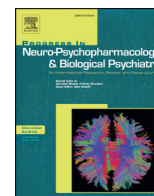




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The level of the zinc homeostasis regulating proteins in the brain of rats subjected to olfactory bulbectomy model of depression



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ABSTRACT

Background: Zinc transporters (ZnTs) and metallothioneins (MT) are important in maintaining Zn homeostasis in the brain. The present study was designed to find out whether alterations in ZnTs and MTs are associated with the pathophysiology of depression and the mechanism of antidepressant action.

Methods: Messenger RNA and proteins of ZnT1, ZnT3, ZnT4, ZnT5, ZnT6 and MT1/2 were measured in the prefrontal cortex (PFC) and hippocampus (Hp) of rats subjected to olfactory bulbectomy (OB) (a model of depression) and chronic amitriptyline (AMI) treatment by Real Time PCR and Western Blot/Immunohistochemistry (IHP).

Results: Results in the OB rats showed: increases in the protein levels of ZnT1 in the PFC and Hp and MT1/2 in the PFC; a decrease in ZnT3 protein level in the PFC; no changes in ZnT4, ZnT5 and ZnT6 in the PFC and Hp. IHP labeling revealed increases in the optical densities of ZnT1-IR in the PFC and Hp and decreases in ZnT3 and ZnT4-IR in the PFC of OB rats. Although OB had no effects on gene expression of ZnTs, mRNAs for MT1/2 were increased. Chronic AMI treatment did not influence protein levels of ZnTs and MT1/2 in Sham and OB rats; however decreased mRNA levels of ZnT4 and ZnT5 in PFC and ZnT1, ZnT3, ZnT4 and ZnT6 in Hp of Sham rats and normalized OB induced increase in MT1/2 gene expression.

Conclusions: Changes in ZnTs and MT1/2 suggest altered cortical distribution of Zn in the OB model which further supports the hypothesis that Zn dyshomeostasis may be involved in the pathophysiology of depression.

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1. Introduction

Zinc is the second most abundant trace element in the human body, essential for life. It is involved in a variety of biological processes as a structural, catalytic and intracellular signaling component (Marger et al., 2014). These diverse roles are possible due to the functions of different proteins regulating Zn homeostasis. These proteins are

membranous transporters (ZnTs); members of the Zip family Zn-transporters and metallothioneins.

The ZnT zinc transporter family is composed of 10 members (ZnT1–ZnT10) while the ZIP (Zrt-, Irt-like protein) transporter family has 14 members (ZIP1–ZIP14) (Kambe et al., 2015). Genes encoding ZnTs are assigned to the Solute Carrier family 30A (SLC30A) but ZIP encoding genes are assigned to the Solute Carrier family 39A (SLC39A) (1–14). ZnTs and ZIPs have opposing functions. While, ZnTs reduce the intracellular Zn concentration to nanomolar levels through the export of Zn from cytosol into the extracellular space or into the intracellular compartments, ZIP proteins increase intracellular Zn level by transporting it from the extracellular space or from intracellular organelles back into the cytoplasm (Palmiter and Findley, 1995; Liuzzi and Cousins, 2004). The efficacy of Zn transport depends on the density of Zn transporters in the subcellular components. In turn, the pattern of expression and localization of Zn transporters correlates with the Zn present in the cells (Liuzzi and Cousins, 2004). The third group of Zn homeostasis-regulating proteins are the metallothioneins (MTs) that function to immediately coordinate intracellular Zn levels (Kimura and Kambe, 2016).

Abbreviations: AMI, amitriptyline; GABA, gamma-aminobutyric acid; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Hp, hippocampus; IHP, immunohistochemistry; IR, immunoreactivity; MT, metallothionein; NMDA, N-methyl-D-aspartic acid; NO, nitric oxide; OB, olfactory bulbectomy; PFC, prefrontal cortex; SDS, sodium dodecyl sulphate; SLC30A, Solute Carrier family 30A; SLC39A, Solute Carrier family 39A; SOD1, superoxide dismutase; TGN, Golgi network; TRPML1, transient receptor potential mucolipin 1; TXRF, total reflection X-ray fluorescence; ZnT, zinc transporter; ZIP, Zrt-, Irt-like protein transporter.

