Short communication

Anxiolytic-like activity of zinc in rodent tests

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Abstract:
Because zinc deficiency induces depression and anxiety-like behavior in rodents, we examined the effects of zinc administration in several tests by measuring anxiolytic activity in mice and rats. We now report that zinc significantly increased the number of entries into the open arms in the elevated plus maze in rats. Moreover, zinc treatment significantly increased the number of punished crossings in the four-plate test and attenuated stress-induced hyperthermia (SIH) in mice. However, no effect of zinc administration was observed in the elevated plus maze test in mice. This lack of effect in the latter case was probably due to the substantial zinc-induced reduction in locomotor activity by the doses used in mice. The present data demonstrate for the first time the anxiolytic-like activity of zinc in rodents and may indicate that zinc could be used as a novel therapeutic/adjunct agent in anxiolytic therapy.

Key words:
zinc, elevated plus maze, stress-induced hyperthermia, four-plate test, mice, rats, anxiolytic activity

Introduction

Zinc (Zn^{2+}) ions rank among the most abundant trace elements in the body. They regulate the function of numerous structural, transcriptional and enzymatic proteins that play important roles in brain function [4, 7, 22]. A substantial fraction of zinc ions is located inside synaptic vesicles of glutamatergic terminals and is released in a calcium-dependent manner [1]. Moreover, Zn^{2+} is co-released with glutamate [33], in-hibits the glutamate ionotropic N-methyl-D-aspartate (NMDA) receptor complex [3] and inhibits $GABA_A$ receptors [21]. The highest levels of zinc in the central nervous system (CNS) are present in areas known to be important in depression and anxiety, including the cerebral cortical regions, the hippocampus, most amygdaloid nuclei and the lateral septum [4]. Zinc deprivation influences brain zinc homeostasis and leads to alteration in behavior, learning, mental function and susceptibility to epileptic convulsions [22, 30]. Several studies have indicated that zinc is in-

volved in the pathophysiology of depression and in the mechanism of action of antidepressants [5, 11, 12, 16]. Preclinical investigations have shown that zinc produces antidepressant-like effects in animal tests (i.e., the forced swim test and the tail suspension test) and in models of depression (i.e., olfactory bulbectomy, chronic unpredictable stress and chronic mild stress) [6, 9, 15, for review 14, 20, 24]. There are also some clinical data showing that zinc supplementation may enhance antidepressant therapy in patients with unipolar depression [13, 23]. Moreover, some preclinical data indicate a correlation between zinc deficiency and an increase in anxiety-like behavior in animal tests of anxiety, i.e., the open field test and the plus maze test in rats and the novelty suppressed feeding test in mice [31, 34].

In the present study, we have investigated the effect of acute administration of zinc in the following anxiety tests: the elevated plus maze test in mice and rats, the four-plate test in mice and the stress-induced hyperthermia (SIH) test in mice.

Materials and Methods

Animals and drug treatment

All procedures were conducted according to the NIH Animal Care and Use Committee Guidelines and were approved by the Ethical Committee of the Institute of Pharmacology, Polish Academy of Sciences, Collegium Medicum Jagiellonian University in Krakow, and Medical University of Lublin.

The experiments were performed on male Wistar rats (220–280 g) and male albino Swiss mice (22–26 g). The animals were kept under a natural day/night cycle at room temperature (21–23°C), with free access to food and water. Each experimental group consisted of 6–15 animals. Experiments were conducted between 9:00 a.m. and 2:00 p.m. by an observer who was blinded to the treatment.

Zinc hydroaspartate (Farmapol, Poznań, Poland) dissolved in aqua pro injectione or vehicle (control) was administered intraperitoneally (*ip*) in a volume of 2 ml/kg or 10 ml/kg to rats or mice, respectively, 45 min before the test. These doses were selected based on previous behavioral data [9, 13].

