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Research article

Post-sensitization treatment with rimonabant blocks the expression of cocaine-induced behavioral sensitization and c-Fos protein in mice

Eduardo A.V. Marinho[a,*,1], Alexandre J. Oliveira-Lima[a,1], Thais S. Yokoyama[b], Renan Santos-Baldaia[b], Luciana T.C. Ribeiro[c], Marilia A. Baldaia[c], Raphael Wuo da Silva[b], Andre Willian Hollais[b], Fernanda Talhati[c], Beatriz Monteiro Longo[b], Lais Fernanda Berro[d,**], Roberto Frussa-Filho[c,d,2]

[a] Department of Health Sciences, Universidade Estadual de Santa Cruz, Rod. Ilhéus/Itabuna, Km 16, 45662-0 Ilhéus, BA, Brazil
[b] Department of Physiology, Universidade Federal de São Paulo, R. Botucatu, 862, São Paulo, SP, Brazil
[c] Department of Pharmacology, Universidade Federal de São Paulo, R. Botucatu, 862, São Paulo, SP, Brazil
[d] Department of Psychobiology, Universidade Federal de São Paulo, R. Botucatu, 862, São Paulo, SP, Brazil

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ABSTRACT

CB1 receptor antagonists have been shown to prevent acute and long-term behavioral effects of cocaine. Here we evaluate the effectiveness of the CB1 receptor antagonist rimonabant to modify sensitized responses to cocaine. Mice were treated with saline or cocaine injections in a 15-day intermittent sensitization treatment and subsequently treated with either vehicle, 1 or 10 mg/kg rimonabant in the drug-associated environment for 8 consecutive days. Animals were then challenged with saline and cocaine in the open-field apparatus on subsequent days to evaluate the expression of conditioned and sensitized effects to cocaine. c-Fos protein expression was evaluated in the nucleus accumbens (NAcc), ventral tegmental area (VTA), basolateral amygdala (BLA), medial prefrontal cortex (mPFC) and caudate-putamen (CPu) after the last (cocaine) challenge. Previous treatment with 10 mg/kg rimonabant blocked the expression of conditioned hyperlocomotion and behavioral sensitization to cocaine, but not acute cocaine-induced hyperlocomotion. These behavioral effects were accompanied by significant changes in c-Fos expression in the brain reward system. Chronic cocaine sensitization blunted a subsequent acute cocaine-induced increase in c-Fos protein in the NAcc, effect that was reversed by previous treatment with rimonabant. Treatment with 10 mg/kg rimonabant also attenuated the significant increase in c-Fos expression in the CPu, mPFC and BLA induced by previous chronic sensitization with cocaine. Our findings add to the evidence that drugs targeting CB1 receptors are good candidates for the treatment of cocaine abuse and provide further insights into the mechanisms underlying endocannabinoid signaling within the brain reward system in the context of cocaine abuse.

1. Introduction

All psychoactive drugs causing addiction in humans activate the mesocorticolimbic system by increasing dopamine availability in the nucleus accumbens (NAcc) (for review see Koob and LeMoal, 2001). This effect, responsible for modulating the reinforcing mechanisms of drugs of abuse in humans, also elicits locomotor stimulation in rodents, with repetitive administration intensifying this response (Stewart and Badiani, 1993). This phenomenon, called behavioral sensitization, is thought to share neuroadaptations with addiction to drugs of abuse and craving in humans (Robinson and Berridge, 1993).

Neuroadaptations in the mesocorticolimbic system resulting from repeated drug administration are influenced by associations between the neuropharmacological effects of drugs and the environmental context in which the drugs are being administered (Badiani and Robinson, 2004; Michel et al., 2003). Through multiple pairings, environmental cues acquire conditioned reinforcing properties *via* associative learning (Buffalari and See, 2010), what in the behavioral sensitization paradigm is expressed as a conditioned hyperlocomotion when rodents are exposed to the drug-associated environment in the

* Correspondence to: E.A.V. Marinho, Departamento de Ciências da Saúde, Universidade Estadual de Santa Cruz, Rod. Ilhéus/Itabuna, Km 16, 45662-0 Ilhéus, BA, Brazil.
** Correspondence to: L.F. Berro, Departamento de Psicobiologia, Universidade Federal de São Paulo, Rua Napoleão de Barros, 925, 04024-002 São Paulo, SP, Brazil.
E-mail addresses: edumarinho@hotmail.com (E.A.V. Marinho), berro.lf@gmail.com (L.F. Berro).
[1] The first two authors contributed equally to this study.
[2] This paper is in memory of Dr. Roberto Frussa-Filho, who dedicated his entire life to Science, because a man is alive while his name is still spoken.

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absence of the drug (Berro et al., 2014).

The resulting long-term memories can be responsible for the ability of drug-associated environmental cues to reinstate extinguished drug-seeking behavior or to lead to relapse into drug use (Crombag and Shaham, 2002; Everitt et al., 2001). Therefore, an improved treatment strategy for drug abuse should combine extinction therapy with pharmacotherapy to modulate learning during treatment and prevent relapse. We have previously established a protocol in which therapeutic drugs are administered in the drug-associated context aiming to modify sensitized responses induced by a repeated treatment with drugs of abuse in mice (Oliveira-Lima et al., 2015, 2017).

The search for medications development for cocaine abuse has originally focused on the dopaminergic system. However, most recent investigations have explored other neural systems that play a role in addictive processes. Evidence suggests that endocannabinoids are critically involved in drug addiction (Le Foll and Goldberg, 2005), with the cannabinoid CB1 receptor participating in drug reward and cue reactivity (Lupica et al., 2004). These receptors are densely expressed in the mesolimbic dopaminergic pathway (Tsou et al., 1998), and activation of cannabinoid CB1 receptors is required for the expression of cocaine-induced rewarding effects in rodents (Xi et al., 2008). In fact, a previous study from our group demonstrated that treatment with the CB1 receptor antagonist rimonabant blocked cocaine-induced hyperlocomotion and behavioral sensitization in mice (Marinho et al., 2015).

