Behavioural Brain Research 269 (2014) 15–19

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

D1-like antagonist blocks conditioned place preference induced by ejaculation in male rats

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HIGHLIGHTS

- SCH 23390 (D1 antagonist) blocks the conditioned place preference induced by ejaculation.
- SCH 23390 does not modify locomotion and have a transient effect on sexual behavior.
- SCH 23390 affects only the first coital interaction.
- SKF 38393 (D1 agonist) induces a conditioned place preference and it is blocked by SCH 23390.
- Our results suggest that dopamine is involved in the sexual reward induced by ejaculation.

ARTICLE INFO

Article history:
Received 2 July 2013
Received in revised form 7 March 2014
Accepted 14 April 2014
Available online 23 April 2014

Keywords:
Male sexual behavior
SCH 23390
SKF 38393
Conditioned place preference
D1-like receptor

ABSTRACT

Mating behavior, particularly ejaculation, induces a state of sexual reward, which is evaluated by the conditioned place preference test. Several studies have shown that opioid receptors are involved in inducing the state of sexual reward, mainly because this state is blocked with naloxone, a mu opioid receptor antagonist. Dopamine has been implicated in sexual motivation, coital behavior and sexual reward, however, some experiments show that D2-like or non-specific dopaminergic antagonists are not capable of blocking the conditioned place preference induced by ejaculation; therefore, the role of dopamine on sexual reward has not been demonstrated, or has been frequently discarded. We show that a dose of SCH 23390 (a specific dopamine D1-like receptor antagonist), which does not modify locomotion, blocks the conditioned place preference induced by ejaculation and the conditioned place preference induced by SKF 38393 (D1-like agonist). Our results indicate that dopamine, across the D1-like receptors, is involved in the sexual reward induced by ejaculation.

1. Introduction

Sexual behavior can be divided into 3 stages: precopulatory behavior, characterized by searching and ano-genital investigation of the sexual partner; copulatory behavior, where males present mounts, intromissions and ejaculations; and postcopulatory behavior, characterized by sexual inactivity, also called the refractory or post ejaculatory period. These stages correlate with sexual motivation, sexual performance and sexual reward. Sexual motivation can be assessed during any stage, but during the precopulatory stage, the sexual motivation for a receptive female is enhanced compared to the other stages. Sexual performance is assessed only during the copulatory phase. The postcopulatory phase is associated to the presence of a positive affective state (reward state) induced by mating, i.e., the sexual reward. In male rats ejaculation generates a reinforcing state [1], and in females the sexual reward state is generated after receiving 10 or 11 intromissions [2,3]. This generation of a positive affective state has been identified by use of the conditioning place preference (CPP) test [1,4–6].

Using the CPP test, it has been observed that naloxone (an opioidergic antagonist) blocks the positive affective state induced by copulation in male [1] and female rats [7]. On the other hand, it was found that the dopamine system participates in motivated behavior [8,9]. Dopamine exerts its effects either through their D1-like (D1 and D5) and D2-like (D2, D3 and D4) receptors [10,11]. Some D2 dopamine antagonists have been used to determine their role in generating the positive affective state induced by mating; e.g., it was observed that at low doses, pimozide does not alter the sexual

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http://dx.doi.org/10.1016/j.bbr.2014.04.026
0166-4328/

reward induced by ejaculation in male rats [1]. Also, raclopride, a D2-like antagonist and flupentixol, a potent D2 and D3 antagonist with few effects on D1 and no effect on D5 receptors [12], did not block sexual reward in females [13]. Therefore, the role of dopamine in generating a positive affective state during mating has been ruled out, although there is no evidence regarding the specific antagonistic effects of the D1-like receptor family to assess the rewarding effect induced by sexual behavior. As a result, we decided to use SCH 23390, a specific antagonist of the D1-like receptors [14] to evaluate its effects on the ejaculation-induced reward state using the CPP test.

2. Methods

Seventy sexually inexperienced male Wistar rats (250–300 g) from our colony were used in this experiment. Subjects were kept in a room with reverse light cycle of 12/12 h (light on at 23:00 h) and they had free access to filtered water and food (Purina 5001). All experiments were approved by the local animal care committee and were carried out in accordance with the "Reglamento de la Ley General de Salud en Materia de Investigación para la Salud" of the Mexican Health Ministry that follows NIH guidelines.

Eight of the 70 males were used for a dose–response curve of the antagonist, 20 were used to evaluate the effect of antagonist on reward induced by a dopaminergic agonist (10 in SKF 38393 group and 10 in SCH 23390 + SKF 38393 group), 30 were used to determine the effect of the antagonist on the sexual reward state (3 groups: SCH ($n = 9$), mating ($n = 10$), and SCH + mating group ($n = 11$)), and 12 (equally distributed between groups) did not reach the criterion of CPP test (8 males) or did not ejaculate within the required parameters (4 males) (see below) and were therefore eliminated from the experiment.

