A preliminary study on the possible effects of the acute zinc administration on the anxiety-like behaviour

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ABSTRACT

Zinc ions are trace elements in the human body with key regulatory role over numerous structural, transcriptional and enzymatic proteins essential for the correct function of the central nervous system. In the present work we were interested in determining the possible relevance of acute zinc administration on the anxiety-like behaviour. Our initial results described here show a promising relevance for zinc in anxiety-related context and advocate for further research, considering oxidative stress related mechanisms.

KEYWORDS:

Zinc, biotechnological, anxiety, oxidative stress.

INTRODUCTION

Zinc ions are some of the most abundant trace elements in the human body. Among many important functions, zinc ions regulate numerous structural, transcriptional and enzymatic proteins that play important roles in the correct function of the central nervous system (1,2,3). Therefore, zinc is considered to be essential for brain development function (3).

Up to date there is not a clear consensus regarding the effect of a zinc deficient diet on the brain. On one hand, zinc homeostasis in the brain is known to be strictly regulated by

the brain barrier system, and, therefore, is not easily affected by a zinc deficient diet (4,5). However, certain studies have demonstrated that some neuronal functions are impaired by a diet low in zinc (6).

For example, learning and memory are impaired before any decrease in zinc concentration can be observed in the brain of experimental animals fed a zinc-deficient diet. Specifically, one study showed that zinc concentration in the brain of rats did not decrease after a 4-week zinc deprivation, whereas a significant impairment in the learning behaviour of passive avoidance was

observed (7). Thus, the significance of zinc homeostasis in the brain neuronal function is still not fully understood.

Regarding the importance of zinc in a healthy diet and the possible negative outcomes of zinc deficient diet, approximately 50% of the world population does not get an optimal quantity of zinc, according to K. Brown et al. (8).

It has been demonstrated that zinc deficiency in children is a real nutritional and health problem in both developing and developed countries (10). This should raise serious concerns, especially when the evidence from animals experimental studies indicates that zinc deprivation during periods of rapid development critically impairs behaviour and brain function, in addition to brain development (11, 12).

One of the main behavioural symptoms of zinc deficiency is lethargy, characterized by reduced activity and responsiveness (13). Furthermore, zinc supplementation has been shown to improve neuropsychological behaviours in school-age children (14, 15).

However, the mechanisms underlying brain impairments in zinc deficiency are still unclear and further analysis on the relationship between neuropsychological behaviour and neuronal function in zinc-deficient animal models are necessary to a better understanding.

The possible correlation between zinc level and anxiety, could be implied by that the highest levels of zinc in the central nervous system are found in areas known to be important in anxiety, including the cerebral cortical part of the brain, the hippocampus, most amygdaloid nuclei and the lateral septum (16).

In addition, although zinc deprivation does not seem to influence brain zinc homeostasis, however zinc deprivation leads to alteration in behaviour, learning, mental function and may cause epileptic convulsions (18).

Several studies have indicated that zinc is also involved in the pathophysiology of depression and in the mechanism of antidepressants (19, 20, 21, 22). Animal models-based investigations have suggested that zinc produces similar effects to those of known antidepressants in various animal tests such as the forced swim test and the tail suspension test (23, 24, 25).

Furthermore, there are clinical data showing that zinc supplementation may present benefits for patients who suffer from unipolar depression (26, 27).

In the present study, we have investigated the effect of acute administration of zinc in rats in the elevated plus maze test.

MATERIALS AND METHODS

Animals and drug treatment

The experiments were performed on male Wistar rats (220–280 g). The animals were kept under a natural day/night cycle at room temperature (22–25°C), with free access to food and water. The design of our study had 5 experimental groups, consisting of 8 animals each: the control group and 4 groups of zinc-treated rats which received increasing zinc hydroaspartate doses of 8.125 mg/kg, 16.25 mg/kg, 32.5 mg/kg and 65 mg/kg respectively.

Experiments were conducted between 9:00 a.m. and 2:00 p.m. by an observer who was blinded to the treatment.

Zinc hydroaspartate dissolved in aqua pro injections or vehicle (control) was

administered intraperitoneally in a volume of 2 ml/kg, 45 min before the test.

These doses were selected based on previous data from literature (28, 29).

