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Anabolic-androgenic steroids cycle administration decreases anxious-like behavior but does not affect long-term memory acquisition in rats

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ABSTRACT. The steroid hormones are lipids in nature, which play crucial roles in several metabolic and behavioral pathways in mammals. Drug therapy uses sterol hormones for treating some disturbances linked with its deficiency; however, the illicit use of these hormones by amateur and elite athletes to enhance performance or body appearance may lead to several health issues. In this study we evaluated the anxious-like behavior and the long-term memory acquisition of male rats undergoing sedentary life-style or physical effort, with or without anabolic-androgenic steroids (ASC) treatment. The results showed a decrease in anxious-like behavioral levels in rats that received ASC treatment associated or not with physical effort, but this treatment did not affect the acquisition of long-term memory at the dose and experimental model assessed.

Keywords: androgens; physical exercise; memory; anxiety.

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Introduction

It is well established that testosterone and nandrolone have antagonistic effects on behavior. Nandrolone seems to cause anxiety, but the memory acquisition capacity is still unclear (Costine, Oberlander, Davis, Penatti, Porter, Leaton, & Henderson, 2010; Magnusson, Hänell, Bazov, Clausen, Zhou, & Nyberg, 2009). On the other hand, testosterone seems to be able to decrease anxiety levels and impair the memory acquisition (Skoett, Bandak, Kreilberg, Lauritsen, & Daugaard, 2018). Besides, these steroids have been misused throughout the world to improve athletes' performance (Kanayama, & Pope Jr, 2018).

The literature reports about androgenic hormones and their effects on human tissues but there is a lack of studies linking the long-term memory acquisition and anxiety with the administration schedules of anabolic-androgenic steroids (ASC) adopted by athletes. In the present study we used Open Field Test (OF) and Step-down Type Passive Avoidance Test (PA) to detect changes in anxious-like behavior and long-term memory in rats experimental model using an administration schedule of ASC.

Material and methods

We carried out the experiments on four groups of 10 rats each, which were sixty days old and belonged to System Pathogen Free Program (Unochapecó Biotherium, Brazil). The animals had access to food and water *ad libitum* and were housed in single cages with a light and dark cycle of 12 hours each, at 22 ± 2°C. All experiments were accomplished following the Principles of Laboratory Animal Care and the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research. The protocols were approved by the Ethics Committee of the Contestado (Case 745/09) and complied with the requirements from the Federal Council of Veterinary Medicine. At the end of experiments, the animals' euthanasia was performed adopting CO_2 chamber protocol, according to Brazilian College of Animal Experimentation. The four groups were divided as follows: sedentary group plus ASC (SA), trained group plus ASC (TA), sedentary group plus vehicle (canola oil) (SV), and trained group plus vehicle (TV). ASC (testosterone decanoate, testosterone fenilpropionate, testosterone isocaproate, testosterone propionate - Durateston®; and nandrolone

decanoate - Deca-durabolin®) used in this study is the same used by athletes (Neto, 2005), with some changes to adjust to the animals' metabolism (Table 1). The administrations were performed in *soleus* muscle once week for eight weeks. The animals from TA and TV groups were submitted to three daily sessions of ten minutes each, with five minutes of interval between them. The swimming pool used had 1.8 m in diameter, 50 cm height and 38 cm depth, with water temperature at 30°C.

Table 1. Administration schedule of ASC in rats.

Week	Anabolic steroids doses – 0.3 mL	
	Deca-durabolin®	Durateston®
1	2.14 mg	5.36 mg
2	2.14 mg	5.36 mg
3	2.14 mg	2.68 mg
4	2.14 mg	2.68 mg
5	1.1 mg	1.34 mg
6	1.1 mg	1.34 mg
7	0.54 mg	0.66 mg
8	0.26 mg	0.66 mg

The spontaneous motor activity of animals was assessed using Open Field Task (OF) according to Mannewitz, Bock, Kreitz, Hess, Goldschmidt, Scheich, & Braun (2018) with some modifications. Complementarily, the OF test was also used to assess the anxious-like behavior (Darbra & Pallarès, 20110). The OF apparatus consisted of a circular arena 60 cm in diameter surrounded by acrylic walls with 50 cm high. Four quadrants divided the base of the arena, each with three subareas, delimited with black lines on a white floor. The illumination was maintained by white light (fluorescent bulb, 100 lux on the OF arena). The OF apparatus was cleaned with 30% ethyl alcohol before and after each rat occupied it. During the test sessions, the animals were placed individually on the center of the OF arena in order to enable to explore it for six minutes. Total area entries (number of times the animals crossed an area with the four paws), central crossing and number of central and outlying areas entries, stationary behavior in central and outlying areas were counted. All observations were performed between 10:00 and 12:00 a.m.