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A growing body of work suggests a link between Zn dyshomeostasis and neurological, neurodegenerative and mood disorders including depression [for review (Szewczyk, 2013; Prakash et al., 2015)]. Clinical studies have long shown that depressed patients exhibit a lower serum Zn level than psychiatrically normal controls and that Zn supplementation may enhance antidepressant therapy in depressed patients (Nowak et al., 2003a; Siwek et al., 2009; Siwek et al., 2010; Ranjbar et al., 2013; Swardfager et al., 2013; Ranjbar et al., 2014; Nowak, 2015). Additionally, preclinical studies show that Zn exerts antidepressant-like activity in different tests and models of depression and that Zn deficiency induces depression-like symptoms in animals (Krocza et al., 2000; Krocza et al., 2001; Rosa et al., 2003; Nowak et al., 2003b; Cunha et al., 2008; Tassabehji et al., 2008; Szewczyk et al., 2009; Tamano et al., 2009; Whittle et al., 2009; Szewczyk et al., 2010; Mlyniec et al., 2012; Doboszewska et al., 2015a; Doboszewska et al., 2015b) and humans (Amani et al., 2010; Jacka et al., 2012; Maserejian et al., 2012).

One of the most widely used animal models of depression is the olfactory bulbectomy model (OB), characterized by both face and predictive validity (Czeh et al., 2016). Removal of the olfactory bulbs induces changes in endocrine, immune, and neurotransmitter systems, as well as behavioral alterations that resemble the symptoms observed in patients with major depression (van Riesen et al., 1976; Kelly et al., 1997; Song and Leonard, 2005; Hendriksen et al., 2015). The olfactory bulbs together with the hippocampus and amygdala are a part of the limbic region of the brain responsible for emotions and memory. It is believed that the behavioral alterations observed after OB result from dysfunctions or/and compensatory mechanisms of the cortical-hippocampal-amygdala circuits, the same neuroanatomical regions that are dysfunctional in patients with MDD (Price and Drevets, 2012). In animals subjected to OB hyperactivity, changes in social behavior, deficits in learning and memory, and changes in taste-aversion behavior have been observed (Kelly et al., 1997; Song and Leonard, 2005). Moreover, chronic administration of antidepressants reverses the behavioral, endocrine, immune, and neurotransmitter changes that occur after bulbectomy [for review (Kelly et al., 1997; Song and Leonard, 2005)]. Our earlier studies showed that both acute and chronic administration of Zn reduced the OB-induced hyperactivity in rats and the number of trials needed for the learning passive avoidance in OB rats (Nowak et al., 2003b).

The present study was designed to examine zinc transporters (ZnTs) and metallothioneins (MTs) – proteins that regulate brain Zn homeostasis, in the OB model of depression. We measured mRNA and protein levels of ZnT1, ZnT3, ZnT4, ZnT5 and ZnT6 which function primarily to reduce intracellular Zn availability. All of these transporters are expressed in the brain and may play important roles in the pathophysiology of neurodegenerative disorders and in the aging process (Beyer et al., 2012; Bosomworth et al., 2013; Lyubartseva et al., 2010a; Whitfield et al., 2015). We also measured the levels of MT1 and MT2 which serve as intracellular zinc reservoirs (Kimura and Kambe, 2016) and together with ZnTs regulate cellular levels of Zn. We performed our studies in the prefrontal cortex (PFC) and hippocampus (Hp) of Sham and OB rats. Functional and structural changes in these brain structures have been implicated in the memory and cognitive deficits observed in OB rats (Song and Leonard, 2005) and in the pathophysiology of human depression (Kupfer et al., 2012). The hippocampus and cortex are also the brain regions where the highest Zn concentrations are found (Frederickson et al., 2000). The effect of chronic treatment with an antidepressant drug (amitriptyline (AMI)) on mRNA and protein levels of ZnTs and MTs in both Sham and OB rats was also studied.

