Hormones and Behavior 66 (2014) 585–590

Contents lists available at ScienceDirect

Hormones and Behavior

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

Regular article

Anabolic–androgenic steroids and appetitive sexual behavior in male rats

Jessica Y. Kim, Ruth I. Wood *

Department of Cell and Neurobiology, Keck School of Medicine, University of Southern California, Los Angeles, CA 90033, USA

ARTICLE INFO

Article history:
Received 3 April 2014
Revised 26 August 2014
Accepted 29 August 2014
Available online 6 September 2014

Keywords:
Anabolic agents
Androgen
Food reward
Operant behavior
Sexual behavior, animal

ABSTRACT

Anabolic–androgenic steroids (AAS) increase libido and sexual behavior, but the underlying behavioral mechanisms are unclear. One way AAS may enhance expression of sexual behavior is by increasing the willingness to work for sex. In the present study, sexually-experienced male rats received daily injections of testosterone at supraphysiologic doses (7.5 mg/kg in water with 13% cyclodextrin) or vehicle and were tested for appetitive sexual behavior measured by operant responding for access to an estrous female. Initially, rats were trained in their home cage to respond on a nose-poke under a 10-min fixed-interval schedule for food reward. Once rats achieved stable response rates, the food was replaced by a female, followed by mating for 10 min. There was no effect of testosterone on operant responding for food (28.1 ± 4.4 responses/10 min for testosterone, 30.6 ± 4.3 for vehicle) or sex (35.0 ± 4.0 responses/10 min for testosterone, 37.3 ± 5.2 for vehicle). However, rats made significantly more responses for sex than for food ($p < 0.05$), and responses for food and sex were positively correlated among individuals ($R^2 = 0.6$). Additional groups of rats were trained to respond on a lever for the female under a 2nd-order schedule of reinforcement, where 5 responses opened a door to show the female for 5 s. After 15 door openings, the male gained access to the female. There was no effect of testosterone on time to complete 75 responses: 38.4 ± 7.8 min for vehicle controls vs 43.3 ± 6.6 min for testosterone-treated rats ($p > 0.05$). These findings suggest that chronic high-dose testosterone does not enhance appetitive drive for sexual behavior.

Introduction

Anabolic–androgenic steroids (AAS) are performance-enhancing substances. Misuse of AAS by athletes is widely acknowledged, but potential health risks are not well-understood. These include not only cardiovascular, hepatic and reproductive dysfunction, but also alterations in brain and behavior (Pope et al., 2014a). Many AAS users meet the Diagnostic and Statistical Manual of Mental Disorders (DSM) criteria for psychoactive substance dependence, including continued use despite negative side effects, and withdrawal symptoms when steroids are discontinued (Brower et al., 1991). However, unlike other illicit drugs, AAS have only a limited capacity to cause acute intoxication or other immediate physiologic responses (Kanayama et al., 2009). However, there is concern that AAS may have a negative impact not only on steroid users, but also on those around them. Steroid use has been implicated in enhanced aggression (Conacher and Workman, 1989; Pope and Katz, 1990; Pope et al., 1996; Schulte et al., 1993), known popularly as "roid rage". AAS also promote excessive and inappropriate sexual behavior (Choi and Pope, 1994; Moss et al., 1993). Clinical investigations of sexual response in human volunteers receiving injections of AAS have observed positive mood including sexual arousal and desire (Anderson et al., 1992; Choi and Pope, 1994; Daly et al., 2001; Hannan et al., 1991; Moss et al., 1993; Pope et al., 2000; Su et al., 1993). From a clinical perspective, the potential for AAS to enhance sexual performance is not problematic. However, the potential for AAS to facilitate sexual violence and non-consensual intercourse is a concern (Schulte et al., 1993).

