Behavioural Processes 113 (2015) 81–85

Contents lists available at ScienceDirect

Behavioural Processes

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

Short report

The effect of the anabolic steroid, nandrolone, in conditioned place preference and D1 dopamine receptor expression in adolescent and adult mice

Freddyson J. Martínez-Rivera[a], Eduardo J. Natal-Albelo[b], Namyr A. Martínez[c], Roberto A. Orozco-Vega[d], Oscar A. Muñiz-Seda[b], Jennifer L. Barreto-Estrada[a,*]

[a] Department of Anatomy and Neurobiology, School of Medicine, Medical Sciences Campus, University of Puerto Rico, San Juan, PR 00936, USA
[b] Department of Biology, Río Piedras Campus, University of Puerto Rico, San Juan, PR 00936, USA
[c] Department of Physiology, School of Medicine, Medical Sciences Campus, University of Puerto Rico, San Juan, PR 00936, USA
[d] Department of Mathematics and Physics, Cayey Campus, University of Puerto Rico, PR 00736, USA

ARTICLE INFO

Article history:
Received 30 September 2014
Received in revised form
17 December 2014
Accepted 17 January 2015
Available online 20 January 2015

Keywords:
Anabolic-androgenic steroids
Reward
Adolescents
D1 dopamine receptors
Nucleus accumbens

ABSTRACT

Adolescents and adults engage in anabolic-androgenic steroid (AAS) misuse seeking their anabolic effects, even though later on, many could develop neuropsychological dependence. Previously, we have shown that nandrolone induces conditioned place preference (CPP) in adult male mice. However, whether nandrolone induces CPP during adolescence remains unknown. In this study, the CPP test was used to determine the rewarding properties of nandrolone (7.5 mg/kg) in adolescent mice. In addition, since D1 dopamine receptors (D1DR) are critical for reward-related processes, the effect of nandrolone on the expression of D1DR in the nucleus accumbens (NAc) was investigated by Western blot analysis. Similar to our previous results, nandrolone induced CPP in adults. However, in adolescents, nandrolone failed to produce place preference. At the molecular level, nandrolone decreased D1DR expression in the NAc only in adult mice. Our data suggest that nandrolone may not be rewarding in adolescents at least during short-term use. The lack of nandrolone rewarding effects in adolescents may be due, in part to differences in D1DR expression during development.

1. Introduction

Anabolic-androgenic steroids (AAS) are considered drugs of abuse, being misused at supraphysiological doses by an increasing number of athletes and adolescents (Bahrke et al., 1998). The use of AAS by teenagers has been a primary public health concern because of their potential side effects during a period where remodeling of the brain and behavioral maturation occurs. The lifetime prevalence of AAS misuse for adolescents has been estimated to be 4–6% in males and 1.5–3% in females (Harmer, 2010). This and other epidemiological reports support studies that link early initiation of AAS misuse with increased risk for psychiatric dysfunction, steroid dependence, as well as a higher risk to engage in the use of other illicit drugs (Kanayama et al., 2009). However, during the last decade, studies that assessed rewarding properties of AAS have been mainly done in adult animal models. In fact, a growing body of work in adult rodents shows that androgen compounds have hedonic and reinforcing effects (Clark and Henderson, 2003; Wood, 2004). These effects have been observed using different behavioral paradigms, such as conditioned place preference (CPP) or self-administration (Clark and Henderson, 2003; Wood, 2004). Recently, we have demonstrated, in adult mice, that pharmacological doses of the anabolic steroids, testosterone (T) propionate (0.075, 0.75 and 7.5 mg/kg) or nandrolone (0.75 and 7.5 mg/kg but not 0.075 mg/kg), shifted place preference during the CPP (Parrilla-Carrero et al., 2009). Still, the rewarding properties of AAS during adolescence have remained undetermined.

The AAS-induced rewarding effects seem to be mediated directly or indirectly by the corticomesolimbic dopamine reward system (Triemstra et al., 2008). This circuit, which is critical for regulating reward-related associative learning (Kindlundh et al.,

Abbreviations: AAS, anabolic androgenic steroids; AR, androgen receptor; CNS, central nervous system; CPP, conditioned place preference; DAT, dopamine transporter; D1DR, D1 dopamine receptor; D2DR, D2 dopamine receptor; ER, estrogen receptor; IP, intraperitoneal; MPN, medial preoptic nucleus; NAc, nucleus accumbens; T, testosterone; PFC, prefrontal cortex; PN, postnatal; NP, non-preferred; VTA, ventral tegmental area.

* Corresponding author. Tel.: +1 787 758 2525x2562; fax: +1 787 767 0788.
E-mail address: jennifer.barreto1@upr.edu (J.L. Barreto-Estrada).

