Available online at www.sciencedirect.com
ScienceDirect
Hormones and Behavior 50 (2006) 410–416
Hormones and Behavior
www.elsevier.com/locate/yhbeh

Physical provocation of pubertal anabolic androgenic steroid exposed male rats elicits aggression towards females

Rebecca L. Cunningham, Marilyn Y. McGinnis *

Department of Biology, The University of Texas at San Antonio, San Antonio, TX 78249, USA

Received 28 December 2005; revised 11 May 2006; accepted 11 May 2006

Abstract

Human studies suggest that anabolic androgenic steroid (AAS) users are aggressive towards women. This study used a rat model to evaluate whether AAS potentiated aggression towards females and the conditions under which this occurs. Gonadally intact pubertal male rats received one of the following AAS treatments (5 mg/kg s.c. 5 days/week for nine weeks): testosterone (T), stanozolol (S), testosterone + stanozolol (T + S), or vehicle control. Each rat was tested with 3 conspecific stimuli: ovariectomized females (OVX), estrogen only females (E), and estrogen + progesterone females (E + P). The response to physical provocation was tested under three conditions: without physical provocation, provocation of the experimental male, and provocation of the conspecific female. Provocation was a mild tail pinch. Both aggressive and sexual behaviors were measured during each test. In the absence of physical provocation, AAS males were not aggressive towards females. However, provocation significantly increased aggression in males treated with testosterone but only towards OVX females. In the presence of E or E + P females, all animals displayed sex behavior, not aggression. Thus, factors such as the nature of the AAS and the hormonal status of the females are important in determining whether male rats will be aggressive towards females. However, the most salient factor determining aggression towards females is the presence of provocation in combination with high levels of testosterone.

Keywords: Testosterone; Stanozolol; Sex behavior; Puberty; Tail pinch; Adolescence; Mounting behavior; Violence; AAS; Dominance

Introduction

High doses of anabolic androgenic steroids (AAS) have been shown to have various behavioral effects. It has been reported that human male AAS abusers have affective disturbances, such as irritability, mood swings, hostility, and increased aggression (Anderson et al., 1992; Galligani et al., 1996; Hannan et al., 1991; Pope and Katz, 1994; Su et al., 1993; Tricker et al., 1996). This heightened aggression by AAS users has been shown to be displayed towards other men (Archer, 1991; Ehrenkranz et al., 1974; Katz and Pope, 1990). Interestingly, it has also been documented that AAS users are violent towards females (Choi and Pope, 1994).

Studies using animal models have reported that pubertal AAS exposure enhances aggression. For example, male hamsters given a 'cocktail' consisting of three combined AAS (testosterone, nandrolone, and boldenone) throughout puberty were more aggressive toward other male hamsters (Harrison et al., 2000; Melloni et al., 1997). In rats, pubertal exposure to chronic high doses of AAS testosterone resulted in heightened aggression towards intact males, whereas stanozolol inhibited aggression (Farrell and McGinnis, 2003). One of the most striking findings from the animal studies is that adult male rats exposed to the AAS, testosterone, are more aggressive towards castrated male rats when provoked by a mild tail pinch (McGinnis et al., 2002). This is significant because typically male rats are not aggressive towards non-threatening conspecifics such as castrated males or females (Blanchard et al., 1984; DeBold and Miczek, 1984). However, the effects of AAS on aggression towards females have not been studied.

Alarmingly, AAS use is increasing among teenagers (Burnett and Kleiman, 1994; DuRant et al., 1995; Melia et al.,

* Corresponding author. Fax: +1 210 458 5493.
E-mail address: Marilyn.McGinnis@utsa.edu (M.Y. McGinnis).

0018-506X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.yhbeh.2006.05.002

1996; NIDA, 2002). Adolescent boys abusing AAS self-report increased displays of aggression (Dukarm et al., 1996; Olweus et al., 1980). Because puberty is a time when maturational changes in the brain and behavior are taking place (Primus and Kellogg, 1990; Sisk et al., 2003), and pubertal AAS exposure has been shown to influence behavior, chronic pubertal AAS exposure may have long-lasting effects (NIDA, 2002; Primus and Kellogg, 1990; Wu, 1997).

The purpose of the present study was to determine (a) whether pubertal males exposed to AAS are more aggressive towards females, (b) whether ovarian hormonal cues influence aggression towards females, and (c) and if physical provocation affects aggression towards females. Two widely abused AAS were examined: testosterone and stanozolol (Mottram and George, 2000; Pope and Katz, 1994). These AAS were administered throughout puberty. Our hypothesis was that male rats would be aggressive toward females, but that this would be determined by the hormonal condition of the female and the presence of a physical provocation.

Methods

Animals

Gonadally intact male and female Long Evans rats were purchased from Charles River Laboratory (Wilmington, MA). Experimental male animals were obtained on postnatal day 35. Females used as stimulus animals were in the weight range of 225–250 g. Animals were housed in a temperature-controlled room (23°C) in standard Plexiglas cages (25 × 20 × 18 cm). The animals had ad libitum access to food and water, and the lights were maintained on a 12:12 reversed light/dark cycle, with lights off at 1200 h. Experimental procedures were performed in accordance with National Institutes of Health's guidelines for animal care and use.

