

Cocaine-induced locomotor activity is enhanced by exogenous testosterone

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Received 23 August 2001; received in revised form 19 February 2002; accepted 16 April 2002

Abstract

Anabolic–androgenic steroids are synthetic derivatives of testosterone, which are increasingly abused by adolescent populations who also abuse psychoactive substances. All these compounds lead to complex behavioral syndromes and the effects of their interactions remain unclear. The main aim of the present study was to determine the influence of testosterone on the locomotor activity-promoting effect of cocaine on male mice in an open field. In the three experiments, animals received two injections: firstly, testosterone or peanut oil, and secondly, cocaine or saline solution. In Experiments 1 and 2, testosterone (or oil) and cocaine (or saline) were injected 45 and 10 min, respectively, prior to activity recording. In the first experiment, we studied the effects of testosterone (2 mg/kg) on locomotor activity induced by different doses of cocaine (2, 4, 8, 10 or 12 mg/kg). In Experiment 2, we explored the effects of supraphysiological doses of testosterone (2, 6, 10 or 14 mg/kg) on animals treated with 10 mg/kg cocaine. Finally, in the third experiment, 14 mg/kg testosterone or vehicle was administered 15, 30, 45 or 75 min before activity data collection to animals that received 10 mg/kg cocaine or saline. Testosterone itself had no effects on spontaneous locomotor activity and, as was expected, cocaine increased locomotor activity dose-dependently. Given together, testosterone enhanced the cocaine-induced hyperactivity although not dose-dependently, the highest effects being found 45 min after testosterone injection. The present study confirmed the existence of an interaction between testosterone and cocaine at the central nervous system. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: Anabolic–androgenic steroids; Testosterone; Cocaine; Polyabuse; Locomotor activity

1. Introduction

Anabolic–androgenic steroid (AAS) abuse leads to a complex syndrome that includes a variety of physiological and psychological symptoms. Cardiovascular disorders, hypogonadism and hepatic injuries have been described as physiological side effects of abuse. Effects on positive mood such as euphoria, feeling of well being or increased self-esteem have been reported with early use. Additionally, the psychotoxic effects associated with prolonged abuse such as increased aggression, psychotic episodes, sexual disturbances, insomnia, dependence and, after ceasing its administration, depressive manifestations have also been reported [2,5,26,45].

The effects of these compounds, which are synthetic derivatives of natural testosterone, on behavior could be a consequence of the action on the classical (lateral septal, medial preoptic, ventromedial hypothalamic and medial amygdaloid nuclei) as well as on the nonclassical (frontal cortex, locus coeruleus, hippocampus, substantia nigra and midbrain central gray) androgen target brain structures. While the classical androgen target sites have been related to the labeled sex-dimorphic social traits such as dominance, competition and aggressive and sexual behavior, the non-classical ones have been related to psychosis, addiction and affective properties [22,27,29].

Recent studies, using a conditioned place preference paradigm (CPP), have shown that peripheral, intraaccumbens and intramedial preoptic area administration of testosterone have rewarding properties in gonadally intact [1,7,20,34,35,39] and gonadectomized [11] male rats. These affective properties of testosterone seem to be related to dopaminergic neurotransmission; in fact, as Schroeder and

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Packard [39] have shown, the injections of a mixed dopamine (DA) D1/D2 receptor antagonist blocked the acquisition of testosterone CPP.

The misuse of AAS is, increasingly, concomitant to the use of other drugs of abuse [4]. DuRant et al. [13], in a descriptive study, found that adolescent AAS users are more likely to be engaged in using one or more other drugs such as cocaine, marijuana, alcohol and tobacco. The strongest factor covarying with AAS use was that of cocaine, which explained the highest percent of the variation in the frequency of use. It has been suggested that this fact could be related to the possible shared mechanisms involved in the actions of AAS and other mood-altering substances.

In order to further elucidate this phenomenon, the main aim of the present study was to determine a possible interaction between testosterone and cocaine on behavior. Specifically, the influence of an acute administration of testosterone on the locomotor activity-promoting effect of cocaine on male mice was investigated.

2. Materials and methods

The use and care of animals and the experimental protocol applied in this study complied with the European Community Council Directive of November 24, 1986 (86/609/ECC).

2.1. Subjects

Male Swiss–Webster mice, purchased from Janvier España (Madrid, Spain), were housed in groups of four per cage, with standard laboratory food and water available ad libitum. Animals, which were 6 weeks old and weighed 27–30 g, arrived at the laboratory separately for each experiment and were maintained on a reversed light/dark schedule (lights on from 1300 to 100 h, local time) under controlled temperature (22 ± 1 °C).

