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Chronic nandrolone decanoate exposure during adolescence affects emotional behavior and monoaminergic neurotransmission in adulthood

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ABSTRACT

Nandrolone decanoate, an anabolic androgen steroid (AAS) illicitly used by adult and adolescent athletes to enhance physical performance and body image, induces psychiatric side effects, such as aggression, depression as well as a spectrum of adverse physiological impairments. Since adolescence represents a neurodevelopmental window that is extremely sensitive to the detrimental effects of drug abuse, we investigated the long-term behavioral and neurophysiological consequences of nandrolone abuse during adolescence. Adolescent rats received daily injections of nandrolone decanoate (15 mg/kg, i.m.) for 14 days (PND 40–53). At early adulthood (PND 68), forced swim, sucrose preference, open field and elevated plus maze tests were performed to assess behavioral changes. In vivo electrophysiological recordings were carried out to monitor changes in electrical activity of serotonergic neurons of the dorsal raphe nucleus (DRN) and noradrenergic neurons of the locus coeruleus (LC).

Our results show that after early exposure to nandrolone, rats display depression-related behavior, characterized by increased immobility in the forced swim test and reduced sucrose intake in the sucrose preference test. In addition, adult rats presented anxiety-like behavior characterized by decreased time and number of entries in the central zone of the open field and decreased time spent in the open arms of the elevated plus maze. Nandrolone decreased the firing rate of spontaneously active serotonergic neurons in the DRN while increasing the firing rate of noradrenergic neurons in the LC.

These results provide evidence that nandrolone decanoate exposure during adolescence alters the emotional profile of animals in adulthood and significantly modifies both serotonergic and noradrenergic neurotransmission.

1. Introduction

Anabolic androgenic steroids (AAS), including testosterone and its derivatives, such as nandrolone decanoate, enhance body mass and improve physical performance (Sjoqvist et al., 2008). AAS abuse was first confined to adult athletes and bodybuilders, but in the past two decades, AAS appear to also have become widely used among adolescent males (Kindlundh et al., 2001). It has been reported that 4–6% of high school males have admitted to the use or abuse of AAS at some time in their life (Bahrke and Yesalis, 2004). Despite the beneficial physical effects sought by AAS users, numerous adverse side effects are known, including psychological effects ranging from marked aggression to depressive mood swings and anxiety (Pope and Katz, 1994; Thiblin et al., 1999; Trenton and Currier, 2005; van Amsterdam et al., 2010). Accordingly, a study from Malone et al. (1995) reported that past AAS abusers had significantly more psychiatric diagnoses than nonusers or current abusers.

While aggressive-related behaviors after AAS exposure in animals confirm human studies (Lumia and McGinnis, 2010), conflicting results remain concerning behavioral states related to

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depression and anxiety. Indeed, data in the literature concerning depressive-like behavior in AAS-treated animals are scarce and contradictory. Matrisciano et al. (2010) described increased immobility in nandrolone-treated adult male rats in the forced swim test, whereas Ambar and Chiavegatto (2009) reported decreased immobility in adult male mice. Concerning anxiety, some studies have shown anxiolytic-like or no effects after chronic nandrolone treatment in adult male mice (Ambar and Chiavegatto, 2009; Celerier et al., 2006) and rats (Kouvelas et al., 2008), while others have presented opposing results in the same behavioral paradigms (Minkin et al., 1993; Rocha et al., 2007) in adult male rats. These discrepancies could be due to the differences in species used. Moreover, most studies were performed in adult animals and only recently, Ricci et al. (2012) reported anxiety-like behavior after adolescent exposure to AAS in adult Syrian hamsters.

Besides behavioral observations, studies have highlighted that chronic nandrolone treatment increases dopamine concentration and its metabolites in the prefrontal cortex and decreases serotonin (5-HT) concentration in the limbic forebrain (Kurling et al., 2005) as well as 5-HT innervation (Ricci et al., 2012). Acute injection of nandrolone in adult rats also leads to changes in the noradrenergic system, by increasing extracellular concentration of noradrenaline (NA) and its metabolites in the hypothalamus (Tamaki et al., 2003). AAS act directly on androgen receptors (AR) and indirectly on estrogen (ER) receptors (Penatti et al., 2009). AR are widely expressed in both the male rat dorsal raphe nucleus (DRN) and the locus coeruleus (LC) (Hamson et al., 2004), where most of the serotonergic and noradrenergic neurons are located, respectively. ER alpha and beta instead are scarcely expressed in male rat DRN (Sheng et al., 2004), while ER beta are present in LC (Yamaguchi and Yuri, 2012). Remarkably, these two monoaminergic nuclei are directly involved in the pathophysiology of depression (Belmaker and Agam, 2008).

Brain development is punctuated by constant neuroplastic changes paralleled by a peaking of emotional instability, partial anhedonia, disproportionately extensive reckless, novelty-seeking, sensation-seeking, and risk-taking behaviors (Spear, 2000). Imaging studies have ascertained that the dynamic neuroanatomical modifications occurring in the brain throughout adolescence enhanced the risk of developing psychiatric disorders (Paus et al., 2008), thus reinforcing the concept that adolescence is a critical time for the adverse impact of drugs of abuse on the monoaminergic systems (Bambico et al., 2010; Labonte et al., 2012).

In this study, we aimed to characterize the long-term behavioral and neurobiological consequences of adolescent exposure to the AAS nandrolone, using behavioral paradigms predictive for depressive-like state and in vivo electrophysiological recordings in the DRN and LC of anaesthetized rats. We hypothesize that behavioral impairment due to nandrolone treatment could be linked to changes in serotonergic and noradrenergic tonic activity.

