The Anabolic Androgenic Steroid Testosterone Propionate Decreases Recognition Memory in Adult Male Rats

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Abstract: Although there are therapeutic applications of the Anabolic Androgenic Steroids (AAS), the predominant use implies the illicit self-administration by athletes and adolescents. In this respect, AAS abuse is associated with untoward effects on brain and behavior. Exposure to supraphysiological doses of AAS leads to changes in anxiety and aggression, but their effects on cognitive functions are poorly understood. We investigated the effects of the AAS testosterone propionate (TP), acutely or chronically administered in supraphysiological doses, on memory in rats. Experiment I - Adult male Wistar rats were treated once with vehicle (Control group, n = 5) or TP (10 mg/kg; n = 5). Behavioral experiments were performed 60 minutes after the single injection, and included the evaluation of spatial working memory and recognition memory. Experiment II - The rats received repeated daily administration of vehicle (n = 6) or TP (n = 7) for 40 days. Behavioral experiments started 23 hours after the last injection. After behavioral procedures, the animals were euthanized, the blood was collected for biochemical analyses and testicles were removed and weighted. Regarding the behavioral assessment, rats chronically treated with TP presented decreased time exploring the novel object when compared to control group. Rats that were treated acutely showed no significant difference compared with control. Acute or chronic treatments with TP were not effective in promoting changes in spatial working memory. Additionally, chronic treatment with TP induced significantly increases in biochemistry marker the enzyme glutamate pyruvate transaminase (SGPT) and a reduction in testicular weight as compared with control. Even though not interfering with spatial working memory performance, AAS abuse could induce deficit on recognition memory.

Keywords: Androgens, AAS supraphysiological dose, behavior effects, cognitive deficit, long-term AAS treatment, acute AAS treatment, spatial working memory.

INTRODUCTION

The original therapeutic use of the synthetic analogues of testosterone, the anabolic androgenic steroids (AAS), was the enhancement of anabolic potency [1]. However, it is known that AAS (in supraphysiological doses) have a high prevalence of misuse by athletes and adolescents, aiming to improve performance or body image [2, 3, 4]. Some consequences of the chronic steroid misuse that have been described are coronary heart disease, increased blood pressure, and liver dysfunction [5]. These adverse effects vary in intensity depending on age, sex, type of AAS, dosage and duration of use [6, 7, 8]. Besides the development of drug dependence, adverse effects of AASs in the neural function and behavior have been reported, such as aggressive behavior, psychosis and even suicide attempts [9, 10, 11, 12]. In addition, a few reports have shown cognitive impairments [13].

Considering that gonadal hormones play a role in mechanisms related to cognitive function [14, 15], it is expected that AASs exert actions on learning and memory processes. Indeed, studies have shown high density of androgen receptors in brain areas related to these processes [16, 17], as well as of the role of sexual hormones in learning and memory [18]. The studies on this issue were mainly conducted with acute testosterone, administered intra-hippocampally, and have shown that this hormone impairs the performance of rats in spatial water maze [19, 20] and inhibitory avoidance tasks [21, 22]. Other studies have shown impaired performance in different kinds of memory tasks in gonadectomized (GDX) male rodents, which were restored after testosterone administration [23, 24].

On the other hand, there are few studies focusing on the effects of long-term treatment with testosterone or synthetic analogues, which would be of interest considering the pattern of abuse of these substances. Moreover, these studies have shown controversial results, Kouvelas et al. [25] showed impaired performance in an olfactory social memory task in rats treated with nandrolone decanoate. Conversely, other studies did not report any alteration in the acquisition or extinction of a lever-pressing task [26] or performance in the water maze task [27] of the rats treated with same drug.

The mechanism underlying the potential cognitive effects of AAS is not understood. It has been demonstrated that the AAS nandrolone, given chronically, modify the glutamatergic [28], GABAergic [29], dopaminergic and serotonergic [30, 31] systems. From another standpoint, beneficial effects of prolonged testosterone treatment on
neuronal function have also been reported [32], although this neuronal effect did not result in behavioral improvement in senescent rats [33].

