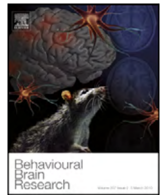
Behavioural Brain Research 214 (2010) 437–442

Contents lists available at ScienceDirect

Behavioural Brain Research

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



Research report

Acute reversible inactivation of the ventral medial prefrontal cortex induces antidepressant-like effects in rats

America Augusto Scopinho[a], Michele Scopinho[a], Sabrina Francesca Lisboa[a], Fernando Morgan de Aguiar Correa[a], Francisco Silveira Guimarães[a], Sâmia Regiane Lourenço Joca[b,*]

[a] Department of Pharmacology, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil
[b] Laboratory of Pharmacology, Department of Physics and Chemistry, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, 14040-903 Ribeirão Preto, SP, Brazil

ARTICLE INFO

Article history:
Received 10 May 2010
Received in revised form 8 June 2010
Accepted 13 June 2010
Available online 19 June 2010

Keywords:
Behavioral despair
Cobalt chloride
Forced swimming
Antidepressant
Prefrontal cortex
Behavior

ABSTRACT

The ventral medial prefrontal cortex (vMPFC) has direct connections to subcortical, diencephalic and brainstem structures that have been closely related to depression. However, studies aimed at investigating the role of the vMPFC in the neurobiology of depression have produced contradictory results. Moreover, the precise involvement of vMPFC anatomic subdivisions, the prelimbic (PL) and the infralimbic (IL) cortices, in regulating depressive-like behavior have been poorly investigated. The forced swimming test (FST) is a widely employed animal model aimed at detecting antidepressant-like effects. Therefore, to further investigate a possible involvement of the vMFPC in depressive-like behavior, rats bilaterally implanted with cannulae aimed at the PL or IL prefrontal cortices were submitted to 15 min of forced swimming (pre-test) followed, 24 h later, by a 5-min swimming session (test), where immobility time was registered. Synaptic transmission in these regions was temporarily inhibited using local microinjection of cobalt chloride at different periods of the experimental procedure (before or after the pre-test or before the test). PL inactivation decreased immobility time independently of the time of the injection. In the IL, inactivation induced a significant antidepressant-like effect when performed immediately before the pre-test or before the test, but not after the pre-test. These results suggest that activation of the vMPFC is important for the behavioral changes observed in rats submitted to the FST. They further indicate that, although both the PL and IL cortices are involved in these effects, they may play different roles.
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1. Introduction

Several pieces of evidence indicate that limbic structures, such as the hippocampus, the hypothalamus, the amygdala, the nucleus accumbens and the prefrontal cortex, are associated with the behavioral changes observed in depressive states [1–3]. The specific role of these brain structures in depression, however, is still unclear.

The prefrontal cortex (PFC) is well known for its role in cognition, affect regulation and social reasoning. In rats, the PFC consists of distinct cortical areas that receive projections from the dorsal medial thalamic nucleus. It is usually divided into three anatomically and functionally distinct subregions: the medial prefrontal cortex (MPFC), the orbitofrontal cortex (OFC) and the lateral or sulcal prefrontal cortex or agranular insular cortex [4–7]. The MPFC can be further divided into ventral (vMPFC: infralimbic-IL and prelimbic-PL cortices), and dorsal (anterior cingulate cortex-ACd) MPFC [8].

The MPFC participates in several higher-order functions, including learning, memory, event association, specific aspects of locomotor activity and spatial navigation, decision making, goal directed behavior and autonomic and endocrine responses to stress [9–16]. Dysfunctions of this cortical area have been suggested to play an important role in the development of stress-related mental illnesses such as anxiety and depression [17–19].

Exposure to stressful stimuli promotes significant activation and plastic changes of the MPFC, which are often correlated with the development of stress-induced behavioral changes [20–23]. Different classes of antidepressants are able to counteract these stress-induced behavioral and morphological changes [22,24,25], further implicating the MPFC in the neurobiology of depression. In agreement with this proposal, depressed patients present abnormal activity of the MPFC [26–28] that is sensitive to antidepressant treatment [29,30].

Despite the large number of studies investigating the responses of the MPFC to stress, its role in pathophysiology of depression is not entirely clear. Data obtained from human studies have produced contradictory results, showing either increased or decreased activation of the MPFC in depressed patients [31]. Moreover, depressive-like behavior in rodents submitted to animal models

* Corresponding author. Tel.: +55 16 36024705; fax: +55 16 36024880.
E-mail address: samia@usp.br (S.R.L. Joca).

