Research Journal of Biological Sciences 3 (12): 1415-1418, 2008
ISSN: 1815-8846

Assessment of Changes in Anxiety and Exploratory Behaviors Following Zinc Supplementation in Rats

[1]Saeid Sobhanirad, [1]Reza Valizadeh, [2]Ali Moghimi and [1]Abdolmansoor Tahmasebi
[1]Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Iran
[2]Department of Biology, Faculty of Sciences, Ferdowsi University of Mashhad, Iran

Abstract: In an experiment the emotional and exploratory behaviors of Wistar rats to dietary zinc supplementation from zinc sulfate monohydrate (ZnS), Zinc oxide (ZnO) and zinc Methionine (ZnM) were studied in an open field test. One hundred-twenty rats were assigned to 10 dietary treatments including: Basal Diet (BD) + 50 mg kg^{-1} ZnS, BD + 100 mg kg^{-1} ZnS, BD + 200 mg kg^{-1} ZnS, BD + 50 mg kg^{-1} ZnO, BD + 100 mg kg^{-1} ZnO, BD + 200 mg kg^{-1} ZnO, BD + 50 mg kg^{-1} ZnM, BD + 100 mg kg^{-1} ZnM, BD + 200 mg kg^{-1} ZnM and Basal diet (without Zn supplementation). These parameters including rearing, number of peripheral squares crossing, number of central squares crossing, hole exploration and defecation were recorded for 5 min. The treatments differed significantly in case of hole exploration and defecation. The results of these experiments indicated that high level of zinc methionine (200 mg kg^{-1}) increased exploratory activity and locomotor activity. The results of this experiment indicated that high level of zinc methionine (200 mg kg^{-1}) reduced exploratory activity and anxiety-like behavior. Further studies are needed to identify the anxiolytic mechanism and the optimum level of Zn supplementation.

Key words: Anxiety behavior, exploratory behavior, open-field, supplemental zinc, rat

INTRODUCTION

Zinc (Zn) is widely distributed throughout the body and plays an essential role in many vital processes. It is the co-factor for several enzymes (e.g., carbonic anhydrase and superoxide dismutase) and influences the metabolism of important hormones such as insulin and gonadotropins (Thompson *et al*., 2003). Approximately, 90% of the total brain zinc exists as zinc metalloproteins, especially in the telencephalon (Takeda *et al*., 2007). Furthermore, recent reports have indicated that zinc deprivation during periods of rapid development critically impairs animal behavior, mental and brain functions (Halas *et al*., 1983, 1986).

Zinc is commonly added at supplemental levels to many formulated animal diets (Sandoval *et al*., 1997). The supplemental zinc has been traditionally added in form of inorganic salts, such as zinc oxide or zinc sulfate. However, considerable reports for feeding organic trace minerals instead inorganic forms to animals for the benefit of higher bioavailability have been recently published (Griffiths *et al*., 2007).

The objective of this study was to evaluate the behavioral responses of young rats to zinc supplementations in both organic and inorganic supplements in an open-field test.

MATERIALS AND METHODS

Diets: The basal diet for young rats was formulated according to Reeves (1997) (AIN-93G purified diet, Table 1) without zinc supplementation. The control group was fed a zinc-adequate diet (40 mg of Zn kg^{-1} of diet DM). The other groups of rats were fed the basal diet supplemented by different supplemental zinc compounds including zinc sulfate monohydrate (ZnS), Zinc oxide (ZnO) and zinc Methionine (ZnM) in the following dietary treatments; Basal diet (BD, Table 1), BD + 50 mg kg^{-1} ZnS, BD + 100 mg kg^{-1} ZnS, BD + 200 mg kg^{-1} ZnS, BD + 50 mg kg^{-1} ZnO, BD + 100 mg kg^{-1} ZnO, BD + 200 mg kg^{-1} ZnO, BD + 50 mg kg^{-1} ZnM, BD + 100 mg kg^{-1} ZnM and BD + 200 mg kg^{-1} ZnM.