Investigating AAS use in humans is complicated by the user's motivation for increased strength and muscle mass (Brower et al., 1991; Kanayama et al., 2009). Animal studies can explore consequences of AAS in an experimental context where appearance and athletic performance are irrelevant. Such studies show that AAS are rewarding (reviewed in Wood, 2008), as demonstrated by self-administration and conditioned place preference (CPP). Furthermore, AAS stimulate social behavior, particularly mating and aggression (Clark and Fast, 1996; Cunningham and McGinnis, 2006, 2007; Farrell and McGinnis, 2003, 2004; McGinnis, 2004; Melloni et al., 1997). In our study of oral testosterone self-administration in hamsters, testosterone stimulated sexual behavior in a dose-dependent manner (Wood, 2002). Other investigators have shown that anabolic steroids reduce the latency to initiate mating, and increase the efficiency of sexual performance in male rats (Farrell and McGinnis, 2003, 2004; McGinnis, 2004).

Up to this point, studies of AAS and mating have mostly focused on the consummatory aspects of sexual behavior (mounts, intromissions, ejaculation). The present study addressed the ability of chronic high-

* Corresponding author at: Department of Cell and Neurobiology, Keck School of Medicine at the University of Southern California, 1333 San Pablo St., BMT 408a, Los Angeles, CA 90033, USA. Fax: +1 323 442 2441.
E-mail address: riw@usc.edu (R.I. Wood).

http://dx.doi.org/10.1016/j.yhbeh.2014.08.009
0018-506X/

dose testosterone to facilitate appetitive sexual behavior. In this regard, the reinforcing effects of mating in males are well-documented (reviewed in Hull et al., 2006). Male rats develop CPP for environments where they have previously mated. They will run rapidly towards a goal box or press a lever on a 2nd-order schedule of reinforcement for access to a female. These responses require gonadal steroids: castrated males do not mate, and show little interest in females. Testosterone restores both the appetitive and consummatory aspects of sexual behavior. Since androgens promote mating, and both mating and androgens are rewarding, we hypothesized that high-dose androgens would enhance operant responding for sexual behavior.

Materials and methods

Animals

Adolescent male Long–Evans rats (4 weeks of age, ca. 200 g BW at the start of the study, Charles River Laboratories, MA) were individually housed under a reversed 14L:10D photoperiod. They remained gonad-intact to approximate AAS use in humans. Female rats used as stimulus animals were ovariectomized via bilateral dorsal flank incision, and received a 4-mm Silastic estradiol implant sc (id: 1.98 mm, od: 3.18 mm; Dow Corning, MI) to maintain chronic physiologic levels of estrogen (Bridges, 1984). To induce estrus, females received 250 μg progesterone in cottonseed oil sc approximately 4 h prior to testing. Females were rotated among the different test males. Behavior was tested under dim red light during the first 4 h of the dark phase when activity peaks. Experimental procedures were approved by USC's Institutional Animal Care and Use Committee and were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, 8th Ed (National Research Council, National Academies Press, Washington DC; 2011).

AAS treatment

Beginning at 5 weeks of age, rats received testosterone (7.5 mg/kg; Steraloids, RI) or vehicle (3% ethanol and 13% cyclodextrin (RBI, MA) in water) by daily sc injection 5 days/week. The 7.5 mg/kg dose approximates a heavy steroid dose in humans, and has been used previously to demonstrate AAS effects on mating and aggression in rats (Clark and Fast, 1996; Clark et al., 1998; Cooper et al., 2014; Wood et al., 2013). Testosterone treatment was initiated in adolescent rats to model human users. A typical AAS user is a young man in his late teens or early 20s (Pope et al., 2014a). Among U.S. high school students, 4–6% of boys have used AAS, comparable to the rates of crack cocaine or heroin use (Johnston et al., 2013). It is estimated that AAS use among men in their 20s is even higher (Pope et al., 2014b).

Experimental design

To determine the effects of chronic high-dose testosterone on appetitive sexual behavior, male rats were trained in their home cage to make operant responses for access to a receptive female. Before training, all males received sexual experience with an estrous female on two occasions for 30 min each. Operant training began after at least 4 weeks of exposure to testosterone or vehicle. Injections of testosterone or vehicle continued throughout training and testing for food reward and for access to a female. Testosterone- and vehicle-treated rats were trained and tested on the same schedule, and there was no effect of testosterone on task acquisition.