Stimulus females were placed into one of three groups and given hormone treatments as described in previous studies (Harding and McGinnis, 2003; McGinnis et al., 1981; Vagell and McGinnis, 1997, 1998): ovariectomized (OVX), ovariectomized and implanted with 1 Silastic capsule (1.47 mm i.d. × 1.96 mm o.d. × 5 mm length, Dow Corning, Midland, MI) containing 10% crystalline estradiol benzoate (Sigma, St. Louis, MO) (E), and a third group consisting of E females that were made sexually receptive (E + P) via a subcutaneous injection of 500 μg progesterone (Sigma, St. Louis, MO) 4 h prior to behavioral testing. The behavioral difference between E and E + P females is that E females are receptive (lordosis) but do not display proceptive (hop-dart and ear wiggling) behaviors toward the males, whereas E + P females are both receptive and proceptive (Beach, 1976).

Anabolic androgenic steroid treatment

Gonadally intact pubescent males were randomly assigned into one of four treatment groups for a total of 8 animals in each group. Testosterone propionate (T), stanozolol (S), or testosterone propionate plus stanozolol (T + S) were subcutaneously injected at a dosage of 5 mg/kg body weight five days a week for nine weeks. This AAS dosage was the same concentration used in prior studies (Breuer et al., 2001; Farrell and McGinnis, 2003; McGinnis et al., 2002). All AAS (Sigma, St. Louis, MO) were dissolved in the vehicle polyethylene glycol 200 (PEG-200). The fourth group consisted of gonadally intact controls injected only with the vehicle. All injections were administered between 0800 and 1000 h. Injections began on postnatal day 40, which is the time of preputial separation and was used as a marker for the onset of puberty (Korenbrot et al., 1977). Injections continued throughout the experiment with behavioral testing occurring during weeks five through nine of AAS exposure (Table 1).

Behavioral tests

All rats were tested as young adults between days 68–105 and during the first 4 h of the dark phase (1200–1600) of the light cycle. Behavior tests were conducted under dim red lighting and videotaped. The males were tested with females in a neutral cage environment using previously described methods for assessing aggression (Farrell and McGinnis, 2004; McGinnis et al., 2002). During testing, both aggression and sex behaviors were scored in the presence of three types of conspecific females: OVX females, E females, and E + P females. The animals were tested in three provocation conditions: no provocation, provocation of the experimental male, and provocation of the female. Provocation was administered as a mild tail pinch (Smith et al., 1997) every 60 s during a 10-min test. Each experimental animal received 9 tests during a 5-week period in a neutral cage with clean bedding beginning at week 5 (Table 1): test 1, OVX conspecific (no tail pinch); test 2, OVX + tail pinch of the experimental male; test 3, OVX + tail pinch of the female; test 4, E conspecific (no tail pinch); test 5, E + tail pinch of the experimental male; test 6, E + tail pinch of the female; test 7, E + P conspecific (no tail pinch); test 8, E + P + tail pinch of the experimental male; and test 9, E + P + tail pinch of the female. The testing order was counterbalanced so that no animal was tested with the same female twice. To assess the impact of provocation, the no provocation test was conducted first to provide a baseline level of aggression. This was followed by provocation of either the male or the female conspecific in a counterbalanced order (McGinnis et al., 2002).

Measurements for sexual behavior consisted of intromission, ejaculation, and postejaculatory interval latencies and frequencies (Farrell and McGinnis, 2004; Vagell and McGinnis, 1998). Aggression measurements consisted of the frequencies of attacks, threats, boxing, and dominance postures initiated by the experimental male rat in a 10-min test. The frequencies of these four behaviors were compiled and then divided by the total number of male rats in each AAS treatment group to create a mean composite aggression score. Because laboratory rats typically show low levels of spontaneous aggression, a mean composite aggression score (rather than individual measures) is frequently used to report aggressive behavior (Albert et al., 1987; Breuer et al., 2001; Christie and Barfield, 1979; Farrell and McGinnis, 2004; McGinnis et al., 2002; Nomura et al., 2002). Attacks were recorded when the experimental rat initiated a series of actions that included a sequence of hip thrusts, attacks, and biting of the female. Threats were scored when a male moved towards a female to strike without making physical contact. Boxing was recorded when both animals were in an upright stance with elevated forepaws and their bodies were in close proximity of each other (Takahashi and Lisk, 1983). Dominance postures were recorded when the male stood over the female rendering it immobilize in a supine position. Normally, mounting behavior (pelvic thrusting) is included in the mean composite aggression score. However, because mounts are typically displayed in both sexual and aggressive contexts, mounting behavior was scored as a separate behavioral measurement. We found that mounts were related to two distinct behavioral repertoires. Either the mounts were accompanied by other sexual behaviors (intromissions, ejaculations) or other aggressive behaviors (attacks, threats, boxing, dominance postures). On this basis, the mounts could be categorized as either aggressive mounts or sexual mounts (Grant and Mackintosh, 1963; Barfield et al., 1972). In instances where males exhibited primarily intromissions within a given test along with one or two aggressive responses, the mounts were classified as sexual mounts. Conversely, if males showed primarily aggressive behaviors with one or two intromissions, the mounts were categorized as aggressive mounts.

Mounting behavior was analyzed by two methods: (1) a separate measure that included all mounts (sexual and aggressive) and (2) as a component of the mean composite aggression score along with the other measures of aggression (attacks, threats, boxing, and dominance postures). These parameters are employed in our standard mean composite aggression score (Breuer et al., 2001; Farrell and McGinnis, 2004; McGinnis et al.,