Elevated plus maze test in rats

The testing procedure was based on a method described by Pellow and File [18]. The plus maze apparatus (an automated device produced by Kinder Scientific) was made of durable, high-density, non-porous black plastic, was elevated to a height of 50 cm and consisted of two open arms (50 × 10 cm) and two closed arms (50 × 10 cm with 30-cm-high walls), which were arranged so that the two arms of each type were opposite each other. The floor of the plus maze was made of infrared-transparent material; no sensors were visible to the mice. The plus maze was placed in a darkened room, and the center of the apparatus was illuminated with a 25 W electric bulb hanging 100 cm above the maze. The plus maze apparatus was connected to PC software by a control chassis. Each rat was gently placed in the center of the plus maze, facing one of the closed arms, immediately after a 5-min adaptation period in a black plastic box (60 × 60 × 35 cm). During a 5-min test period, the automated Kinder Scientific Motor Monitor System recorded the number of entries into the closed and open arms, the time spent in each and the distance (cm) covered by a rat in each type of arm. After each trial, the maze was wiped clean.

Exploratory activity measured in the elevated plus maze in rats

The experiment was performed using a plus maze apparatus (an automated device produced by Kinder Scientific) (see above). During a 5-min test period (i.e., the time equal to the observation period in the elevated plus maze), the automated Kinder Scientific Motor Monitor System recorded the total time spent in the open and closed arms, the total entries, the total distance and X and Y ambulation; changes in these parameters would indicate that treatment with zinc affected general locomotor activity rather than anxiety-like behavior specifically. After each trial, the maze was wiped clean.

Four-plates test

A single mouse was placed gently onto the plate, and each animal was allowed to explore for 15 s. After this time, each time a mouse passed from one plate to another, the experimenter electrified the whole floor for 0.5 s (current intensity 0.8 mA), which evoked

a visible flight reaction from the animal. If the animal continued running, it received no new shock for the following 3 s. The number of punished crossings was counted for 60 s [2].

Modified stress-induced hyperthermia (SIH) test in singly-housed mice

The procedure for the modified stress-induced hyperthermia test was adapted from Van der Heyden et al. [32]. For this assay, body temperature was measured in each mouse at t = 0 min (T_1) and t = 15 min (T_2). Albino Swiss mice were placed into a new cage immediately following measurement of T_1, with the difference in temperature (T_2–T_1) used as a measure of stress-induced hyperthermia. Pilot studies by Spooren et al. [25] demonstrated that a T_2–T_1 interval of 15 min is optimal for the measurement of SIH. A comparison between T_1 in vehicle-treated mice and T_1 in test-compound-treated mice was used to determine whether the agent alone affects body temperature. Rectal temperature was measured to the nearest 0.1°C by an Ellab thermometer. The lubricated thermistor probe (2 mm diameter) was inserted 20 mm into the rectum. The mouse was held at the base of the tail during this determination, and the thermistor probe was left in place until a constant reading was obtained (± 15 s).

Elevated plus maze test in mice

The EPM studies were conducted on mice according to the method of Lister [10]. The EPM apparatus was made of Plexiglas and consisted of two open (30 × 5 cm) and two enclosed (30 × 5 × 15 cm) arms. The arms extended from a central platform of 5 × 5 cm. The apparatus was mounted on a Plexiglas base, raising it 38.5 cm above the floor, and was illuminated by a red light. The test was performed by placing a mouse in the center of the apparatus (facing an enclosed arm) and allowing it to freely explore. The number of entries into the open arms and the time spent in these arms were scored for a 5-min test period. An entry was defined as placing all four paws within the boundaries of the arm. The following measures were obtained from the test: the total number of arm entries; the percentage of arm entries into the open arms; and the time spent in the open arms expressed as a percentage of the time spent in both the open and closed arms. Anxiolytic activity was indicated by increases in the time spent in open arms or in the number of open arm entries.

Locomotor activity in mice

The actimeter consisted of a cylinder (30 cm in diameter, 12 cm high, MultiServ, Lublin, Poland) equipped with two perpendicular infrared light beams located 1.5 cm above the floor. Mice were placed in the actimeter, and locomotor activity (the number of interruptions of light beams) was recorded for a period of 5 min after placing each mouse into the actimeter.