Evaluation of long-term memory acquisition and anxious-like behavior using Step-Down Passive Avoidance Task (PA) was assessed according to Palleria, Antonio, Andreozzi, Citraro, Iannone, Spiga and Russo (2017), with some adjustment. The PA apparatus consisted of a transparent acrylic rectangular cage (30 cm × 30 cm × 40 cm high) comprising a grid floor. The side walls of the chamber were made of steel, while the front wall was made of translucent acrylic to permit observation of the animals. The chamber was cleaned with 30% ethyl alcohol before and after each rat occupied it. The room test was kept without any noise and with lighting of 15 watts on the PA. An electric current (1 Hz, 500 ms, 60V dc) was delivered to the grid floor by an isolated stimulator (Insight®, Brazil). In the training test, each rat was placed on platform located at one side of chamber above the grid floor.

The training session was performed for 180 seconds, and every time the animal descended from the platform with its four paws, an electrical stimulus was triggered for two seconds. The responses to electric stimuli were recorded. The following scores were given based on the responses to the electric stimuli: 3, jumping; 2, vocalization; 1, flinching; 0, no response. The total score was obtained from the sum of each score (Hiramatsu, Mizuno, & Kanematsu, 2006). The test session was performed 24 hours after the end of the treatments, however without electrical stimulus. In the test session, each rat was placed on the platform individually, and the latency to descend from the platform was recorded. A cut-off time of 180 seconds was set. Freezing responses during this period were recorded as immediate freezing. Vertical and horizontal explorative behaviors in PA also were assessed by counting the time that the animals remained on their own posterior paws (vertical) or moving on the platform with all four paws (horizontal). At the end of the test session, each rat was placed back in the cage at room accommodation.

The data from OF was evaluated by one-way analysis of variance followed by Bonferroni's Test. To analyze the data from PA, the one-way analysis of variance followed by Kruskal-Wallis Test was used. The criterion for significance was $p < 0.05$ in all statistical evaluations.

Results

OF shows an increase in anxious-like behavior of the SV, considering the number of central crossing $F(3.2) = 1.00$, $p < 0.05$, central areas entries $F(3.9) = 5.05$, $p < 0.05$, and stationary behavior in central areas,

$F(4.4) = 9.01$, $p < 0.05$ in relation to groups that received treatment (ASC) and or training (Figure 1. A, B, C). Regarding to spontaneous motor activity, it was observed that all animals showed a similar number of total areas covered (Figure 1. D), with no statistically significant differences between groups.

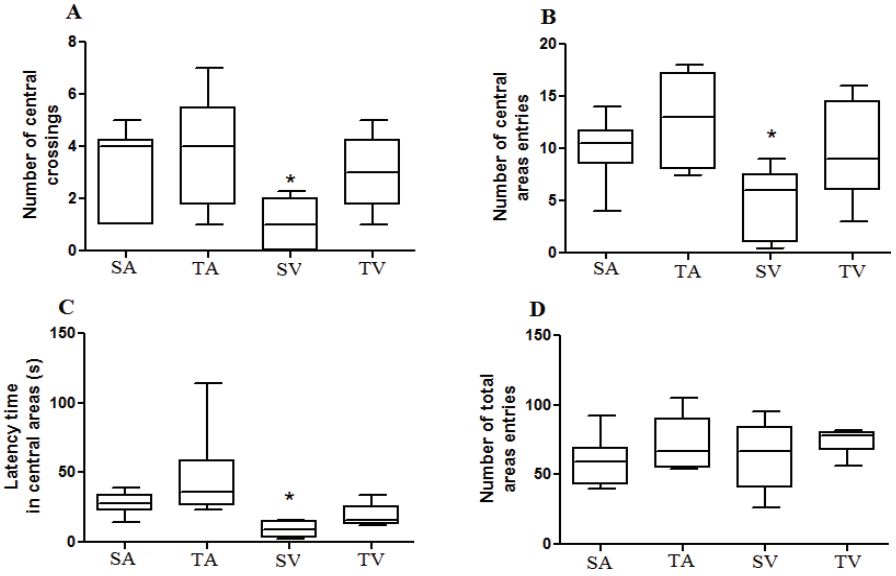

Figure 1. Effect of ASC treatment and training in the behavior of rats observed in the open field test, when A represent the number of central crossing, B the number of central areas entries, C stationary behavior in central areas, D the number of total areas entries. The data were expressed by mean ± SD. The One-Way Analysis of Variance (ANOVA) followed by Bonferroni's test for multiple comparisons was used, n=10, * $p < 0.05$ compared to SV. SA = Sedentary + ASC; TA = Training + ASC; SV = Sedentary + vehicle; TV = Training + vehicle.