In this context, the widespread use of AAS during lifetime is an important concern. In addition to the physiological damage that has been extensively described, evidence suggests that there are also cognitive impairments. However, as mentioned, the results in animal studies are not unequivocal. In order to extend the knowledge about the cognitive effects of prolonged treatment with AAS, we investigated the effects of chronic testosterone propionate (TP) at a supraphysiological dose in rats. We hypothesized that the effects of AAS would extend to different aspects of memory function other than those previously investigated. The animals were tested in the spontaneous alternation task (to evaluate spatial working memory), and the novel object recognition (NOR) task. After the behavioral tests, animals were euthanized and testicular weight and biochemical analyses were obtained. Additionally, behavioral testing in the same paradigms after acute treatment was conducted for comparison.

MATERIALS AND METHODS

Subjects and General Procedures

Three-month-old male Wistar rats (300-350g) were used. Animals were housed individually in plastic cages (30x19x13cm, length x width x high), under conditions of acoustic isolation and controlled airflow and temperature (25 ± 1º C), with a 12h light/12 h dark cycle (lights on at 06:30). Food and water were available ad libitum. All procedures were approved by the local ethics committee. All efforts were made to minimize animal pain, suffering or discomfort.

Treatment

Animals in the control group were injected with sesame oil (Vehicle - VEH) and those in the treated group were injected with 10 mg/kg testosterone propionate (TP - Androgenol®, Herpate Calier, Brazil) diluted in vegetable oil. The injections were administered intramuscularly, and the target hind limbs were alternated. The dose of 1 mg/kg is sufficient to restore endocrine function and reproductive behaviors in gonadectomized male rats [34]. Thus, the dosage of 10 mg/kg used in this experiment can be considered supraphysiological, and was combined with a long-term treatment of 40 days (20 injections) to simulate abuse conditions. The health condition of the animals during the treatment with TP was considered adequate: no deaths occurred during the treatment and no wounds were observed in the injection sites. Additionally, repeated oil injection did not seem to interfere with animals’ general behavior (as suggested by absence of effect in the locomotion - Table 1). Moreover, no changes were observed in the weight of the rats throughout the treatment (data not shown).

Experimental Design

The animals were handled daily for 10 min during seven days before the beginning of the experimental procedures. Afterwards, the rats were randomly assigned to one of the groups: Control or PT-treated.

Table 1. Effects of 10 mg/kg of Testosterone Propionate (TP) or Sesame Oil, Vehicle (VEH) in Rats Submitted on Spontaneous Alternation Task

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatment</th>
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<tbody>
<tr>
<td></td>
<td>VEH</td>
</tr>
<tr>
<td><strong>Acute Administration</strong></td>
<td></td>
</tr>
<tr>
<td>Alternations (%)</td>
<td>34.46 ± 7.29</td>
</tr>
<tr>
<td>Total number of entries in the arms</td>
<td>24.75 ± 2.65</td>
</tr>
<tr>
<td>Distance travelled (m)</td>
<td>23.34 ± 2.54</td>
</tr>
<tr>
<td><strong>Chronic Administration</strong></td>
<td></td>
</tr>
<tr>
<td>Alternations (%)</td>
<td>44.73 ± 4.15</td>
</tr>
<tr>
<td>Total number of entries in the arms</td>
<td>33.00 ± 3.01</td>
</tr>
<tr>
<td>Distance travelled (m)</td>
<td>38.16 ± 4.12</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. (Independent samples t-test).

Experiment I - Effects of Acute Administration of Testosterone

Each animal received a single intramuscular injections of sesame oil (VEH; n = 5) or injections of 10 mg/kg testosterone propionate (TP; n = 5). This day was considered day 1-I.

On day 1-I, sixty minutes after the single administration, rats were individually placed in this central space in a plus-maze to spontaneous alternation task.

On day 2-I (24h after the single injections), rats were exposed to the open field for habituation for 10 min. After 24h and 48h (on days 3-I and 4-I), the training and test sessions of the novel object recognition task were performed as described above, respectively.

Experiment II - Effects of Chronic Administration of Testosterone

Rats received intramuscular injections of sesame oil (VEH; n = 6) or 10 mg/kg testosterone propionate (TP; n = 7) every other day for 40 days (20 injections). The first injection day of the Experiment II, was considered day 1-II. On day 39, rats were individually submitted to the spontaneous alternation task.