The first experiment used a 10-minute fixed-interval (FI) schedule of reinforcement (FI-10), according to the methods of Wood et al. (2013) as modified from Scott et al. (1994) and Fish et al. (2008). A second experiment provided access to the female on a 2nd-order schedule of reinforcement (2nd-order FR), using modifications of Everitt et al. (1987). In Everitt et al. (1987), access to the reinforcer (estrous female) was paired with a neutral conditioned stimulus (CS, a stimulus light) activated by operant responses on a lever. In the present study, access to the reinforcer (estrous female) was paired with a sexually-salient CS (brief visual presentation of the female) activated by operant responses on a lever. The assumption was that a sexually-salient CS would enhance rates of operant responding under 2nd-order FR. To facilitate training because copulatory behavior is impaired when males are mated daily (Everitt et al., 1987), rats were initially trained in daily sessions to respond for a small food reward (Froot Loops, Kellogg's, Battle Creek, MI). To compare appetitive and consummatory sexual behaviors in both experiments, mating was recorded on videotape during presentation of the female, and was scored by an observer blinded to the treatment groups. Measures of sexual behavior included the number of mounts + intromissions, and latency to the first intromission and ejaculation.

FI-10

An operant conditioning panel containing a nose-poke with stimulus light (Med Associates, VT) was introduced into the home cage 10 min after injection of testosterone or vehicle ($n = 7$ each). The reward (initially a Froot Loop, later an estrous female rat) was present behind a perforated Plexiglas screen adjacent to the nose-poke, permitting transmission of visual, auditory and olfactory stimuli. Responses on the nose-poke were recorded and reinforced on a FI schedule, as in previous studies of aggressive motivation in mice (Fish et al., 2008) and rats (Wood et al., 2013). The initial FI was 30 s, subsequently increased to 1 min, and by 1-min increments thereafter until a 10 min FI was reached. Testing for food reward on the FI-10 schedule continued until response rates stabilized (8 days of testing at FI-10 for both testosterone- and vehicle-treated rats). At this point, an estrous female was substituted for the Froot Loop behind the Plexiglas screen, with access to the female for 10 min as the reinforcer. Testing continued twice weekly until behavior stabilized. In addition, sexual behavior of each male was videotaped on one occasion upon presentation of the female at the end of the 10-min FI. In 3 subsequent trials, males were tested with an anestrous female.

Data were analyzed using JMP 9.0 statistical software (SAS Institute, NC), and $p < 0.05$ was considered significant for all analyses. Effect sizes for significant relationships from unpaired comparisons, pairwise comparisons, and ANOVAs were estimated using Cohen's *d* and $\eta 2_p$ using online tools (http://www.cognitiveflexibility.org/effectsize/ and http://www.campbellcollaboration.org/escalc/html/EffectSizeCalculator-SMD-main.php). Operant responses for food during initial training in vehicle- and testosterone-treated rats were compared by RM-ANOVA with increasing FI as the repeated measure. Operant responses for access to food or females in each male were averaged over the last 3 days of testing. Individual responses were then averaged across the two experimental groups (vehicle and testosterone), and compared by Student's t-test. Measures of sexual behavior (numbers of mounts + intromissions, latency to the first ejaculation) in vehicle- and testosterone-treated rats were likewise compared by Student's t-test.

2nd-order FR

As with FI-10, an operant conditioning panel containing a lever with stimulus light (Med Associates, VT) was introduced into the home cage 10 min after injection of testosterone or vehicle ($n = 9$ each). Unlike FI-10, the panel separating the male from the Froot Loop or estrous female was opaque, with an automated guillotine door that opened to a perforated Plexiglas screen. Responding on the lever opened the guillotine door for 5 s, thereby providing access to visual, olfactory and auditory stimuli from the female. As with FI-10, rats were trained first to respond for food in 5 trials/day. For each trial, the door opened after 1 response on the lever (FR1), and the rat was rewarded for each door opening (FR1:1). The number of door openings