At the test session of the PA, all animals from different groups did not step down from the platform throughout the session period. However, the groups that were treated with ASC (SA and TA) showed a better explorative behavior, both vertically ($H = 29.4$, $p < 0.001$) as horizontally ($H = 29.7$, $p < 0.001$), when compared to the groups without ASC treatment (Figure 2. A, B). Furthermore, both groups SV and TV showed a higher stationary behavior on the platform than the groups SA and TA ($H = 29.7$, $p < 0.001$). The sum of score (jumping, vocalization, flinching, and no response) was performed throughout the training session and there was no difference between groups, that is, all individuals were subjected to same stimulus and showed similar behaviors (Figure 2. D).

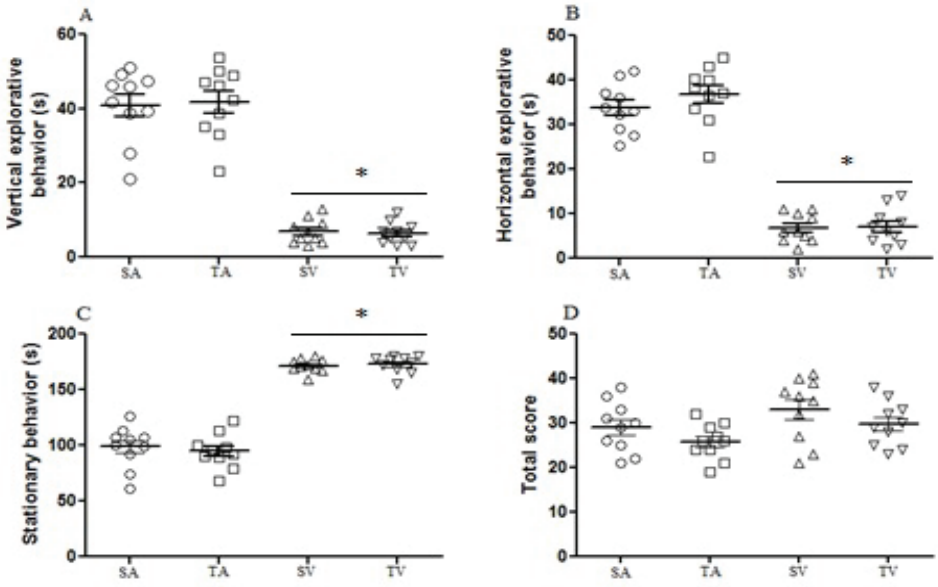

Figure 2. Effect of ASC and or training on anxious-like behavior of rats undergoing to passive avoidance task, when **A** spent time (seconds) in the vertical explorative behavior, **B** horizontal explorative behavior, **C** stationary behavior, **D** total score of jumping, vocalization and flinching in training session. The data were expressed by mean ± SD. The one-way ANOVA followed by Kruskal-Wallis Test was used, n=10, * $p<0.05$ compared to SV. SA = Sedentary + ASC; TA = Training + ASC; SV = Sedentary + vehicle; TV = Training + vehicle.

Discussion

The anxious-like behavior may be evaluated experimentally using the OF Test. However, in this study we include the PA as a tool to evaluate anxious-like behavior, beyond its classic use as a memory tool assessment.

It is well established that OF test is able to outline an indirect correlation between ambulation in central and outlying areas with the levels of anxiety, that is, as greater the ambulation in central areas, the anxious-like behavior is admitted as minor than those with high ambulation in outlying areas in experimental models using rodents (Guilloux et al., 2011).

In this study, the data from OF suggest that the treatment with ASC and the physical exercise program were able to decrease the anxious-like behavior when compared to the sedentary group. Furthermore, the increase in the latency time in central areas, as observed, suggest a minor anxious-like behavior level, since this behavioral pattern reflects a decrease in defensive behavior in a new environmental context (Lima, Blanco, Santos Júnior, Coelho, & Mello, 2008).