Fifteen female rats were used as stimulus; they were bilaterally ovariectomized (Ovx) under ketamine/xylazine anesthesia two weeks before the experiments. Ovx females were injected with 20 μg of estradiol benzoate and with 400 μg of progesterone 52 and 4 h, respectively, before sexual behavior tests.

The D1-like receptor antagonist used was R-(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride (SCH 23390) from Tocris Bioscience. The D1-like receptor agonist used was (±)-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol hydrobromide (SKF 38393) from Tocris.

Open field test

Automatized open field apparatus for testing of locomotion was used. It consists of an acrylic box (45 cm each side × 37 cm high), which has a device on the bottom that shines light beams forming a grid, and a computer counts the frequency at which the beams are interrupted. We first determined the dose of SCH 233390 that does not affect motor activity in the male rats. Eight subjects were used, and SCH 23390 was administered i.p. in a Latin-square design, 1 min before testing. Two and 4 days before the beginning of the experiment, two habituation sessions to the testing box were done. The measures of locomotion were registered at 5, 10, 15, 20 and 25 min after administration of 0, 2.5, 5 and 10 μg/kg of SCH 23390 dissolved in saline. A period of 2–3 day resting period elapsed between the different doses.

Sexual behavior test

Males were placed in an acrylic box (50 cm × 35 cm × 30 cm) with a receptive female. The time of the first mount (mount latency, ML), intromission (IL) and ejaculation was registered. Ejaculation latency (EL: time of ejaculation – IL), the number of mounts (NM) and the number of intromissions (NI) were recorded. Mounts can be present with or without pelvic movements, only mounts with pelvic thrusting were counted. Subjects without sexual behavior were removed from the groups.

2.1. Effect of D1-like antagonist on reward induced by ejaculation

Conditioning place preference (CPP) was performed in a rectangular plastic box (96 cm long × 28 cm wide × 32 cm high), which was divided in three compartments. The end compartments (38 cm long × 28 cm wide × 32 cm high) are connected by a central neutral compartment (20 cm long × 28 cm wide × 32 cm high) that has one clear acrylic wall and three gray walls. Both end compartments communicate with the central compartment through sliding doors and are different from each other. One compartment is black (walls and floor) and one wall was slightly moistened with 2% acetic acid. Another compartment is white (walls and floor) and clean wood shavings are placed on the floor. Before pretest, males were habituated for 5 min to the box in two days. During pretest, the subject is placed in the central compartment, the doors are removed and the rat is free to move among the three compartments. For 10 min, the time spent in each end compartment is recorded. The compartment where the male spends more time is called the preferred compartment, while the compartment where the male spends less time is called the non-preferred or reinforced compartment. A criteria need to be reached during pretest: subjects need to remain in each end compartment for at least 45 s, five subjects did not reach this criterion and were eliminated from the experiment. Sexually inexperienced (naive) male rats, were divided into three groups: (1) males treated with SCH 23390 ($n = 9$), (2) males that mated until ejaculation, previously treated with saline ($n = 10$), and (3) males that mated until ejaculation previously treated with SCH 23390 ($n = 11$). One day after pretest (day 2 of conditioning), the animals were placed in the preferred compartment for 30 min directly from their home cage. On day 3, 20 min after i.p. administration of 2.5 μg/kg of SCH 23390, males from group 1 were introduced in the non-preferred compartment for 30 min directly from their home cage. Males from group 2 were injected i.p. with 0.2 mL of saline, and males from group 3 were injected i.p. with 2.5 mcg/kg of SCH 23390. One minute later, they were placed in the mating box with a receptive female; immediately after ejaculation, males were gently placed in their non-preferred compartment for 30 min. The mating test ended at the time of ejaculation, however, if a male did not ejaculate after being with the receptive female for19 min, the test ended and was repeated a day later. If a subject spent 19 min with a receptive female without ejaculating, it was eliminated from the experiment (four males were eliminated under this criteria). On days 4 and 6, subjects were placed in the preferred compartment for 30 min directly from their home cage. On days 5 and 7, males were placed in the non-preferred compartment in identical conditions as on day 3. Then, after being in the preferred and the non-preferred compartment 3 times each, on day 8 of conditioning (test), time spent in both compartments was evaluated in a similar way as during pretest. To be able to consider that a positive affective state has been induced, it is necessary to observe two significant increases in: (1) the time in the non-preferred compartment and (2) the preference index (time in the non-preferred compartment divided by time in both compartments). Both parameters need to be significantly higher on the test in comparison to the pretest.

2.2. Conditioned place preference test in animals treated with SCH 23390 plus SKF 38393

In order to test the efficiency of D1-like antagonist to block the D1-like receptors, an additional experiment was performed. 20 subjects were habituated to CPP box and the pretest was performed