0.001$) in aged mice compared to corresponding vehicle-treated groups.

Pairwise comparison of age-related effects revealed a significant increase in locomotor activity at 2.5 mg/kg of TP ($t(18) = 60.67$, $P < 0.001$) and 5 mg/kg of TP ($t(18) = 37.64$, $P < 0.001$) in prepubertal mice compared to aged mice with acute behavioral tests and a significant decrease at 2.5 mg/kg of TP ($t(18) = 56.43$, $P < 0.001$) and 5 mg/kg of TP ($t(18) = 4.63$, $P < 0.002$) with subchronic behavioral tests; however, there was no significant difference in the groups administered vehicle in either prepubertal or aged animals. Comparison of effects due to duration of administration (acute behavioral test vs subchronic behavioral tests) revealed a significant increase in locomotor activity at 2.5 mg/kg of TP ($t(18) = 75.11$, $P < 0.001$) and 5 mg/kg of TP ($t(18) = 27.53$, $P < 0.001$; acute vs subchronic behavioral tests) in prepubertal mice and a significant decrease in locomotor activity at 2.5 mg/kg of TP ($t(18) = 37.16$, $P < 0.001$) and 5 mg/kg of TP ($t(18) = 2.16$, $P < 0.045$; acute vs subchronic behavioral tests) in aged mice.

Figure 3 shows the effect of TP on rearing activity. MANOVA revealed a significant main effect of TP dose ($F(2,108) = 18.6$, $P < 0.001$), duration of administration ($F(1,108) = 78.9$, $P < 0.001$), and age ($F(1,108) = 41.4$, $P < 0.001$), with strong interactions between TP × duration of administration ($F(2,108) = 13.4$, $P < 0.006$), TP dose × age ($F(2,108) = 121$, $P < 0.001$), duration of administration × age ($F(1,108) = 35.6$, $P < 0.001$), and TP dose × duration of administration × age ($F(2,108) = 67.1$, $P < 0.001$). Pairwise comparison of testosterone dose against vehicle following behavioral tests conducted after first dose (acute behavioral tests) of either vehicle or TP revealed a significant increase in rearing at 2.5 ($t(18) = 25.92$, $P < 0.001$) and 5 mg/kg ($t(18) = 26.40$, $P < 0.001$) in prepubertal mice and a decrease at 2.5 ($t(18) = 10.82$, $P < 0.001$) and 5 mg/kg ($t(18) = 3.30$, $P < 0.004$) in aged mice compared to corresponding vehicle-treated groups. Tests conducted after last dose (subchronic behavioral tests) of vehicle or TP showed an decrease in rearing at 2.5 ($t(18) = 31.68$, $P < 0.001$) and an increase at 5 mg/kg ($t(18) =$

41.79, $P < 0.001$) in prepubertal mice and a decrease at 2.5 ($t(18) = 5.33$, $P < 0.005$) and 5 mg/kg ($t(18) = 3.41$, $P < 0.031$) in aged mice compared to corresponding vehicle groups.

Comparison of age-related effects revealed a significant increase in rearing activity at 2.5 ($t(18) = 18.38$, $P < 0.001$) and 5 mg/kg ($t(18) = 13.83$, $P < 0.001$) of TP in prepubertal mice compared to aged mice with acute behavioral tests and a significant decrease at 2.5 ($t(18) = 8.31$, $P < 0.001$) and an increase at 5 mg/kg ($t(18) = 43.31$, $P < 0.001$) in prepubertal compared to aged mice following subchronic behavioral tests; however, there was no significant difference in the groups administered vehicle in either prepubertal or aged animals. Comparison of effects due to duration of administration (acute behavioral test vs subchronic behavioral tests) revealed a significant increase in rearing activity at 2.5 ($t(18) = 19.61$, $P < 0.001$) and 5 mg/kg ($t(18) = 11.67$, $P < 0.001$; acute vs subchronic behavioral tests) in prepubertal mice. A significant decrease in rearing activity at 2.5 ($t(18) = 8.48$, $P < 0.001$) and an increase at 5 mg/kg ($t(18) = 16.22$, $P < 0.001$; acute vs subchronic behavioral tests) was seen in aged mice.

