Pharmacological Reports 69 (2017) 456–461

Contents lists available at ScienceDirect

Pharmacological Reports

journal homepage: www.elsevier.com/locate/pharep

Short communication

Antidepressant activity of zinc: Further evidence for the involvement of the serotonergic system

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ARTICLE INFO

Article history:
Received 7 November 2016
Accepted 11 January 2017
Available online 19 January 2017

Keywords:
Zinc
5-HT1ARs
Dopamine
Serotonin

ABSTRACT

Background: The present study sought to further evaluate the role of the serotonergic system especially the postsynaptic 5-HT1A receptors (5-HT1AR) in the mechanism of antidepressant action of zinc.
Methods: Messenger RNA (mRNA), protein level, and 5-HT1AR density as well as the rate of monoamine (dopamine, DA, and serotonin) metabolism in the prefrontal cortex (PFC) and hippocampus (Hp) of rats subjected to acute and chronic (21 days) zinc (5 mg Zn/kg) treatment were measured.
Results: Acute or chronic zinc treatment did not induce any changes in 5-HT1AR mRNA levels in the PFC or Hp of rats. However, chronic zinc treatment induced increases in both 5-HT1AR protein levels and density of 5-HT1A receptor binding sites in the Hp of rats. Chronic zinc treatment also increased tissue levels of serotonin metabolite and turnover in the rat Hp. On the other hand, DA, DOPAC, HVA tissue levels increased while DOPAC/DA and 3MT/DA decreased in the PFC of rats after chronic zinc treatment. Acute treatment induced increases only in tissue levels of DOPAC, and DOPAC/DA.
Conclusions: Our results confirm that the antidepressant effects of zinc are mediated in concert with the modulation of the serotonergic system including postsynaptic 5-HT1ARs and allude to a possible involvement of dopaminergic neurotransmission in this action.

Introduction

Zinc is thought to be involved in the pathophysiology and treatment of depression. Recent studies showed significant antidepressant-like effects of zinc after acute and chronic administration in several tests and models of depression (for review see [1]). Zinc's antidepressant activity is thought to be associated with the modulation of NMDA/glutamate-mediated neurotransmission (for review see [2]). The role of serotonergic neurotransmission in this action has also been suggested. For instance, the additive effects of zinc and selective serotonin reuptake inhibitors (citalopram and fluoxetine) in the forced swim test (FST) have been demonstrated [3,4]. Moreover, the depletion of serotonin by *para*-chlorophenylanine (PCPA) completely blocks the antidepressant-like effects of zinc in the FST [5], indicating a requirement for an intact serotonergic system for these effects to occur. Additionally, earlier studies, using a selective 5-HT1AR antagonist (WAY 100635) demonstrated the important role of these receptors in the antidepressant action of zinc in the FST [5,6]. Recently, both the binding and behavioural studies [6] showed the direct modulatory effects of zinc at the 5-HT1AR and indicated a concentration-dependent dual mechanism of zinc action at 5-HT1ARs, with potentiation at a low dose and inhibition at a high dose. The *in vivo* studies further show that zinc can modulate both presynaptic and postsynaptic 5-HT1ARs [6].

Based on the above data and the fact that adaptive changes in the serotonergic system are generally believed to underlie the therapeutic effect of antidepressant drugs, the present study was

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http://dx.doi.org/10.1016/j.pharep.2017.01.008
1734-1140/

designed to further evaluate the role of zinc in the modulation of the serotonergic system using neurochemical, biochemical and radioligand binding assays.

Material and methods

Animals

Experiments were carried out with male Sprague-Dawley rats, (Charles, River, Germany), kept under standard laboratory conditions of lighting (light phase: 7:00–19:00) and temperature (21±2 °C), with free access to water and food. All procedures were performed according to the guidelines of the National Institutes of Health Animal Care and Use Committee with approval from the Ethics Committee of the Institute of Pharmacology PAS in Krakow. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Drug administration and tissue collection

Zinc (dose refer to mg Zn/kg) was administered intraperitoneally (*ip*) as zinc hydroaspartate (Farmapol, Poland) either acutely (30 min before decapitation) or chronically for 21 days (last dose administered 24 h before decapitation). Controls were treated with 0.9% NaCl and are indicated as Veh on the graphs. Animals were sacrificed under non-stress conditions by rapid decapitation following which brains were rapidly removed. Both the PFC and Hp were dissected on an ice-cold glass plate, frozen on dry ice and stored at −80 °C until required.

