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Short communication

Zinc treatment induces cortical brain-derived neurotrophic factor gene expression

Gabriel Nowak[a,b,*], Beata Legutko[a], Bernadeta Szewczyk[a], Mariusz Papp[a], Marek Sanak[a], Andrzej Pilc[a,c]

[a] *Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343, Cracow, Poland*
[b] *Department of Pharmacobiology, Collegium Medicum, Jagiellonian University, Cracow, Poland*
[c] *Institute of Public Health, Collegium Medicum, Jagiellonian University, Cracow, Poland*

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Abstract

Most of antidepressants induce expression of the gene coding for brain-derived neurotrophic factor (BDNF) in the hippocampal/cortical neurons. Recent data indicate antidepressant-like activity of zinc in animal models. We now report that chronic treatment with zinc induced an increase in cortical but not hippocampal BDNF mRNA level (Northern blot). Tranylcypromine, a classic antidepressant, increased BDNF mRNA level in both examined brain regions. This is the first demonstration that zinc increases the BDNF gene expression, which is the effect shared by most of clinically effective antidepressants.

Keywords: Zinc; BDNF; Cortex; Hippocampus

1. Introduction

Zinc is an endogenous neuromodulator of glutamate (mainly N-methyl-D-aspartatic acid—NMDA) receptors which may be involved in the psychopathology and treatment of depression (Nowak and Szewczyk, 2002). Zinc exhibits antidepressant-like effects in the forced swim and tail suspension tests and is also active in olfactory bulbectomy and chronic mild stress animal models of depression (Kroczka et al., 2001; Nowak et al., 2003b; Rosa et al., 2003, our unpublished data). All these data strongly suggest possible antidepressant activity of zinc in human depression. In fact, our preliminary clinical study demonstrated beneficial effect of zinc supplementation in antidepressant therapy (Nowak et al., 2003a).

According to the recently proposed hypotheses, the brain-derived neurotrophic factor (BDNF) is involved in the mechanism of antidepressant action as one of the main targets of antidepressants (Nibuya et al., 1995).

Antidepressant drugs or electroconvulsive therapy induced an increase in hippocampal (and cortical) BDNF mRNA level (Nibuya et al., 1995). Although there are some discrepancies in antidepressant dosing and treatment schedules, which are appropriate for affecting BDNF, undoubtedly, antidepressants influence BDNF gene expression (Nibuya et al., 1995; Coppel et al., 2003).

Since most effects of antidepressants on BDNF gene expression were demonstrated after prolonged treatment, in the present study we investigated the effect of chronic (2-week) treatment with zinc on BDNF mRNA level in the rat cerebral cortex and hippocampus. In order to control experimental conditions, we also determined the effect of a classic antidepressant drug, an inhibitor of monoamine oxidase, tranylcypromine.

2. Materials and methods

2.1. Animals

All procedures were conducted according to the guidelines of the National Institutes of Health Animal Care and Use Committee, and were approved by the Ethics Com-

* Corresponding author. Department of Pharmacology, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343, Cracow, Poland. Tel.: +48-12-6623215; fax: +48-12-637-4500.
E-mail address: nowak@if-pan.krakow.pl (G. Nowak).

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mittee of the Institute of Pharmacology, Krakow. The experiments were carried out on male Wistar rats (180–220 g). The animals were kept under a natural day–night cycle with free access to food and water. Zinc (hydroaspartate, Farmapol, Poznan, Poland) at a dose of 65 mg/kg (11.5 mg Zn/kg), tranylcypromine (Sigma) at a dose of 10 mg/kg or vehicle (0.9% sodium chloride) were administrated i.p. once a day for 14 days. Twenty-four hours after the last treatment, the animals were decapitated, their cortices (frontal cortices) and hippocampi were dissected and immediately frozen.

2.2. Determination of BDNF mRNA

The procedure for determination of BDNF mRNA levels was performed according to Legutko et al. (2001). Briefly, total RNA was extracted by chaotropic lysis (TRIzol Reagent, Life Technologies) following manufacturer's protocol. Northern blot analysis was performed with 7 μg of total RNA, separated on 1% denaturing agarose-formaldehyde gel, transferred subsequently to nylon membrane (Nytran s, Schleicher and Schuell) and immobilized by ultraviolet (UV) radiation. A probe for rat BDNF was generated by polymerase chain reaction (PCR) from cDNA, using primers: 5′-ACT-CTG-GAG-AGC-GTG-AAT-GG-3′ and 5′-CAG-CCT-TCC-TTC-GTG-TAA-CC-3′, the 470 bp product was cloned into pCRII TA cloning vector. Insert cut with enzyme EcoRI was random primer-labeled with P^{32}dCTP, and purified (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° and exposed. The same filters stripped off BNDF probe (washed three times in 0.1 × SSC/0.1% SDS at 100° for 10 min), were re-hybridized for β-actin cDNA probe (Clontech) to normalize RNA loading. Northern blots were quantified using a digitalized autoradiographs (PhosphorImager, Image Gauge 4.0, Fuji).

2.3. Data analysis

Group differences were assessed using analysis of variance (ANOVA) followed by Dunnett's test.

