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Administration of an anabolic steroid during the adolescent phase changes the behavior, cardiac autonomic balance and fluid intake in male adult rats

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HIGHLIGHTS

- Few data are available on chronic impact of anabolic–androgenic steroids (AAS).
- AAS increased aggressiveness in the pubertal phase of rats.
- AAS increased exploration and anxiety-related behavior in adult rats.
- High sympathetic modulation occurred in adult rats treated with AAS when adolescent.

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ABSTRACT

Few data are available on adolescent users because most behavioral studies on anabolic–androgenic steroids (AAS) abuse have been performed in adults. Studies evaluating the impact of long-term effects of AAS abuse on the prepubertal phase are even more uncommon. Accordingly, this study was developed to test the hypothesis that changes induced by the use of AAS during the adolescent phase may be noted in the adult phase even when the AAS treatment cycle is discontinued. Therefore, not only behavioral changes but also possible autonomic and electrolyte disorders were evaluated. For this purpose, we used male prepubertal, 26-day-old (P26) Wistar rats that were treated with vehicle (control, n = 10) or testosterone propionate (TP; 5 mg/kg intramuscular (IM) injection, AAS, n = 10) five times per week for 5 weeks, totaling 25 applications during the treatment. Aggression tests were performed at the end of the cycle (P54–56), whereas open-field tests (OFTs), elevated plus maze (EPM) behavioral tests and measurements of heart rate variability (HRV), fluid intake and pathology were conducted in the adult phase (P87–92). The AAS group showed greater aggressiveness in the pubertal phase and higher levels of horizontal and vertical exploration and anxiety-related behavior in the adult phase than the control group ($P < 0.05$). HRV tests showed an increase in sympathetic autonomic modulation, and hydroelectrolytic assessment showed lower basal intake levels of hypertonic saline than the control group ($P < 0.05$), without statistically significant changes in the basal intake of water. These data together suggest that the use of AAS during the prepubertal phase induces behavioral, autonomic and hydroelectrolytic changes that manifest in the adult phase even when treatment is discontinued in late adolescence in rats.

1. Introduction

Anabolic–androgenic steroids (AAS) are synthetic derivatives of testosterone with four-ring structures consisting of 19 carbon atoms. There are approximately 60 different AAS available, and these molecules vary in their chemical structure and, as a result, in their metabolic and physiological effects [1,2]. Some studies report that more than one million Americans have used AAS [3]. Although some athletes use AAS to be successful, use is not restricted to them, and AAS abuse in adolescents has dramatically increased [4]. This is related to the supposed enhancement of physical appearance and athletic performance by AAS [5].

The 2003 Youth Risk Behavior Surveillance System showed that 5.3% to 6.8% of adolescents have used AAS at some point in their lives. Furthermore, it has been reported that adolescents are more susceptible to the deleterious effects of AAS [5]. These side effects include unprovoked and indiscriminate aggression [6] and impulsiveness [7].

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Experimental studies also corroborate the highly aggressive behavior induced by AAS injected during adolescence. For example, Farrell and McGinnis [8] showed that male rats exposed to AAS during puberty were more aggressive towards male rats (resident or intrusive opponent) during aggressive encounters [9].

Cardiovascular dysfunction, including sudden cardiac death, is one of the main side effects associated with AAS abuse, both in AAS users and animal models of AAS abuse, concomitant with the behavioral changes observed [10–13].

Although several studies have shown direct cardiac changes induced by AAS, including ventricular hypertrophy associated with diastolic dysfunction [14,15], thrombosis and myocardial infarction [16,17], indirect effects of AAS on heart morphology and function are difficult to exclude. Thus, the cause and effect relationship between the heart and other tissues remains unclear in the literature. For example, an increase in peripheral resistance/blood pressure has long been observed in studies of experimental models of AAS abuse [18,19].

Cardiac arrhythmias and sudden death are also associated with AAS abuse [20,21], and some authors argue that cardiac electrophysiological changes may not only be attributed to myofibrillar disorders, tissue necrosis and fibrosis, among other factors, but other indirect mechanisms may also exist that cause these effects on heart excitability [22]. Therefore, identifying the extra-cardiac sites affected by AAS abuse may bring new perspectives and help establish the main mechanisms underlying cardiac abnormalities induced by AAS.

Pereira-Junior et al. [22] first reported cardiac autonomic dysfunction as a potential mechanism underlying cardiac dysfunction and sudden death associated with AAS abuse in adult rats [22]. This is unsurprising when considering that sympathetic hyperactivity is a mechanism known to generate cardiac arrhythmias, given the decreased ventricular fibrillation threshold [23].

