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Short communication

Chronic unpredictable stress-induced reduction in the hippocampal brain-derived neurotrophic factor (BDNF) gene expression is antagonized by zinc treatment

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Abstract:
Preclinical data indicate the antidepressant activity of zinc and the involvement of the brain-derived neurotrophic factor (BDNF) in this mechanism. The present study investigates the effect of chronic (16 days) combined treatment with zinc (15 mg/kg zinc hydroaspartate) and imipramine (5 mg/kg) in chronic unpredictable stress (CUS) on the BDNF mRNA level in the rat brain. Moreover, serum zinc concentrations were also assessed. CUS induced a significant reduction in the BDNF mRNA level in the hippocampus by 21% but had no effect in the frontal cortex. Repeated treatment with zinc induced a significant increase in the BDNF mRNA level in the hippocampus in the unstressed animals by 12% and as in the chronically stressed animals by 14%, compared to the appropriate controls. Imipramine treatment did not affect this factor. However, combined treatment of zinc and imipramine induced a 12% elevation of the BDNF mRNA level in the stressed but not in the unstressed rats. CUS induced a 19% reduction in the serum zinc concentration, whereas combined treatment of zinc and imipramine reduced this concentration by 24% in the unstressed and increased it (by 20%) in the stressed animals. These results indicate that: 1) CUS induces a reduction in the BDNF gene expression with a concomitant diminution of serum zinc concentration and 2) the CUS-induced reduction in the BDNF gene expression is antagonized by chronic treatment with zinc.

Key words:
chronic unpredictable stress, chronic treatment, zinc, imipramine, BDNF

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Introduction

Depression is a major psychiatric disorder that is associated with high rates of suicide and is considered to be one of the most important causes of human disability [23]. The mechanisms of the psychopathology of depression are multifaceted; moreover, the available antidepressant drugs are not entirely efficient and may have undesirable side effects [14]. Therefore, the search for new antidepressant therapies is an area of considerable interest.

During the last several years, many articles have been presented that indicate an important role of zinc in the psychopathology and therapy of depression. This trace element acts as a cellular signaling molecule, being present in many regions of the mammalian central nervous system, especially in the cerebral cortex and hippocampus [9]. Synaptically-released zinc modulates glutamatergic, γ-aminobutyric acid (GABA) and glycinergic transmission [22].

Zinc deprivation influences brain zinc homeostasis and leads to behavioral disturbances, such as anorexia, dysphoria, impaired learning and cognitive function [44]. Clinical observations demonstrated a reduced serum zinc concentration in the depressed patients [19, 21, 27, 40], which was normalized after successful antidepressant therapy [20, 21, 36, 40]. Moreover, zinc supplementation was effective at enhancing the efficacy of antidepressant therapy in patients with major depression [28, 39]. Recent studies have also demonstrated the antidepressant-like activity of zinc, both in preclinical tests (the forced swim test and tail suspension test) and some models of depression (olfactory bulbectomy, chronic unpredictable stress and chronic mild stress) [5, 12, 13, 29, 34, 41, 45].

The precise molecular mechanisms underlying the pathophysiology of depression and therapeutic efficacy of antidepressant drugs and zinc are currently not well understood. Several studies suggest that alterations in the brain-derived neurotrophic factor (BDNF) gene expression may play a role in the pathophysiology and treatment of depression. Clinical data have reported that serum BDNF levels are decreased in depressed patients and that they can be normalized by antidepressant administration [2, 10]. Furthermore, animal studies have reported that exposure of rats to stress (an important factor in the etiology of depression) can result in decreased (by 12.5–25%) hippocampal BDNF mRNA levels [25, 32]. Conversely, chronic antidepressant treatment, as well as repeated electroconvulsive seizures, were reported to enhance the BDNF level in the rat hippocampus [15, 33, 38]. Recent studies also demonstrated that chronic zinc administration induced an increase in the cortical [4, 8, 26] or hippocampal [41] BDNF levels in rats.