2. Materials and methods

2.1. Animals and housing

All procedures involving the use of animals were carried out according to the National Institute of Health Animal Care and Use Committee guidelines and approved by the Ethical Committee of the Institute of

Pharmacology, Krakow. Experiments described here were carried out on male Sprague-Dawley rats (220–250 g; 7 weeks old). Animals were maintained on a normal day-night cycle (light phase 7:00–19:00) and temperature (19–21 °C) with ad libitum access to food and water.

2.2. Olfactory bulbectomy – surgical procedure

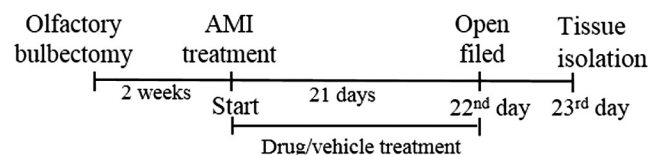
Olfactory bulbectomy was carried out as previously described by Pochwat et al. (2015b). Briefly, 1 week after arrival in the laboratory, animals were subjected to bilateral olfactory bulbectomy under anaesthesia (ketamine (100 mg/kg)/xylazine (10 mg/kg)). Metoxicam (0.05 mg/kg, s.c.) was given as an analgesic and anti-inflammatory drug 1 h before and two days after surgery. Following exposure of the skull, burr holes were drilled using the following coordinates 7 mm anterior to the bregma and 2 mm (on either side) from the middle line, i.e. at a point corresponding to the posterior margin of the orbit of the eye. Olfactory bulbs were removed by suction, and burr holes filled with a hemostatic sponge (Ferrosan, Poland) following which skin around surgery area was sutured. Sham-operated animals were similarly treated, but with bulbs left intact. Animals were allowed to recover for two weeks following surgery. During this period, animals were handled daily by the experimenter to eliminate any aggressiveness that would otherwise develop (Leonard and Tuite, 1981). On day 15, antidepressant therapy was initiated. Amitriptyline (AMI, 10 mg/kg) was administered (intraperitoneally; *i.p.*) chronically once daily for 21 days. Control animals were treated with vehicle (0.9% sodium chloride). AMI and vehicle were injected at a constant volume of 2 ml/kg. Twenty-four hours after the last dose of chronic (21 days) of AMI injections (day 22nd) the open field test was performed. After the test, animals were injected once more and 24 h later (day 23rd) brain tissue was collected (Scheme 1). Only rats with completely removed olfactory bulbs but without significant damage to the frontal cortex (as visually assessed post-sacrifice) and rats showing hyperactivity in the open field test (Fig. 1), were selected for further studies.

2.3. Open field test

The open field test was performed as described by Pochwat et al. (2015b) using an “open field” apparatus (an arena 90 cm in diameter, divided into 10 cm squares by faint yellow lines). The arena was surrounded by a 75 cm high aluminum sheet. All measurements during the experimentation were carried out in a darkened room. Illumination was provided by a 60-W bulb positioned 90 cm above the floor. Animals were placed individually in the center of the open field and allowed to freely explore it for 3 min. The behavior of interest included ambulation scores which is defined as the number of sector lines crossed (once a line had been crossed with all four paws, Fig. 1). Results are expressed as group means \pm SEM ($n = 13$ –16 per group). Differences between groups were analyzed using two way analysis of variance and post hoc Newman-Keuls multiple comparisons test.

2.4. Tissue collection

Animals were sacrificed by rapid decapitation 24 h after the last dose of AMI (day 23; Scheme 1). For mRNA and protein analyses brains were rapidly removed with the PFC and Hp dissected out on an ice-cold glass



Scheme 1. Schedule of the experimental procedure.

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