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Research report

The role of the GPR39 receptor in zinc deficient-animal model of depression

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HIGHLIGHTS

- Zinc deficiency causes depressive-like behaviour.
- Zinc deficiency induces a decrease in expression of the BDNF protein.
- Zinc deficiency induces a decrease in expression of the GPR39 receptor.
- Impaired GPR39 neurotransmission is a possible mechanism of the pathophysiology of depression.

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ABSTRACT

During the last decade it has been shown that zinc may activate neural transmissions via the GPR39 Zn^{2+}-sensing receptor, which can be involved in the regulation of neuronal plasticity. According to the neurotrophic hypothesis of depression, decreased brain derived neurotrophic factor (BDNF) levels in depressed patients play a key role in the pathogenesis of this disorder. BDNF, similarly as zinc, is known to be involved in the process of neuron survival and the regulation of neuronal plasticity. The aim of the present study was to determine whether the administration of a 6-week diet deficient in zinc would cause depressive-like behaviour and if such behavioural alterations would correlate with changes in the expression of the BDNF protein and GPR39 receptor. In the first part of the present study the animal behaviour after a 6-week zinc-deficient diet, in the forced swim test (FST) was investigated. In the second part expression of the GPR39 and BDNF protein level in the frontal cortex was measured using the Western Blot method. Administration of a zinc-deficient diet for 6 weeks increased immobility time in the FST by 24%, so exerted depression-like behaviour. A biochemical study showed a significant reduction in GPR39 (by 53%) and BDNF (by 49%) protein expression in the frontal cortex in mice receiving the zinc deficient diet for 6 weeks. Our study provides evidence that the GPR39 Zn^{2+}-sensing receptor may be responsible for lowering the BDNF protein level and in consequence may be involved in the pathogenesis of depression.

1. Introduction

Depression is an increasingly common mood disorder, which often end up committing suicide. Lack of proper medical treatment is due to the fact, that the pathogenesis of the disease still remains unclear. Last two decades have provided evidence suggesting that unipolar depression may be associated with inflammation, cell-mediated immune (CMI) activation, oxidative and nitrosative stress (O&NS) and neuroprogression, term used to describe progressive neuroanatomical dysfunctions in depression including neurodegeneration, increased neuronal apoptosis, reduced neurogenesis and lowered neurotrophic factors [see [1–3]; for a review]. Studies of Maes et al. showed correlation between the immuno-inflammatory and neuroprogressive theory of depression with lower serum zinc observed in depressed patients [4,5]. There is strong evidence that depression is accompanied by lower serum zinc [4,6–8]. These changes are explained as a consequence of the sequestration of zinc-metallothionein and increased synthesis in

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the liver of the metallothioneins, which are induced by interleukin-1β (IL-1β) and elevated interleukin-6 (IL-6), observed in depressed patients as well [9, see [1]; for a review]. Another studies showed correlation between the immuno-inflammatory theory of depression and lower serum zinc, in patients with chronic fatigue syndrom (CFS). They found negative correlation of low serum zinc status and increase in the alpha2 protein fraction, and positive correlation to decrease in the expression of mitogen-induced CD69+ (a T cell activation marker) on CD3+ as well as CD3+ CD8+ T cells [10]. Maes et al. proposed lower serum zinc as a state marker in treatment resistant depression and as the first reported that lower serum zinc was significantly related to immune/inflammatory markers, such as CD4+/CD8+ T-cell ratio (negative), serum albumin and transferin (positive) and serum IL-6 (negative) [4,5].

Results coming from preclinical [11,12] and clinical [8,13,14] studies showed zinc as an enhancer of common antidepressant therapy. Several studies showed better response to common antidepressants, supplemented with zinc [8,13]. This clinical efficacy of zinc in treating depression may in part be explained by the anti-oxidative properties of zinc [see [1]; for a review]. According to review of Leonard and Maes [1] activation of oxidative and nitrosative pathways may contribute to depression, causing damage to DNA, mitochondria, proteins, functional intracellular signalling molecules involved in the pathophysiology of depression. Chronic zinc deprivation may result in an increased sensitivity to O&NS, while its administration increases the antioxidant capacity [see [1]; for a review].

There is growing evidence of the participation of zinc in mood disorders. Zinc is one of the most important trace elements required for cell division and differentiation via replication, transcription and protein synthesis [15], and as the last two decades have shown, in proper neurotransmission as well. Zn^{2+} is selectively stored in, and released from, the presynaptic vesicles of a specific type of neuron called 'gluzinergic' [16], because of the co-release with glutamate. Most gluzinergic neurons are located at cortical or limbic structures, directly connected to the pathophysiology of depression.

There are three different pools of zinc in the brain. The first is histochemically reactive, the other one exists as a zinc metaloproteins [17], and the last is released as a free zinc [16,18]. After it's release from presynaptic terminals, zinc affects and modulates various receptors, mainly the glutamatergic NMDA one. Authors referring to the glutamatergic theory of depression [19,20], explained the antidepressant action of zinc via inhibition of the NMDA receptor [21–23]. Besides NMDA, zinc influences other types of receptors, such as the AMPA, metabotropic (mGluR) and GABA receptors.