Data analysis

The data were evaluated by a one-way analysis of variance (ANOVA) followed by the Newman-Keuls Multiple Comparison test. A p value less than 0.05 was considered significant.

Results and Discussion

Preclinical and clinical evidence from the last ten years indicates that zinc is involved in the pathophysiology of depression and in the mechanism of action of antidepressants [26–28]. Its antidepressant effects have been demonstrated in a number of animal tests and models of depression and in clinical studies. Moreover, it was recently demonstrated that zinc deficiency induces depression- and anxiety-like behavior in rodents [31, 34].

In this study, we examined for the first time the anxiolytic-like effects of acute treatment with zinc hydroaspartate in the following animal tests commonly used to predict the potential anxiolytic activity of a drug in humans: the elevated plus maze test in rats and mice, the four-plate test in mice and the stress-induced hyperthermia test in mice.

The effects of acute treatment with zinc hydroaspartate on rats in the elevated plus maze are shown in Table 1. In this test, zinc hydroaspartate was administered at doses of 65, 32.5, 16.25 and 8.125 mg/kg. Zinc given at doses of 32.5 and 16.25 mg/kg significantly increased (up to 42% and 39%, respectively) the percentage of entries into the open arms [ANOVA: $F_{(4,87)} = 4.6271$, p = 0.00196]. However, the percentage of time spent in the open arms did not reach the level of statistical significance. The anxiolytic-like effect of zinc hydroaspartate in the elevated plus maze test seems to be specific, as it did not sig-

Tab. 1. The effect of zinc hydroaspartate on percent of time spent in open arms, percent of open arms entries and exploratory activity in the elevated plus maze test in rats

Compound	Dose (mg/kg)	Percent of time spent in open arms	Percent of open arms entries	Total distance (cm)	Total entries
Vehicle	–	18.0 ± 2.4	29.0 ± 2.5	3499 ± 123	24.0 ± 1.5
Zn	8.125	20.6 ± 5.1	31.8 ± 5.4	3475 ± 332	22.4 ± 3.7
	16.25	22.6 ± 3.4	42.1 ± 3.6**	2977 ± 245	18.4 ± 2.6
	32.5	23.2 ± 2.5	39.4 ± 2.7*	3154 ± 217	20.7 ± 2.0
	65.0	16.9 ± 4.8	22.2 ± 5.1	3857 ± 199	25.8 ± 3.7

Zinc hydroasparatate was administrated *ip* 45 min before the test. Data were assessed by a one-way ANOVA followed by the Newman-Keuls Multiple Comparison test. Each column represents the mean ± SEM of 12–15 rats per group. * $p < 0.05$, ** $p < 0.02$ *vs.* vehicle-treated control group

nificantly affect the exploratory activity of the animals (total entries and total distance). The remaining two doses of the compound (65 and 8.125 mg/kg) did not produce any effects characteristic of anxiolytics in this test.
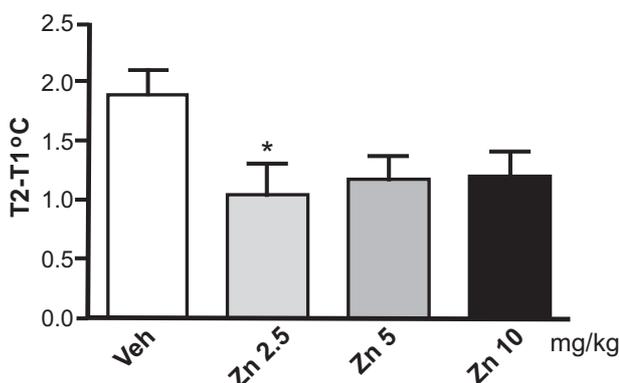
The effects of acute treatment with zinc hydroaspartate on mice in the four-plate test are shown in Figure 1. Zinc hydrosapartate at a dose of 10 mg/kg significantly increased the number of punished crossings [ANOVA: $F_{(2,25)} = 4.317$, $p = 0.0245$]. The higher dose of this compound had no effect in the test.

