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Cocaine-induced genital reflexes in paradoxical sleep deprived rats: Indications of mediation by serotonin receptors

Monica L. Andersen *, Isabela B. Antunes, Sergio Tufik

Department of Psychobiology-Universidade Federal de São Paulo, Escola Paulista de Medicina (UNIFESP/EPM), Brazil

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Abstract

As paradoxical sleep deprivation (PSD) modifies cocaine-induced genital reflexes (penile erection [PE] and ejaculation [EJ]) and since cocaine is a serotonin (5-HT) reuptake inhibitor, we hypothesized that 5-HT also plays a role in these genital reflexes in PSD male rats. After a 4-day period of PSD each group was administered with serotonergic drugs prior to cocaine and placed in observation cages. The selective 5-HT_1 agonist (8-OH-DPAT) completely abolished PE events whereas the antagonist (pindolol) did not produce significant effects in the number of animals displaying PE. It was found that both drugs reduce the frequency of PE. There were no significant effects on the number of animals that ejaculated or in its frequency after pindolol although both parameters were reduced by the agonist at the highest doses (2 and 4 mg/kg, SC). Pretreatment with the 5-HT_2 agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) (0.12; 0.5 and 1 mg/kg, SC) significantly reduced the number of rats displaying PE and all doses reduced both PE and EJ frequencies. The number of animals displaying PE after 5-HT_2 antagonist (ketanserin) pretreatment at 1 and 2.5 mg/kg doses was significantly decreased in relation to vehicle rats and all doses reduced PE frequency. 5-HT_2 compounds at any dose did not affect the number of animals ejaculating, but the frequency was significantly reduced by all doses of DOI and by 1 to 5 mg/kg doses of ketanserin. Taken together, the results suggest that serotonergic receptors play an important role in genital reflexes induced by cocaine in sleep deprived males.

Keywords: cocaine; DOI; DPAT; ketanserin; pindolol; serotonin receptors; sexual behavior; sleep deprivation

1. Introduction

A great deal of experimental evidence indicates that sexual behavior in male rats is stimulated by treatments that decrease brain serotonin (5-HT) concentration. For instance, *p*-chlorophenylalanine (PCPA), a synthesis inhibitor, enhances sexual activity (Malmnas and Meyerson, 1971). Conversely, the augmentation of serotonergic activity also elicits spontaneous erectile and/or ejaculatory responses (Berendsen and Broekkamp, 1987).

The existence of multiple receptors for 5-HT in brain tissue is well established (Glennon, 1990). Studies employing receptor subtype selective ligands have suggested a complex influence of 5-HT on the male sexual behavior, with the various subtypes of central receptors subsuming differing roles in the expression of sexual behavior (Foreman et al., 1989; Gorzalka et al., 1990, 2001; Ahlenius and Larsson, 1998). It has been repeatedly demonstrated that the 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT), facilitates sexual behavior of male rats (Ahlenius and Larsson, 1989; Gorzalka et al., 1990) by decreasing the number of intromissions to ejaculation (Ahlenius et al., 1981). The arylpiperazine 5-HT_{1A} partial agonists buspirone (Mathes et al., 1990) and ipsapirone (Fernandez-Guasti et al., 1989) likewise appear to be facilitatory.

In contrast to the attention given to the role of 5-HT_1 receptors in male sexual behavior, relatively few studies have addressed the effects of the 5-HT_2-selective subtype receptor. The 5-HT_2 selective antagonist ketanserin has been reported to inhibit male rat sexual behavior, suggesting a facilitatory role of this subtype receptor activation (Mendelson and Gorzalka, 1985). However, the administration of selective 5-HT_2 agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) has

* Corresponding author. Department of Psychobiology, Universidade Federal de São Paulo, Rua Napoleão de Barros, 925, Vila Clementino, SP-04024-002, São Paulo, Brazil. Tel.: +55 11 2149 0155; fax: +55 11 5572 5092.
E-mail address: mandersen@psicobio.epm.br (M.L. Andersen).

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been found to potently inhibit male sexual responses and this effect was reversed by 5-HT_2 antagonists like ketanserin (Foreman et al., 1989; Klint et al., 1992; Watson and Gorzalka, 1991). The inhibition of copulation by DOI in the male rat may be related to the activity at 5-HT_2 receptors. The investigation into the efficacy of agents in reversing this inhibition may provide a behavioral bioassay for effects of 5-HT_2 receptor activation or blockade.