0166-4328/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2010.06.018

predictive of antidepressant activity can be either facilitated or attenuated by lesion or inactivation of the MPFC [32–34]. The large variability among these studies could be due to different target areas and extension of the MPFC lesions [27,35–37], since dorsal regions of the MPFC (ACd) have been linked to motor behaviors, while the vMPFC (PL and IL) has been associated with diverse emotional, cognitive, and mnemonic processes [8,35]. Moreover, recent evidence has also shown that the IL and PL are cytoarchitectonically distinct and that they can play different roles in emotional [33,38] endocrine [37], autonomic [16] and cognitive [39,40] responses to stress.

Therefore, the aim of the present study was to investigate the effects of temporary acute inactivation of synaptic transmission within the IL or PL by local microinjection of cobalt chloride ($CoCl_2$), at different time points with respect to stress exposure, in rats submitted to the forced swimming test, an animal model predictive of antidepressant activity. Microinjection of $CoCl_2$ was used to cause temporary inhibition of selected brain areas, since it competitively reduces calcium presynaptic influx and leads to a reversible inhibition of neurotransmitter release [41]. This technique has the advantages, compared to other deactivation techniques, of being reversible and sparing fibers of passage, therefore allowing a more precise evaluation of a given brain structure in regulating specific functions over time.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 240–260 g at the beginning of each experiment were housed in pairs in a temperature-controlled room (24 ± 1 °C) under standard laboratory conditions with free access to food and water and a 12 h light/12 h dark cycle (lights on at 06:30 a.m.). Procedures were conducted in conformity with the Brazilian Society of Neuroscience and Behavior guidelines for the care and use of laboratory animals, which are in compliance with international laws and politics. The protocols described herein have been approved by the local Ethical Committee and all efforts were made to minimize animal suffering.

2.2. Drugs

The following drugs were used: cobalt chloride ($CoCl_2$; Sigma, St. Louis, MO, USA), tribromoethanol (Aldrich, St. Louis, MO, USA) and urethane (Sigma, St. Louis, MO, USA). $CoCl_2$ was dissolved in sterile artificial cerebrospinal fluid (ACSF: 100 mM NaCl; 2 mM Na_3PO_4; 2.5 mM KCl; 1 mM $MgCl_2$; 27 mM $NaHCO_3$; 2.5 mM $CaCl_2$; pH 7.4).

2.3. Experimental procedures

2.3.1. Stereotaxic surgery and intracerebral drug administration

Seven days before the experiment the animals were anaesthetized with 2,2,2-tribromoethanol (10 mg/kg, i.p.) and fixed in a stereotaxic frame. After scalp anesthesia with 2% lidocaine, the skull was surgically exposed and stainless steel guide cannulae (26 G) were implanted bilaterally in the PL or IL using a stereotaxic apparatus (Stoelting, Wood Dale, IL, USA). Coordinates for cannula implantation into the PL (AP = +3.3 mm from bregma; L = +1.9 mm from the medial suture, V = −2.4 mm from the skull with a lateral inclination of 22°) and IL (AP = +3.3 mm from bregma; L = +2.7 mm from the medial suture, V = −3.2 mm from the skull with a lateral inclination of 24°) were selected from the rat brain atlas of Paxinos and Watson [42]. The cannulae tips were 1 mm above the site of injection and the cannulae were attached to the skull bone with stainless steel screws and acrylic cement. An obturator inside the guide cannulae prevented obstruction. After surgery, the animals received a poly-antibiotic (Pentabiotico®, Fort Dodge, Brazil), with streptomycins and penicillins, to prevent infection and a nonsteroidal anti-inflammatory, flunixine meglumine (Banamine®, Schering Plough, Brazil), for post-operation analgesia.

The needles (33G, Small Parts, Miami Lakes, FL, USA) used for microinjections were 1 mm longer than the guide cannulae and were connected to a 2 μL syringe (7002-H, Hamilton Co., Reno, NV, USA) through PE-10 tubing. A volume of 200 nL/side was injected in 1 min using an infusion pump (Kd Scientific, USA). The movement of an air bubble inside the polyethylene catheter confirmed drug flow.

