Dopaminergic and noradrenergic drugs revert cocaine-induced erection in paradoxical sleep-deprived rats

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ABSTRACT

Objectives: Evidence suggests that paradoxical sleep deprivation induces dopaminergic supersensitivity and noradrenergic subsensitivity. Previous studies have demonstrated that 96 hours of paradoxical sleep deprivation enhance cocaine-induced penile erection and ejaculation. The present study investigated the influence of the dopaminergic agonist apomorphine and noradrenergic antagonist propranolol in reverting genital reflexes potentiated by cocaine. Methods: Rats were exposed to 96 hours of paradoxical sleep deprivation and received acute and chronic intraperitoneal injections of apomorphine (120, 240 and 480 μg/kg) or propranolol (2, 4 and 8 mg/kg) before being challenged with cocaine (7 mg/kg). Acute or chronic apomorphine (240 and 480 μg/kg) administration reduced the proportion of animals that displayed erection. Results: Acute propranolol administration induced a decrease in the proportion of animals displaying penile erection only at the lowest dose. Penile erection frequency decreased after acute and chronic propranolol treatment. Conclusions: This study indicated that dopaminergic supersensitivity and noradrenergic subsensitivity have critical roles in the regulation of sexual reflexes.

Keywords: Sleep deprivation; Cocaine; Sexual behavior, animal; Ejaculation/drug effects; Penile erection/drug effects; Genitalia, male/drug effects; Dopamine/therapeutic use; Apomorphine/therapeutic use; Propranolol; Animals; Rats

INTRODUCTION

There is great interest in the consequences of paradoxical sleep deprivation (PSD) techniques. Most consequences are thought to be controlled by central neurotransmitter systems and have been implicated in several behavioral alterations. Therefore, it is reasonable to predict that the behavioral alterations observed after PSD result from changes in the modulation of brain neurotransmitters occurring after selective sleep loss.

Experiments involving PSD employing the water tank technique have shown that this procedure alters behaviors that can be induced by drugs, acting on many neurotransmitter systems. A number of studies have reported a marked behavioral supersensitivity to directly- and indirectly-acting dopamine agonists after being subjected to a period of PSD. These findings have led to the hypothesis that modification in sensitivity to a dopaminergic drug by PSD is due to functional hyperactivity of the dopaminergic system. Furthermore, downregulation of the noradrenergic system has been reported.

Among the behavioral alterations investigated in PSD rats, sexual behavior has drawn increased attention in recent years. Our laboratory group has consistently studied...
the stimulatory effect of PSD in male rats on genital reflexes such as penile erection (PE) and ejaculation (EJ). It has been shown that dopaminergic drugs such as cocaine\textsuperscript{(12)}, methamphetamine\textsuperscript{(13)} and other dopaminergic agonists\textsuperscript{(14,15)} enhance the effect of PSD. Additionally, noradrenergic mechanisms have been reported to be involved in PE induced by sleep deprivation and cocaine\textsuperscript{(16)}.

Pharmacological studies have been performed in an attempt to identify the neurotransmitter systems involved in the regulation of sexual behavior in male rats. Although the effect of dopaminergic agonists in facilitating male sexual behavior is well documented\textsuperscript{(17-20)}, the role of noradrenergic transmission is less clear and has received less attention.

Considering that dopaminergic\textsuperscript{(5,8)} and noradrenergic\textsuperscript{(11)} receptor sensitivity is altered by PSD, that both systems are related to sexual function\textsuperscript{(17)}, and that dopamine and noradrenaline play relevant roles in genital reflexes induced by cocaine in PSD rats, the present study was designed to determine whether a dopaminergic agonist (apomorphine) and noradrenergic antagonist (propranolol) could revert the occurrence of spontaneous genital reflexes potentiated by cocaine in PSD rats.

**METHODS**

**Subjects**

Naïve male Wistar rats were bred and raised in the animal facility of the Department of Psychobiology of the Escola Paulista de Medicina of Universidade Federal de São Paulo (UNIFESP). The animals were housed in a colony maintained at 22°C with 12:12 h light:dark cycle (lights on at 07:00 a.m.) and allowed free access to food and water inside standard polypropylene cages. Rats were maintained and treated in accordance with the guidelines established by the Ethical and Practical Principles of the Use of Laboratory Animals\textsuperscript{(21)}. The experimental protocol was approved by the Ethical Committee of UNIFESP (CEP N. 482/02).