Cocaine is a potent psychomotor stimulant that affects several neurotransmitters in the brain (Wise, 1998). Although increased dopaminergic transmission is generally considered to be the primary mediator of the behavioral effects of cocaine, there are lines of evidence pointing to a relevant role of 5-HT (Walsh and Cunningham, 1997). While PCPA potentiated cocaine-induced locomotor activity and rearing (Herges and Taylor, 1999; Scheel-Kruger et al., 1977) the pretreatment with 5-HT precursor reduced the stimulant effects of cocaine (Pradhan et al., 1978). In addition, cocaine increases 5-HT concentration in the nucleus accumbens (Muller et al., 2002).

Although progress has been made in understanding the neurobiological basis of cocaine effects in rats, further studies are needed at the behavioral level of interaction between cocaine and sleep deprivation, a consequent event related to cocaine use. In this context, we have been consistently studying the effect of paradoxical sleep deprivation (PSD) in the occurrence of spontaneous genital reflexes in male rats. The combination of acute cocaine administration and PSD caused a marked change in the percentage of rats displaying genital reflexes events like penile erection (PE) and ejaculation (EJ), backed by the fact that none of the control rats displayed these behaviors (Andersen et al., 2003, 2004).

In an attempt to elucidate the mechanisms behind these genital events induced by cocaine in PSD males, we have been investigating the neurochemical basis of such behaviors. Systemic administration of noradrenergic (Andersen et al., 2005a), GABAergic (Andersen and Tufik, 2004) and dopaminergic drugs alter the expression of genital reflexes, suggesting PE and EJ are episodes of a complex chain of events that are under the influence of several systems.

Since 5-HT has been considered to be one of the mediators involved in the regulation of sexual behavior and based on the evidence that 5-HT can modulate the activity of dopaminergic neurons in the brain areas (Kelland et al., 1993; Parsons and Justice, 1993), and that cocaine is a 5-HT reuptake inhibitor and finally that the increased synaptic 5-HT may contribute, in part, to the stimulating effects of cocaine, we hypothesized that 5-HT also plays a role in the cocaine-induced genital reflexes in PSD male rats.

2. Methods

2.1. Subjects

Naïve male Wistar strain rats were bred and raised in the animal facility of the Department of Psychobiology, Universidade Federal de São Paulo. The animals were housed in a colony maintained at 22 °C with 12:12 h light–dark cycle (lights on at 0700 h) and allowed free access to food and water inside standard polypropylene cages. The rats were 3 months old and weighed 339.8±13.9 g at the beginning of the experiment. Rats used in this study were maintained and treated in accordance with the guidelines established by the Guide for the Care and Use of Laboratory Animals. The experimental protocol was approved by the Ethical Committee of UNIFESP (CEP N. 482/02).

2.2. Drugs

All drugs were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Cocaine (7 mg/kg) was mixed with sterile saline immediately before testing. The solution was injected intraperitoneally (IP) in a volume of 1 mL/kg. Four doses of each serotonergic drug were administered permitting the derivation of dose-response for the percentage and frequency of genital reflexes (N=10/per dose, except for pindolol 0.5 mg/kg and 2 mg/kg whose N=9). The 5-HT_{1A} drugs were the agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) and the antagonist pindolol. The 5-$HT_{2A/C}$ drugs were the agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and the antagonist ketanserin. The data produced by pilot tests indicated that the chosen protocol design did not result in motor impairment and led to genital reflexes.

All serotonergic drugs were dissolved in drops of Tween and sterile saline. Drugs were prepared fresh before each test session, and were administered intraperitoneally for 8-OH-DPAT and ketanserin, and subcutaneously (SC) for pindolol and DOI. 8-OH-DPAT was administered 15 min (IP, Ahlenius et al., 1981); pindolol 30 min (SC, Berendsen and Broekkamp, 1987); DOI 20 min (SC, Ahlenius and Larsson, 1998) and ketanserin 60 min (IP, Watson and Gorzalka, 1991) once prior to cocaine injection. The doses, the route and latencies of administrations were selected based on the above studies. This protocol is in accordance with our experimental design established in previous studies (Andersen et al., 2004; Andersen and Tufik, 2004). No animal received more than one experimental treatment.

2.3. Paradoxical Sleep Deprivation (PSD)

The animals were submitted to PSD over a period of 96 h using the modified multiple platform method. This period of PSD was chosen since it has been shown that the most genital reflexes are produced during this span of time (Andersen et al., 2003). Ten rats are placed inside a tilled water tank (123×44×44 cm), containing 14 circular platforms, 6.5 cm in diameter, in water up to within 1 cm of their upper surface. The rats could thus move around inside the tank by jumping from one platform to another. When they reached the paradoxical phase of sleep, muscle atonia set in and they fell into the water and woke. Throughout the study, the experimental room was maintained under controlled temperature (23±1 °C) and a 12 h light–dark cycle (lights on 0700 h–1900 h). The cage control group was maintained in the same room as the experimental rats for the duration of the study and showed normal sleep patterns.