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ACUTE FLUOXETINE ADMINISTRATION DIFFERENTIALLY AFFECTS BRAIN C-Fos EXPRESSION IN FASTED AND REFED RATS

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Abstract—In the present study we investigated the effect of acute fluoxetine administration on the expression of c-Fos in the rat brain under two different metabolic conditions: fed and fasting states. Wistar male rats, weighing 220±30 g, received i.p. injections of saline solution or fluoxetine (10 mg/kg), and were killed 2 h later. The brains were removed after transcardiac perfusion with phosphate-buffered saline followed by paraformaldehyde, and were then processed for immunohistochemistry. Fos-like immunoreactivity was quantified by a computerized system. Fasted animals faced an 18-h suppression of food intake, while fed groups were submitted to an initial 14-h period of fast followed by a 4-h period in which food was freely available. Both in fasting and fed states, fluoxetine-treated animals presented a significant increase in c-Fos expression in hypothalamic areas, limbic structures, circumventricular areas, and in mesencephalic and rhomboencephalic regions, as compared with saline-treated controls. The quantitative comparison of data obtained from fasted and fed animals showed that fasted rats treated with fluoxetine presented a higher c-Fos expression in the ventromedial hypothalamus and the paraventricular nuclei compared with the fed group, while in fluoxetine-treated fed rats c-Fos expression was higher in the arcuate nuclei, medial amygdala, locus coeruleus and dorsal raphe nuclei, as compared with fasted, fluoxetine-treated animals. These data indicate that the metabolic condition of the animals significantly modifies fluoxetine-induced brain c-Fos expression, suggesting that visceral and behavioral fluoxetine effects may be influenced by the metabolic state of the individual. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: serotonin reuptake inhibitors, Fos-IR, serotonin, fed state, fasting state.

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Abbreviations: AP, area postrema; Arc, arcuate nuclei; BSTL, bed nucleus of the stria terminalis, lateral division; CeA, central amygdala; DMN, dorsomedial nuclei of the hypothalamus; DRN, dorsal raphe nuclei; Fos-IR, c-Fos-immunoreactive nuclei; LC, locus coeruleus; LH, lateral hypothalamus; LPBN, lateral parabrachial nuclei; LSV, lateral septal nuclei, ventral part; MeA, medial amygdala; MnR, median raphe nucleus; NTS, nucleus of the solitary tract; OVLT, organum vasculosum of the lamina terminalis; PAG, periaqueductal gray matter; PBS, phosphate-buffered saline; PVN, paraventricular nuclei; Sch, suprachiasmatic nuclei; SFO, subfornical organ; SON, supraoptic nuclei; VMH, ventromedial hypothalamus.

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Brain serotonin pathways constitute a chemically and anatomically well-defined neurotransmitter system that possesses several families of serotonin receptors, exerts numerous physiological actions and is pharmacologically modified by a multitude of exogenous agents (Barnes and Sharp, 1999). The midbrain raphe nuclei that originate the central serotonergic system spread widely into most brain regions, making serotonin and serotonin receptors ubiquitously present throughout the CNS (Steinbusch and Nieuwenhuys, 1981).

Central serotonin synthesis, release, receptor binding and activity may be affected by the animal's feeding/metabolic state. Circulating free tryptophan and brain tryptophan levels, crucial parameters influencing brain serotonin synthesis (Knott and Curzon, 1972), are increased during fasting, and hypothalamic serotonin release is enhanced during feeding (Schwartz et al., 1989). These metabolic-related changes in serotonin activity in the CNS suggest that animals may present different responses to a particular serotonergic agent during the fast and fed states.

Fluoxetine, a selective serotonin reuptake inhibitor (SSRI), is one of the most clinically used serotonergic agents. Previous work has demonstrated that the effects observed after acute fluoxetine administration may be considerably different from those seen after its chronic use (Lino-de-Oliveira et al., 2001). Indeed, acute administration of fluoxetine promotes anxiety, agitation and nervousness (Amsterdam et al., 1994), while the opposite effects are seen after its chronic use.

The acute biochemical effects of fluoxetine are a consequence of a sudden increase in synaptic serotonin availability, and acute fluoxetine administration may be a valuable pharmacological tool for studying the effects of a rapid increase in serotoninergic activity promoted by the activation of the various serotonin receptor subtypes normally stimulated by synaptic serotonin release.

We have recently demonstrated that acute fluoxetine administration induces a significant increase in plasma glucose levels in fasted rats (Carvalho et al., 2004). In view of these findings with respect to the possible differential status of the brain serotonin system in different metabolic states, in the present study we decided to investigate whether different brain areas could be activated by acute fluoxetine administration, in rats during fasting and fed states, by analyzing c-Fos expression as an indicator of neuronal activity.

EXPERIMENTAL PROCEDURES

Animals

We have used adult Wistar male rats weighing 220±30 g kept under controlled light (lights on from five AM to seven PM) and

temperature (22–24 °C) conditions. The animals had free access to water and laboratory chow (Nuvital Nutrientes Ltd., Curitiba, Brazil) in the days prior to the experiments. They were handled daily for 5 days, in order to minimize stress during the experimental sessions. The experimental protocols were conducted according to the regulations established by the National Institutes of Health (USA) and were approved by a local committee (Ethical Committee on the Use of Laboratory Animals, Osvaldo Cruz Foundation, Bahia, Brazil) that analyzes ethical aspects of research in laboratory animals. In all experiments every effort was made to minimize animals suffering and pain.

