Hormones and Behavior 69 (2015) 132–138

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Regular article

Anabolic/androgenic steroid administration during adolescence and adulthood differentially modulates aggression and anxiety

Thomas R. Morrison, Lesley A. Ricci, Richard H. Melloni Jr. *

Behavioral Neuroscience Program, Department of Psychology, 125 Nightingale Hall, Northeastern University, 360 Huntington Avenue, Boston, MA 02155, USA

ARTICLE INFO

Article history:
Received 24 September 2014
Revised 3 January 2015
Accepted 28 January 2015
Available online 3 February 2015

Keywords:
Anabolic–androgenic
Steroids
AAS
Adolescence
AAS withdrawal
Anxiety
Aggression
Developmental AAS exposure
Elevated-plus maze

ABSTRACT

Anabolic/androgenic steroid (AAS) use remains high in both teens and adults in the U.S. and worldwide despite studies showing that AAS use is associated with a higher incidence of aggression and anxiety. Recently we showed that chronic exposure to AAS through adolescence increases aggression and decreases anxious behaviors, while during AAS-withdrawal aggression is lowered to species-normative levels and anxiety increases. AAS exposure is known to differentially alter behaviors and their underlying neural substrates between adults and adolescents and thus the current study investigated whether exposure to AAS during adulthood affects the relationship between aggression and anxiety in a manner similar to that previously observed in adolescents. Male hamsters were administered a moderate dose of AAS (5.0 mg/kg/day × 30 days) during adolescence (P27–56) or young adulthood (P65–P94) and then tested for aggression and anxiety during AAS exposure (i.e., on P57 or P95) and during AAS withdrawal (i.e., 30 days later on P77 or P115). Adolescent exposure to AAS increased aggressive responding during the AAS exposure period and anxiety-like responding during AAS withdrawal. Neither behavior was similarly influenced by adult exposure to AAS. Adult AAS exposure produced no difference in aggressive responding during AAS exposure (P95) or AAS withdrawal (P115); however, while AAS exposure during adulthood produced no difference in anxiety-like responding during AAS exposure, adult hamsters administered AAS were less anxious than vehicle control animals following AAS withdrawal. Together these data suggest that the aggression and anxiety provoking influence of AAS are likely a developmental phenomenon and that adult exposure to AAS may be anxiolytic over the long term.

Introduction

Abuse of anabolic/androgenic steroids (AAS) has remained a concern for decades, yet its use has risen in recent years worldwide (Harmer, 2010; NIDACapsules, 2007) despite strong evidence for negative acute and long-term physical, psychological, and behavioral consequences. While the most common behavioral side effect of AAS use is increased aggression in adult (Kouri et al., 1995; Pope and Katz, 1994; Pope et al., 2000; Su et al., 1993) and youth populations (Beaver et al., 2008; Dabbs et al., 1991; Johnson et al., 1989; Johnson, 1990; Mattsson et al., 1980; Olweus et al., 1980), there is also a high incidence of anxiety-related disorder diagnoses in AAS users (Bahrke et al., 1990; Johnson, 1990; Pagonis et al., 2006a, 2006b; Pope and Katz, 1994), particularly during withdrawal from AAS use (Bahrke et al., 1990; Brower, 2002; Corrigan, 1996; Lindqvist et al., 2007; Malone et al., 1995; Malone and Dimeff, 1992; Perry et al., 1990). Clinical data reflect this incidence showing marked increases in both aggression and anxiety in AAS users (Hall et al., 2005; Pagonis et al., 2006a; Pope et al., 2000; Su et al., 1993); suggesting that AAS exposure may promote the development of both negative behavioral phenotypes simultaneously. While a number of preclinical studies have investigated the link between AAS use and the expression of aggression (Farrell and McGinnis, 2004; Lumia et al., 1994; McGinnis et al., 2002a, 2002b; Melloni and Ricci, 2010) less is known regarding the relationship between AAS exposure and anxiety. AAS exposure produces variable effects on anxiety-like responding in animal models (Agis-Balboa et al., 2009; Aikey et al., 2002; Bitran et al., 1993; Fernández-Guasti and Martínez-Mota, 2005; Ricci et al., 2012; Rocha et al., 2007), despite consistent evidence for an anxiolytic effect of testosterone (Bing et al., 1998; Frye et al., 2008; Toufexis et al., 2005; Zuloaga et al., 2008). Though evidence between androgen insensitive mice and rats suggests that this inconsistency may involve species differences (Zuloaga et al., 2008, 2011), it is also plausible that inconsistent findings can be explained by the age of AAS administration since no preclinical study has investigated the effects of AAS administration on the emergence of these two behaviors during AAS exposure and withdrawal in both adolescent and adult populations — especially as it pertains to experimental, novice use.

