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Chronic nandrolone administration induces dysfunction of the reward pathway in rats

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ABSTRACT

Data in animal models and surveys in humans have revealed psychiatric complications of long-term anabolic androgenic steroid abuse. However, the neurobiochemical mechanisms behind the observed behavioral changes are poorly understood.

The aim of the present study was to investigate the effects of nandrolone decanoate on emotional behavior and neurochemical brain alterations in gonadally intact male rats.

The behavioral reactivity to the elevated plus maze and the social interaction test was used to assess anxiety-related symptoms, and the sucrose preference test was used to evaluate anhedonia. Dopaminergic, serotonergic and noradrenergic transmissions were also evaluated in selected brain areas.

The chronic administration of nandrolone, at 5 mg kg^{-1} injected daily for 4 weeks, induced the loss of sweet taste preference, a sign of anhedonia and dysfunction of the reward pathway. The behavioral outcomes were accompanied by reductions in the dopamine, serotonin and noradrenaline contents in the nucleus accumbens. Alterations in the time spent in the open arms and in the social interaction test were not found, suggesting that nandrolone did not induce an anxiogenic profile. No differences were revealed between the experimental groups in the amygdala in terms of the neurotransmitters measured.

Our data suggest that nandrolone-treated rats have a depressive, but not anxiogenic-like, profile, accompanied by brain region-dependent changes in dopaminergic, serotonergic and noradrenergic neurotransmission. As anabolic androgenic steroid dependence is plausibly the major form of worldwide substance dependence that remains largely unexplored, it should be highlighted that our data could contribute to a better understanding of the altered rewards induced by nandrolone treatment and to the development of appropriate treatments.

1. Introduction

Although originally developed for clinical purposes, anabolic androgenic steroids (AAS) are currently used not only among high-profile and non-professional athletes as performance enhancing drugs but also among the general population, especially adolescents, to improve physical appearance and increase self-esteem [1,2]. Thus, the adverse effects of AAS rapidly became of significant concern from a public health perspective, outside of sports. In this regard, several studies have reported numerous peripheral side effects [3].

Moreover, central nervous system processing and behavioral output have been reported to be altered. Both data in animal models and large population surveys in humans have shown that AAS misuse could induce numerous adverse psychiatric effects, including the lack of impulse control, labile mood with quick swings and depression [see [4] for review], although AAS-induced alterations have been shown to be dependent upon the type of AAS administered. However, constellations of personality traits have been related to several biological parameters.

Until now, the neurobiochemical mechanisms behind the observed behavioral changes have been poorly understood, although the wide range of altered behaviors observed in animals subjected to AAS administration suggests a widespread involvement of different signaling systems in the brain.

In this regard, recent animal studies have found that signaling mediated by both classical neurotransmitters and neuromodulatory peptides in the brain is significantly altered and that these alterations could be related to different AAS-induced behavioral

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changes [5,6]. Brain opioid peptides have been demonstrated to be involved in the addictive behavior after AAS misuse [see [7] for review]. Matrisciano and co-workers recently showed that repeated injections of AAS, at doses considered equivalent to those abused by humans, induced behavioral changes and alterations in neurotrophin levels suggestive of a depressed state in rats [8].

Three systems considered of particular interest are the neurotransmitters dopamine (DA), 5-hydroxytryptamine (5-HT) and noradrenaline (NA). They have an evident role in shaping mood and behavior. In particular, sub-chronic treatment with nandrolone alters DA metabolism in brain regions involved in the development of drug dependence, such as the nucleus accumbens (NAc) [9]. Moreover, dysfunction in 5-HT neurotransmission has been associated with disinhibitory behavior [6,10], and early AAS exposure showed anxiogenic effects during withdrawal partly modulated by 5-HT signaling [11]. Elevated levels of hypothalamic noradrenergic and serotonergic amines were also found [12]. Our previous study supported the idea of numerous neurochemical alterations in the brain after the chronic administration of AAS. We found alterations in the DA, 5-HT and NA contents in several brain areas of stanozolol-treated rats [13].

Thus, the aim of the present study was to characterize the neurobehavioral and neurochemical profiles induced by nandrolone decanoate. As reported by the World Anti-Doping Agency, after stanozolol, nandrolone is the second most used prohibited drug in all sports [14].

