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Research report

Significance of serum glucocorticoid and chelatable zinc in depression and cognition in zinc deficiency

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ABSTRACT

Dietary zinc deficiency elicits neuropsychological symptoms and cognitive dysfunction. To pursue the mechanisms of these symptoms, in the present study, the relationship among serum glucocorticoid, chelatable zinc in the synaptic cleft and brain function based on behavior was examined in young rats fed a zinc-deficient diet for 4 weeks. Serum glucocorticoid level was significantly increased in zinc-deficient rats. However, the induction of in vivo dentate gyrus LTP and object recognition memory were not affected in zinc-deficient rats. Chelatable zinc levels were decreased in the stratum lucidum of the hippocampal CA3, but not in the molecular layer of the dentate gyrus. It is reported that dentate gyrus LTP and object recognition memory are affected in clioquinol (30 mg/kg)-administered rats, in which chelatable zinc is significantly decreased in the molecular layer of the dentate gyrus. Thus, the significant decrease in chelatable zinc in the molecular layer of the dentate gyrus may be required for object recognition memory deficit in zinc deficiency. On the other hand, the time of grooming in the open-field test was decreased in zinc-deficient rats. Immobility time in the forced swim test was increased in zinc-deficient rats, but not in clioquinol-administered rats, in which chelatable zinc was more markedly decreased than in zinc-deficient rats, suggesting that the lack of chelatable zinc does not increase depression-like behavior. These results suggest that the chronic increase in serum glucocorticoid level is involved in the increase in depression-like behavior rather than the decrease in chelatable zinc after 4-week zinc deficiency.

1. Introduction

Approximately 80% of the total brain zinc exists as zinc metalloproteins. The rest mainly exists in the presynaptic vesicles and is histochemically reactive (chelatable) as revealed by Timm's sulfide-silver staining method [1,2]. The removal of zinc transporter-3 (ZnT-3) protein, which is responsible for the movement of zinc from the cytoplasm into synaptic vesicles, results in a 20% reduction of the total amount of zinc in the brain [3]. Chelatable zinc is released along with neuronal activity; there is a large number of evidence on zincergic neurons that sequester zinc in the presynaptic vesicles and release it in a calcium- and impulse-dependent manner [4,5]. In the rat brain, Timm's stain is hardly observed just after the birth and its intensity increases with brain development [6,7], indicating that chelatable zinc is involved in not only brain growth but also brain function. However, the role of chelatable zinc in cognitive function and synaptic plasticity such as long-term potentiation (LTP) that is the cellular mechanism for memory is poorly understood.

Approximately 50% of the world population does not get adequate zinc and is at the risk of zinc deficiency [8]. Zinc deficiency in children is a nutritional and health problem in both developing and developed countries [9–11]. The evidence from experimental animals indicates that zinc deprivation during periods of rapid development critically impairs behavior and brain function, in addition to brain development [12]. Lethargy (reduced activity and responsiveness) is a characteristic in zinc deficiency [13].

Dietary zinc deficiency readily decreases serum zinc level [14] and the increase in chelatable zinc is suppressed by zinc deficiency during periods of hippocampal development [15]. The insufficiency of chelatable zinc may enhance susceptibility to epileptic seizures and glutamate excitotoxicity in zinc-deficient young mice and rats [16–18]. On the other hand, dietary zinc deficiency increases serum glucocorticoid level via activation of the hypothalamo–pituitary–adrenocortical (HPA) system [19,20] prior to the significant change (insufficiency) in chelatable zinc levels [14,21,22]. Learning behavior of passive avoidance is affected in zinc-deficient rats, in which chelatable zinc levels in the hippocampus are not decreased [23]. Lupien et al. [24] reports that basal cortisol elevation may cause hippocampal damage and impair hippocampus-dependent learning and memory in humans.

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Furthermore, it has been reported that abnormal glucocorticoid secretion that is induced by stress is associated with neuropsychological symptoms such as depression [25–27]. The HPA system is affected in approximately 50% of human depressives [28–30]. Therefore, it is possible that chronic increase in serum glucocorticoids is a key factor to induce cognitive dysfunction and neuropsychological symptoms such as depression in zinc deficiency.