Rats were treated in accordance with the guidelines of the animal bioethics of the Act on Animal Experimentation and Animal Health and Welfare from Romania and all procedures performed in studies involving animals were in compliance with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

Elevated plus maze test

The testing procedure was based on a method described by Pellow and File (30). The apparatus, consisted of two open arms (50 × 10 cm) and two closed arms (50 × 10 cm with 30-cm-high walls), arranged in a “+” shape, elevated to a height of 50 cm and made of durable, high-density, non-porous black plastic. The 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. Each rat was gently placed in the centre 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 time in open arms was recorded. The ratio (%) of time spent in the open arms to total time spent in any arm was calculated as the standard anxiety index. After each trial, the maze was wiped clean.

RESULTS

Post hoc comparisons between all the 5 groups showed a significant difference regarding the percentage of time spent in the open arms, $F(1, 38) = 447.550$, $p < 0.001$.

Significant differences concerning the time spent in the open arms were also observed between the control group and the 8.125 mg/kg of zinc hydroaspartate group ($p = 0.044$), between the control group and the 16.25 mg/kg of zinc hydroaspartate group ($p = 0.011$), and between control and the 32.5 mg/kg of zinc hydroaspartate group ($p = 0.001$).

No significant difference regarding the time spent open arms was found between the control group and the group which received the maximum dosage of 65 mg/kg of zinc hydroaspartate ($p = 0.063$).

Furthermore, no other statistically significant differences were found between any possible pair of the experimental groups.

Figure 1. The differences regarding the time spent in open arms (in percent) between the 5 experimental groups. The results are displayed as means $\pm$ SEM for $n=8$ rats for each experimental group.

Discussion

When we first started the study, the main goal of our research team was to discover if a zinc treatment for two weeks will influence the anxiety-like behaviour of rats in the elevated plus maze. Furthermore, we assumed that a decrease in the anxiety-like behaviour of the rats would be proportional with an increase in the dosage of zinc treatment. In addition, we also wanted to ascertain if an upper threshold exist, for the hypothesised improvement in the rats' anxiety related behaviour. In other words, we searched for the optimal zinc hydroaspartate dosage, equivalent to the maximum improvement in the time spent in the open arms.

The results from the statistical analysis showed that 3 out of the 4 dosages were efficient in reducing the anxiety-like behaviours in rats. More specifically, post hoc analysis showed that the rats from the control group spent significantly less time in the open

arms of the elevated plus maze compared to the rats which received the lower doses of zinc of 8.125 mg/kg ($p=0.044$), 16.25 mg/kg ($p=0.011$) and 32.5 mg/kg of ($p=0.001$). However, the maximum dosage of zinc hydroaspartate (65 mg/kg) did not improve significantly the time in the open arms in the treated rats when compared to the control group ($p=0.063$). These results suggest that a dose of 8.125 mg/kg of zinc hydroaspartate is sufficient for inducing significant improvements in anxiety-like behaviour of rats.

Increasing dosage of the zinc does not seem to significantly reduce the anxiety levels in rats. Furthermore, a high dose of 65mg/kg of zinc seem to have no significant effect on the anxiety related behaviour of the rats.

In addition, the assertion that a low dose of zinc is sufficient for optimal results in reducing anxiety levels in rats is also supported by our results that showed no

significant differences between the groups that received the lower dose of 8.125 mg /kg of zinc the groups receiving higher doses of 16.25 mg/kg and 32.5 g mg/kg and the group that received the highest dose of mg/kg of zinc.

The results of our study are similar to those reported by previous studies on the possible correlation between anxiety and zinc supplementing in animal models, which demonstrated that zinc is involved in the pathophysiology of anxiety (31-33).

Also, the zinc supplementation has been demonstrated in preclinical studies to decrease anxiety-like behaviour.

Furthermore, two recent studies showed that a rodents fed a zinc deficient diet for a prolonged period of time exhibited a significantly higher rate of anxiety-like behaviours (34, 35).

Whereas the correlation between an optimal zinc level and a low level of anxiety has been

described, the mechanism responsible for the anxiolytic action of zinc is still unknown. Different authors have suggested various theories to support this correlation. Thus, J. Kotlinska (36) states that the beneficial action of zinc on anxiety may be related to the modulation of glutamate and GABA systems (36).

Examining the zinc antidepressant-like activity, other authors have speculated that the anxiolytic effect of zinc may be related to its antagonism to NMDA and mGluR5 glutamate receptors (37, 38).

A third theory suggests serotonergic receptors to play an essential role in the anxiolytic effect of zinc (39). Clearly, future studies are needed to elucidate the exact mechanisms that underlie this apparent anxiolytic effect of zinc, while these data could also exert an important scientific and possible therapeutically relevance.

CONCLUSIONS

These initial results described in the present study suggest a promising relevance for zinc in anxiety-related and also advocate for further theoretical research in this area considering oxidative stress related mechanisms.

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