**Paradoxical sleep deprivation**

The procedure consisted of placing ten rats in a tiled water tank (123 x 44 x 44 cm) for 96 hours. The tank contained 14 platforms (6.5 cm in diameter) rising 1 cm above the water surface, thus allowing the rats to move around by leaping from one platform to another. At the onset of each paradoxical sleep episode, the animal experiences a loss of muscle tone and falls into the water, thus being awakened. When platforms of this size are used, previous work from this laboratory demonstrated that paradoxical sleep is completely abolished during the four-day period\textsuperscript{(22)}. Slow wave sleep is significantly reduced but does not lead to rebound sleep. It therefore seems appropriate to refer to these animals as being paradoxical sleep deprived rather than being exclusively deprived of sleep. The cage control group was maintained in the same room as the experimental rats throughout the study and showed normal patterns of paradoxical sleep, slow wave sleep and wake. Throughout the study, the experimental room was maintained under controlled temperature and a light:dark cycle. Food and water were available ad libitum, with chow pellets and water bottles provided on a grid located on the top of the tank. The water in the tank was changed daily throughout the PSD period. The duration of 96 hours of PSD was chosen since it has been shown that most genital reflexes are produced during this period of time\textsuperscript{(12)}.

**Drugs**

Cocaine hydrochloride (Sigma Chemical Co., St. Louis, MO, USA) was freshly dissolved in a vehicle of 0.9% sterile saline solution for injection. Apomorphine and propranolol (Sigma Chemical Co., St. Louis, MO, USA) were freshly dissolved in a vehicle of 0.9% sterile saline solution and two drops of Tween. The injection volume for all systemic studies was 1 mL/kg, and the route of administration was intraperitoneal injection.

**Experimental design**

The rats were subjected to PSD (n=10-11/group). In acute treatment, each group was administered with apomorphine or propranolol and was returned immediately to the tank. One hour later, the animals were administered intraperitoneally with cocaine (7 mg/kg) and placed immediately in the observation cages for the evaluation of genital behaviors. In chronic treatment, rats were administered with apomorphine (120, 240 and 480 μg/kg) or propranolol (2, 4 and 8 mg/kg) twice per day (09:00 a.m. and 4:00 p.m.) every day during the four days of the PSD protocol. The PSD + cocaine group was pretreated with sterile saline. Rats were randomly distributed into seven distinct treatment groups (apomorphine: APO and propranolol: PRO): (1) PSD+coc; (2) PSD+APO120; (3) PSD+APO240; (4) PSD+APO480; (5) PSD+PRO2; (6) PSD+PRO4; (7) PSD+PRO8. At the end of the PSD period, rats were placed in wire mesh cages for behavioral observations.

**Penile erection evaluation**

The animals were observed in experimental wire mesh cages (15 x 31 x 26 cm) containing neither water nor food. The behavioral observations were carried out between 08:00 a.m. and 11:00 a.m. in a temperature-controlled room by trained observers with inter-rater reliability established in previous studies. The observers were unaware of group assignments of the rats under observation.

Penile erection (PE) was counted only when the rat stood on its hind limbs, bent its body forward, bent its
head down to reach the genital area, held and licked its penis in full erection and displayed hip movements. The erect penis was always visible. The proportion of rats displaying genital behaviors, the frequency of PE (total number of genital reflexes divided by the number of rats) and latency (time elapsed between the injection to the first genital reflex) were assessed for 60 minutes. Each animal was tested only once.

Statistical analysis
For statistical analysis of the proportion of animals displaying PE (expressed in percentage), the Chi-square method was used to assess differences between groups. Homogeneity of variance was assessed by the Bartlett test, and normal distribution of the data was assessed by the Kolmogorov-Smirnov test. When the Bartlett test showed absence of homogeneity of variance, data were square transformed. One-way analysis of variance (ANOVA) was used to analyze the frequency and latency data to determine possible group effects. In order to make specific group comparisons, the post hoc Tukey test was performed. Values shown are expressed as mean ± standard error of mean (SEM). The value of p<0.05 was used as the criterion for statistical significance.

RESULTS

Effects of acute and chronic apomorphine administration
Figure 1 shows the effect of acute and chronic apomorphine administration in PSD+coc rats. As expected, a single cocaine injection (7 mg/kg) increased the proportion and frequency of PE after four days of PSD. Chi-square analysis indicates that there was no significant difference in the proportion of PE in rats that received the lowest dose of apomorphine (120 μg/kg). However, the high doses (240 and 480 μg/kg) administrated acutely and chronically reduced PE compared to the PSD+coc group, as showed in Figure 1A.

As shown in Figure 1B, one-way ANOVA revealed significant group effects in the frequency of PE after acute and chronic apomorphine treatment \([F_{(6,66)}=5.61, p<0.001]\). Tukey post hoc test showed that acute (240 and 480 μg/kg) and chronic (all doses) apomorphine administration decreased the frequency of PE as compared to PSD+coc rats (p<0.05). No statistically significant alteration was found in latency to first erection after acute or chronic apomorphine administration \([F_{(6,35)}=1.58, p=0.18]\) (data not shown).

Effects of acute and chronic propranolol administration
In PSD+coc rats, chronic propranolol administration (2 mg/kg) induced a decrease in PE proportion. In the other propranolol groups, there was no significant altera-
tion in the proportion of animals displaying erection when compared to PSD+coc animals, as shown in Figure 2A.