On day 40-II (24h after the last injection), rats were submitted to the habituation session for the object recognition task, and the training session was conducted on day 41-II. The test session was conducted 24h later (on Day 42-II).

Behavioral Tasks

Recognition Memory - Novel Object Recognition (NOR) Task

Each animal was placed in the central region of an open field arena (diameter = 1.0 m) and were given 10 min of free exploration to habituate (without any objects). After 24h, in a 5-min training session, the animals were placed in the field with two identical objects (objects A1 and A2), diametrically
positioned in opposite sides of the field and fixed on the floor. In the test session, conducted 24h later, a novel object (B) replaced one of the previous objects (A) and the rats were allowed to explore them for 5 minutes. The objects used were approximately the same size and had different colors and shapes. Exploratory behavior (touching with the nose and/or forepaws, sniffing or biting each object) was recorded. The exploration ratio [time spent exploring the novel object B / (time exploring familiar object A + time exploring the novel object B)] was calculated for each animal. The maze and the objects were cleaned thoroughly after each session with a 5% ethanol solution. All behavioral tests were held between 09:00 a.m. and 11:30 a.m.

Spatial Working Memory - Spontaneous Alternation (SA) Task

The apparatus used was a wooden plus-maze placed 50cm elevated from the floor, with four enclosed arms (47cm×16cm×34cm) extending from a central platform (16cm×16cm). The animals were individually placed in this central space with access to all arms and allowed free exploration for 10 min. The behavior of the animals was recorded by a video camera positioned above the maze and analyzed by video-tracking software (Any-maze, Stoelting, USA). The four arms were designated A, B, C and D, and the amount and sequence of entries in the arms were registered. An entry was defined by the crossing of the four paws through the entrance of the arm. An alternation was computed when the animal explored the 4 individual arms consecutively, in any order, without repeating an arm choice. Percentage of alternation was calculated by number of registered alternations /number of possible alternations. General locomotor activity was evaluated by the total number of entries and the distance travelled.

Biochemical Analysis

On day 42-II, after the test session of NOR task, blood samples (5 ml) were collected into heparinized tubes after rapid decapitation. Centrifugation (1000 rpm at 4°C for 10 min) separated serum from blood. The serum obtained was used to estimate the levels of glutamate oxaloacetate transaminase (SGOT), glutamate pyruvate transaminase (SGPT) [35] and bilirubin [36]. The increase in the levels of liver enzymes is frequent among steroid users [37].

Gonadal Weight

After decapitation, testicles were dissected and weighted. For data analysis, testicular weight was normalized considering the total body weight.

Ethical Statement

The authors declare that all experiments reported in this article received formal approval of the local ethical committee (CEUA-UFRN, protocol 024/2009), and followed the law for the use of animals in scientific research (Brazilian law number 11.794). Moreover, the study was conducted in compliance with the standards set forth in the eighth edition of Guide for the Care and Use of Laboratory Animals published by the National Academy of Sciences, Washington, D.C). All efforts were made to keep the number of animals used to a minimum, as well as pain, suffering or discomfort.

Statistical Analyses

The independent samples t test was used to analyze differences between the groups VEH or TP. In the novel object recognition task, within-subject comparisons for percentage of time to explore old x new objects were conducted with paired-samples t tests. Data are displayed as mean ± standard error (S.E.M), and p < 0.05 was considered to reflect significant differences. The SPSS 18 for Windows software was used to perform the statistical analysis.

RESULTS

Experiment I - Effects of Acute TP Treatment

Novel Object Recognition Task

No changes in locomotor activity were found in the habituation session [t(8) = 0.377, p = 0.716 (Fig. 1A)]. In the test session, there was no effect of treatment on new object exploration ratio [t(8) = 0.439, p = 0.672 (Fig. 1C)]. Both groups showed augmented exploration of the new object compared to old object [Control group, t(4) = 4.624, p = 0.010] and TP treated group [t(4) = 3.967, p = 0.017] (Fig. 1E).

