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Sex-specific effect of the anabolic steroid, 17α-methyltestosterone, on inhibitory avoidance learning in periadolescent rats

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ABSTRACT

The illicit use of anabolic androgenic steroids (AAS) has gained popularity among adolescents in the last decade. However, although it is known that exposure to AAS impairs cognition in adult animal models, the cognitive effects during adolescence remain undetermined. An inhibitory avoidance task (IAT) was used to assess the effect of AAS (17α-methyltestosterone; 17α-meT-7.5 mg/kg) in male and female periadolescent rats. A single injection of 17α-meT immediately before the footshock produced significant impairment of inhibitory avoidance learning in males but not females. Generalized anxiety, locomotion, and risk assessment behaviors (RAB) were not affected. Our results show that exposure to a single pharmacological dose of 17α-meT during periadolescence exerts sex-specific cognitive effects without affecting anxiety. Thus, disruption of the hormonal milieu during this early developmental period might have negative impact on learning and memory.

1. Introduction

In spite of being prohibited by law, anabolic androgenic steroids (AAS) are often taken in high doses by athletes to increase muscle mass and enhance athletic performance. In the last decades, illicit use of AAS by adolescents has generated significant concern (Castillo and Comstock, 2007; Mulcahey et al., 2010); particularly because adolescence is a developmental period characterized by an increase in sex hormones when reproductive, neural, and behavioral maturation occurs (Blakemore et al., 2010; Sisk and Foster, 2004). In addition to the well-known physiological effects of AAS (Hartgens and Kuipers, 2004), these compounds have been associated with multiple psychological disorders in humans, such as anxiety, aggression, or mood disorders (for review see Graham et al., 2008; Maravelias et al., 2005). In animals, anxiety and aggression-like behaviors have been shown to be affected by AAS (Oberlander and Henderson, 2012), as well as changes in sexual behavior, learning, and memory (for review see Clark and Henderson, 2003).

Studies support that gonadal hormones modulate learning and memory, but considerable debate exist regarding their effects on cognitive function. Specifically, acute (single injection following training; 1 mg/kg) or chronic (5 weeks through Silastic capsules) testosterone administration enhances cognitive performance on the inhibitory avoidance task (IAT) in adult male rats (Edinger et al., 2004; Frye et al., 2010; Frye and Seliga, 2001). On the other hand, studies have demonstrated that a single intracerebral administration of several doses of exogenous testosterone (10, 20, 40, 80, and 120 μg/0.5 μl) caused a dose-dependent impairment of spatial memory (Naghdi et al., 2001, 2003), as well as acquisition, consolidation and retrieval of inhibitory avoidance learning (Harooni et al., 2008). Similar to the endogenous compounds, studies have shown that high doses of synthetic androgens (nandrolone: 15 mg/kg), when given in daily subcutaneous injections for either 6 weeks (Kouvelas et al., 2008) or 14 days (Magnusson et al., 2009) can also impair cognition, although a single acute injection of the same steroid (4 mg) showed cognition facilitation (Vázquez-Pereyra et al., 1995). Therefore, as reflected by the diversity of these results, it seems that the lack of consistency between androgens effects on IAT might depend on different experimental paradigms that might include class of androgens, dose, exposure duration, as well as route of administration.

To date, available data regarding AAS effects on cognitive behaviors is limited to adult animals, whereas their effects around adolescence have not been described. Prepubertal sex differences in learning and memory in rats have received limited attention (Frankola et al., 2010; Grissom et al., 2012), although it is known that males outperform females in specific cognitive tasks and vice versa in both humans and rodents (Andreano and Cahill, 2009).

Here we tested the effect of the AAS, 17α-methyltestosterone (17α-meT), on the IAT using a periadolescent animal model.

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The periadolescent period is the age around the time of sexual maturation when age-specific behavioral and physiological changes are evident (for review see Spear, 2000). In rats it is defined as approximately, 30–42 postnatal days (PN-30–42). 17α-meT is a Class III AAS with an alkylation at C-17 and with restricted potential of metabolizing substrates for aromatization (Penatti et al., 2009). In fact, 17α-meT cannot be aromatize to 17β-estradiol, the most potent form of mammalian estrogenic steroids, and the main product of aromatase. Nevertheless, it can be converted to the 17α-isomer of estradiol (Pawlowski et al., 2004), which binds weakly to estrogen receptors and exhibits weaker estrogenic activity (Shughrue et al., 1997). Thus, the use of this drug will diminish confounding effects that might result from potent estrogenic metabolites. We choose to administer an acute AAS injection by several reasons. The most important was to establish the minimum AAS exposure required to attain cognitive negative effects, given that such adverse effects are usually associated with chronic or continuous androgen exposure. To address this question is of great significance when studying subjects undergoing developmental changes, as this is the case in adolescence. Even though scarce studies have been done that test acute AAS exposure, it has been shown that a single androgen injection is sufficient to cause behavioral changes such as increased punished licks (Bing et al., 1998), and to modulate short and long-term memory (Vázquez-Pereyra et al., 1995). In addition, acute drug exposure is more compatible for the 3-day IAT, a short behavioral test that is very convenient to determine AAS-induced cognitive effects around adolescence without having effects in adulthood. Besides acquisition on inhibitory avoidance learning, AAS effects on generalized anxiety and risk assessment behaviors (RAB) were also evaluated. We hypothesized that a disturbance in the normal hormonal milieu by a pharmacological dose of AAS will have sex-specific effects on cognition in periadolescent rats.

