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Research article

Effect of acute imipramine administration on the pattern of forced swim-induced c-Fos expression in the mouse brain

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HIGHLIGHTS

- Forced swim stress for mice increased c-Fos in hypothalamus and brain stem.
- Swim-induced c-Fos increased in additional regions after imipramine treatment.
- Forced swim test for rats and mice may reflect different aspect of antidepressants.

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ABSTRACT

The forced swim test (FST) has been widely used for the preclinical evaluation of antidepressant drugs. Despite considerable differences in the protocol, equivalence of the FST for rats and mice has been rarely questioned. Previous research on the FST for rats revealed that repeated administration of antidepressant drugs attenuates the c-Fos response to swim stress in the hypothalamus and limbic regions. However, few studies have made similar investigations using the FST for mice. In the present study, we explored the mouse brain through immunohistochemistry staining for c-Fos after acute administration of imipramine or saline with or without a subsequent swim session. Imipramine enhanced the c-Fos density in regions of the central extended amygdala, while forced swim stress increased c-Fos expression in some hypothalamic (the ventrolateral preoptic nucleus and dorsomedial nucleus) and brain stem regions, which is consistent with previous reports. In contrast to previous literature with rats, swim stress brought a significant increase in c-Fos expression in the lateral septal nucleus and some other regions in the hypothalamus (the intermediate hypothalamic area, the paraventricular and arcuate nucleus) only in the imipramine-pretreated group, which has not been observed previously. In the arcuate nucleus, double immunostaining revealed that c-Fos was rarely co-expressed with proopiomelanocortin or tyrosine hydroxylase regardless of imipramine treatment. The present results suggest that the activation of several regions in the lateral septum and the hypothalamus underlies antidepressant-like effect in the mouse FST.

1. Introduction

Depression consists of disturbances in emotional, homeostatic, and cognitive functions, and there is a worldwide concern about its immense impact on quality of life in individuals and economic burden upon society [1]. While the available antidepressant drugs show limited efficacy [2], an insufficient understanding of the pathophysiology of depression has hampered the development of novel therapeutic agents. Clinical and experimental studies suggest abnormal functioning of the medial prefrontal cortex and related limbic structures in depressive patients [3].

The forced swim test (FST) is a widely used tool for the screening of potential antidepressant drugs in preclinical studies [4]. It is a reliable and convenient behavioral test that assesses the antidepressant-like effects of a given agent as the decrease of immobility of rats or mice in the water. Regarding its neuronal correlates, some research groups investigated the pattern of brain activation during the FST through c-Fos immunohistochemistry [5–8]. According to their reports, antidepressant pretreatment attenuated c-Fos response to swim stress in the limbic forebrain, hypothalamus, and related regions, all of which have been implicated in the pathophysiology of depression.

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However, there are some differences in the protocols of the FST for rats and mice. While the standard protocol of rat FST consists of two swim sessions (at interval of a day) and repeated administrations of the antidepressant drug, mouse FST is comprised of a single swim session after acute administration of the compound to be tested [9–11]. Nevertheless, whether FST for rats and mice involve the same mechanisms has been rarely questioned, and the extent to which they share neuronal mechanisms remains unknown. Given that all of the above-mentioned studies that demonstrated attenuation of the c-Fos response used rats receiving repeated (or chronic) administration of antidepressants, it remains unknown whether these results may also be observed for mice in the FST.

In the present study, we investigated the effects of the mouse FST protocol, which includes an acute antidepressant administration and a single swim session, on the pattern of neuronal activation using immunohistochemistry labeling for c-Fos. We chose imipramine, a canonical tricyclic antidepressant, and the BALB/c mouse, which is being increasingly used as a stress-vulnerable inbred strain [12]. Through examination of forty brain regions, we examined the neuronal correlates of an antidepressant-like effect and determined whether antidepressant pretreatment attenuated the c-Fos response in stress-related regions following FST in the mouse.

2. Materials and methods

2.1. Animals

Eight-week-old male mice of the BALB/c (BALB/cByJJcl) strain were purchased (Kyudo Co., Saga, Japan), were housed in groups of 2–4 per cage and were kept under standard controlled laboratory conditions (12-h light/dark cycle with lights on at 8:00 AM, 25 ± 2 °C, 50% humidity, pelleted food and water *ad libitum*). To facilitate adaptation to novel surroundings, the mice were transported to the testing room 18–24 h prior to the experiment. All experimental protocols were approved by the ethics committees of Kyushu University (Permit Number: A25-087-02). All procedures were performed in accordance with the ethical standards of the institution and the Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology.