Few data exist on the impact of nandrolone treatment on anxiety and depressive behavior, with even less on the aminergic systems as neurochemical correlates for behavioral alterations in selected brain areas. Therefore, here we investigated the effects of chronic nandrolone on the behavioral reactivity to the elevated plus maze (EPM) [15] and social interaction tests [16], two "gold standard tests" for anxiety-related behaviors. Additional testing of anhedonia, a cardinal symptom of depression [17], was performed using the sucrose preference test.

Moreover, we investigated the neurochemical alterations induced by chronic nandrolone treatment, studying key biological systems relevant for anxiety- and depression-induced alterations of neural plasticity, such as the DA, 5-HT and NA contents in two brain regions predominantly involved in regulating emotional responses, the NAc and amygdala (Amy).

2. Materials and methods

2.1. Animals

Male Wistar rats (Harlan, S. Pietro al Natisone, Udine, Italy) weighing 225–250 g were used. They were housed at constant room temperature (22 ± 1 °C) and relative humidity (55 ± 5%) under a 12-h light/dark cycle (lights on from 7:00 AM to 7:00 PM). During normal housing conditions, food and water were freely available. The procedures involving animals and their care were conducted in conformity with the institutional guidelines of the Italian Ministry of Health (D.L. 116/92), the Declaration of Helsinki, the Guide for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council 2004), the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Adequate measures were taken to minimize the number of animals used and their pain or discomfort.

2.2. Animal treatment

Different groups of gonadally intact, experimentally naive male rats were randomly assigned according to the experimental protocols. For each experimental group, rats received a subcutaneous (s.c.) injection of nandrolone decanoate (5 mg kg^{-1}, Sigma, Milan, Italy) or vehicle (polyethylene glycol, PEG, 1 ml kg^{-1}, Carlo Erba Reagents, Milan, Italy) once daily, 5 days per week for 4 weeks. The dosage was chosen on the basis of the previously described model [18]. The animals were weighed daily; at the end of the treatment, they were sacrificed, and the testis weight was assessed. Each experimental procedure was conducted before 2:00 PM, 24 h after the last injection of nandrolone decanoate, and different sets of animals were used for each behavioral test (n = 10/group) and for the neurochemical analysis (n = 8/group).

2.3. Sucrose preference test

The sucrose preference test was performed in the animal's individual cages, where non-food-deprived rats were given a 48-h two-bottle exposure, one containing a 2% sucrose solution and the other containing water according to the protocol previously described by Monteggia et al [19].

To prevent a potential locational drinking preference, the position of the bottles was changed after 24 h. At the end of the test, the bottles were removed and weighed to determine the amount of sucrose solution or water consumed. The preference for the sucrose solution was calculated as the percentage of sucrose solution ingested relative to the total amount of liquid consumed.

2.4. Social interaction test

The social interaction procedure was adapted from File et al. [16]. The test was performed in a circular open arena (made of dark plastic; diameter 60 cm; height 31 cm), unfamiliar to the animals and placed in a highly lit room. To increase social interaction, the rats were single-housed for 5 days before the test [20]. On the day of testing, all rats were weighed, and pairs were assigned on the basis of weight and treatment (nandrolone-treated rat was tested with a vehicle-treated partner on the basis of weight, ensuring that they did not differ by more than 10 g). The animals were marked on their back and placed head to head simultaneously in the arena, and their behavior was recorded for 10 min by a camera mounted vertically above the test arena. During quantification, the observer, who was blinded to the experimental conditions, scored the total time that each rat spent performing the following behaviors: *sniffing* (sniffing several body parts of the other rat, including the anogenital region), *following* (moving towards and following behind the other rat around the arena), *climbing* (climbing over and under the conspecific's back), *aggressive behavior* (including kicking and boxing) and *exploratory behavior* (rearing and wall rearing).

2.5. Elevated plus maze test

The experimental procedures were performed according to Pellow et al. [15]. Briefly, the Plexiglas apparatus consisted of two opposite open arms (50 × 10 cm) without side walls and two closed arms (50 × 10 × 40 cm) extending horizontally at right angles from a central area (10 × 10 cm). The maze was situated in a separate brightly lit room illuminated with four, 32-W fluorescent overhead lights that produced consistent illumination within the room. The apparatus had similar levels of illumination on both the open and closed arms. The maze was elevated to a height of 50 cm in the lit room. At the beginning of the experiment, the rat was placed in the central platform facing an open arm and allowed to explore the maze for 5 min. The following parameters were analyzed: number of entries into the open and closed arms and the time spent in the open arms. An arm entry was counted when the hindpaws were placed on the open arm. Principal component