Spontaneous Alternation Task

No significant differences were found on spontaneous alternation performances [t(7) = 1.367, p = 0.214], number of arm entries [t(7) = 0.206, p = 0.842] and distance travelled [t(7) = 0.076, p = 0.941] between control and experimental groups (Table 1).

Experiment II - Effects of Chronic TP Treatment

Novel Object Recognition Task

No changes in locomotor activity were observed in the habituation session [t(11) = 0.719, p = 0.487 (Fig. 1B)]. Rats treated with 10 mg/kg TP presented significantly decreased exploration of the new object compared to VEH in the test session [t(7) = 3.869, p = 0.006 (Fig. 1D)]. In addition, the exploration of the new object by the VEH (but not TP) group was greater than the exploration of the old object [t(7) = 4.22, p = 0.024 (Fig. 1F)]. In this task, the data of four animals (two VEH and two TP) were removed from the analysis due to absence of object exploration in the training session.

Spontaneous Alternation Task

No significant differences were found on spontaneous alternation performances [t(11) = 1.019, p = 0.33], number of arm entries [t(11) = 0.302, p = 0.76] and distance travelled [t(11) = 0.859, p = 0.41] between control and experimental groups (Table 1).

Endocrine Effects

The protocol of TP treatment used in the present study showed significant effects in testicular weight. Indeed, when compared to VEH, this administration reduced relative
testicular weight in relation the total body weight \[t(11) = 4.33, \ p = 0.001\] (Fig. 2). The administration of TP also caused significant increase in marker enzyme SGPT compared with the control group \[t(11) = 2.715, \ p = 0.02\] (Table 2). However, no significant differences were detected in levels of SGPO and serum bilirubin.

**DISCUSSION**

As mentioned, the AAS are used aiming improvements on performance and body image, usually through the illicit self-administration that is associated with untoward effects on brain and behavior. Moreover the AAS has become a widespread drug abuse problem [1, 2, 38]. Thereby, due to
The involvement of steroid hormones with cognitive functions, the prolonged use may promote impairment in these processes. In this context, aiming to investigate the effects of continuous exposure to supraphysiological levels of androgen steroids, chronically and acutely testosterone propionate (TP)-treated rats were tested in two different memory tasks.

**Table 2. Effects of Testosterone Propionate (TP) on Different Biochemical Parameters in the Serum of Rats**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
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<tbody>
<tr>
<td></td>
<td>VEH</td>
<td>TP</td>
<td></td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.45 ± 0.25</td>
<td>0.49 ± 0.22</td>
<td></td>
</tr>
<tr>
<td>Direct</td>
<td>0.31 ± 0.20</td>
<td>0.36 ± 0.22</td>
<td></td>
</tr>
<tr>
<td>SGPT (U/ml)</td>
<td>49.00 ± 4.42</td>
<td>84.29 ± 11.38*</td>
<td></td>
</tr>
<tr>
<td>SGOT (U/ml)</td>
<td>137.83 ± 13.87</td>
<td>121.00 ± 18.50</td>
<td></td>
</tr>
</tbody>
</table>

SGPT, glutamate pyruvate transaminase in the serum; SGOT, glutamate oxaloacetate transaminase in the serum. Values are expressed as mean ± S.E.M. (*p < 0.05 compared to vehicle; Independent samples t-test).

Regarding the effects of TP on the performance of rats in the spontaneous alternation (SA) task (Experiment I and II) (Table 1), these findings corroborate studies in which spatial working memory was not altered after administration of a high dose of one of the three AAS compounds (17α-methyltestosterone, testosterone cypionate or methandrostenolone) [39]. The spontaneous alternation behavior comprises the tendency for rodents and other animals to alternate their nonreinforced choices of arms in a maze at successive opportunities [40]. This task has been used as a simple test of spatial working memory. Thus, the absence of effect observed in rats treated with TP (acute or chronically administered) is possibly because this task is not complex enough to verify the effects of treatment.