The aim of the present study was to examine the expression of the BDNF mRNA in the hippocampus and frontal cortex and serum zinc level of rats exposed to chronic unpredictable stress and repeated co-treatment with zinc hydroaspartate and imipramine. The chronic unpredictable stress (CUS) is an animal model of depression, which belongs to the well-validated models [11, 31]. In the CUS paradigm, rats are subjected to a variety of different stressors, which lead to behavioral changes resembling clinical depression, such as motor activity deficits, reduced food and water consumption and decreases in responsiveness to rewarding stimuli [11, 31].

Materials and Methods

Animals and drug treatment

The experiments were performed on male Wistar rats (weighting initially 180–200 g). The animals were housed under controlled laboratory conditions (12-h light/dark cycle, constant temperature: 22 ± 2°C and humidity: 60 ± 2%) with food and water freely available except as described below for the chronic unpredictable stress group. All experimental procedures were conducted between 8:00 a.m. and 1:00 p.m. The animals were divided into two matched groups (chronically stressed or unstressed rats).

Zinc hydroaspartate (Farmapol) and imipramine (Sigma-Aldrich) were used in the experiments. The tested drugs were dissolved in saline (0.9% NaCl) and injected intraperitoneally (*ip*) once daily for 16 days. The control rats were treated with vehicle. The drugs or saline were given to both unstressed and chronically stressed rats.

Imipramine at a dose of 5 mg/kg was given 1 h before every stress session and zinc hydroaspartate at the dose of 15 mg/kg 1 h before the imipramine administration.

The doses of imipramine and zinc were previously examined and were effective in behavioral and biochemical experiments [5, 33].

All experimental procedures were conducted according to the NIH Animal Care and Use Committee Guidelines and were approved by the Ethics Committee of the Medical University of Lublin.

CUS procedure

CUS procedure was a variant of the method of Katz et al. [11]. The rats were subjected once daily to the following kinds of unpredictable stressors: 20 s exposure to electric footshock (3 mA, 0.2 s duration every 2 s), 2 h periods of immobilization at 20°C or at 4°C, 5 min exposure to an electric bell, 3 min periods of swimming in cold water (12°C) or 5 min periods of illumination (80 ± klx) and 48 periods of food deprivation (with water *ad libitum*). Each stressor was repeated 2 times during the 16-day period.

Serum zinc determination

For zinc determination, the rats were euthanized by decapitation 48 h after the last session of chronic stress. Trunk blood was collected, and serum was separated, frozen and stored at –20°C for 1 month before analysis. Each sample was wet-digested with nitric acid and hydrogen peroxide (microwave digestion, Milestone MLS-1200 Mega Microwave Digestion System). The zinc concentration in serum was determined using flame atomic absorption spectrometry. The equipment used was a Pye Unicam SP-9 800 AA Spectrophotometer with deuterium background correction (air flow – 4.2 l/min, acetylene flow – 1.2 l/min, analytical wavelength – 213.9 nm). Relative standard deviation (RSD) of the method (the whole analytical procedure: digestion + zinc determination) did not exceed 2.4%. Mean recovery of zinc was 99% (SD 0.78). The aqueous standard for serum was $Zn(NO_3)_2$ (Zinc Standard Solution, Merck, Germany). Seronorm™ Trace Elements (Sero ASBillingstad, Norway) was used as a serum control for the zinc measurement.

Northern blot analysis of BDNF mRNA

Forty-eight hours after the last session of chronic stress, the animals were killed, and their brains were removed. Dissected brain structures (the frontal cortices and hippocampi) were frozen immediately on dry ice and stored at –80°C for 2–6 weeks before analysis. BDNF mRNA levels were measured according to Legutko et al. [17]. The total RNA was extracted using TRIzol Reagent (Life Technologies) following the manufacturer's protocol. Northern blot analysis was performed with 10 μg of the total RNA, separated on a 1% denaturing agarose–formaldehyde gel, transferred subsequently to a nylon membrane (Nytran, Schleicher and Schuell) and immobilized by ultraviolet (UV) radiation. A probe for the rat BDNF was generated by polymerase chain reaction (PCR) from cDNA, using primers: 5'-ACTCTGGAGAGCGTGAATGG-3' and 5'-CAGCCTTCCTTCGTGTAACC-3'. The 470 bp product was cloned into the pCRII TA cloning vector. The cloned insert was isolated by a restriction digest with EcoRI and radio-labeled with α-[^{32}P]dCTP by random priming. The probe was purified with Prime-It RmT (Stratagene). Hybridization was performed in Church's buffer at 65°C overnight. Hybridized filters were washed for 30 min in 2× saline–sodium citrate (SSC) buffer/0.1% sodium dodecyl sulfate (SDS) at room temperature and 30 min in 0.1×SSC/0.1% SDS at 55°C and exposed. Following exposure, the filters were stripped (washed three times in 0.1×SSC/0.1% SDS at 100°C for 10 min), and re-hybridized with a β-actin cDNA probe (Clontech) to normalize the RNA loading. Northern blots were analyzed quantitatively with a PhosphorImager (Image Gauge 4.0, Fuji).

