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Research report

Fluoxetine modulates hippocampal cell signaling pathways implicated in neuroplasticity in olfactory bulbectomized mice

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HIGHLIGHTS

- OB causes behavioral and neurochemical changes that mimic depressive symptoms.
- OB induced a significant increase in ERK1/CREB/BDNF pathway in the hippocampus.
- Fluoxetine effects were associated with a reduced ERK1/2 and CREB phosphorylation and BDNF levels.

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ABSTRACT

The olfactory bulbectomy (OB) animal model of depression is a well-established model that is capable of detecting antidepressant activity following chronic drug therapy, and the surgery results in behavioral and biochemical changes that are reminiscent of various symptoms of depression. In the present study, we investigated the degree to which 14 days of p.o. administration of the classic antidepressant fluoxetine (10 mg/kg) were able to reverse OB-induced changes in behavior (namely, hyperactivity in the open-field test and reduced motivational and self-care behaviors in the splash test) and in the activation of hippocampal cell signaling pathways that are thought to be involved in synaptic plasticity. OB caused significant increases in ERK1 and CREB (Ser^{133}) phosphorylation and in the expression of BDNF immunocontent, all of which were prevented by fluoxetine administration. Moreover, fluoxetine administration also caused a significant decrease in ERK2 phosphorylation in mice that had undergone OB. Neither Akt nor GSK-3β phosphorylation was altered in any experimental condition. In conclusion, the present study shows that OB can induce significant behavioral changes that are accompanied by the activation of hippocampal signaling pathways, namely the ERK1/CREB/BDNF pathway, which is involved in the synaptic plasticity. Conversely, fluoxetine prevented these OB-induced behavioral changes and avoided the activation of ERK1/CREB/BDNF in the hippocampus. Taken together, our results extend the data from the existing literature regarding OB-induced behavioral and neurochemical changes, and suggest a possible underlying mechanism that can account for the antidepressant effect of fluoxetine in this model.

1. Introduction

Depression is a debilitating disease with a high prevalence and social cost [1]. Selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine, are among the most widely prescribed antidepressant drugs [2]. Despite their well-established efficacy, the molecular mechanisms underlying the therapeutic activity of SSRIs remain unclear. Neurobiological theories have suggested that the efficacy of fluoxetine may be due to alterations in various signaling pathways that regulate cellular plasticity and survival [3]. Fluoxetine treatment increases the rate of adult hippocampal cell proliferation, but acute administration of the drug does not [4]. This idea concurs with the neurotrophin hypothesis of depression, which involves an increase in the expression of brain-derived-neurotrophic factor (BDNF) that results from the

Abbreviations: ANOVA, analysis of variance; BDNF, brain-derived-neurotrophic factor; CaMK, Ca^{2+}/calmodulin-dependent protein kinase; CREB, cyclic-AMP responsive-element binding protein; ERK, extracellular signal-regulated kinases; FST, forced swimming test; GSK-3β, glycogen synthase kinase-3β; LTD, long-term depression; LTP, long-term potentiation; PI-3K, phosphatidylinositol 3′-kinase; MAPK, mitogen-activated protein kinase; OB, olfactory bulbectomy; PKA, protein kinase A; PKB, protein kinase B; PKC, protein kinase C; SSRIs, selective serotonin reuptake inhibitors; 5-HT, serotonin; TCAs, tricyclic antidepressants; TrkB, tropomyosin-related kinase B; TST, tail suspension test.

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augmented neural activity elicited by antidepressant medications [5]. BDNF activates a variety of signaling cascades, including the phosphatidylinositol 3′-kinase (PI-3K)-Akt [6] and the extracellular signal-regulated kinase 1 and 2 (ERK1/2) pathways [7]. All of these pathways, in addition to several others that promote neuronal survival, converge on the activation of cyclic-AMP responsive-element binding protein (CREB), which is a transcriptional regulator. A number of growth factors and hormones have been shown to stimulate cellular gene expression in the presence of CREB that has been activated by the phosphorylation of Ser^{133} [8]. Several lines of evidence have shown that depressive disorders are related to the activation of glycogen synthase kinase-3β (GSK-3β) by a mechanism dependent on the enzyme dephosphorylation [9]. Protein kinases, such as Akt/protein kinase B (PKB), protein kinase A (PKA), and protein kinase C (PKC), negatively regulate the activity of GSK-3β by phosphorylating it at the N-terminal Ser^{9} residue [9]. In general, inhibiting GSK-3β activity prevents apoptosis; increased GSK-3β activation is proapoptotic. GSK-3β has also recently been identified as a common target of SSRIs, tricyclic antidepressants (TCAs), and antipsychotics [10–12].

The antidepressant efficacies of different classes of compounds are currently assessed by the tail suspension test (TST) and the forced swimming test (FST) [13]. However, a major drawback of these behavioral tools is that antidepressant activity is detected following acute drug administration, whereas several days or weeks may elapse before a therapeutic effect is observed when these drugs are used in the treatment of clinical depression [14]. A widely used model of depression is the olfactory bulbectomy (OB). This model allows for the detection of antidepressant activity almost exclusively following chronic antidepressant treatment, and it often fails to demonstrate antidepressant activity following acute antidepressant treatment [15]. In terms of construct validity, the OB model has been proposed as an animal model of depression because it induces alterations in behavior, and in the endocrine, immune, and neurotransmitter systems that reproduce many of the behavioral and biological symptoms that are commonly observed in depressed patients [16,17].