Therefore, adverse effects on the electrophysiological responses to sympathetic hyperactivity may contribute to the development of a proarrhythmic substrate [24]. An excess of androgens has already been related to increased sympathetic tone [25]. Together, these data led us to recognize that AAS abuse may trigger chain events that eventually cause sudden death induced by sympathetic hyperactivity.

It has not been studied thus far whether the possible AAS-induced autonomic imbalance persists throughout the adult life period of rats treated with AAS during the adolescent phase. In this study we evaluated the effects of testosterone not only on heart rate variability but also on fluid intake and the behavior of adult rats treated with testosterone during the adolescent life period.

Our hypothesis is that AAS abuse during this critical period of time may alter not only animal behavior in the adolescent phase but also affect the behavior, heart function and hydroelectrolytic balance in the adult phase of rats because adolescence is a critical period of brain development, partly due to increased hormone levels [26].

2. Material and methods

The present study was conducted according to the "Guide for the Care and Use of Laboratory Animals" (NIH Publication No. 85–23, revised in 1996), and the experimental protocols were approved by the Ethics Committee on Animal Use of the Federal Rural University of Rio de Janeiro in accordance with Brazilian law (Law No. 11.794 of October 8, 2008).

2.1. Animals

Forty intact male Wistar rats, with initial body weights between 110 and 130 g, were used in the present study. All animals were housed in cages (four to five animals/cage) under controlled temperature (22 ± 2 °C), with daily exposure to a 12-hour light–dark cycle (lights off at 7:00 pm) and free access to water and standard chow.

In addition, older and heavier male animals, initially with body weights of approximately 450 g (n = 10), remained housed throughout the experiment with ovariectomized females weighing approximately 250 g (n = 10), except during the aggression test, at which time the females were removed from the box immediately before the interaction between males intended to stimulate their partner's territorial aggressiveness (see below). Males in the company of ovariectomized females were housed in plastic boxes (35 × 50 × 35 cm). To stimulate territorial aggressiveness, these males received training (five times per week) with other sparring animals (lighter and younger; approximately 200 g and 50 days old) for 10 min daily, with weekly rotations of their respective opponents, for four consecutive weeks.

2.2. Experimental design

Prepubertal (postnatal day 26, P26) male rats were randomly distributed into two groups: the TP group (AAS, n = 20) received testosterone propionate (TP; Perinon®, Perini-Brasil Laboratory, 5 mg kg^{-1} body weight), and the control group (Control, n = 20) received an equal volume of vehicle (corn oil); both were given intramuscularly (always between 10:00 and 11:00 am) as five injections per week for 5 weeks as previously described [27]. The dose of AAS used in the present study is equivalent to the dose used in humans to reach a high muscle weight gain during AAS abuse. The treatments were discontinued after 5 weeks of AAS or vehicle injections to assess the long-term effects of AAS administered during the prepubertal/pubertal phase of rats. The aggression tests were conducted in the final week of treatments (P54–56) in 20 animals (n = 10/group), whereas the behavioral, heart rate variability (HRV) and fluid intake tests (all described next) were performed in other 20 rats (n = 10/group) during their adult life (P84–90), ~3–4 weeks after the final day of treatment in both groups. The reason for using different subsets of animals in aggression test and behavioral, autonomic and hydroelectrolytic studies is because aggressive stimulus could influence the other parameters and lead to misleading results. Postmortem study (pathology) was performed at the end of the protocol (P92) as shown in Fig. 1.

2.3. Behavioral studies

2.3.1. Aggression tests

The aggression test consisted of placing the intruder and resident rats alone for 10 min in a clean polypropylene box with a closed, high and transparent acrylic lid, without the availability of food and water. The aggressiveness of the intrusive rat was observed during the test using a scoring system, wherein a score was tallied each time the rat performed one of the following tasks: 1. Attack threat, 2. Attacks by bites, 3. Attacks by sideways kicks, 4. Attacks by boxing, 5. Pelvic pressure or 6. Position of dominance. The tests were performed at the beginning of the last week of the TP cycle and were recorded for further individual analysis off-line [27].

2.3.2. Open-field test (OFT)

We used a quadrangular open field (100 × 100 × 30 cm) split into 25 equal quadrants to assess the exploratory activity of animals as previously reported by our group [28]. Briefly, the experimental assay started at 7 pm and was conducted in a dark room with a red light. Each animal was individually placed in the center of the open field at the beginning of the test, and the following parameters were recorded: 1. Locomotor activity (the number of times the animal invaded with all four legs in one of the arena fields): counted as the total number of squares crossed; 2. Rearing (animal standing on his hind legs with the torso perpendicular to the floor and his head pointing upwards, touching or not touching the walls of the open field with the forefeet); 3. Time of inactivity: recorded as total time without movement; and 4. Grooming episodes (number of movements performed with one or both front or hind legs towards the mouth or head, which may be