2.3.1.1. Forced swimming test (FST). The procedures for the FST were similar to those first described by Porsolt et al. [43]. Animals were initially placed individually to swim in plastic cylinders (30 cm diameter by 40 cm height containing 25 cm of water at 24 ± 1 °C) [44] for 15 min (pre-test). They were then removed and allowed to dry in a separate cage before returning to their home cages. Twenty-four hours later the animals were submitted to a 5 min session of forced swim (test). During this session the total amount of time in which animals remained immobile (except for small limb movements necessary for floating) were recorded by an observer that was blind to the treatments. The water was changed after each trial to avoid the influence of alarm substances.

2.3.2. Open field

In order to rule out the possible involvement of unspecific motor changes in the results obtained in the FST, an independent group of rats were treated and submitted to the open field test. The experiments were carried out in a circular arena (72 cm in diameter with a 50 cm high Plexiglas wall) located in a sound-attenuated, temperature-controlled (25 ± 1 °C) room, illuminated with three 40 W fluorescent bulbs placed 4 m above the apparatus. The rats were videotaped inside the arena and their position was captured by AnyMaze software (Stoelting, Wood Dale, IL, USA), which detects and calculates the distance moved by the animals each minute.

2.3.3. Histological analysis

After the behavioral tests, the animals were sacrificed under deep urethane anesthesia (1.25 g/kg, intraperitoneally) and perfused through the left ventricle of the heart with isotonic saline followed by 10% formalin solution. Subsequently, a dental needle was inserted through the guide cannula and 200 nL of Evan's blue dye was injected into the PL and IL as a marker. The brains were removed and, after a minimum period of 3 days immersed in a 10% formalin solution, 40 μm sections were obtained in a Cryostat (Cryocut 1800). The injection sites were identified on diagrams from Paxinos and Watson's atlas [42]. Rats that received injections outside the target area were excluded from analysis.

2.4. Experimental design

2.4.1. Experiment 1: effects of $CoCl_2$ injection into the PL of rats submitted to the forced swimming test

Animals were randomly assigned to one of three independent groups: the first group received bilateral microinjections into the PL of 200 nL of either ACSF or 1 mM of $CoCl_2$ [16] 10 min before the pre-test session (ACSF: n = 7 and $CoCl_2$: n = 7); the second group received microinjections into the PL immediately after the end of the pre-test session (ACSF: n = 7 and $CoCl_2$: n = 7), the third group received microinjections into the PL 10 min before the test session (ACSF: n = 7 and $CoCl_2$: n = 7).

2.4.2. Experiment 2: effects of $CoCl_2$ injection into the IL of rats submitted to the forced swimming test

Similar to experiment 1, except that the animals received the microinjections into the IL instead of the PL (injections before pre-test, ACSF: n = 5 and $CoCl_2$: n = 5, injections after pre-test, ACSF: n = 5 and $CoCl_2$: n = 5, injections before test ACSF: n = 5 and $CoCl_2$: n = 5).

2.4.3. Experiment 3: effects of $CoCl_2$ injection into the PL of rats submitted to the open field

Animals received bilateral microinjection (200 nL) of either ACSF (n = 4) or 1 mM of $CoCl_2$ (n = 6) into the PL and were submitted to an open field 10 min later for motor behavior evaluation.

2.4.4. Experiment 4: effects of $CoCl_2$ injection into the IL of rats submitted to the open field

Similar to experiment 3, except that the animals received microinjections into the IL instead of the PL (ACSF: n = 4; $CoCl_2$: n = 5).

2.5. Statistical analysis

The results of the FST for each region (PL or IL) were analyzed using a two-way ANOVA (factors: treatment and injection time). Post hoc analyses were done using the Bonferroni test. The results of the distance travelled in the OF during each minute for each region (PL or IL) were analyzed using a two-way ANOVA (factors: treatment and time) and the total distance moved by the *Student's t*-test. Probability <0.05 was accepted as significant.

3. Results

3.1. Determination of microinjection sites

Representative photomicrographs of coronal brain sections depicting bilateral microinjection sites in the PL or the IL of representative animals are presented in Fig. 1. Moreover, diagrammatic representation showing microinjection sites of ACSF and $CoCl_2$ are also shown in Fig. 2.

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