The effects of acute treatment with zinc hydroaspartate on stress-induced hyperthermia (SIH) in mice are shown in Figure 2. SIH was significantly attenuated by zinc treatment [ANOVA: $F_{(3,34)} = 2.902$, $p = 0.0489$]. *Post-hoc* comparison showed that zinc was significantly effective in reducing SIH only at a dose of 2.5 mg/kg.

In the plus maze test in mice, zinc did not influence the number of open arms entries and time spent on the open arms (data not shown). On the other hand, zinc hydroaspartate at doses of 2.5, 5, 10 mg/kg significantly decreased the locomotor activity (the number of animal movements measured by the actimeters, Table 2 [ANOVA: $F_{(3,35)} = 19.09$, $p < 0.0001$]).

As shown above, we have observed anxiolytic-like effects of zinc in most of the animal tests used: it altered the number of shocks accepted in the four-plate test in mice even though it decreased the locomotor activity of animals; it also significantly reduced stress-induced hyperthermia and increased the percentage of open arms entries in the elevated plus maze
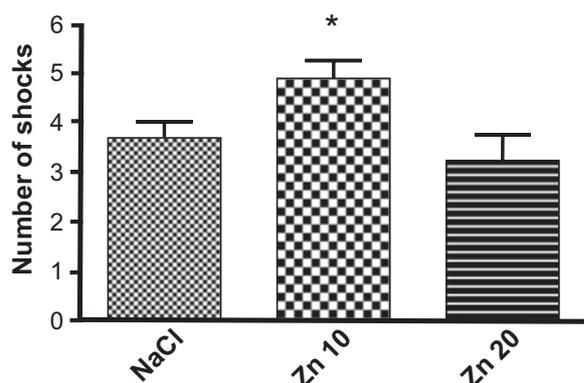
Fig. 1. The effect of zinc hydroaspartate on the number of shocks accepted in the four-plate test in mice. Zinc hydroaspartate was administrated *ip* 45 min before the test. Data were assessed by a one-way ANOVA followed by the Newman-Keuls Multiple Comparison test. Each column represents the mean ± SEM of 8–10 mice per group. * $p < 0.05$ *vs.* vehicle-treated control group

Fig. 2. The effect of zinc hydroaspartate on stress-induced hyperthermia (SIH) in mice. Zinc hydroaspartate was administered *ip* 45 min before the test. Data were assessed by a one-way ANOVA followed by the Newman-Keuls Multiple Comparison test. Each column represents the mean ± SEM of 8–10 mice per group. * $p < 0.05$ *vs.* vehicle-treated control group

Tab. 2. Effect of zinc hydroaspartate administration on locomotor activity in mice

Compound	Dose (mg/kg)	Locomotor activity/5 min
Vehicle	–	115.0 ± 8.5
Zn	2.5	$78.0 \pm 7.8*$
	5.0	$55.1 \pm 3.7*$
	10.0	$43.1 \pm 7.6*$

Zinc hydroaspartate was administrated *ip* 45 min before the test. Data were assessed by a one-way ANOVA followed by the Newman-Keuls Multiple Comparison test. Each column represents the mean ± SEM of 8–10 mice per group. * $p < 0.05$ *vs.* vehicle-treated control group

test in rats but not in mice. The lack of an effect in mice was probably due to the substantial zinc-induced reduction in locomotor activity.

The mechanism responsible for the anxiolytic action of zinc is probably related to the modulation of glutamate and/or GABA-ergic systems. Based on the results of experiments examining the mechanism of zinc antidepressant-like activity, we can speculate that the anxiolytic activity of zinc (like organic NMDA receptor antagonists, e.g., [8, 19]) may be related to its antagonism of NMDA and/or mGluR5 glutamate receptors [17 for review]. However, zinc action through GABA-ergic or serotonergic receptors cannot be disregarded [29]. Further studies are needed to evaluate the exact mechanism(s) responsible for the anxiolytic activity of zinc.

Overall, our data show for the first time the anxiolytic-like effects of zinc hydroaspartate, as evaluated by three tests in rats and mice. These data should encourage further examination of anxiolytic zinc activity and may indicate that zinc could be used as a novel therapeutic/adjunct agent in anxiolytic therapy.

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