Data analysis

Data were evaluated using GraphPad Prism software ver. 4.0. The results of the experiments are expressed as group means ± SEM. The obtained data were evaluated by two-way ANOVA followed by Bonferroni's *post-hoc* test. A value of $p < 0.05$ was considered as statistically significant.

Results and Discussion

The recent hypotheses of depression and the mechanism of action of antidepressants include alterations of the neurotrophic factors, particularly of BDNF [24] (see [3] for review). It was proposed that depression demonstrates low levels of the BDNF expression in the hippocampus or cortex, whereas antidepressants increase/normalize the depression-induced effect [7]. Because depression seems to be induced/precipitated

by stress, accordingly stress-induced reductions in brain BDNF expression are in line with this hypothesis [6, 35]. However, not all kinds of stress reduce BDNF expression [3]. What is important, the different stress procedures (the well validated animal models of depression), differently affect BDNF expression [1, 16, 18, 37].

Zinc exhibits antidepressant-like activity in animal screening tests and models of depression (see [43] for review). The mechanism of zinc's antidepressant activity may be related to the enhancement of BDNF expression [4, 8, 26, 41, 42].

In the present study, we demonstrated that the CUS procedure (a modification of Katz et al. [11]) reduced

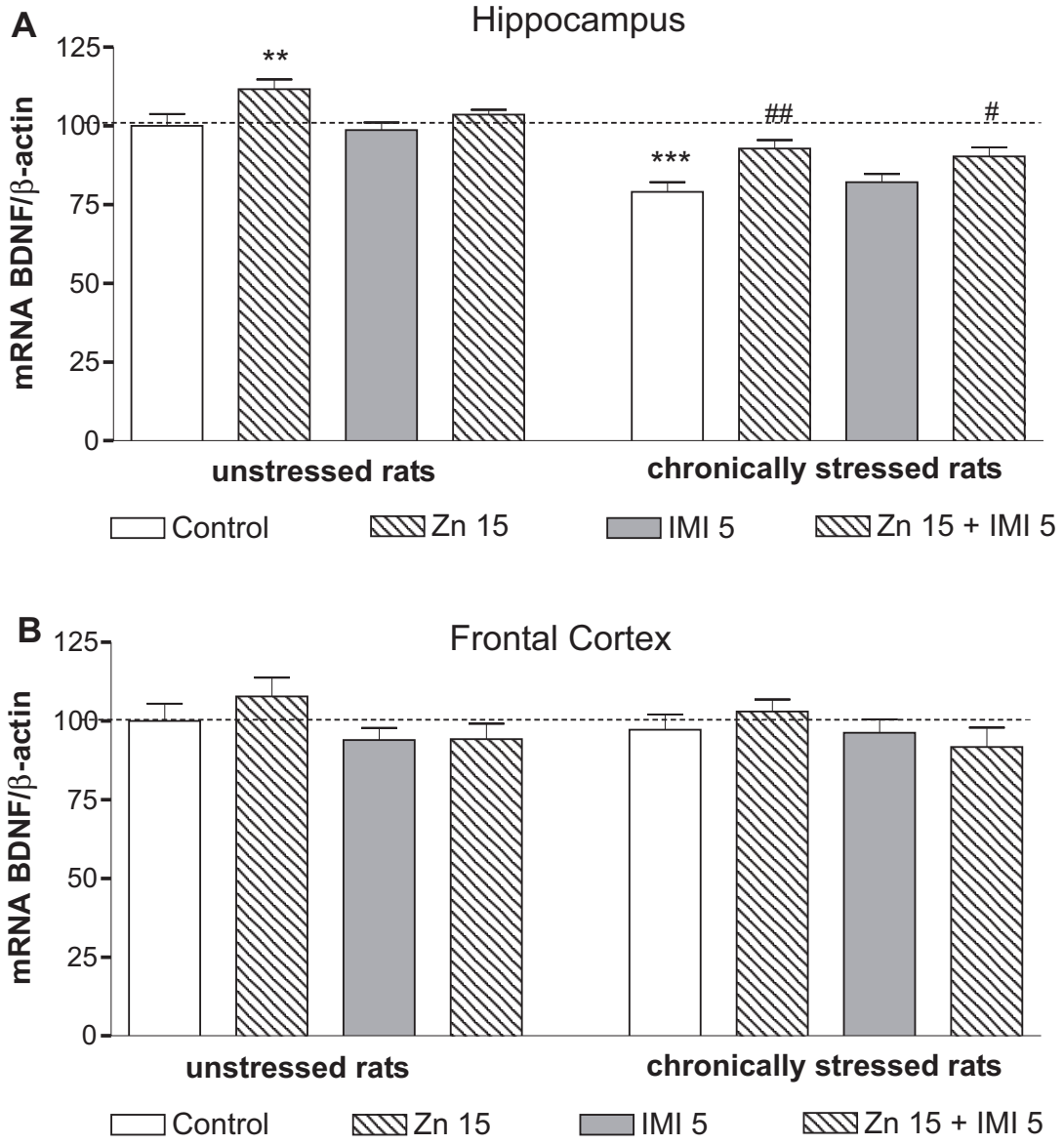

Fig. 1. The effect of chronic unpredictable stress (CUS) and chronic zinc and/or imipramine administration on the BDNF mRNA level in the rat hippocampus (**A**) and frontal cortex (**B**). Zinc hydroaspartate at a dose 15 mg/kg (Zn 15), imipramine at a dose 5 mg/kg (IMI 5) or saline (control) was administered once daily, for 16 days. Imipramine and saline were given 1 h before every stress session and zinc hydroaspartate 1 h before imipramine administration. The results are presented as a percentage of the control (BDNF/β-actin ratio) and expressed as group means ± SEM of 8–10 animals per group. Two-way ANOVA revealed a significant effect of stress $F(1,66) = 78.14$, $p < 0.0001$, significant effect of treatment $F(3,66) = 9.22$, $p < 0.0001$ with no interaction $F(3,66) = 0.72$, $p = 0.5422$ for the hippocampus (**A**) and no effect of stress $F(1,68) = 0.30$, $p = 0.5849$, no effect of treatment $F(3,68) = 2.35$, $p = 0.0803$ and no interaction $F(3,68) = 0.18$, $p = 0.9093$ for the frontal cortex (**B**). ** $p < 0.01$, *** $p < 0.001$ *vs.* control unstressed rats; # $p < 0.05$, ## $p < 0.01$ *vs.* control chronically stressed rats (Bonferroni's multiple comparison test)