Figure 4 shows the effect of TP on grooming behavior. MANOVA revealed a significant main effect of TP dose ($F(2,108) = 4253$, $P < 0.001$), duration of administration ($F(1,108) = 202$, $P < 0.001$), and age ($F(1,108) = 1378$, $P < 0.001$), with strong interactions between TP dose × duration of administration ($F(2,108) = 246$, $P < 0.006$), TP dose × age ($F(2,108) = 4127$, $P < 0.001$), duration of administration × age ($F(1,108) = 938$, $P < 0.001$), and TP dose × duration of administration × age ($F(2,108) = 210$, $P < 0.001$). Pairwise comparisons of the effect of testosterone dose against vehicle in behavioral tests after first dose (acute behavioral tests) of either vehicle or TP revealed a significant decrease in grooming at 2.5 ($t(18) = 70.94$, $P < 0.001$) and at 5 mg/kg ($t(18) = 94.04$, $P < 0.001$) in prepubertal mice and an increase at 2.5 ($t(18) = 8.58$, $P < 0.001$) and 5 mg/kg ($t(18) = 11.31$, $P < 0.001$) in aged mice compared to corresponding vehicle-treated groups. Tests conducted after the last dose (subchronic behavioral tests) of either vehicle or TP revealed a significant decrease in grooming at 2.5 ($t(18) = 72.17$, $P < 0.001$) and 5 mg/kg ($t(18) = 77.25$, $P < 0.001$) in prepubertal mice and at 2.5 mg/kg in aged mice compared to corresponding vehicle-treated groups. However, in aged mice at 5 mg/kg, no significant difference was seen.

5. DISCUSSION

Endogenous or exogenous testosterone influences brain behavior and chemistry through mechanisms that are both due to its organizational and activational effects.38 Concerning its effects, a number of human and animal studies have been published, with several conflicting and puzzling results largely due to variability in dosing, route of administration, state of the gonads, gender, and timing, all of which could considerably alter outcome.38 We set out to investigate the effects exogenous testosterone in gonadally-intact prepubertal and aged male mice with a view to testing the hypothesis that aging modulates the influence of exogenous testosterone on neurobehavior and serum testosterone levels.

The results of this study revealed alterations in behavioral response of gonadally intact male mice to i.p. injection of TP, which were influenced not only by age (prepubertal vs aged) but also by duration of administration (effects seen when behavioral tests were conducted after 1st dose compared to after the 21st dose of TP [acute versus subchronic]). The prepubertal period in male rodents extend from PNDs 28 to 70.39,40 This period however can be characterized using varying physiological parameters, such as physical features, levels of sex steroid, the attainment of sexual maturity, and reproductive capability.41 These variations make comparisons of research results difficult.

In this study, all animals administered TP gained weight over the 21-day period, although prepubertal mice gained more weight than aged mice at both doses. Studies have shown that administration of androgenic steroids could result in weight gain. The results seen in aged mice corroborate the results by Davies et al who reported a decrease in weight in male obese Zucker rats following testosterone supplementation. Several human studies have also reported weight loss in elderly men on hormone replacement therapy. Testosterone therapy has been associated with weight reduction and increase in lean body mass, in obese elderly men with primary hypogonadism on hormone replacement therapy. Weight changes seen in prepubertal mice could be attributable to prepubertal growth spurts.48 In aged mice, a decrease in total body fat may be a plausible explanation for the changes seen, as an inverse association has been described between levels of testosterone and accumulation of body fat.

6. CONCLUSION

Testosterone (endogenous or exogenous) has been reported to exert several behavioral traits in both human and animal studies, and these effects are mediated not only through the androgen or estrogen receptors but also through rapid nongenomic effects. In this study, administration of testosterone resulted in increase in horizontal locomotion and rearing in prepubertal mice with acute administration and a decrease with repeated administration, while in aged

mice, the reverse was the case. Grooming behavior however showed a decrease in prepubertal mice and an increase in aged mice with acute administration, while with repeated administration, it decreased in both prepubertal and aged mice. Results of behavioral tests after acute TP administration in prepubertal mice and repeated administration of TP in aged mice point to cerebral cortical stimulation. It was also observed that repeated administration of testosterone in prepubertal rats had inhibitory effects on cerebral cortical stimulation. Central excitatory effect seen in prepubertal mice after acute administration could have been mediated via glutamatergic and dopaminergic systems. A few studies have indicated that testosterone supplementation particularly within the adolescence period may regulate dopamine neurotransmission. Purves-Tyson reported that exogenous testosterone during adolescence (between PNDs 45 and 60) in male rats increased dopamine synthesis and stimulated expression of dopamine receptor 2 messenger RNA (DR2 mRNA) in the midbrain. Dopaminergic transmission in the nucleus accumbens may have increased with testosterone supplementation since increase in dopaminergic transmission in the nucleus accumbens is associated with locomotor hyperactivity and increases in the caudate nucleus with increased rearing activity.

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