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Antidepressant treatment reduces Fos-like immunoreactivity induced by swim stress in different columns of the periaqueductal gray matter

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Abstract

Antidepressant treatment attenuates behavioral changes induced by uncontrollable stress. The periaqueductal gray matter (PAG) is proposed to be a brain site involved in the behavioral responses to uncontrollable stress and antidepressant effects. The main goal of the present study was to investigate the effect of antidepressant treatment on the pattern of neural activation of the PAG along its mediolateral and rostrocaudal subregions after a forced swim stress episode. Male Wistar rats were sub-acutely treated with desipramine (a selective noradrenaline re-uptake blocker, three injections of 10 mg/kg in 24 h) or clomipramine (a non-selective serotonin and noradrenaline re-uptake blocker, three injections of 10 mg/kg in 24 h) and submitted to the forced swimming test (FST). Two hours after the test their brain were removed for Fos immunohistochemistry. Fos-like immunoreactivity (FLI) in rostral, intermediate and caudal portions of dorsomedial (dmPAG), dorsolateral (dlPAG), lateral (lPAG) and ventrolateral (vlPAG) PAG were quantified by a computerized system. The FST session increased FLI in most parts of the PAG. Previous treatment with desipramine or clomipramine reduced FLI in all columns of the PAG. FLI in the PAG correlated positively with to the immobility time and negatively with to climbing behavior scored during the test. These results indicate that neurons in the PAG are activated by uncontrollable stress. Moreover, inhibitory action of antidepressants on this activity may be associated with the anti-immobility effects of these drugs in the FST.

Keywords: *c-fos*; Immunohistochemistry; Depression; Aversion; Uncontrollable stress

1. Introduction

Uncontrollable stress is proposed to play a pivotal role in the development of affective disorders in humans [27]. In laboratory animals, uncontrollable stress usually induces passive emotional coping characterized by conservation-withdrawal strategies [26,27]. Most of the animal models of depression are based on behavioral changes induced by this kind of stress that are attenuated by antidepressant treatment [12,13,26]. Although there is a bulk of information about the acute pharmacological effects of the antidepressant drugs (e.g., blockade of serotonin and/or noradrenaline re-uptake [28]), the mechanisms responsible for their behavioral effects in animal models and human depression remain controversial (for review [21]). An extensive circuitry seems to underlie these behavioral effects [13,18]. Thus, understanding the role of each component of this circuitry could help to clarify how different kinds of antidepressant drugs produce similar behavioral effects. In this regard, the expression of immediate early gene such as *c-fos* could be a helpful tool to elucidate distinct brain circuitries, since different stimuli such as stress, drugs, seizure, pain, light or odors are able to induce *c-fos* expression in specific brain regions (for review [22]).

Uncontrollable stress has been shown to induce *c-fos* expression in multiple brain areas [2,18,20]. Among them the midbrain periaqueductal gray matter (PAG) shows Fos protein expression after restraint [18] or swimming [2] stress. Neurons located in

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this region are proposed to play a role in several visceral, somatic and emotional functions (for review [8,9]). Anatomical, neurochemical and functional criteria have been used to divide the PAG into dorsomedial (dmPAG), dorsolateral (dlPAG), lateral (lPAG) and ventrolateral (vlPAG) longitudinal columns extending from posterior commissure to the ventral tegmental nucleus (for review [1]). These columns may play a differential role on stress responses. For example, stimulation and lesion studies of the dorsal and lateral portions of PAG suggest their involvement in the elicitation of active behaviors when the animal is exposed to a controllable stress or inhibition of passive behavioral responses elicited by uncontrollable stress [14,16]. Stimulation of the vlPAG, on the other hand, produces passive emotional coping, as also elicited by uncontrollable stress [15]. The flight reactions induced by electrical or chemical stimulation of the dorsal PAG are attenuated by antidepressant drugs [29]. However, these same drugs decrease immobility time in the forced swimming test (FST) [11,12,26]. It is possible, then, that antidepressants could have different effects in distinct regions of the PAG.

The purpose of the present study was to investigate the pattern of Fos protein expression along of mediolateral and rostrocaudal subregions of the PAG after uncontrollable stress in the presence of antidepressant drug treatments. To achieve this objective, rats were submitted to the FST, with or without previous sub-acute treatment with antidepressant drugs, and Fos expression was analyzed in the different columns of the PAG. The FST [11,12,17,26], an animal model of depression, makes use of the forced swimming stress to produce passive emotional coping characterized by increased periods of immobility posture during a subsequent swimming test. Sub-acute treatment with antidepressant drugs that inhibit the re-uptake of serotonin and/or noradrenaline impairs the stress-induced effects in the FST [11,12,26].

2. Materials and methods

2.1. Animals

Four-month-old male Wistar rats (300–400 g, $N = 5$–7/group) were housed in groups, with free access to water and food, kept under a 12 h light–dark cycle (lights on at 6:00 a.m.) and controlled temperature (23 ± 1 °C). All procedures were carried out according to international standards of animal welfare (Brazilian Society of Neuroscience and Behavior, Act 1992) and the guidelines of the local Committee for Animal Care in Research (# 081/CEUA and 23080.001156//2001-50/UFSC).