The aim of the present study was to evaluate the effects of the CB1 receptor antagonist rimonabant on a post-sensitization protocol to modify sensitized responses induced by a repeated treatment with cocaine. Given the rapid induction by cocaine of c-Fos protein expression (see Kufahl et al., 2009 and references within), a marker for stimulus-elicited brain activity (Harlan and Garcia, 1998), we investigated if the post-sensitization treatment with rimonabant would affect the reward circuitry as indicated by modulating cocaine-induced c-Fos expression.

2. Methods

2.1. Animals

Three-month-old Swiss male mice from our own colony were used. Animals weighing 35–40 g were housed under controlled temperature (22–23 °C) and light (12 h light, 12 h dark; lights on at 6h45a.m.) conditions. Food and water were available *ad libitum* throughout the experiments. Animals were maintained according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1996). The Institutional Ethical Committee of UNIFESP approved the experimental procedures (protocol #470/07).

2.2. Drugs

Cocaine-HCl (Sigma®) was diluted in 0.9% saline solution. Rimonabant (Sanofi-Aventis®) was dissolved in a solution of saline + 1% Tween 80 + 3% propylene glycol, which was used as vehicle solution (Veh). All solutions were administered intraperitoneally at 10 ml/kg of body weight. The selected dose range of rimonabant and cocaine was based on previous studies from our group (Marinho et al., 2014, 2015).

2.3. Open-field evaluation

Locomotor activity was measured in an open-field apparatus as described previously (Chinen and Frussa-Filho, 1999). The apparatus consisted of a circular wooden arena (40 cm in diameter and 50 cm high) with an open top and a floor divided into 19 squares. Hand-operated counters were used by an observer who was blind to the treatment to score total locomotion frequencies (total number of any squares entered) during the 10-min sessions.

2.4. Experimental procedures

2.4.1. Experiment 1

Effects of rimonabant on a post-sensitization protocol to modify sensitized responses and c-Fos expression induced by a repeated treatment with cocaine.

2.4.1.1. Locomotor activity. Eighty-four male mice were given a 10-min habituation period in the open field on 2 consecutive days after a saline i.p. injection. Basal locomotor activity was measured on day 2. Seven groups of animals were formed (n = 12 for each group), which were statistically equivalent with respect to the basal levels of locomotor activity. Twenty-four hours after the second habituation day, the behavioral sensitization procedure began. Four groups of animals received an i.p. injection of saline (Sal) and the remaining groups were treated with 10 mg/kg cocaine (Coc) 5 min prior to being placed in the open-field apparatus every other day for 15 days (3th to 17th days; Sensitization Phase). During the alternate non-sensitization days, mice were left undisturbed in their home-cages. On days 3 and 17 animals were observed for the quantification of their locomotion frequency.

Forty-eight hours after the last injection of the sensitization phase, the post-sensitization protocol began (19th day). For 8 consecutive days (19th to 26th days) 12 animals from the Sal group received daily saline i.p. injections (Sal–Sal). Another group of 12 animals from the Sal group received daily vehicle i.p. injections (Sal-Veh) and the remaining mice in the Sal group received daily i.p. injections of rimonabant (Rim) at the doses of 1 (Sal–Rim1, n = 12) or 10 mg/kg (Sal–Rim10, n = 12). Cocaine-sensitized groups underwent the same procedure (Coc–Veh, Coc–Rim1 and Coc–Rim10 groups, n = 12 per group). Thirty minutes after each administration of saline, vehicle or rimonabant, animals were individually exposed to the open-field arena for 10-min sessions (Post-Sensitization Phase). On days 19 and 26 animals were observed for the quantification of their locomotion frequency.

Four days after the last post-sensitization day (30th day), all animals received an i.p. saline injection and were placed in the open-field apparatus 5 min later for locomotion frequency quantification. Two days after the Saline challenge, animals were tested for the expression of cocaine-induced behavioral sensitization (day 32). Except for the Sal-Sal group, which received a saline i.p. injection, all animals received an i.p. injection of 10 mg/kg cocaine and were placed in the open-field apparatus 5 min later for locomotion frequency quantification.

2.4.1.2. c-Fos protein expression. Ninety minutes after the last behavioral test (Cocaine Challenge), 6 animals per group were randomly selected and deeply anesthetised and perfused through the heart with 50 ml of saline solution (9% NaCl) followed by 200 ml of 4% paraformaldehyde at 4 °C. The brains were removed and post-fixed in 4% paraformaldehyde for 1 day, and then in isotonic solution of sucrose (30%) to cryoprotection. Brains were then sectioned in cryostat (Leica CM1850). Coronal brain sections (30-μm thick) were made between bregma 1.98 and bregma − 3.88 mm, according to the stereotaxic coordinates of the mouse brain atlas. Sections were selected (6 per animal) to quantify the c-Fos expression by immunohistochemistry using rabbit anti-c-Fos (1:3000, Calbiochem) as primary antibody. Free-floating sections were washed in PBS and incubated separately overnight with the primary antibody diluted in PBS. After incubation, the sections were washed in PBS and incubated with biotinylated anti-rabbit secondary antibody for 2 h (1:600, Vectastain Vector), followed by incubation in the ABC kit solutions (Vectashield, Vector, Burlingame, CA, EUA) for 1.5 h. The sections were stained with diaminobenzidine (DAB, Sigma-Aldrich Corporation, St. Louis, EUA) and mounted on slides and sealed with coverslips.