http://dx.doi.org/10.1016/j.beproc.2015.01.008
0376-6357/

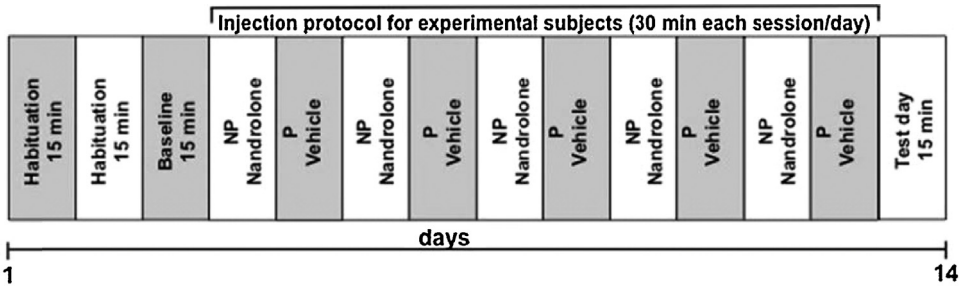

Fig. 1. Experimental design and injection protocol for the CPP in nandrolone-treated animals. The CPP was performed for 14 days. On days 1–2, animals were habituated to the CPP chambers for 15 min. On day 3 (baseline), animals were allowed to move freely between the compartments for 15 min. The time spent in each compartment was immediately calculated. During days 4–13 (conditioning), nandrolone-treated animals received alternate injections of drug (days 4, 6, 8, 10, 12) or vehicle (days 5, 7, 9, 11, 13) while placed for 30 min in the non-preferred (NP) or preferred (P) side, respectively. Control animals received daily injections of vehicle, whether they were restricted to the NP or P side of the chamber. On day 14 (test day), injection-free animals were allowed to move freely between the compartments, and the time spent in the NP side was recorded for 15 min.

2004), is mainly composed of the ventral tegmental area (VTA), nucleus accumbens (NAc) and prefrontal cortex (PFC; Koob and Volkow, 2010). In this context, rewarding stimuli lead to increments of dopamine release to the NAc from the VTA. Although D1 and D2 dopamine receptors (D1DR and D2DR) are key players for the signaling process of reward, the D1DR are the most critical in reward-related learning (Beninger and Miller, 1998). In this line of evidence, Kindlundh et al. (2001, 2003),) have shown that nandrolone altered proteins and transcripts of the dopamine receptors within the striatum and NAc. Moreover, accumbal administration of flupentixol, a D1/D2 dopamine receptor antagonist, blocked the expression of androgen-induced CPP (Packard et al., 1998).

In the present study, the rewarding properties of nandrolone, one of the most abused AAS (Lood et al., 2012), were assessed using an adolescent animal model. Furthermore, we addressed the molecular modulation of nandrolone in the NAc through the expression of D1DR. Knowing that adolescents engage in high reward motivated behaviors (Wahlstrom et al., 2010) due to developmental changes in the striatum that confer reward hypersensitivity (Galvan, 2010), we hypothesized that exposure to nandrolone during this developmental stage will induce: (i) an increase in place preference, and (ii) an increase in the levels of accumbal D1DR protein expression.

2. Materials and methods

2.1. Subjects

C57Bl/6 male mice were purchased from Charles River and housed individually in the Animal Resources Center with food and water available ad libitum. Animals were kept on a reverse 12:12 h light/dark cycle. The behavioral experiments were performed during the dark phase of the cycle, in accordance with the Institutional Animal Care and Use Committee (IACUC) of the University of Puerto Rico, Medical Sciences Campus.

2.2. Androgen administration

Similar to previous experiments, adolescent and adult experimental groups received intraperitoneal (IP; Hoseini et al., 2009; Witzmann, 1988) injections of nandrolone (7.5 mg/kg; 19-nortestosterone; Sigma Chemical Co., St. Louis, MO), whereas control groups received IP injections of vehicle, which consisted of sesame oil (0.02 cc/10 g of body weight). As 1 mg/kg of AAS is sufficient to restore endocrine function and reproductive behaviors in rodents (Clark and Barber, 1994), the dosage used in this experiment reflects a high dose. Since periadolescence in rodents extends from postnatal (PN) day 28 to PN-60, (Spear, 2000), nandrolone injections alternated with vehicle injections, were started at PN-33 until PN-42 (Fig. 1). Furthermore, to confirm our previous experiment in adults (Parrilla-Carrero et al., 2009), adult male mice (PN 88–97) were also tested following the same CPP injection protocol as in adolescents.

2.3. Conditioned place preference (CPP)

This behavioral paradigm was performed as previously described (Parrilla-Carrero et al., 2009) with some modifications. In brief, the apparatus consisted of an acrylic cage separated in two compartments by an opening that can be closed by a removable guillotine door. The chambers comprised of two sides. One side of the chamber has smooth flooring, lateral walls with black and white lines, and anterior/posterior white walls. The other side has grated-texture flooring, lateral walls with black and white checkers, and anterior/posterior black walls.

The CPP was performed using a biased design and under semi dark conditions. Briefly, the CPP protocol lasted 14 days and consisted of four phases (Fig. 1): habituation (days 1–2), baseline (day 3), conditioning (days 4–13) and test day (day 14). During the baseline (15 min), animals were recorded for the time spent in each side of the chamber to determine the non-preferred (NP) side (where the drug will be injected during conditioning). Thereafter, animals were randomly assigned to control or experimental groups. The conditioning phase consisted of a 30 min session per day. The experimental group received nandrolone injections (days 4, 6, 8, 10, 12) while they were restricted to the NP side, or vehicle injections while restricted to the preferred (P) side (days 5, 7, 9, 11, 13). The control group received daily vehicle injections whether they were restricted to the NP or P side of the chamber. Overall, both groups received a total of 10 injections. On the test-day, all animals were tested for the time spent in the NP-side in the absence of further vehicle or nandrolone injections. The time in the CPP compartments was gathered using the Any-Maze tracking system (Stoelting Co., IL). Data were obtained as the time (s) the animals spent in the P and NP sides during the test-day. The time spent in the NP side between control and experimental animals was reported and statistically compared.

2.4. Western blot analysis

2.4.1. Sample preparation

Nandrolone (7.5 mg/kg) and vehicle treated adolescent and adult mice were sacrificed by rapid decapitation, and the NAc rapidly dissected as previously described (Sharma and Fulton, 2012). Tissue was homogenized and lysed using CelLytic MT with SIGMAFAST™ Protease Inhibitor Tablets (Sigma–Aldrich, MO).