From another standpoint, some findings indicated acute intrahippocampal injections of testosterone have benefits for memory performance in rats [22]. In agreement, a correlation between spatial impairment and testosterone loss in aged mice was demonstrated, and treatment with exogenous testosterone counteracted this impairment [41, 42]. Further, there is evidence of an organizational action of these hormones in the development of neuronal circuitries related to cognitive function. Indeed, a study showed that testosterone enhances learning and memory in rats when given at critical periods of development [41]. In summary, studies with testosterone (or its derivatives) and spatial working memory in animals have shown controversial results.

In the case of the present study, the absence of effects of TP in SA task in the results obtained cannot be attributed to motor alterations, since the treatment did not cause changes in motor functions when compared to rats treated with untreated in both the SA and NOR tasks (Table 1). Our data on locomotor activity corroborate previous studies that demonstrated lack of motor alterations in gonadally-intact rodents after chronic exposure to AAS [43, 44, 45].

We also investigated the effects of AAS in the novel object recognition (NOR) paradigm. The chronic treatment with TP 10 mg/kg was able to cause a deficit in the index of recognition of novel object when compared to the control group (Fig. 1D). Few previous investigations focused the effects of AAS chronic treatment on recognition memory. For example, recognition of a juvenile in a social recognition task was decreased by chronic administration of nandrolone decanoate (15mg/kg), although this treatment did not impair the acquisition of the information [25]. In line with this result, nandrolone decanoate also delayed the extinction learning in rats tested in an aversively-motivated task (passive avoidance) [46]. On the other hand, Aubele and coworkers [47] found a deficit in a spontaneous object recognition task (performed with a 1.5 h delay) after gonadectomy in adult male rats, providing evidence of a modulatory role of these hormones in recognition memory processes.

It is relevant to mention that different mechanisms could be related to the modulatory role of endogenous hormones as opposed to the effects of supraphysiological doses of androgens on the cognitive function. Indeed, there is an improvement of cognitive function when low doses of androgens are administered, while higher doses can cause cognitive impairments [33]. In line with this reasoning, in studies using the Morris Water Maze showed that testosterone and flutamide impaired de performance of rats after infusions in the CA1 hippocampal subregion [19, 48]. Interestingly, this pattern of bidirectional results has also been found to the effects of estrogens administration in ovariectomized female rats [49].

The mechanisms behind a specific impairment in recognition memory (reported here), or in the complex effects for cognitive function in general, remain unclear. There is evidence of modulation of several neurotransmission systems by steroid hormones and/or their active metabolites. Regarding the effects related to memory processes, an action via the cholinergic transmission has been indicated [50]. In addition, the chronic treatment with
nandrolone interferes with the glutamatemic transmission [28]. Moreover, GABA-, dopamine- or serotonin-mediated neurotransmissions can also be modulated by AAS [51, 52, 53]. Further investigation on the mechanisms related to memory impairment of object recognition due to AAS is needed.

Regarding physical (non-behavioral) effects, AAS compounds usually produce typical marked effects like reduction in testicles’ weights [5]. In this study, testicular weight of animals receiving TP was reduced compared to the control group (Fig. 2). The alterations in the gonadal weight are likely due to the well-established actions of AAS compounds on the negative feedback loop (hypothalamic-pituitary-gonadal axis) which results in changes in endogenous testosterone production [54]. Also, increased levels of enzyme GPT, which often indicates liver damage possibly caused by high doses of TP. The spectrum of hepatic injury is ample. Elevated levels of alkaline phosphatase, as well as elevation of liver transaminases are perhaps the more frequently described abnormalities [55, 56]. Some studies have also demonstrated evidence for enhanced blood aminotransferase activities after AAS use in bodybuilders [56]. Thus, besides the behavioral cognitive effects, these biochemical and weight analysis overall confirmed the effectiveness of the protocol used in the present study in inducing typical consequences of AAS abuse.

CONCLUSION

In conclusion, data reported here extents the studies showing that AASs affects the cognitive functions in rats. Specifically, we have demonstrated that chronic administration of TP resulted in significant reductions of performance in novel object recognition task. Further research is needed to determine the extent to which high levels of androgens may influence different aspects of cognitive functions, as well as the mechanisms underlying these effects.

CONFLICTS OF INTEREST

The authors confirm that there is no conflict of interest regarding the present work.

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