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Research report

Antidepressant activity of fluoxetine in the zinc deficiency model in rats involves the NMDA receptor complex

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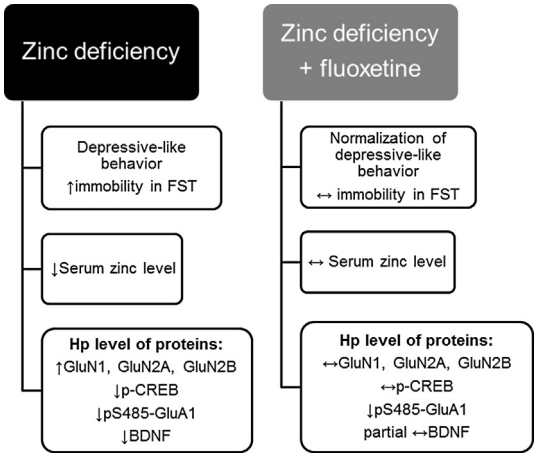
HIGHLIGHTS

- The effects of zinc deficiency (ZnD) and fluoxetine (FLX) treatment were examined.
- FLX counteracts ZnD-induced depression-like behavior in the FST.
- FLX counteracts ZnD-induced changes in GluN1, GluN2A, GluN2B and p-CREB in the Hp.
- Our data support ZnD-model of depression with improved predictive validity.

GRAPHICAL ABSTRACT

The effects of fluoxetine on zinc deficiency induced alterations. Legend: FST – forced swim test; Hp – hippocampus; ↔ – normalization.



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ABSTRACT

The zinc deficiency animal model of depression has been proposed; however, it has not been validated in a detailed manner. We have recently shown that depression-like behavior induced by dietary zinc restriction is associated with up-regulation of hippocampal N-methyl-D-aspartate receptor (NMDAR). Here we examined the effects of chronic administration of a selective serotonin reuptake inhibitor, fluoxetine (FLX), on behavioral and biochemical alterations (within NMDAR signaling pathway) induced by zinc deficiency. Male Sprague Dawley rats were fed a zinc adequate diet (ZnA, 50 mg Zn/kg) or a zinc deficient diet (ZnD, 3 mg Zn/kg) for 4 weeks. Then, FLX treatment (10 mg/kg, i.p.) begun. Following 2 weeks of FLX administration the behavior of the rats was examined in the forced swim test (FST) and the spontaneous locomotor activity test. Twenty four hours later tissue was harvested. The proteins of NMDAR (GluN1, GluN2A and GluN2B) or AMPAR (GluA1) subunits, p-CREB and BDNF in the hippocampus (Western blot) and serum zinc level (TXRF) were examined. Depression-like behavior induced by ZnD in the FST was sensitive to chronic treatment with FLX. ZnD increased levels of GluN1, GluN2A, GluN2B and decreased pS485-GluA1, p-CREB and BDNF proteins. Administration of FLX counteracted the zinc

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restriction-induced changes in serum zinc level and hippocampal GluN1, GluN2A, GluN2B and p-CREB but not BDNF or pS845-GluA1 protein levels. This finding adds new evidence to the predictive validity of the proposed zinc deficiency model of depression. Antidepressant-like activity of FLX in the zinc deficiency model is associated with NMDAR complex.

1. Introduction

Since the first report on antidepressant-like properties of the N-methyl-D-aspartate receptor (NMDAR) antagonists [1] multiple lines of evidence have linked dysfunction of the glutamatergic system to the pathophysiology of depression. Drugs acting on glutamatergic targets show promise as novel antidepressants [2,3]. Selective serotonin reuptake inhibitors (SSRIs) are among the first-line medications for major depressive-disorder (MDD) [4]. Of note, chronic administration of antidepressants, which primarily act as serotonin and/or noradrenaline reuptake inhibitors, affects NMDAR expression and/or activity [5,6]. Thus, conventional antidepressants and NMDAR antagonists may share a common endpoint for antidepressant activity of dampening NMDAR function [5]. A mechanism has been proposed whereby administration of antidepressants leads to modulation of NMDAR indirectly through cAMP response element binding protein (CREB) and brain-derived neurotrophic factor (BDNF) whereas NMDAR antagonists attenuate its function directly [5]. In addition to NMDARs, another class of ionotropic glutamate receptors, alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptors (AMPARs), is implicated in the pathophysiology of depression and mechanism of antidepressant activity. AMPAR activation is known to produce antidepressant-like effects in preclinical paradigms [7] and is involved in the antidepressant-like effects of NMDAR antagonists [8]. Moreover, similarly to NMDAR, AMPAR is a target for conventional antidepressants [9,10], e.g. chronic treatment with an SSRI fluoxetine (FLX) increases phosphorylation of GluA1, a major subunit of AMPAR, on Serine-845 [11].