Animals: One-hundred twenty weaned male Wister rats (4 weeks old, 112±13 g body weight) were randomly assigned to one of the above-mentioned treatments. The animals were housed in the stainless still cages located in a light (the lighting from 07:00-19:00), temperature (22±2°C) and moisture controlled room. Rats were given free access to pelleted diets and distilled water throughout the experiment (60 days). The experiment took

Corresponding Author: Saeid Sobhanirad, Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Iran

Table 1: Composition of the basal diet

Ingredient	Amount (g kg^{-1})
Cornstarch	370
Soybean oil (no additives)	80
Skim milk powder	350
Cellulose	50
Sucrose	100
AIN-93G Mineral mix	35
AIN-93G Vitamin mix	10
L-Cystine	3
Choline bitartrate (41.1%)	2
Tert-Butylhydroquinone (TBHQ), mg	14

place under quiet conditions and low light (50 lux). The experimental conditions were adjusted according to the rules of local committee for animal ethics.

Apparatus: The open-field apparatus was made of fiberglass with a green floor (divided by black lines into 25 squares of 20×20 cm) and 40 cm high translucent walls (100×100×40 cm). There were holes in the center of each square and in the corners and the middles of lines of central squares. The open-field was previously validated to measure behavioral indices of anxiety in rats (Gavioli and Calo, 2006).

Procedures: On the day 60, each rat was individually placed in the specific point of open-field box and the following parameters were registered for 5 min: The number of hole exploration, number of the head entering into the holes. The number of defecation. All the floors of this apparatus were cleaned with ethyl alcohol and dried with paper towels.

Statistic: The data were statistically analyzed by one-way analysis of variance, with the significance level of $p<0.05$. When a main factor was significant, the post hoc comparisons were performed using Duncan test.

RESULTS

Figure 1 showed the hole exploration during the 5 min test periods. The number of hole exploration was significantly increased by adding ZnM to the diet at a level of 200 mg kg^{-1}, compared the control (11.91 vs. 6.33). There was also a significantly increasing in numbers of defecation by adding ZnM at a level of 200 mg zinc kg^{-1} diet as shown in Fig. 2. Elevated zinc can lead to adverse gastrointestinal effects (Houston *et al*., 2001), thus the increased defecation is predictable. The numbers of peripheral squares crossing, the number of central squares crossing and the number of rearing by rats fed all zinc supplements at the levels of 50, 100 and 200 mg of Zn kg^{-1} of diet are shown in Table 2. There were no significant differences between theses factors for all zinc supplements.

Table 2: Mean (±SEM) number of peripheral squares crossing, number of central squares crossing and number of rearing by rats fed zinc supplements at the zinc levels of 0, 50, 100 and 200 mg kg^{-1} of diet dry matter

	Zinc level (mg kg^{-1})			
	0	50	100	200
No. of peripheral squares crossing				
ZnS	70.91±6.79	59.33±6.79	72.66±6.79	63.58±6.79
ZnO	70.91±6.43	72.41±6.43	70.25±6.43	71.00±6.43
ZnM	70.91±5.38	82.08±5.38	77.75±5.38	70.00±5.38
No. of central squares crossing				
ZnS	1.83±0.72	1.58±0.72	2.50±0.72	2.08±0.72
ZnO	1.83±0.77	2.25±0.77	2.16±0.77	2.83±0.77
ZnM	1.83±0.78	3.16±0.78	1.91±0.78	1.33±0.78
No. of rearing				
ZnS	16.66±2.02	14.75±2.02	15.50±2.02	16.58±2.02
ZnO	16.66±1.93	17.83±1.93	18.41±1.93	20.16±1.93
ZnM	16.66±2.15	20.91±2.15	21.25±2.15	14.51±2.15