Interestingly, serum zinc levels are decreased in patients with major depression [31,32]. The decrease in serum zinc is normalized by effective antidepressant treatment [33]. Antidepressant therapy can be improved by zinc supplementation [34]. Siwek et al. [35] report that serum zinc is a state marker of depression. However, the mechanisms of neuropsychological symptoms in zinc deficiency remain to be solved in addition to those of cognitive dysfunction. To pursue the mechanisms of these symptoms, in the present study, the relationship among serum glucocorticoid, chelatable zinc in the synaptic cleft and brain function based on behavior was examined in young rats fed a zinc-deficient diet for 4 weeks.

2. Materials and methods

2.1. Diets and chemicals

Control (52.8 mg Zn/kg) and zinc-deficient (0.37 mg Zn/kg) diets were purchased from Oriental Yeast Co. Ltd. (Yokohama, Japan). ZnAF-2, a membrane-impermeable zinc indicator, was kindly supplied from Sekisui Medical Co., LTD (Ibaraki, Japan). The fluorescent indicator was dissolved in dimethyl sulfoxide (DMSO) and then diluted with artificial cerebrospinal fluid (ACSF), which was composed of 119 mM NaCl, 2.5 mM KCl, 1.3 mM $MgSO_4$, 1.0 mM NaH_2PO_4, 2.5 mM $CaCl_2$, 26.2 mM $NaHCO_3$, and 11 mM D-glucose (pH 7.3). Clioquinol (5-chloro-7-iodo-8-hydroxyquinoline; CQ), a lipophilic chelator for zinc, was dissolved in 20% dimethyl sulfoxide in olive oil.

2.2. Experimental animals

Male Wistar rats (3 weeks old) were purchased from Japan SLC (Hamamatsu, Japan). They were housed in the breeding rooms under the standard laboratory conditions (23 ± 1 °C, 55 ± 5% humidity) and had access to tap water and food *ad libitum*. Feeding the zinc-deficient diet was begun at 4 weeks of age. Rats were fed the diet for 4 weeks and used for experiments. The lights were automatically turned on at 8:00 and off at 20:00. All experiments were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of the University of Shizuoka that refer to American Association for Laboratory Animals Science and the guidelines laid down by the NIH (*NIH Guide for the Care and Use of Laboratory Animals*) in the USA. Averaged body weight (87 ± 2 g) of rats fed the zinc-deficient diet for 4 weeks was about 40% of that (213 ± 7 g) of the control rats.

2.3. Serum corticosterone concentration

Blood samples were collected from the common carotid arteries of diethyl ether-anesthetized rats. The collection was performed in the morning (10–11 o'clock) and quickly finished within 2 min. Blood samples were kept on ice and centrifuged for 10 min (5500 rpm, 4 °C). Corticosterone concentration in the serum obtained was determined by a corticosterone [^{125}I] RIA kit (MP Biomedicals, Inc., Irvine, CA).

2.4. Hippocampal slice preparation and zinc imaging

Rats were anesthetized with ether and decapitated. The brain was quickly removed and immersed in ice-cold ACSF. Transverse hippocampal slices (400 μm) were prepared using a vibratome ZERO-1 (Dosaka Kyoto, Japan) in an ice-cold ACSF. Slices were then maintained in a holding chamber at room temperature for at least 1 h. All solutions used in the experiments were continuously bubbled with 95% O_2 and 5% CO_2.

For extracellular zinc imaging, the hippocampal slices were transferred to a recording chamber filled with 10 μM ZnAF-2 in ACSF. The fluorescence of ZnAF-2 (excitation, 488 nm; monitoring, 505–530 nm) was measured in the hippocampus by using a confocal laser-scanning microscopic system LSM 510 (Carl Zeiss), equipped with the inverted microscope (Axiovert 200 M, Carl Zeiss). Region of interest was set in the molecular layer of the dentate gyrus, the stratum lucidum of the CA3.