2.2. Drugs

4-Androsten-17 β -ol-3-one testosterone (Sigma, Madrid, Spain) was dissolved in peanut oil (Guinama, Valencia, Spain) at a concentration of 2, 6, 10 or 14 mg/10 ml. Animals were treated with 2, 6, 10 or 14 mg/kg testosterone, which is within a range of doses commonly used by abusers [25,43]. Each mouse was injected subcutaneously with testosterone or vehicle (peanut oil).

Cocaine (Sigma) was dissolved in physiological saline solution (0.9% NaCl) at a concentration of 2, 4, 8, 10 or 12 mg/10 ml. Experimental subjects were injected intraperitoneally with 2, 4, 8, 10 or 12 mg/kg, with controls receiving saline solution. The volume injected to each animal depended on its weight: body weight/100.

2.3. Apparatus

The open field apparatus consisted of a transparent glass cylinder 25 cm in diameter and 30 cm high with a floor divided into four identical quadrants by two intersecting lines drawn on the floor. A locomotion score (count) was assigned each time an animal crossed over from one quadrant to another with all four legs.

2.4. Procedure

One week after the arrival at the laboratory, mice were randomly allocated to treatment groups ($n=9-12$ per group). Testosterone or vehicle was injected 15, 30, 45 or 75 min before the recording of activity, whereas cocaine or saline solution was always injected 10 min before data registration. Animals were transferred from the housing to the experimental room 30 min before drug treatment.

During test sessions, mice were individually placed in the open field chambers for 20 min. Individual measures of locomotor activity were recorded only for the last 10-min period. These delays were chosen to decrease the effects of animal handling and the environmental novelty of the open field [12]. The behavioral room was illuminated by a dim light and maintained at 21 ± 1 °C, with the external noise attenuated. All tests were conducted between 1330 and 1730 h.

In the first experiment, we studied the effects of a single administration of 2 mg/kg testosterone or peanut oil 45 min before evaluation on animals that received different doses (2, 4, 8, 10 or 12 mg/kg) of cocaine or saline solution. In Experiment 2, we explored the effects of an acute administration of testosterone (2, 6, 10 or 14 mg/kg) or peanut oil 45 min before evaluation on animals that received 10 mg/kg cocaine or saline solution. This dose of cocaine was selected because, in the first experiment, its combination with testosterone was the most efficient at increasing spontaneous locomotor activity in this particular test design. Finally, 14 mg/kg testosterone or vehicle was injected 15, 30, 45 or 75 min prior to activity recording in combination with 10 mg/kg cocaine. This dose of testosterone was selected due to the fact it is within the range that the majority of the studies, which focus on the physical and behavioral effects of AAS abuse, usually employ to mimic the moderate range of abuse in humans.

2.5. Statistical analyses

Data were analyzed by means of two-factor ('Injection 1' and 'Injection 2') analyses of variance (ANOVA) for the main effects and their interactions. Fisher's least significant difference tests were performed to evaluate the differences between means. Values of P equal to or less than .05 were considered statistically significant. All statistical analyses were carried out using SPSS statistical package [32].

3. Results

3.1. Experiment 1

A two-factor ANOVA revealed a statistically significant effect of Testosterone [$F(1,11)=11.1$, $P<.001$], a significant effect of Cocaine [$F(5,11)=36.50$, $P<.001$] and a significant Testosterone–Cocaine interaction [$F(5,11)=2.55$, $P<.03$]. Pairwise comparisons using the Fisher's least significant difference tests showed that all animals that received cocaine plus peanut oil displayed higher locomotor activity dose-dependently, in comparison with controls ($P<.005$). Additionally, testosterone did not stimulate locomotor activity when compared with vehicle. However, in this study, the activity-promoting effect of cocaine was significantly enhanced by 2 mg/kg testosterone in those animals treated with 4 and 10 mg/kg ($P<.005$) (see Fig. 1).

3.2. Experiment 2

A two-factor ANOVA showed a significant effect of the doses of Testosterone [$F(4,9)=5.48$, $P<.001$] on the activity-promoting effect of 10 mg/kg cocaine [$F(1,9)=426.79$, $P<.001$] and a significant effect of the Testosterone \times Cocaine interaction [$F(4,9)=4.75$, $P<.002$]. The posthoc tests indicated testosterone enhancement of the cocaine-induced locomotor activity at all the doses ($P<.01$). There were no differences in locomotor activity between animals that received testosterone or vehicle within the respective groups (see Fig. 2).