2.2. Drugs

Desipramine hydrochloride (10 mg/kg, Sigma Chemical, St Louis, USA) and clomipramine hydrochloride (10 mg/kg, Sigma Chemical, St Louis, USA) were dissolved in sterile isotonic saline and administered intraperitoneally (i.p.) at three distinct periods: immediately after the pre-test, 5 and 1 h before the test session (sub-acute treatment). The doses and treatment regime were chosen based on previous studies from the literature [12,26].

2.3. Procedures

2.3.1. Forced swimming test (FST)

The FST [12,17,26] consists in placing the rats individually, in two 24 h apart occasions (pre-test and test sessions), into a cylindrical tank (PVC, 20 cm × 40 cm) containing clean water at 25 °C (25 cm deep). After the pre-test (15 min) or the test (5 min) swimming session, they were taken out of the water and allowed to dry under a lamp (40 W, 15 min) before returning to their home cages where they remained undisturbed until the moment of the perfusion.

Animals were submitted to sub-acute antidepressant treatment that has previously been described as effective in the FST [12,26]. They received three i.p. injections of clomipramine (10 mg/kg, $n = 7$), desipramine (10 mg/kg, $n = 7$), or saline (1 ml/kg, $n = 6$) before the test session. All animals were sacrificed 2 h after the test. A control group ($n = 5$) was submitted only to the pre-test session being sacrificed 24 h later. The test sessions were videotaped and subsequent analyses of the total time of animals remain immobile or show active behavior (climbing or swimming) was performed as described by Lino-de-Oliveira et al. [16,17].

2.3.2. Immunohistochemistry

Animals were anesthetised (ketamine, 80 mg/kg plus xilazine, 75 mg/kg (v/v); 1 ml/kg, i.p.) and transcardially perfused with saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were removed and post fixed over 2 h in paraformaldehyde (4%) and stored for at least 48 h in 30% sucrose for cryoprotection. Coronal sections (40 μm) were obtained in a cryostat (Criocut 1800, Reichrt-Jung, Heidelberg, Germany) and processed for Fos immunohistochemistry, as described by Lino-de-Oliveira et al. [18]. Briefly, tissue sections were successively washed and incubated overnight at room temperature with rabbit polyclonal immunoglobulin G (1:10,000 into PBS, sc-52, Santa Cruz Biotechnology, Santa Cruz, CA, USA), which was raised against an amino acid sequence of the N-terminal region of the peptide and specifically recognizes Fos protein. Sections were then processed by the avidin–biotin immunoperoxidase method (Vectastain ABC kit, Vector Lab, Burlingame, CA, USA). The product of the reaction was revealed by adding the chromogen 3,3-diaminobenzidine tetrahydrochloride (Sigma Chemical, St Louis, USA) and visualised as a brown product inside neuronal nuclei. The omission of Fos antibody was used as a control of the assay to avoid false positive results [18]. For each division of the PAG the number of Fos-positive nuclei (or Fos-like immunoreactivity, FLI) was counted from a fixed area size (0.2 mm^2) using a computerized image analysis system (Image-Pro Plus) as previously described [18]. A fixed area size was employed in order to avoid changes in the FLI resulting of changes in region dimensions over the rostrocaudal axis [18]. Darker objects with areas between 10 and 80 μm^2 were identified as Fos-positive nuclei. The system was calibrated to ignore background staining. The whole analysis was performed by a trained observer (RMWO) that was blind to the treatment groups. Positive objects visually recognized as spurious were eliminated from the analysis. The PAG sections were delineated (Fig. 1) according to Canteras and Goto [6] and Bellchambers et al. [2]. Neuroanatomical sites were identified with the help of the Paxinos and Watson's atlas [24]. Taking the bregma as the reference point, the rostral portion containing dmPAG, dlPAG, and lPAG was represented by levels −5.3 to −5.8 mm; the intermediate portion containing the dmPAG, dlPAG, and lPAG subregions was considered from levels −6.04 to −7.04 mm and the caudal portion (containing all of the subregions including the vlPAG) was represented by levels −7.3 to −8.0 mm (Fig. 1). The results are expressed as the number of positive cells in each area corrected by the number of sections analyzed.

2.3.3. Statistical analysis

Fos-like immunoreactivity and behavioral data were analyzed by one-way ANOVA followed by the Duncan test for multiple comparisons. A correlation analysis between the number of Fos-like immunoreactive cells in different stereotaxic levels (rostral, intermediate and caudal) of dmPAG, dlPAG, lPAG, or vlPAG and the time spent in immobility during FST was performed using the Spearman test. The significance level was set at $p < 0.05$.

3. Results

Compared to saline-treated animals, sub-acute treatment with clomipramine or desipramine significantly decreased the immobility time ($F(2,17) = 6.24$, $p = 0.01$, Duncan's test, $p < 0.05$,