Figure 2B shows the effects of acute and chronic propranolol administration on PE frequency in rats challenged with cocaine. The ANOVA followed by Tukey test \( F_{5,651} = 3.72, p<0.004 \) revealed that the PE frequencies observed in the acute and chronic propranolol groups were significantly lower than PSD+coc rats. Examination of PE latency and one-way ANOVA revealed no significant difference between the groups after acute or chronic propranolol treatment \( F_{(6,47)} = 1.04, p=0.41 \) (data not shown).

**DISCUSSION**

These results demonstrate that acute and chronic apomorphine administration in rats challenged with cocaine reduces the proportion of animals displaying erection and PE frequency. Acute propranolol administration induced a decrease in PE proportion only at the lowest dose. However, PE frequency decreased after both acute and chronic propranolol treatment.

Several systems play critical roles in the regulation of male sexual behavior. It was recently demonstrated that dopaminergic agonists have facilitator properties for PE and may also enhance sexual drive\(^{17}\). Dopamine has facilitator effects on sexual motivation, copulatory proficiency and genital reflexes. It is well recognized that sexual stimulation leading to PE is controlled by different areas in the brain\(^{23}\). Apomorphine induces dose-dependent increases in PE in PSD-treated rats \(^{13}\) but does not modify EJ. In addition, the response to apomorphine decreased by increasing the drug doses. This biphasic effect of apomorphine has been previously shown\(^{24-26}\).

Studies suggest that the adrenergic system may exert a relevant function in male copulatory behavior\(^{27}\). Systemic administration of subtype selective adrenoreceptor agonist and antagonist allows discrimination of the roles of these receptors in the modulation of sexual behavior in male rats. Previous investigations have found that agonist and antagonist \(\alpha\)-1, \(\beta\)-1, \(\beta\)-2 and antagonist \(\alpha\)-2 reduce genital reflexes, and that only \(\alpha\)-2 agonist does not induce alterations in sexual behavior\(^{16}\).

Cocaine, considered by most to be the world’s most addictive drug, produces its powerful euphoric effect by acting on monoamine neurons to produce acute psychomotor activation and long-term changes, including psychosis and addiction. These effects are the result of cocaine-induced activity in dopaminergic pathways that project to the neostriatum and nucleus accumbens\(^{28}\). Although cocaine has a variety of pharmacological actions, one of its major activities is binding the DA transporter to prevent dopamine uptake into presynaptic neurons, thus increasing synaptic concentrations of dopamine\(^{29}\). This leads to behavioral effects in animals and humans. However, an effect of cocaine on the noradrenergic system cannot be excluded\(^{30}\).

In rats, cocaine administration induces a significant increase in stereotypical behavior\(^9\). PSD induces significant but heterogeneous effects in animals such as increased grooming. PSD has no effect on stereotypical behavior, locomotion or anxiety-like behaviors, but it significantly decreases rearing behavior. PSD potentiates the action of cocaine on stereotypical behaviors, suggesting alteration of central mechanisms. Thus, the behavioral effects of cocaine may be modified by PSD.

PSD induces a marked increase in percentage of rats displaying genital reflex events like PE and EJ after acute cocaine injection\(^{12}\). Evidence indicates that PSD results in dopaminergic supersensitivity\(^{5,8,31}\) and noradrenergic subsensitivity\(^{11}\) and that central compounds alter the effects of PSD in rats\(^{5,7}\). Our group has observed increased stereotypical behavior induced by apomorphine\(^9\) and cocaine\(^9\) after 96 hours of PSD. The effects of cocaine\(^9\) and amphetamine\(^{12}\) in PSD rats are consistent with previous findings that suggest participation of dopaminergic supersensitivity in behavioral alterations induced by PSD\(^5,7\). Facilitation of sexual behavior by cocaine\(^{12}\) and other dopaminergic drugs in PSD rats\(^{10}\) in addition to the present findings suggests that dopaminergic \(D_2\) receptor supersensitivity may account for the PSD-induced effects on behavior altered by cocaine.

The aim of this study was to investigate whether a dopaminergic agonist and a noradrenergic antagonist could revert the occurrence of spontaneous genital reflexes potentiated by cocaine in PSD rats. The findings demonstrated that rats receiving acute and chronic treatment with apomorphine (\(D_2\)\(>D_1\) dopamine receptors nonselective agonist) and propranolol (nonselective \(\beta\)-receptor antagonist) display reversion of dopaminergic supersensitivity and subsensitivity of the \(\beta\)-adrenergic receptor induced by PSD.

In summary, the present findings confirm our previous work, demonstrating that the combination of sleep deprivation and cocaine evokes a marked increase in genital reflexes in male rats, which suggests critical involvement of dopaminergic and noradrenergic systems in this phenomenon. Since sexual behavior and sleep are complex behaviors that result from a well-synchronized string of neurochemical events that in turn trigger a network of neurotransmitters, it can be assumed that any disruption of these elements may promote significant and broad consequences, such as peripheral and central nervous system alterations rather than modifying only two neurotransmitter receptor pathways.

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