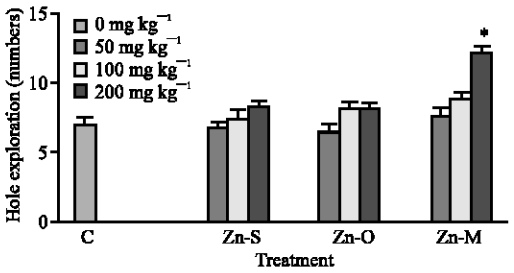

Fig. 1: The number of hole exploration (mean ± SEM) by rats fed zinc sulfate (ZnS), zinc oxide (ZnO) and zinc methionine (ZnM) compared to the control group. Data are expressed as mean values (±SEM) from each group of minimum 12 rats. *p<0.05

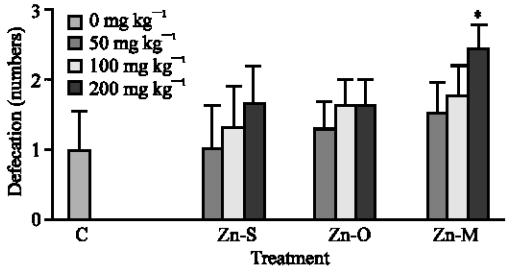

Fig. 2: The number of defecation (mean±SEM) by rats fed zinc sulfate (ZnS), zinc oxide (ZnO) and zinc methionine (ZnM) compared to the control group. Data are expressed as mean values (±SEM) from each group of minimum 12 rats. *p<0.05

DISCUSSION

It was found that increasing levels of zinc supplements in the diet lead to reduction in exploratory behavior and locomotor activities by the rats. Zinc sulfate is a highly available and absorbable supplement. Zinc oxide is less soluble and available for absorption. In

addition, several investigators have reported that bioavailability of organic forms of zinc in the diet of various animals are higher in comparison with the inorganic zinc forms, probably due to their different metabolism (Spears and Weiss, 2008).

The increase in releasing of effective intermediators in the ventral hippocampus when the animals confront a novel environment such as elevated plus-maze can influence the Hypothalamic-Pituitary-Adrenal (HPA) axis activity (Yusim *et al*., 2000) and lead to performing different behavioral responses. There are many reports (*in vitro* and *in vivo*) regarding the direct and indirect influences of HPA on emotional and anxiety-like behaviors. The studies provide additional evidence that supplementation with one metal can have effects on the status of other metals in the brain and body and these interactive effects likely play a role in cognition (Chrosniak *et al*., 2006) although, its severity is related to many factors such as animal age, sex, species and nutritional status and composition of the diet. Elevated zinc can also lead to adverse gastrointestinal effects (Houston *et al*., 2001; Højberg *et al*., 2005), thus the increased defecation is predictable.

The major problem is that the (already modest) effects seen can not be univocally attributed to zinc. Indeed the effects of methionine per se have been underestimating. L-Methionine is the precursor of S-adenosyl-L-methionine (SAMe) whose relation to mood is suggested by several lines of evidence. SAMe is one of the better studied of the natural remedies. SAMe is a methyl donor and is involved in the synthesis of various neurotransmitters in the brain. Low SAMe concentrations have been observed in the cerebrospinal fluid of depressed persons. Conversely, there is a positive correlation between an increase in plasma SAMe concentrations and an improvement in depressive symptoms (America and Milling, 2008). The activity of the enzyme methionine adenosyltransferase, which is involved in the synthesis of SAMe, has been shown to be low in depressed schizophrenic patients but high in manic patients (Shippy *et al*., 2004). To appear the roles of methionine, we can suggest having control group using only methionine supplemented diet.

In comparison the effects of organic and inorganic zinc supplements it was resulted that high level of zinc methionine (200 mg kg^{-1}) reduced exploratory activity and anxiety-like behavior. It was concluded that more experiments with lower levels of zinc methionine related to these activities are required.

CONCLUSION

We provide evidence that organic zinc supplements at high level of zinc may be useful for the treatment of anxiety. Further studies are needed to identify the anxiolytic mechanism and the optimum level of Zn supplementation.

ACKNOWLEDGMENT

Authors wish to thank the Iran National Science Foundation for financially supporting this study and Javaneh Khorasan Company for preparation the pelleted diets.

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