Previous reports have shown that hippocampal pyramidal neurons in rats that had been subjected to OB were less vulnerable to excitotoxic injury [18], which suggests that the procedure may affect neurotrophic factor-mediated signaling in the hippocampus. However, the cellular and molecular mechanisms underlying the observed neuroprotective effects are not well understood. Therefore, the present study aimed to investigate the effects of OB on the modulation of various signaling targets that are associated with hippocampal synaptic plasticity, including Akt, GSK-3β, ERK1/2, CREB, and BDNF. Moreover, this study also evaluated the degree to which fluoxetine administration was able to reverse OB-induced alterations in both behavior and hippocampal signaling parameters.

2. Methods

2.1. Animals

Female Swiss mice (2 months old and weighing 30–40 g) were housed in an environment that was maintained at room temperature (20–22 °C) with a 12:12 h light:dark cycle (lights on at 07:00 h) and were given free access to food and water. Prior to testing, the cages were kept in the experimental room for 24 h to allow the animals to acclimatize. All of the experimental manipulations were conducted between 9:00 and 17:00 h, and each animal were only used once. All of the procedures used in the present study were performed in accordance with the guidelines set forth in the NIH Guide for the Care and Use of Laboratory Animals and were approved by the local Ethics Committee. Every effort was made to minimize both the number of animals used in the experiments and the degree to which each animal suffered.

2.2. Surgical procedure

After a 2-week acclimatization period, bilateral OB was performed using the suction method that has been described previously [19]. The animals were randomly divided into two groups; one group underwent OB and the other underwent sham operations. Briefly, the mice were anesthetized with a combination of xylazine (6 mg/kg) and ketamine (100 mg/kg) diluted in saline given by intraperitoneal (i.p.) route. To expose the skull, an incision was made in the overlying skin, after which holes were drilled on both sides of the midline. The olfactory bulbs were then bilaterally aspirated using a blunt hypodermic needle (1.0–1.2 cm long with a rounded tip that was 0.80–1.2 mm in diameter) attached to a 10-ml syringe that was used to create suction. Care was taken to avoid damaging the frontal cortex. To stop the bleeding, the holes were filled with swabs and covered with dental cement. A 70% alcohol solution was used to eliminate contamination during all of the surgical procedures. Sham-operated animals underwent all of the same surgical procedures, but the olfactory bulbs were left intact. The mice were allowed to recover under a warming lamp to help with body temperature maintenance. Each animal was given 14 days to recover from the surgery prior to undergoing any further treatment.

2.3. Drugs and treatment

Fluoxetine (10 mg/kg, Sigma Chemical Co.) was dissolved in distilled water and administered orally (p.o.) by gavage. A control group received only the distilled water delivery vehicle.

Two weeks after surgery, fluoxetine (10 mg/kg, p.o.) was administered once a day for 14 days. Each animal was assigned to one of the following groups: (a) sham/vehicle, (b) sham/fluoxetine, (c) OB/vehicle, and (d) OB/fluoxetine. The sham/vehicle and sham/fluoxetine groups were considered the control groups, and each group comprised 9–12 mice.

A diagram of the complete experimental schedule is given in Fig. 1.

2.4. Open-field test

In preclinical assays, the OB model of depression reproduces psychomotor agitation that is consistent with that which is observed in agitated depression [20]. The mice were evaluated using the previously described open-field paradigm to assess the effects of undergoing an OB on locomotor activity [21]. The test was performed during each of 3 consecutive time periods: pre-surgically, 2 weeks after surgery, and post-treatment (2 weeks after the initiation of fluoxetine treatment or p.o. administered water). The number of crossings (squares that were crossed with all 4 paws of an animal) that occurred during a 6-min session was counted. The apparatus was cleaned with a 10% ethanol solution between tests to remove any traces of each animal.

2.5. Splash test

Twenty-four hours after the final drug administration, the splash test was conducted in accordance with the protocol described by Isingrini and co-workers [22] with minor modifications. This test consists of squirting a 10% sucrose solution onto the dorsal coat of a mouse that has been placed individually in a clear Plexiglass box (9 cm × 7 cm × 11 cm). Because of its viscosity, the sucrose solution soils the fur of the mouse, which causes the animal to initiate grooming behavior. During the 5-min period following the application of the sucrose solution, the latency to grooming and the grooming time spent by each animal were manually recorded as indices of self-care and motivational behavior [23,24]. Between tests, the apparatus was cleaned with a 10% ethanol solution to remove any traces of each animal.

2.6. Western blot

After 2 weeks of treatment, and 24 h after the last oral administration of either fluoxetine or the control vehicle, the mice were decapitated. Their brains were subsequently removed, and the lesions were immediately macroscopically inspected. Any brains with incomplete surgeries or cortical damage were excluded from further analysis. The hippocampus from each brain was then rapidly dissected and placed in cold saline solution. Western blot analyses were performed in accordance with a previously described protocol [25,26]. Briefly, the hippocampal tissue was mechanically homogenized in 400 μl of a solution that contained Tris-base (50 mM, pH 7.0), EDTA (1 mM), sodium fluoride (100 mM), PMSF (0.1 mM), sodium vanadate (2 mM), 1% Triton X-100, and 10% glycerol, after which the homogenate was incubated in ice for 30 min. The lysates were later centrifuged (10,000 × *g* for 10 min, at 4 °C) to eliminate cellular debris, and the supernatants were diluted at a 1:1 (v/v) ratio in a solution containing Tris-base (100 mM, pH 6.8), EDTA (4 mM), 8% SDS, and 16% glycerol. The protein content of the resulting solution was estimated using a previously described method [27], and the protein concentration was calculated using a standard bovine serum albumin curve. To compare the signals that were obtained, identical quantities of protein (70 μg per lane) from each sample were electrophoresed through 10% SDS-PAGE minigels (after the addition of 0.2% bromophenol blue and 8% β-mercaptoethanol) and transferred to nitrocellulose or polyvinylidene fluoride membranes. To verify the efficiency of the transfer process, the gels were stained with a Coomassie blue solution (containing 0.1% Coomassie