For over a decade we have used pubertal male Syrian hamsters (*Mesocricetus auratus*) as an adolescent animal model to investigate

* Corresponding author at: Program in Behavioral Neuroscience, Department of Psychology, 125 Nightingale Hall, Northeastern University, 360 Huntington Avenue, Boston, MA 02155, USA. Fax: +1 617 373 8714.
E-mail address: r.melloni@neu.edu (R.H. Melloni).

http://dx.doi.org/10.1016/j.yhbeh.2015.01.009
0018-506X/

the effects of adolescent AAS exposure on the behavioral neurobiology of two of the most common side effects of AAS exposure, i.e., offensive aggression (DeLeon et al., 2002; Harrison et al., 2000; Melloni and Ricci, 2010) and anxiety (Ricci et al., 2012, 2013). Behavioral data from these studies show that adolescent hamsters repeatedly exposed to moderate doses of AAS (5.0 mg/kg/day) display significant increases in aggression during the adolescent AAS exposure period followed by significant increases in anxiety during withdrawal. Linear regression analysis revealed that the difference in aggressive responding between the AAS exposure and withdrawal periods was a significant predictor of changes in anxiety-like behavior between these two periods, indicating that moderate adolescent AAS exposure has potent aggression- and anxiety-eliciting effects, and that these behavioral changes occur alongside a predictive relationship that exists between these two behaviors over time (Ricci et al., 2013). While these alterations in aggressive and anxiety-like responding exist for adolescents exposed to AAS, it is unknown whether adults exposed to moderate doses of AAS experience similar behavioral side effects in parallel. To determine whether adult exposure to moderate dose AAS alters aggression and anxiety in a manner similar to adolescent exposure, the current study compares the effect(s) of moderate dose AAS administration between adolescents and adults on the expression of aggression during AAS exposure and anxiety during withdrawal.

Methods

Animals

Intact male Syrian hamsters (*M. auratus*) postnatal day 21 (P21; pubertal hamsters) or postnatal day 59 (P59; young adult hamsters) were obtained from Charles River Laboratories (Wilmington, MA), individually housed in polycarbonate cages, and maintained at ambient room temperature (22–24 °C with 55% relative humidity) on a reverse light/dark cycle (14L:10D; lights off at 7:00). Food and water were provided ad libitum. For aggression testing, stimulus (intruder) males of equal size and weight to the experimental animals were obtained from Charles River one week prior to the behavioral test, group housed at 5 animals/cage in large polycarbonate cages, and maintained as above to acclimate to the animal facility. All intruders were evaluated and prescreened for low aggression (i.e., disengage and evade) and submission (i.e., tail-up freeze, flee, and fly-away) one day prior to the aggression test to control for behavioral differences between stimulus animals, as previously described in a number of our studies (Ferris et al., 1997; Grimes and Melloni, 2002; Ricci et al., 2009). Intruders displaying significantly low aggression and/or submissive postures were excluded from use in the behavioral assay. All experimental treatments and behavioral tests described below were administered during the first four hours of the dark cycle under dim-red illumination to control for circadian influences. All studies using live animals were approved by the Northeastern University Institutional Animal Care and Use Committee (NU-IACUC), and all methods used were consistent with the guidelines provided by the National Institute of Health for the scientific treatment of animals.