Drugs

Fluoxetine, diluted in isotonic saline solution, injected intraperitoneally (10 mg/kg), was acquired from Sigma Co., St. Louis, MO, USA. In order to be administered in such concentration fluoxetine solution had to be warmed up (37 °C).

Experimental design

We studied brain c-Fos expression after acute fluoxetine or saline (controls) i.p. administration in both fasted and fed rats. In the groups used to study the effects of acute fluoxetine administration in fasted rats, the animals were fasted for the 18 h prior to the experiments (food access was restricted at 6 PM the day before the experimental session) and the experiments always began at 12 PM. To investigate the role of acute fluoxetine administration in fed rats, the animals were fasted for 14 h (from 6 PM to 8 AM). After this period, they had free access to standard laboratory chow for 4 h (from 8 AM to 12 PM) and the experimental sessions also began at 12 PM. This procedure serves to minimize variations in food intake among individuals immediately before the experimental sessions.

Animal perfusion and histology

In all groups studied, the animals were anesthetized (thionembutal, 50 mg/kg, i.p.) 2 h following the i.p. injections of fluoxetine (10 mg/kg) or isotonic saline solution and perfused transcardially with 400 ml of phosphate-buffered saline (PBS) 0.1 M (pH 7.4) followed by 4% paraformaldehyde (pH 7.4). After these procedures, the brains were removed and stored overnight in the same fixative at 4 °C and then submerged in 30% sucrose solution for at least 2 days. The whole brains were serially sectioned at 40 μm in a cryostat.

Immunohistochemistry

The free floating sections were rinsed three times for 5 min in 0.01 M PBS. After this initial washing step, tissue sections were incubated in PBS containing 1% hydrogen peroxide for 15 min to block endogenous peroxidase activity. Sections were then washed three times again in PBS and incubated in PBS containing 5% normal goat serum for 1 h. Next, this solution was replaced by PBS with 0.3% Triton X-100 (Sigma, St. Louis, MO, USA) and Fos primary antisera diluted 1:4000 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight, at room temperature. The sections were washed three more times with PBS and incubated with a biotinylated rabbit antisheep secondary antibody (Vector Laboratories, Burlingame, CA, USA) diluted 1:400 in PBS, for 1 h at room temperature. Again, the sections were rinsed three times with PBS and finally incubated at room temperature for 60 min with avidin-biotinylated horseradish peroxidase complex (Avidin-Biotin Complex-Kit, Vector) diluted 1:200 in PBS. After a final three washes, sections were incubated in a PBS solution containing 0.02% 3,3′-diaminobenzidine-tetrahydrochloride (DAB; Sigma), 0.08% nickel sulfate and 0.0002% hydrogen peroxide for 10 min. The enzymatic peroxidase reaction was stopped by rinsing the sections with PBS. Immunohistochemistry was carried out simultaneously on the brains of experimental and control animals.

Following these procedures, the brain sections were mounted in chrom-alum-gelatin-coated glass slides and allowed to dry overnight before dehydration by using ethanol solutions of increasing concentrations (50–100%), soaked for 20 min in 100% xylene and coverslipped with Permount (Fischer Scientific International Inc., Springfield, NJ, USA).

A preliminary qualitative analysis identified the brain regions presenting c-Fos expression. In these areas, the number of Fos-positive nuclei was counted under microscopy using a computerized image analysis system (Image-Pro Plus, Media Cybernetic, Silver Spring, MD, USA). The system was calibrated to ignore background staining. Cells containing a nuclear brown–black reaction product were considered positive for Fos immunoreactivity. Neuroanatomical sites were identified with the help of Paxinos and Watson's (1998) atlas.

Statistical analysis

A computer software package (SigmaStat for Windows, Jandel Scientific, San Rafael, CA, USA) was used to carry out one-way analysis of variance for each area studied. The post hoc Student-Newman-Keuls test was used for comparison of each group. The data are presented as mean±S.E.M. Fos-IR cells for each particular area studied. The groups were considered significantly different when $P<0.05$.

RESULTS

The main objective of the present study was to identify brain areas in which Fos expression following i.p. fluoxetine administration quantitatively differed between fasting and fed states. Thus, the only microphotographs and graphs included are those corresponding to the areas in which such fasting/fed state difference was observed. However, the numerical expression of Fos-positive nuclei for every Fos-immunoreactive brain site is shown in Table 1.

Fig. 1 condenses the number of c-Fos-immunoreactive nuclei (Fos-IR) in the hypothalamic areas in which fluoxetine-induced Fos-IR was significantly higher in fasted than in fed animals. In the ventromedial hypothalamus (VMH) and paraventricular nuclei (PVN), fluoxetine-treated fed and fasted animals exhibited a significantly higher Fos-IR when compared with saline-treated controls. It is important to note that, in the VMH, control saline-treated, fasted rats presented a significantly higher Fos-IR than fed animals.

Fig. 2 depicts the number of Fos-IR in areas in which fluoxetine-induced Fos-IR was significantly higher in fed than in fasted animals. Panel A shows that, in the arcuate nuclei (Arc) and medial amygdala (MeA), Fos-IR was significantly increased in fluoxetine-treated animals, compared with saline-treated controls in both fed and fasting states. Control, saline-treated, fed animals presented a significantly higher Fos-IR in MeA, as compared with control, saline-treated, fasted rats. Panel B shows that in dorsal raphe nuclei (DRN) and in locus coeruleus (LC) both in fed and fasted animals, fluoxetine-treated groups presented a significant increase in Fos-IR when compared with saline-treated controls.