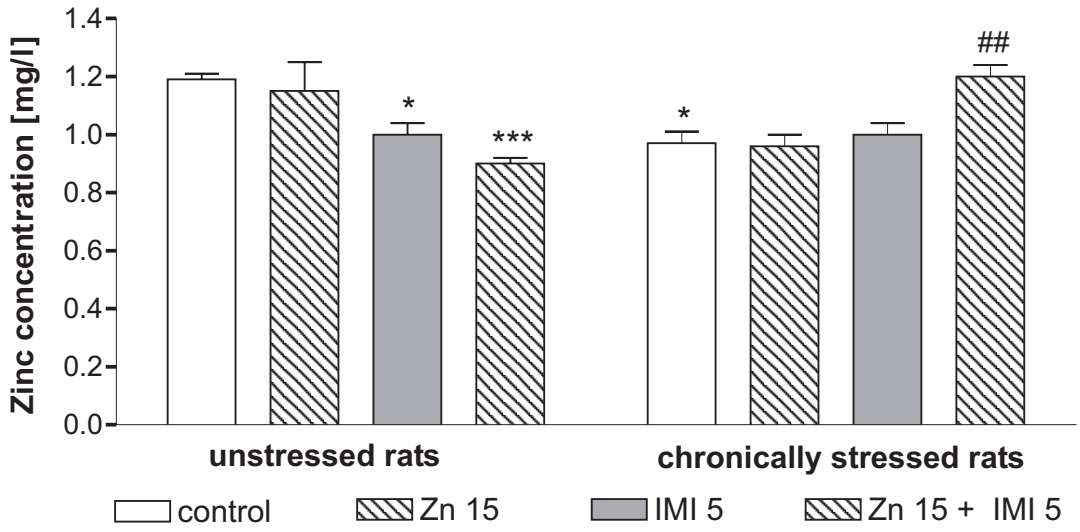

Fig. 2. The effect of chronic unpredictable stress (CUS) and prolonged zinc and/or imipramine administration on serum zinc concentration. Zinc hydroaspartate at a dose 15 mg/kg (Zn 15), imipramine at a dose 5 mg/kg (IMI 5) or saline (control) were administered once daily for 16 days. Imipramine and saline were given 1 h before every stress session and zinc hydroaspartate 1 h before imipramine administration. The results are expressed as group means ± SEM of 6 animals per group. Two-way ANOVA revealed no effect of stress $F(1,40) = 0.64$, $p = 0.4271$, no effect of treatment $F(3,40) = 0.96$, $p = 0.4230$, however, a significant interaction $F(3,40) = 12.16$, $p < 0.0001$. * $p < 0.05$, *** $p < 0.001$ *vs.* control unstressed rats; ## $p < 0.01$ *vs.* control chronically stressed rats (Bonferroni's multiple comparison test)

the mRNA level of BDNF by 21% in the hippocampus but not in the frontal cortex (Fig. 1). Thus, contrary to the other procedure modification of the CUS and to chronic mild stress, which do not alter the hippocampal BDNF levels [1, 16, 18], our data are in good agreement with the BDNF-depression hypothesis [7].

Moreover, zinc (15 mg/kg as zinc hydroaspartate) increased the expression of the hippocampal BDNF mRNA in control (not stressed) as well as in stressed animals (by 12–14%, Fig. 1). Interestingly, combined treatment of zinc plus imipramine (both at doses ineffective at modifying behavior in this model [5]) increased the hippocampal BDNF mRNA in the stressed but not in the unstressed animals (Fig. 1). No effect of examined compounds on the cortical BDNF expression was noticed (Fig. 1B). Thus, the data confirmed that chronic treatment with the low dose of zinc increases the hippocampal BDNF mRNA [41], which is not active behaviorally [5] and suggest that sensitivity of the BDNF gene expression precedes the behavioral manifestation of zinc's antidepressant-like activity in the CUS model.

In the present study, the CUS procedure reduced serum zinc concentration by 19% (Fig. 2). Such an effect was demonstrated previously for CUS but not for chronic mild stress or olfactory bulbectomy models of depression [30]. Similar to our previous studies [27, 29], chronic treatment with zinc or imipramine did not alter the serum zinc concentration (Fig. 2). However, combined treatment with zinc plus imipramine reduced serum zinc concentration in the unstressed rats but increased it the in the stressed rats (Fig. 2). The reduction in serum zinc concentration induced by zinc plus imipramine treatment in unstressed subjects is unexplainable at this time.

On the other hand, increases (normalization) in the serum zinc concentration in the CUS model induced by combined zinc plus imipramine treatment correlate with the antidepressant-like behavioral effect elicited by such combined treatment [5].

To summarize, the present report demonstrates that: 1) CUS induces a reduction in the BDNF gene expression with concomitant diminution of serum zinc concentration, and 2) the CUS-induced reduction in the BDNF gene expression is antagonized by chronic treatment with zinc.

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