There is strong evidence that antagonists of this jonotropic receptor, such as ketamine or traxoprodil, exhibit antidepressant properties [20]. According to the immune-inflammatory theory of depression it should be emphasized that ketamine is a strong anti-inflammatory agent [see [24]; for a review]. Chang et al. [25] found that ketamine inhibited IL-1β release in primary cultured microglia and extracellular signal-regulated kinase (ERK1/2) phosphorylation, causing in part microglial inactivation [25].

Studies over the last decade showed that zinc may activate metabotropic GPR39 receptor [26–29]. The GPR39 receptor is widely expressed in the body, including the central nervous system [30]. It was initially considered that GPR39 is an orphan receptor [31]. Study by Zhang et al. [32] indicated that obestatin is a natural endogenous ligand for this metabotropic receptor, but nobody has confirmed these findings [26,27,33]. Finally it was proved that the natural ligand for the GPR39 receptor is zinc [26–28]. According to Hershfinkel et al. [34] this receptor is capable of sensing extracellular Zn^{2+}, thereby activating diverse signal-transduction pathways. Zinc activates three different signalling pathways via the GPR39 receptor. Study of Yasuda et al. [28] showed GPR39 as a Gq-coupled Zn^{2+}-sensing receptor, acting through the $G_q\alpha$-PLC pathway. Another study showed that GPR39 exhibits very high constitutive activity for the activation of cAMP response element-driven transcription [35], mainly phospho-CREB/CRE pathway. Phospho-CREB is involved both in the adaptive response to stress, as well as, in the physiological and pharmacological regulation of the expression of BDNF [36]. BDNF increase has been reported for selective serotonin reuptake inhibitors (SSRIs), noradrenaline reuptake inhibitors (NRIs), serotonin and noradrenaline reuptake inhibitors (SNRIs) (dual-action antidepressants and tricyclic antidepressants), with monoamine oxidase inhibitors (MAOIs), atypical antidepressants, as well as with electroconvulsive shock treatment, one of the most clinically effective treatments for refractory depression [see [37]; for a review].

In the present study we determine whether the administration of a 6-week diet deficient in zinc would cause depressive-like behaviour and if such behavioural alterations would correlate with changes in the expression of the BDNF protein and the GPR39 Zn^{2+}-sensing receptor. This is the first report showing the participation/exploration of the GPR39 receptor in depressive-like behaviour.

2. Materials and methods

2.1. Animals

3-week-old male CD-1 mice (±16 g) were housed under standard laboratory conditions with a natural day-night cycle, a temperature of 22 ± 2 °C and the humidity at 55 ± 5% as well as access to food and water *ad libitum*. Each experimental group consisted of 7 animals. All of the procedures were conducted according to the National Institute of Health Animal Care and Use Committee guidelines, which were approved by the Ethical Committee of the Jagiellonian University Medical College, Kraków.

2.2. Zinc adequate and zinc deficient diets

Zinc adequate (33.5 mg Zn/kg) and zinc deficient (0.2 mg Zn/kg) diets were purchased from MP Biomedicals (France) and administrated for 6 weeks. After this period of time, the body weight of each mouse was measured.

2.3. Behaviour

2.3.1. Forced swim test (FST)

The studies were carried out on mice according to the method of Porsolt et al. [38]. The FST was performed after 6 weeks of diet. The animals were dropped individually into glass cylinders (height 25 cm, diameter 10 cm) containing 10 cm^3 of water, maintained at 23–25 °C. The mice were left in the cylinder for 6 min. After the first 2 min, the total duration of immobility was measured during the following 4-min test. The mouse was judged to be immobile when it remained floating passively in the water.

2.3.2. Locomotor activity

Locomotor activity was performed after 6 weeks of feeding mice with zinc-deficient diet and was measured with photoresistor actometers (circular cages, diameter 25 cm, two light beams). The animals were individually placed in an actometer and their activity was then measured between 2 and 6 min. The number of crossings of the light beams by the mice was then recorded as the locomotor activity.

2.4. Biochemical study

The animals were killed by rapid decapitation, blood was collected for zinc determination and the frontal cortex was rapidly dissected and stored at −80 °C until the GRP39 and BDNF analysis.

2.5. Zinc assay

Zinc levels were determined in serum. Due to the low volumes, no sample pretreatment procedures were applied. The thawed samples were thoroughly mixed as these were not homogenous, and then analyzed directly by means of the atomic absorption spectrometry (AAS) method. In some instances (the samples with the smallest volume), the electrothermal technique (ET AAS) was used, while for samples with a higher volume, the flame technique (F AAS) was applied.

For both techniques, the determination procedure and instrumental parameters were optimized to obtain the highest possible sensitivity. On the other hand, the procedure was prepared in a way so as to prevent any possible contamination of