2.2. Drug administration

Imipramine (Sigma-Aldrich, St. Louis, MO; 10 mg/kg) was dissolved in 0.9% saline on the test day, which was 7–10 days after the arrival of the mice in the laboratory. The dose was determined on the basis of a previous report [13]. 30 min prior to testing, imipramine was administered intraperitoneally in a volume of 10 ml/kg. The control animals received injections of 0.9% saline (10 ml/kg). After the treatment, the animals were held in their cages and were either left undisturbed or underwent the FST, generating 4 experimental groups; saline-FST(−), imipramine-FST(−), saline-FST(+), and imipramine-FST(+) groups (n = 6–7 for each group).

2.3. The forced swim test

The FST sessions were conducted between 10:00 and 13:00. Thirty minutes after injection, each mouse in the FST groups was placed in a glass cylinder (17 cm in diameter; 25 cm in height) filled with tap water (25 ± 1 °C, 15 cm deep) without a pretest session. Based on the previous study [7], 10 min duration was chosen for more consistent c-Fos induction, instead of 6 min in the standard protocol. The water was changed between subjects. Behavior was recorded with a digital camera from above, and the immobility time during the last 8 min was scored by trained observers blind to the treatment. A mouse was judged to be immobile when making only those movements necessary to keep its head above water.

2.4. Fos immunohistochemistry

Two hours after the onset of the FST session (150 min after injection), the mice were deeply anesthetized with sodium pentobarbital (100 mg/kg) and perfused transcardially with 0.1 M phosphate buffered saline (PBS), followed by 4% paraformaldehyde in PB (pH 7.4). The brains were left *in situ* for 1 h at room temperature and then removed from the skull. After 4 h postfixation in 4% paraformaldehyde, the brains were saturated with 30% sucrose in PBS for 3 days. The entire brains were sectioned into 40-μm coronal slices with a cryostat (Leica CM3050S; Leica Microsystems, Germany). The selected sections were rinsed in PBS and then pre-incubated for 30 min with 3% H_2O_2 in PBS to deplete endogenous peroxidase activity. After pre-incubation, the sections were rinsed with PBS followed by incubation for 1 h in 1% bovine serum albumin in PBS containing 0.3% Triton X-100 and 0.1% sodium azide. Then, they were incubated in anti-c-Fos antibodies (Santa Cruz Biotechnology, Santa Cruz, CA; 1:50,000, rabbit polyclonal) for 3–4 days at room temperature. The sections were then rinsed in PBS and were incubated in biotinylated anti-rabbit antibody (1:200, goat-anti-rabbit IgG, Vector Laboratories, Inc., Burlingame, CA) for 90 min. The sections were rinsed again and then were processed according to the ABC method using a Vector kit (Vectastain Elite ABC Standard Kit, Vector Laboratories; diluted 1:200 in PBS with 0.1% triton X). After rinsing the sections, they were incubated with diaminobenzidine (DAB, Sigma Aldrich; 500 μg/ml) and nickel-ammonium–sulfate (Wako, Japan; 25 mg/ml, dissolved in 0.2 M acetate buffer) producing a black reaction product in cell nuclei and was terminated by rinsing in PBS. The sections were mounted on gelatin-coated slides, dehydrated in ascending concentrations of ethanol, counterstained with neutral red (Wako), and coverslipped with DPX.

2.5. Fos-immunoreactive cell counting

To select the brain regions for semi-quantitative analysis of the c-Fos-immunoreactive cells, we processed and microscopically examined the sections at 120-μm intervals throughout the brain, except for the olfactory bulb, in two mice from each of the four treatment-groups. Additional sections involving the preoptic areas were further examined for anatomical complexity. In addition to the examination of those sections, the findings of preceding studies on forced swimming [5–8,14–20] and other emotional behaviors [21] were also considered to select the brain regions for semi-quantification. Section planes involving the regions of interest were then standardized according to a brain atlas [22], and sections from the rest of the animals were collected by referring to the atlas. One exception is the intermediate hypothalamic area, which was located ventrolaterally to the anterior hypothalamic area, according to Roeling et al. [23]. Table 1 shows the forty brain areas selected for the analysis, with the anterior-posterior coordinates. The brightfield images of the regions of interest were photographed with a digital camera (DP72; Olympus, Japan) attached to a light microscope (BX51; Olympus; 20× objectives). All microphotographs were exported in TIFF format and processed in ImageJ (http://rsbweb.nih.gov/ij/, ver.1.44, National Institutes of Health, USA), converted to grayscale, and adjusted to proper contrast, and the number of immunoreactive nuclei was counted with a certain threshold. After comparing the result of the ImageJ counting with the microscopic examination, a threshold was established and used uniformly for each region. In most regions, a square frame (250 × 250 μm^2) was used to quantify the