Western blot analyses

Tissue preparation and western blot analyses were done as previously described [7]. Briefly, tissue samples from the PFC and Hp were homogenized in 2% solution of sodium dodecyl sulphate (SDS), denatured at 95 °C for 10 min and finally centrifuged for 5 min at 10,000 rpm at 4 °C. Proteins present in the supernatant were fractionated by 10% SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membrane (Invitrogen, Paisley, UK). Membranes were subsequently subjected to: blocking with 1% blocking solution (Roche), incubation (overnight at 4 °C) with rabbit polyclonal anti-5-HT1AR antibody (1:500, Abcam), washing (3 × 10 min) with Tris-buffered saline containing Tween 20 (TBS-T), incubation (30 min) with secondary anti-rabbit- IgG-peroxidase conjugated antibody (1:7000, Roche), washing (3 × 10 min) with TBS-T; incubation with detection reagent (Roche). To check for transfer and loading, β-actin was indicated on each blot (Millipore; 1:8000). The optical densities of the ensuing proteins were measured and analyzed using the Image Gauge v.4.0 software. Results are given as the ratio of the optical density of 5-HT1AR

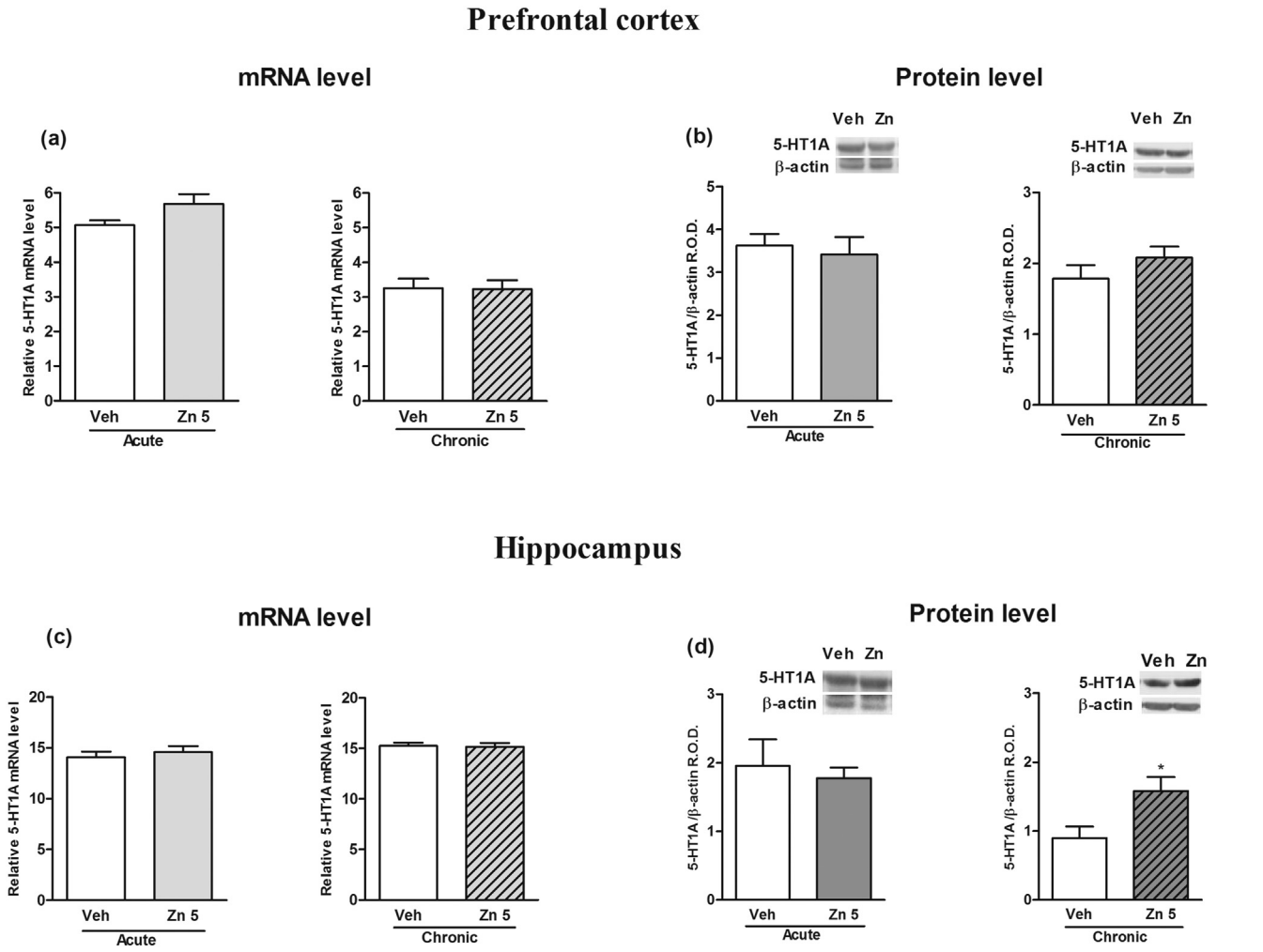

Fig. 1. Analysis of mRNA and protein levels of 5-HT1AR in the PFC (**a, b**) and Hp (**c, d**) of rats subjected to the acute and chronic zinc treatments. Data are expressed as mRNA of 5-HT1A in relation to Gapdh ($2^{-\Delta Ct} \times 10^3$ values ± SEM from 7 to 9 samples) and as protein level of 5-HT1AR in relation to β-actin (ROD values ± SEM from 7 to 8 samples). The data were analyzed by Student *t*-test. $^*p < 0.05$ *vs.* Veh.