Anabolic/androgenic steroid treatment

Postnatal day 27 (P27; pubertal) and postnatal day 65 (P65; adult) hamsters were weighted and received daily subcutaneous (SC) injections (0.1 ml–0.2 ml) for 30 consecutive days (P27–P56, pubertal; P65–P94, adult) of a mixture (or "stack") of 3 commonly used AAS (Hall et al., 2005) in doses consistent with repeated, moderate use in humans as described (see Melloni and Ricci, 2010 for a review). The AAS cocktail was composed of 2 mg/kg testosterone cypionate, 2 mg/kg nortestosterone and 1 mg/kg dihydrotestosterone undecyclate (Steraloids, Newport, RI) dissolved in sesame oil (SO). As a control, a separate set of P27 and P65 male hamsters received daily SC injections (0.1 ml–0.2 ml) for 30 consecutive days (P27–P56 or P65–P94) of SO (vehicle control).

Experimental design

The day following the last AAS injection (P57 or P95), AAS-treated animals (P57, n = 7; P95, n = 10) and SO-treated controls (P57, n = 7; P95, n = 10) were randomly assigned to one of two counterbalanced groups and tested for anxiety-like responding using the elevated plus maze (EPM) test and aggressive behavior using the resident/intruder (RI) test. At the completion of behavioral testing on P57 or P95, animals were placed back into their home cage and withdrawn from AAS (or SO) for 21 days (i.e., until P77 or P115) and then tested again for anxiety and aggression.

Behavior testing

Aggression

Hamsters were tested for offensive aggression using the resident–intruder (RI) paradigm, a well-characterized and ethologically valid model of offensive aggression in Syrian hamsters (Floody and Pfaff, 1977; Lerwill and Makings, 1971). For this measure, a novel intruder of similar size and weight was introduced into the home cage of the experimental animal (resident) and the resident was scored for specific and targeted aggressive responses observed as lateral, flank-directed attacks as previously described (Grimes et al., 2003; Ricci et al., 2006). An attack was scored each time the resident animal would pursue and then either [1] lunge toward and/or [2] confine the intruder by upright and sideways threat; each generally followed by a direct attempt to bite the intruder's dorsal rump and/or flank target area(s). The latency to attack was defined as the period of time between the beginning of the behavioral test and the first attack the residents made toward an intruder. In the case of no attacks, latencies to attack were assigned the maximum latency (i.e., 600 s). Each aggression test lasted for 10 min and was videotaped and scored manually by two observers unaware of the hamsters' experimental treatment. Inter-rater reliability was set at 95%. No intruder was used for more than one behavioral test, and all subjects were tested during the first 4 h of the dark cycle under dim red illumination to control for circadian influences on behavioral responding.

Anxiety

Hamsters were tested for anxiety-related behavior using the elevated plus maze (EPM) test as in our previous studies (Ricci et al., 2012, 2013) and elsewhere (Gannon et al., 2011; Prendergast and Nelson, 2005). The EPM has been used extensively in rodents as a reliable test of anxiety-like responding, with particular use as a sensitive behavioral test to screen for anxiolytic drug effects (Gannon et al., 2011; Pellow et al., 1985; Pellow and File, 1986). The apparatus consisted of two open arms and two closed arms (30 × 5 cm) elevated to a height of 38.5 cm and intersecting in a central platform (5 × 5 cm). The closed arms had black Plexiglas walls (15 cm high) covered with a black Plexiglas lid on the roof. The apparatus was arranged such that the open arms were opposite to each other. Animals were individually placed in the center of the apparatus facing one of the closed arms. The duration of time (s) spent beyond a complete body length in the open arms was calculated for each animal over a 5-minute period. An increase in the duration of time spent in the open arms of the EPM was used as an index of anxiolytic behavior (Lister, 1987; Pellow et al., 1985). Each anxiety test was videotaped and coded by two observers unaware of experimental treatment. Animals were tested during the first four hours of the dark cycle under dim-red illumination to control for circadian influences in behavioral responding.

Statistical analyses

Behavioral results from aggression (i.e., attacks and latency to attack) and anxiety (i.e., number of entries and duration of time spent