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Effects of anabolic androgenic steroids on the development and expression of running wheel activity and circadian rhythms in male rats

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Abstract

In humans, anabolic androgenic steroid (AAS) use has been associated with hyperactivity and disruption of circadian rhythmicity. We used an animal model to determine the impact of AAS on the development and expression of circadian function. Beginning on day 68 gonadally intact male rats received testosterone, nandrolone, or stanozolol via constant release pellets for 60 days; gonadally intact controls received vehicle pellets. Wheel running was recorded in a 12:12 LD cycle and constant dim red light (RR) before and after AAS implants. Post-AAS implant, circadian activity phase, period and mean level of wheel running wheel activity were compared to baseline measures. Post-AAS phase response to a light pulse at circadian time 15 h was also tested. To determine if AAS differentially affects steroid receptor coactivator (SRC) expression we measured SRC-1 and SRC-2 protein in brain. Running wheel activity was significantly elevated by testosterone, significantly depressed by nandrolone, and unaffected by stanozolol. None of the AAS altered measures of circadian rhythmicity or phase response. While SRC-1 was unaffected by AAS exposure, SRC-2 was decreased by testosterone in the hypothalamus. Activity levels, phase of peak activity and circadian period all changed over the course of development from puberty to adulthood. Development of activity was clearly modified by AAS exposure as testosterone significantly elevated activity levels and nandrolone significantly suppressed activity relative to controls. Thus, AAS exposure differentially affects both the magnitude and direction of developmental changes in activity levels depending in part on the chemical composition of the AAS.

Keywords: Anabolic androgenic steroids; Circadian rhythms; Rats; Running wheel activity; SRC-2; Body weight

1. Introduction

Abuse of anabolic androgenic steroids (AAS) has increased dramatically in recent years [1–5]. AAS abuse has become a public health issue, and evidence of numerous undesirable behavioral changes has been reported. For example, AAS use reportedly induces hyperactivity and sleep disturbances [2,5–8]. Since these disorders are associated with changes in circadian rhythmicity [9–12] as well as with human AAS abuse, it is quite plausible that these effects are mediated by AAS-induced changes in biological clock function. Because the human data are largely subjective or based on single case reports and uncontrolled conditions, it is essential to establish animal models to confirm the validity of the findings for humans. Human alterations in circadian rhythms associated with psychiatric disorders are quantitative in nature, typically represented by relatively small but significant differences in phase or amplitude of various daily rhythms [9–12]. Thus, even

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relatively minor changes in circadian rhythm expression can serve as markers of certain psychopathological states [10].

Studies using various mammalian species have demonstrated effects of replacement doses of testosterone on circadian rhythmicity in castrated males [13–16]. For example, testosterone increased activity levels and elevated nocturnal amplitude of activity in castrated male voles [16]. In hamsters, castration increased the variability and duration of running wheel activity [15]. The diurnal rodent, *Octodon degus* exhibited significantly reduced wheel running activity and increased phase angle of entrainment after castration [14]. Castrated male mice displayed a slightly shorter free-running period and increased activity following treatment with exogenous testosterone [13]. These data, along with the human studies discussed above, suggest that chronic exposure to very high doses of androgens such as those employed by human AAS users, may indeed affect circadian rhythms and mean levels of wheel running activity. To our knowledge, the current study is the first to determine the impact of AAS exposure on the chronobiological regulation of activity.

In order to provide a comprehensive assessment of the effects of AAS on the chronobiological regulation of activity, there were three hypotheses that guided this study. The first hypothesis was that exposure to AAS would influence wheel running activity and circadian rhythms. The rational for this hypothesis is predicated on the hyperactivity and altered sleep patterns reported for humans following AAS use [2,5–8], and data showing that endogenous levels of testosterone may influence circadian rhythms in other rodent species [13–17]. The second hypothesis was that AAS with dissimilar chemical characteristics would differentially affect both the level of wheel running activity and circadian parameters. This is based on behavioral research indicating that three of the most commonly abused AAS, testosterone, nandrolone and stanozolol [8,18], all have markedly different effects on a variety of behavior patterns. For example testosterone potentiates aggressiveness, nandrolone has little effect on aggression and stanozolol actually inhibits aggression [19]. The third hypothesis was that chronic AAS exposure would have an impact on the ontogenetic development of wheel running activity and circadian rhythms. This was predicted on the basis of work in *O. degus* showing that the development of adult circadian rhythm patterns is age and hormone dependent [17]. Because our baseline measures of wheel running activity began prior to puberty and continued through adolescence and into adulthood we were able to examine the impact of these three distinct AAS on running wheel activity and circadian rhythms over the course of development.

Several measures were obtained in order to examine how AAS influence circadian rhythms and the expression of wheel running activity. Specifically, we assessed the effects of chronic AAS exposure on running wheel activity in 12:12 LD and constant dim red light (RR), peak time of activity in LD, circadian period in RR and phase-response to a light pulse at circadian time 15 h. These parameters represent central circadian clock properties that play a critical role in the normal expression of circadian rhythms [20]. To better understand the biological consequences of AAS exposure on activity levels and circadian rhythms, we measured body and tissue weights, serum testosterone levels, and two nuclear receptor coactivators, SRC-1 and SRC-2 which have been shown to dramatically enhance the transcriptional activity of nuclear receptors, including androgen receptors (AR) [21–23].

2. Materials and methods

2.1. Subjects and treatment groups

Forty-eight male Long–Evans rats, approximately 25 days old, were purchased from Charles River Labs (North Wilmington, MA, 01887, USA). Each rat was randomly assigned to one of four treatment groups: testosterone propionate (T), nandrolone (N), stanozolol (S) and control (C). Body weights were recorded twice: once when hormone pellets were implanted and again at the time of sacrifice. All procedures were conducted in accordance with the guidelines established for the care and use of laboratory animals by the National Institute of Health.

2.2. Housing

The rats were placed directly into transparent plastic cages, 47×27×20 cm (length×width×height) with ad lib food and water, and a 34 cm diameter running wheel on the day of their arrival in the lab in a 12:12 LD cycle with lights on at 0900 h. Cages were arranged on open racks, alternating by drug treatment in a random block design. Trays suspended below wire mesh cage floors contained crushed corncob bedding. Bedding was changed, and water and food replenished approximately once per week. During 12:12 LD entrainment, light was provided by overhead fluorescent ceiling fixtures. The light intensity at the bottom center of each cage averaged 59+/−7 lx, with no significant difference among the four treatment groups. During free-running circadian period assays and phase-response assays in constant dim red light (RR) the red light was provided by three safelights, each with a number one Kodak red monochromatic filter and a 15 W bulb in a light-tight room measuring 16 ft. long by 8 ft. wide and 8 ft. high. Red light intensity was less than 1 lx in all cages.

2.3. AAS treatment

The three AAS, testosterone (T), nandrolone (N) and stanozolol (S), were chosen for several reasons: 1) they are structurally distinct in their chemical composition, 2) they have differing affinities for the androgen receptor [24], 3) they are markedly different in their behavioral consequences (for review, see [19]), and 4) they are highly abused by humans. Previous studies [25–30] have employed daily subcutaneous injections to provide chronic exposure to high doses of AAS. In order to eliminate the effects of repeated handling and injections of the animals while in the running wheels, we used timed-release pellets (Innovative Research of America, Sarasota, FL, USA) designed to administer 200 mg of hormone at a constant rate for 60 days. This dosage reportedly provides approximately 3.3 mg