3. Results

The effect of chronic treatment with zinc and tranylcypromine on BDNF mRNA level is presented in Fig. 1. Chronic treatment with zinc, similarly to a classic antidepressant, tranylcypromine, increased level of BDNF mRNA in the rat cerebral cortex (ANOVA: $F(2,12) = 8.547$, $P = 0.0049$; Fig. 1A). However, tranylcypromine but not zinc, induced an increase in BDNF mRNA level in the hippocampus (ANOVA: $F(2,12) = 149.9$, $P < 0.0001$; Fig. 1B).

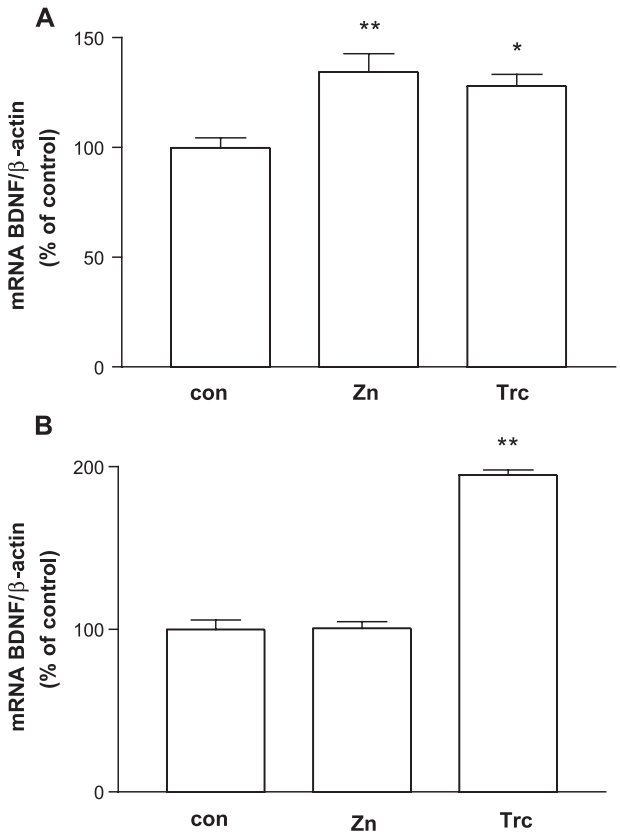

Fig. 1. Effect of chronic treatment with zinc (Zn) and tranylcypromine (Trc) on BDNF mRNA level (Northern blot) in the cortex (A) and hippocampus (B) in rats. The results are expressed as a mean ± S.E.M. of 5 animals/group. *$P < 0.05$, **$P < 0.001$ vs. control (con, Dunnett's test).

4. Discussion

In spite of more then 40 years of research, the mechanism of antidepressant action remains not fully understood. Most adaptive changes, proposed to be "responsible" for neurochemical antidepressant mechanisms are not common for all antidepressant therapies (Vetulani and Nalepa, 2000). Thus, search for common alteration(s) is still in progress. One of the recent loci is the brain-derived neurotrophic factor (BDNF). The first examination of the effect of various antidepressants on BDNF gene expression demonstrated its increase 2–3 h after the last drug administration under chronic (but not acute) treatment schedule (Nibuya et al., 1995). The effect of mianserin, sertraline and desipramine was restricted to the hippocampus, while electroconvulsive shock and tranylcypromine induced this effect in the hippocampus as well as in the cerebral cortex (Nibuya et al., 1995). The more recent paper of Coppel et al. (2003) shows that some antidepressants (fluoxetine, paroxetine, setraline or tranylcypromine) but not desipra-

mine, maprotiline or mianserin, induced an increase in the hippocampal BDNF expression 24 h after chronic treatment, while decreased it at 4 h after acute or chronic treatment. In view of the persistency of antidepressant-induced clinical effect, the effect present 24 h after chronic treatment seems to be a good candidate for "common antidepressant locus". These above-mentioned studies used probes, which do not distinguish between BDNF transcripts. However, when Dias et al. (2003) examined specific I–IV BDNF transcripts, more complex antidepressant-induced alterations of transcripts/brain region/drugs relationships were detected.

In the present paper, we report that chronic treatment with zinc increased level of BDNF mRNA in the rat cerebral cortex. We have also confirmed that tranylcypromine (unlike other antidepressants, Nibuya et al., 1995) increased BDNF gene expression in cerebral cortex also 24 h after the last treatment. The dose of zinc and schedule of treatment used in the present study were examined previously (Nowak et al., 2003b), and were demonstrated to evoke antidepressant-like action in the rat behavioral paradigms (forced swim test, olfactory bulbectomy).

The lack of effect of zinc on BDNF expression in the hippocampus differs from the changes evoked by most of antidepressants in that structure. However, it is possible that other zinc doses and treatment schedules might also cause the hippocampal BDNF alterations. The studies examining effect of different doses and schedules of zinc treatment on BDNF expression are in progress.

Since zinc profoundly affects glutamate transmission (mainly by antagonizing the NMDA receptors; Nowak and Szewczyk, 2002), this glutamate-dependent mechanism is likely to be responsible for zinc-induced changes in BDNF gene expression. The cortical selectivity of NMDA receptor antagonists-induced BDNF gene expression was demonstrated previously. For example, the NMDA receptor antagonist, memantine, induced cortical BDNF gene expression at lower doses than those necessary to produce such effect in the hippocampus (Marvanova et al., 2001).

Summarizing, this is the first demonstration that zinc increases the BDNF gene expression, which is the effect shared by most of clinically effective antidepressants.

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