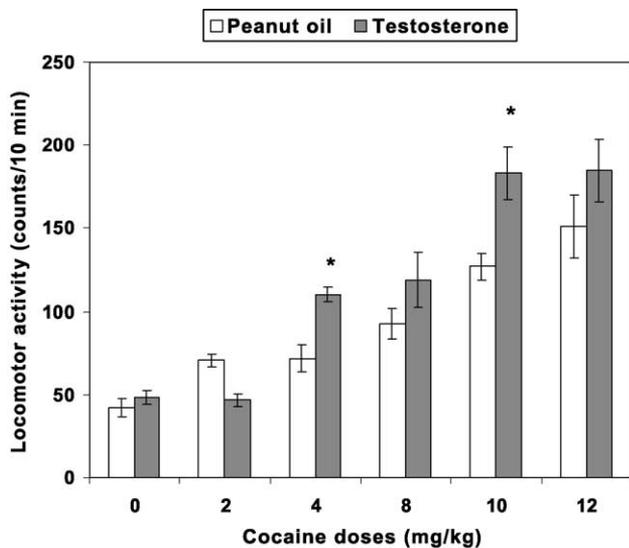


Fig. 1. Effect of testosterone or peanut oil on cocaine-induced locomotor activity. Mean (\pm S.E.M.) locomotor activity (counts in 10 min) for all treatment groups. Mice were pretreated with peanut oil or testosterone (2 mg/kg) 45 min prior to cocaine (2, 4, 8, 10 or 12 mg/kg). * $P<.01$ significantly different from those receiving peanut oil plus the same dose of cocaine.

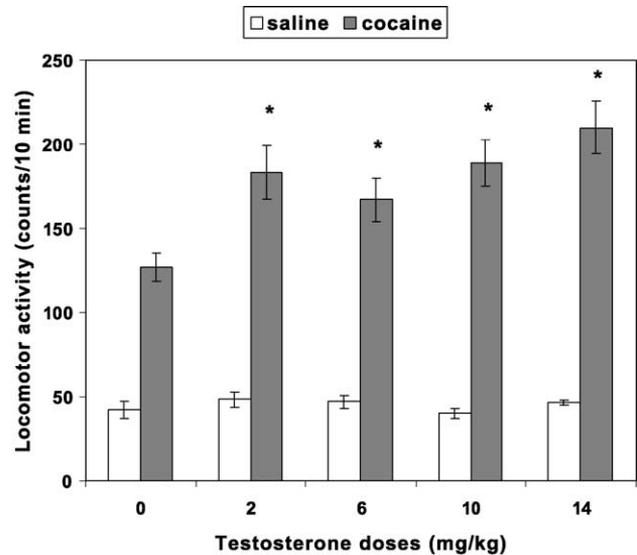


Fig. 2. Effect of different doses of testosterone on locomotion induced by cocaine. Mean (\pm S.E.M.) locomotor activity (counts in 10 min) for all treatment groups. Mice were pretreated with peanut oil or testosterone (2, 6, 10 or 14 mg/kg) 45 min prior to cocaine (10 mg/kg). * $P<.01$ significantly different from those receiving peanut oil plus 10 mg/kg cocaine.

3.3. Experiment 3

A two-factor ANOVA showed a significant effect of the Time interval between both administrations [$F(3,7)=2.81$, $P<.05$] and a significant effect of Time \times Testosterone interaction on locomotor effect of cocaine [$F(3,7)=6.71$, $P<.001$]. The highest promotion of locomotor activity induced by 10 mg/kg cocaine was found 45 min after the injection of 14 mg/kg testosterone ($P<.001$) (see Fig. 3).

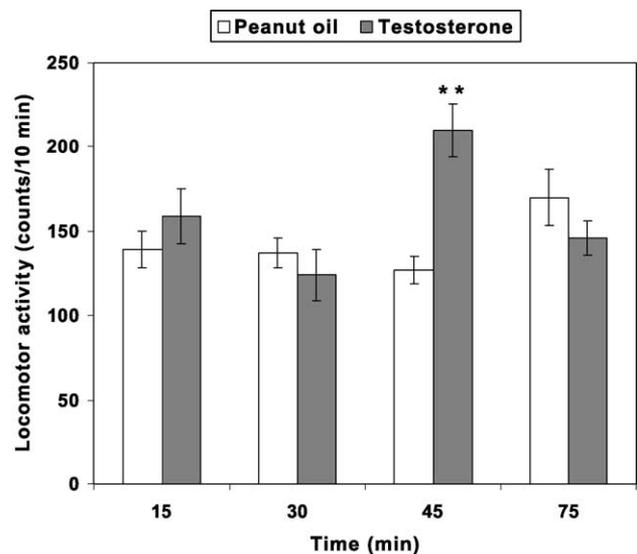


Fig. 3. Time course of testosterone effect on cocaine-induced locomotor activity. Mean (\pm S.E.M.) locomotor activity (counts in 10 min) for all treatment groups. Mice were pretreated with peanut oil or testosterone (14 mg/kg) 15, 30, 45 or 75 min before the cocaine injection (10 mg/kg). * $P<.001$.