2. Materials and methods

2.1. Animals

Gonadally intact male and female Sprague Dawley rats were purchased from Charles Rivers Labs (Wilmington, MA). Animals were received at PN-25, maintained in the vivarium for acclimation for four days after arrival, and housed on a 12:12-h reverse light/dark cycle (lights off at 8:30 A.M.). Food and water was available *ad libitum*. The IAT and EPM were performed during the dark phase of the cycle. All experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Puerto Rico, Medical Sciences Campus.

2.2. Vaginal lavage and smears

For female experiments, daily vaginal lavage, starting at PN-31 was performed at 1:00 P.M. for at least two estrous cycles. Vaginal lavage was performed with a small eyedropper containing 0.25 ml of 0.9% saline. The fluid obtained after the lavage was evenly distributed onto a microscope slide for further cytology analysis under low magnification. We monitored for a normal 4-day cycle, consisting of vaginal smears evaluated for the presence of leukocytes, nucleated epithelial cells, or cornified epithelial cells (Cooper et al., 1993). The vaginal smears are classified as diestrus (predominance of leukocytes and a few scattered cornified epithelial cells), proestrus (round polynucleated epithelial cells), or estrus (cornified epithelial cells). Metestrus is considered a transition out of estrus, characterized by a large number of cornified cells and infiltration of leukocytes.

2.3. Drug

In the first experiment, animals were exposed to a single (acute) dose of 17α-methyltestosterone (17α-meT; 7.5 mg/kg; 0.2 cm^3/kg, i.p.). Thereafter, to deteremine if the effect of 17α-meT (7.5 mg/kg) on the IAT was dose-dependent, lower AAS doses (0.075 and 0.75 mg/kg) were tested. Also, we administered 17α-meT (7.5 mg/kg) for 2 weeks to determine its effect during longer exposure. The high pharmacological dose (7.5 mg/kg) reflects a medium to high dose range of abuse in humans on a milligram per kilogram basis (for review see Clark and Henderson, 2003; Blasberg et al., 1997). 17α-meT was dissolved in 0.9% saline containing 30% cyclodextrin and this vehicle was injected to control animals. Cyclodextrin enhances drug solubility due to the affinity between the drug and the interior of the cyclodextrin host molecules (Chaudhari et al., 2007). Their water soluble aggregates are able to solubilize lipophilic water-insoluble drugs through micelle-like structures (Loftsson et al., 2004). Moreover, no behavioral changes or alteration of gonadal weight have been reported when used as vehicle for 17α-meT or testosterone (Alexander et al., 1994; Barreto-Estrada et al., 2004; Packard et al., 1997). Compounds were purchased from Sigma (St. Louis, MO).

2.4. Inhibitory avoidance task (IAT)

The step-through inhibitory avoidance apparatus (AccuScan Instruments; Columbus, OH) consists of a two-compartment acrylic box (45 cm × 22 cm × 33 cm) with an illuminated compartment connected to a darkened one, by a movable guillotine door and a stainless steel grid floor. During this test, the rat learns to avoid entering the dark compartment once it becomes associated with an aversive stimulus (Jarvik and Kopp, 1967).

Sixty (60) females (cohort I; $n = 40$ and cohort II; $n = 20$) and 19 males were tested on the IAT. Males were tested on the IAT on days PN-40 (habituation), PN-41 (acquisition) and PN-42 (retention), whereas females in each experiment were divided in two cohorts according to estrus cyclicity. Females in cohort I were tested in diestrus during IAT acquisition (Day 2), whereas animals in cohort II were tested while they were in proestrus. Specifically, females were tested on habituation day (cohort I: PN-40 or cohort II: PN-43), acquisition day (cohort I: PN-41 or cohort II: PN-44) and retention (cohort I: PN-42 or cohort II: PN-45). The difference in age between female rats in the two cohorts is due to the days needed for the females to reach the proestrus stage according to observations in vaginal lavages. Statistical analysis between females in PN-40 vs. females in PN-43 did not find any significant difference, thus the two cohorts were analyzed together.

In general, rats were placed in the dark compartment of the chamber during 2 min, and then transferred to the home cage for one additional minute. Thereafter, rats were placed again in the illuminated side of the chamber and the latency to enter to the dark compartment was recorded. Animals were removed from the chamber after crossing to the dark compartment. During Day 2 (acquisition phase), rats were injected with either 17α-meT or vehicle, and immediately placed in the dark compartment with the door closed. A mild electric footshock (0.3 mA; 3 s) was delivered through the grid floor. The retention test was carried out 24 h later. Rats were placed in the illuminated compartment with the posterior body parts facing the guillotine door. Immediately the door was opened. Rats were allowed to enter the dark compartment and crossover latency was measured as an index of avoidance learning. The maximum entry latency allowed in the retention test was 300 s.