2. Materials and methods

2.1. Animals and treatments

Pregnant Sprague–Dawley female rats (Charles River, Canada) were housed individually in polycarbonate cages and maintained under standard laboratory conditions [12-h light/dark cycle, temperature 20 ± 2 °C]. Water and food were available *ad libitum*. Male pups were weaned on postnatal day 21 (PND 21). At PND 40 male rats received either 15 mg/kg intramuscular (i.m.) injections of nandrolone decanoate (Deca-Durabolin®, purchased from La Farmaceutica, Castronno, VA) or vehicle (peanut oil). The dose of nandrolone was chosen according to the literature, and corresponds to heavy use of nandrolone (Lindblom et al., 2003; Pope and Katz, 1994). Rats were injected from PND 40 to 53, corresponding to the adolescent period (Spear, 2000).

With this protocol we mimicked human beings using nandrolone mostly during adolescence, but showing mental disorders later in life. This protocol was successfully applied to cannabis (Bambico et al., 2010) and amphetamine (Labonte et al., 2012) adolescent use. Furthermore, the dose (40–50 times greater than those used clinically) was chosen in order to mimic the doses used by human steroid abusers.

All groups were then subjected to a resting period of 14 days or longer since nandrolone decanoate has a half-life of 5.4 days in rats when administrated i.m. (van der Vies, 1985). Behavioral and electrophysiological experiments were conducted (PND 68–85) under drug-free conditions (see Fig. 1 for a scheme of the experimental design). Different cohorts of animals were used for behavioral and electrophysiological experiments. All procedures were approved by the McGill University Animal Care Center and the Canadian Office of Controlled Substances, and were performed in accordance with the guidelines released by the Italian Ministry of Health (D.L.116/92) and (D.L.111/94-B), and the European Community directives regulating animals research (86/609/EEC), with all efforts made to minimize the number of animals used and their suffering.

2.2. Body weight monitoring

At PND 40 rats were weighed and divided into two equivalent groups according to their body weight (199.9 ± 5.3 g and 207.3 ± 5.5 g for the vehicle and nandrolone treated group, respectively). Body weight was monitored twice a week to assess the consequences of the treatments (PND 40–53) and continued until the beginning of the experiments (PND 68).

2.3. Behavioral testing

Behavioral tests were performed on separate groups of animals between PND 68 and PND 72.

2.3.1. Forced swim test (FST)

Animals were tested in a modified version of the FST with only one session of swimming as previously reported (Realini et al., 2011), because we aimed at measuring an existing behavioral deficit induced by the pharmacological manipulation. Briefly, rats were placed in a clear 50 cm tall, 20-cm-diameter glass cylinder filled to 30 cm with 25 °C water for 15 min. The session was videotaped for later analysis of the following parameters: immobility (time spent floating, moving only when necessary to keep head above the water), swimming (active swimming movements, climbing (forceful thrashing movements with forelimbs against the walls of the cylinder). An experimenter blind to the treatment groups analyzed the videos.

2.3.2. Sucrose preference test (SPT)

Sucrose preference test (*SPT*). Rats were tested for preference of a 1% sucrose solution, using a two-bottle choice procedure as reported in Rubino et al. (2008). Animals' preference was evaluated for one night. Briefly, animals were housed singly during the night of the test: they had free choice between the sucrose bottle and the bottle of tap water. The following morning the amount of sucrose and water consumed was evaluated as grams of liquid consumed, and sucrose preference index was thus calculated: [Sucrose (g)/(Sucrose (g) + Water (g))] × 100.

2.3.3. Open field (OF)

The open field arena is a Plexiglas box (60 × 60 × 40 cm) virtually divided into four compartments and a central squared area (15 cm × 15 cm). A video camera was mounted above the open field arena. Animals were transported to the experimental room 1 h before the session started. At the beginning of the test session, each rat was placed in the same corner of the open field and allowed to explore the arena for 10 min while it was videotaped. The number of entries, the time spent in the center zone and the total distance traveled were monitored.

2.3.4. Elevated plus maze (EPM)

The elevated plus-maze comprised 2 open arms (50 cm × 10 cm) and 2 enclosed arms (50 cm × 10 cm × 40 cm) that extended from a common central platform (10 cm × 10 cm). The apparatus, constructed from gray iron, was elevated 50 cm above floor level. Testing was conducted in a quiet room under dim light (30 lux) during the early light phase (9 AM–1 PM) of the light cycle. For all sessions, rats were individually placed on the central platform facing an open arm and allowed to freely explore the maze for 5 min. The maze was thoroughly cleaned between each session with acetic acid 0.1%. Behavioral parameters comprised both conventional spatiotemporal and ethological measures (Rodgers et al., 1997). Conventional measures were the frequencies of open and closed arm entries (arm entry = all 4 paws into an arm) and the time spent in open, closed, and central parts of the maze. Ethological measures comprised frequency scores for head-dips (HD: exploratory movement of head/shoulders over the side of the maze), stretched attend postures (SAP: exploratory posture in which the body is stretched forward then retracted to the original position without any forward locomotion) and closed arm returns (CAR: exiting closed arm with forepaws only and doubling back into the same arm).

2.4. In vivo electrophysiological recordings of serotonergic and noradrenergic neurons

Rats were randomly assigned to one of two groups, one exclusively for serotonergic recordings and the other exclusively for NA recordings, alternately conducted