4. Discussion

In the present work, testosterone contributed to increase the cocaine-induced enhancement of activity although not dose-dependently, which indicates a possible “plateau” effect. In the first experiment, 2 mg/kg testosterone enhanced the activity induced by 4 and 10 mg/kg cocaine, whereas there were no statistically significant differences between animals that received 12 mg/kg cocaine plus testosterone and those treated with 12 mg/kg cocaine plus peanut oil. According to the results shown in Fig. 1, although the locomotion among 12 mg/kg-treated mice is slightly higher than in the 10-mg/kg group, the lack of statistical significance of these differences seems to be due to a higher activity among the controls in the 12-mg/kg group. With respect to the temporal interval between injections, the highest effects were found 45 min after testosterone injection, which coincides with the time in which the highest serum testosterone levels have been found after exogenous administration [3]. The mechanisms involved in the “testosterone–cocaine” interaction effect could be those participating in the activation of the reward system. Thiblin et al. [42] have found that male rats receiving one of four different AAS (testosterone, nandrolone, methandrostolone and oxymetholone) showed an increment in DA and serotonin (5-HT) metabolism in the striatum and hippocampus, respectively. These effects could be due to an increased neuronal activity in these brain regions, which regulate affective, emotional and motivational behavior. Thus, the positive effects of AAS on mood are likely to reflect the DA neuronal activity at the mesolimbic system, which is connected to reinforcement behavior.

Consequently, cocaine and testosterone could share the locus of action in their affective properties. In fact, other studies using different AAS and a different duration of administration have shown a similar interaction. For example, the chronic treatment with an AAS for 14 consecutive days had no effect on the reward of intracranial self-stimulation, although a longer treatment with a cocktail of several AAS altered the sensitivity of the brain to amphetamine treatment [9]. Hruska and Silbergeld [16] reported an increment of the dopaminergic sensitivity and receptor density in male rats after estradiol administration and, as is well known, androgens modulate behavior through organizational or activational effects either directly or via the action of its metabolites estradiol or dihydrotestosterone [33]. To our knowledge, only one study has explored the action of androgen administration on the locomotor activity induced by cocaine [23]. In that study, they found that long-term treatment with testosterone prevented the enhancement of locomotor activity produced acutely by cocaine in male rats. The differences between our study and that of Long et al. [23] could be due to the different duration of the treatment, which in their case led to a possible down-regulation of D2 receptors, that probably did not occur with a single testosterone injection. In fact,

Kindlundh et al. [19] have found that a chronic administration of AAS down-regulated D1 and D2 receptor densities in the caudate–putamen and the nucleus accumbens.

The present experiments revealed no direct effects of a wide range of doses of testosterone on spontaneous locomotor activity in male mice, as was found in previous experiments testing different doses and time courses [37]. Several studies using animal models have shown decrements or no changes in spontaneous locomotor activity displayed by gonadally intact male rodents after single or repeated injections of either an individual steroid (testosterone propionate, nandrolone decanoate or stanozolol) or a mixture of several AAS [28,30,37]. Conversely, the use of testosterone for medical purposes at therapeutic doses has induced euphoria and increased energy in young and elderly populations [8,38,40,41]. Additionally, some heavy AAS abusers have frequently reported maniac and hypomaniac episodes, hyperactivity and decreased fatigue [5,15,21,36].

On the contrary and, as was expected, cocaine enhanced locomotor activity dose-dependently. The activity-promoting effects of cocaine are well known and are caused by DA increments in the nigrostriatal and mesoaccumbens system [6,18]. Its influence on other androgen-dependent behaviors such as aggression has been studied in the last decade. This latter action seems to be due to increases in noradrenaline and DA neurotransmitters and decreases in 5-HT levels [10,14,17,24,31,44]. Additionally, these monoamines are also affected by androgen action at the central nervous system [42].

In summary, the present study gives support to the hypothesis of an interaction between AAS and other psychoactive substances, which probably involves the rewarding system. Further research on interactions between AAS and psychoactive substances other than testosterone and cocaine would be necessary to explore the mechanism at the central nervous system and its connection with the rewarding system. For this purpose, it could be interesting to test the long-term administration of AAS and the action of DA agonists and antagonists.

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