It is well known that testosterone and nandrolone have a synergistic anabolic effect. However, there is an antagonism concerning the behavioral effects. In fact, testosterone has an anxiolytic effect, while the nandrolone provides an anxiogenic effect (Rocha, Calil, Ferreira, Moura, & Marcondes, 2007). Thus, the dosing regimen of the ASC adopted in this study seems to have favored the anxiolytic effect of the testosterone. This is possibly supported by the distinct behavioral pattern among sedentary groups in the presence or absence of ASC administration, since the vehicle administration revealed a decrease in ambulation and latency time in the central areas. On the other hand, the ASC administration showed the opposite.

Moreover, in rodent models, the administration of testosterone propionate is able to reduce physiological stress response (Quinn, Hitchcott, Umeda, Arnold, & Taylor, 2007), which are attributed to changes in dendritic morphology and gene expression in stress-responsive brain areas (Romeo, Staub, Jasnow, Karatsoreos, Thornton, & McEwen, 2005), including a modulatory effect on serotonin and γ-aminobutyric acid systems (Robichaud, & Debonnel, 2005), which are directly involved in maintaining a non-anxious behavioral status.

On the other hand, the practice of physical exercises has proved effective in reducing symptoms associated with anxiety and depression, even in subjects clinically diagnosed (Verschueren, Eskes, Maaskant, Roest, Latour, & Reimer, 2018), besides to improve the cognitive performance. These evidences are corroborated by the increasing in the neurotransmitter levels and changes in brain plasticity and epigenetic mechanisms through the physical exercise programs (Mandolesi, Polverino, Montuori, Foti, Ferraioli, Sorrentino, & Sorrentino, 2018).

With regard to locomotor activity, we can see (Figure 1D) that all groups explored a total similar area, i.e., regardless of treatment used, there was no interference in this parameter by the ASC treatment and or physical exercises, at least in the model and experimental conditions performed. These data reinforce the less anxious-like behavior observed in non-sedentary groups, since it was not possible to establish any relationship between the locomotor capacity and the anxiety level of the animals.

In PA test session, none of the animals stepped down from the platform, regardless of group that they belonged. However, it was observed an increase in both the vertical and horizontal explorative behaviors in animals that received the ASC administration (VA and TA groups). On the other hand, there was an increase of stationary behavior in the groups not treated with ASC (SV and TV groups). These data seem to corroborate with the testosterone effect, which decrease the anxious-like behavior.

Furthermore, the findings from the PA suggest that all groups were able to acquire long-term memory, which allows us to suggest that the ASC did not cause cognitive impairment related to the capacity of long-term memory acquisition, at least in the dose regimen and experimental model used.

The influence of testosterone on learning processes and the acquisition of various types of memory, in different experimental models, have been reported throughout the years. It is well known that testosterone and its metabolites, dihydrotestosterones, are able to modulate the behavioral biochemical metabolism, which reflects, among other effects, on the maintenance of the net hippocampal synaptic density, considered an important factor to allow the memory acquisition and learning (Murakami et al., 2018).

In addition, there are indications that the improvement of some kinds of memory acquisition is mediated through the enzymatic conversion of testosterone into estradiol by the aromatase in the brain. Besides,

testosterone and nandrolone affect the nerve growth factor (NGF) in brain by different mechanisms. NGF is known by regulating memory, learning, and defensive behavior (Tanichi et al., 2018). Thus, an unbalance in the concentration of NGF could lead to an impairment in neurobehavioral status. In particular, Nandrolone seems to interfere with NGF transport and/or its utilization by forebrain neurons, inducing to changes in expression of NGF low-affinity receptor (p75-NGFr) and therefore, to NGF accumulation (Tirassa, Thiblin, Ågren, Vigneti, Aloe, L.,& Stenfors, 1997, Dragica, Jovana, & Gvozden,, 2018).

However, the concentration range responsible for such unbalance is not clearly explained. In this study, although we have used high dosages of androgens, there was no impairment in memory acquisition or increase levels of anxiety in the animals.

Furthermore, nandrolone plays an interesting role in hippocampal metabolism, increasing the NMDA receptor phosphorylation (subunits NR2A and NR2B) as well as the ERK1/2 phosphorylation. These molecular targets are involved in memory acquisition and its evocation (Rossbach, Steensland, Nyberg, & Le Grevès, 2007). Curiously, nandrolone treatment in supraphysiological doses is able to reduce the expression of choline acetyltransferase in the basal forebrain and impair the spatial memory in rats, besides inducing resistance to the extinction process of learning and memory (Rivas-Arancibia & Vazquez-Pereyra, 1994).

Conclusion

In summary, findings suggest that ASC, at the amount and experimental model assessed, was able to decrease the anxious-like behavior, but does not impair the long-term memory acquisition.

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