2.5. Dentate gyrus LTP

Dentate gyrus LTP was recorded under anesthesia as reported previously [36]. Male rats were anesthetized with chloral hydrate (400 mg/kg) and placed in a stereotaxic apparatus. A bipolar stimulating electrode and a monopolar recording electrode made of tungsten wire were positioned stereotaxically so as to selectively stimulate the perforant pathway while recording in the dentate gyrus. The electrode stimulating the perforant pathway fibers was implanted 8.0 mm posterior to the bregma, 4.5 mm lateral, 3.0–3.5 mm inferior to the dura. A recording electrode was implanted ipsilaterally 4.0 mm posterior to the bregma, 2.3 mm lateral and 3.0–3.5 mm inferior to the dura. All the stimuli were biphasic square wave pulses (200 μs width) and their intensities were set at the current that evoked 40% of the maximum population spike (PS) amplitude. Test stimuli (0.05 Hz) were delivered at 20 s intervals to monitor PS.

At the beginning of the experiments, input/output curves were generated by systematic variation of the stimulus current (0.1–1.0 mA) to evaluate synaptic potency. After stable baseline recording for at least 30 min, LTP was induced by delivery of high-frequency stimulation (HFS; 10 trains of 20 pulses at 200 Hz separated by 10 s). PS amplitudes (test frequency: 0.05 Hz) were averaged over 120-s intervals and expressed as percentages of the mean PS amplitude measured during the 30-min baseline period perfused with ACSF prior to LTP induction.

2.6. Object recognition memory

Rats were placed for 10 min into an open field, which was a 70 cm × 60 cm arena surrounded by 70 cm high walls, made of a black-colored plastic. Twenty-four hours after open field exploration, rats were trained and tested in a novel object recognition task as reported previously [37]. Training in the object recognition task took place in the same area used for the open field exploration. The open field exploration was thus used as a context habituation trial for the recognition memory task. The object recognition test requires that the rats recall which of two earthenware objects they had been previously familiarized with. Twenty-four hours after arena exploration, training was conducted by placing individual rats into the field, in which two identical objects (objects A1 and A2; sake bottle) were positioned in two adjacent corners, 15 cm from the walls. Rats were left to explore the objects for 5 min. Rats were not used for the test when the total of the object exploration time was less than 20 s. In the test 24 h after training, the rats explored the open field for 3 min in the presence of one familiar (A) and one novel (B; cup) object. All objects presented similar textures, colors and sizes, but distinctive shapes. A recognition index calculated for each rat was expressed by the ratio $T_B/(T_A + T_B)$ [T_A = time spent to explore the familiar object A; T_B = time spent to explore the novel object B]. Between trials the objects were washed with 70% ethanol solution. Exploration was defined as sniffing or touching the object with the nose and/or forepaws. Sitting on the object was not considered as exploration. To check the preference for the novel object in the test, the novel object (cup) was changed with the familiar object (sake bottle). No preference for the objects was confirmed in the initial experiment.

2.7. Open field

Behavior and locomotor activity of rats were assessed in the open-field test. Each rat was placed in an arena (70 cm × 70 cm × 64 cm) made of a black-colored wooden box, in which it has never been placed. The arena was illuminated with three overhead lights (40 W each). Behavior of each rat in the arena was recorded with a video camera. Behavior and locomotor activity of rats were measured for 5 min and two persons independently measured the times of rearing and line crossing, and the time of glooming. The averaged values were used.

2.8. Forced swim test

The forced swim test was performed according to the procedure reported previously [38,39] to assess neuropsychological behavior in stressful and novel environment. The forced swim test was performed in a plastic tank (diameter, 45 cm; height, 79 cm) containing water (23–24 °C) to a depth of 30 cm. Rats were individually placed into the plastic tank for 5 min. Behavior of rats during the test was recorded with video camera to measure immobility time exactly. Rats were judged to be immobile when they remained floating passively in the water. Two persons independently measured immobility time and the averaged time was used.

2.9. Statistical analysis

Grouped data are expressed as the mean ± SEM. For statistical analysis, Student's *t*-test was used for comparison of the means of paired or unpaired data.

3. Results

3.1. Dentate gyrus LTP and object recognition memory in zinc deficiency

Dentate gyrus LTP was compared between the control and zinc-deficient rats. Dentate gyrus LTP was induced to almost the same extent (Fig. 1). In object recognition memory, there was no significant difference in exploratory time between the control and