Zinc ions, which inhibit NMDAR and potentiate AMPAR ([12,13,71]), are among modulators of ionotropic glutamate receptors with antidepressant properties [14–16]. Antidepressant-like activity of zinc was demonstrated in a number of preclinical tests and models of depression [17–21,22,70]. Moreover, it was shown that zinc supplementation may be beneficial in depressed patients as an augmentation strategy (in adjunction to tricyclic antidepressants [23,24]) or SSRIs [23,25] or as a stand-alone intervention for depression [26,27].

Recently, the knowledge of prevalence of zinc deficiency and its impact on health has grown [28]. It is estimated that 17% of the world's population is at risk of inadequate zinc intake [29] and zinc deficiency is ranked among the leading risks for both mortality and the burden of disease [30]. Noteworthy, zinc deficiency belongs to the group of medical conditions with psychiatric manifestations [31]. A link between severe nutritional zinc deficiency and depression has been known for many years. Disturbance of mood is a feature of acrodermatitis enetropathica, a rare inherited form of zinc deficiency [32]. Depression, amidst other symptoms of zinc deficiency, occurs in patients on total parenteral nutrition without added zinc and improves dramatically when oral or parenteral zinc supplements are administered [33,34]. A link between reduced intake of zinc and depression has been recently shown in large, cross-sectional, population-based epidemiological studies [35,36]. Moreover, recent meta-analysis study demonstrated that MDD is associated with a lower concentration of zinc in peripheral blood [37]. It should be noted that most of the studies included in the meta-analysis reported the concentration of zinc in blood of depressed patients to be within normal laboratory reference ranges, however it was often near the lower boundary of the normal range [37]. Although the first prospective study examining the association of zinc intake and depression risk indicated a modest, but significant, inverse correlation between zinc intake and depression in a cross-sectional setting, the 20-year prospective follow-up observations have suggested that a low dietary zinc intake may not precede depression in initially depression-free men [38]. Yet, there were some limitations to the study, e.g., the study sample comprised exclusively men, furthermore, depression was defined in the prospective setting as a hospital admission due to depressive disorder. In contrast, in a prospective study of both men and women, a low dietary zinc intake emerged as a risk factor for depression [39].

Data from rodent studies further support a causative role of dietary zinc restriction in the induction of depression-like symptoms [40–44] or anhedonia [40,45]. Based on these findings, zinc deficiency was proposed as an animal model of depression [42,46,47]. We have previously shown that depression-like behavior induced by dietary zinc restriction is associated with changes in NMDAR signaling pathway. Up-regulation of GluN2A and GluN2B subunits of NMDAR with concomitant decreased expression of phosphorylated CREB (p-CREB) and BDNF proteins in the hippocampus (Hp) were observed following zinc regimen [48]. Here we tested whether chronic treatment with an SSRI, FLX, would rescue behavioral and biochemical deficits induced by zinc restriction. In addition to GluN2A and GluN2B subunits of NMDAR, p-CREB and BDNF we focused on GluN1 subunit of NMDAR as well as GluA1 subunit of AMPAR and phosphorylated on Serine-845 GluA1 (pS845-GluA1).

2. Material and methods

2.1. Animals and diet

All procedures were conducted according to the National Institutes of Health Animal Care and Use Committee guidelines and were approved by the Ethics Committee of the Institute of Pharmacology, Krakow. The experiments were carried out on male Sprague Dawley rats; animals were derived from Charles River Laboratories (Germany) at the age of 4 weeks and habituated to the laboratory conditions for 1 week prior to use. During the habituation phase, the rats were fed a standard diet with 35 mg Zn/kg. Following the habituation phase, the animals were divided into ZnA and ZnD groups; that were fed a zinc adequate diet (ZnA) of 50 mg Zn/kg or a zinc deficient diet (ZnD) of 3 mg Zn/kg, respectively, for 4 weeks. All of the diets were purchased from Altromin GmbH (Lage, Germany). The animals were housed 5 per cage in a controlled environment (temperature 22 ± 2 °C, 12 h light/dark cycle, 40–50% humidity) with free access to food and water.

2.2. Drug administration

Following 4 weeks of the diet, the ZnA and ZnD groups were further divided into subgroups, that received (in addition to the diet) once daily fluoxetine hydrochloride (FLX) (a generous gift from ICN Polfa Rzeszów S.A.) at the dose of 10 mg/kg of body weight, dissolved in sterile 0.9% sodium chloride solution (0.9% NaCl) or 0.9%