BioMetals (2006) 19:565–572
DOI 10.1007/s10534-005-6145-9

Responsiveness to kainate in young rats after 2-week zinc deprivation

Atsushi Takeda*, Hiromasa Itoh, Haruna Tamano & Naoto Oku
*Department of Medical Biochemistry, School of Pharmaceutical Sciences, University of Shizuoka, 52- 1 Yada, Shizuoka, 422-8526, Japan; *Author for correspondence (Tel: +81-54-264-5700; Fax: +81-54-264-5705; E-mail: takedaa@u-shizuoka-ken.ac.jp)*

Received 9 November 2005; Accepted 21 December 2005

Key words: calcium, hippocampus, kainate, seizure, zinc

Abstract

On the basis of the evidence of the enhanced susceptibility to kainate-induced seizures in young rats fed a zinc-deficient diet for 4 weeks, the relationship between zinc release from hippocampal neuron terminals and seizure susceptibility was studied in young rats fed the zinc-deficient diet for 2 weeks. Timm's stain, with which histochemically reactive zinc in the presynaptic vesicle is detected, was not attenuated in mossy fibers and other areas in the hippocampus after 2-week zinc deprivation, whereas the attenuation was observed after 4-week zinc deprivation. Extracellular zinc concentration was not also decreased after 2-week zinc deprivation, unlike the case after 4-week zinc deprivation. To check the capacity for zinc release from neuron terminals after 2-week zinc deprivation, the hippocampus was excessively stimulated with 100 mM KCl. The increase in extracellular zinc concentration of zinc-deficient group was significantly more than that of control group. These results suggest that zinc release from hippocampal neuron terminals is not affected by 2-week zinc deprivation. On the other hand, the latency in myoclonic jerks of zinc-deficient group was significantly shorter than in the control group after treatment with kainate, while the latency in clonic convulsions was not different between the two groups. Intracellular fura-2 signal, a calcium indicator, was significantly higher in the hippocampal CA3 areas of zinc-deficient group 4 s after delivery of kainate to dentate granule cells. These results suggest that susceptibility to kainate-induced seizures is altered prior to the decrease in extracellular zinc concentration and zinc release from neuron terminals in zinc-deficient young rats. The alteration of calcium signaling seems to be involved in the susceptibility in zinc deficiency.

Introduction

Cortical regions of the brain are stained by Timm's sulfide–silver method, which is used to detect histochemically reactive zinc in the presynaptic vesicles (Danscher 1981). The zinc is sequestered in zinc-containing glutamatergic neuron terminals and released in a calcium- and impulse-dependent manner (Frederickson 1989). Zinc serves as a neuromodulator in glutamatergic and GABAergic neurotransmitter systems: zinc decreases extracellular glutamate concentration in the hippocampus, while it increases extracellular GABA concentration there (Takeda *et al.* 2003a, 2004). Zinc released into synaptic cleft may be important to modulate the balance of inhibition-excitation in the synapse (Buhl *et al.* 1996; Cohen-Kfir *et al.* 2005). In the hippocampus, histochemically reactive zinc exists in both the terminals of mossy fibers, which originate from dentate granular cells, and Schaffer collaterals, which originate from CA3 pyramidal cells (Frederickson & Danscher 1990). Mossy fibers form unusually large excitatory synapses with the proximal dendrites of CA3 pyramidal cells (Chicurel & Harris 1992). Mossy fibers also form morphologically distinct synaptic contacts with a population of inhibitory interneurons in the CA3 region (Acsady *et al.* 1998). All of mossy fiber terminals contain zinc in the presynaptic vesicles (Sindreu *et al.* 2003), while

approximately 45% of Schaffer collateral terminals, which project to the CA1, also contain it.

Temporal lobe epilepsy is the most common type of epilepsy in adults. The hippocampus is thought to be an epileptic focus in temporal lobe epilepsy (Ojemann 1987). Many researches pointed out that zinc homeostasis in the brain is linked with the etiology and manifestation of epileptic seizures (Sterman *et al*. 1988; Buhl *et al*. 1996; Takeda 2001). Kainate is an agonist of glutamate receptor subtypes and the kainate-treated mice and rats are an experimental model of temporal lobe epilepsy (Ben-Ari 1985). They have been used to understand the relationship between zinc movement in the brain and epileptic seizures. Zinc concentration in the hippocampus is decreased in kainate-treated rats (Assaf & Chung 1984) and kainate-induced seizures are linked with the decrease in zinc concentrations in the brain including the hippocampus (Takeda *et al*. 2003b).

On the other hand, 4-week zinc deprivation, which attenuates Timm's stain in the brain of young (8-week-old) rats and mice, enhances susceptibility to kainate-induced seizures (Takeda *et al*. 2003c, d). An *in vivo* microdialysis experiment demonstrates that the increase in glutamate release associated with the lack of increased GABA release in the hippocampus is a possible mechanism for the enhanced susceptibility to kainate-induced seizures in zinc deficiency (Takeda *et al*. 2003d). However, it is unknown whether the decrease in zinc release from neuron terminals in the hippocampus is linked with the enhanced seizure susceptibility in zinc deficiency. Zinc transporter 3 knockout mice are more sensitive than control mice to kainate-induced seizures, suggesting that the net effect of hippocampal zinc on acute seizures *in vivo* is inhibitory (Cole *et al*. 2000).

Biological half-lives of zinc in the hippocampus of young rats are around 4 weeks (Takeda *et al*. 1995). Zinc turnover in the brain may be strictly restricted in zinc deficiency. Thus, zinc concentration in the hippocampus is not appreciably decreased after 4-week zinc deprivation (Takeda et al. 2003c). However, Timm's stain in the hippocampus is decreased after treatment with kainate (Takeda *et al*. 2003b), implying excessive release of zinc and glutamate from glutamatergic neurons. In hippocampal slices prepared from mice after 4-week zinc deprivation, the enhanced excitability of mossy fibers was evidenced by stimulation with 100 mM KCl and was linked with alteration of intracellular calcium signaling (Takeda et al. 2005).

In the present study, the relationship between zinc release from hippocampal neuron terminals and seizure susceptibility was studied in young rats fed the zinc-deficient diet for 2 weeks, in which zinc concentration in the presynaptic vesicle may be affected minimally. Furthermore, the involvement of intracellular calcium signaling in seizure susceptibility was studied in hippocampal slices from the zinc-deficient rats.

Materials and methods

Chemicals

Control (44 mg Zn/kg) and zinc-deficient (2.7 mg Zn/kg) diets were purchased from Oriental Yeast Co. Ltd. (Yokohama, Japan). Artificial cerebrospinal fluid (ACSF) used as a perfusate was composed of 124 mM NaCl, 2.5 mM KCl, 2.0 mM $CaCl_2$, 1.0 mM $MgCl_2$, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$ and 10 mM D-glucose (pH 7.3).

Experimental animals

Male Wistar rats (4 weeks old) were purchased from Japan SLC (Hamamatsu, Japan) and then fed a control and zinc-deficient diet for 2 or 4 weeks. They were housed under the standard laboratory conditions (23 ± 1 °C, 55 ± 5% humidity) and had access to tap water and diet *ad libitum*. The lights were automatically turned on at 8:00 and off at 20:00. All experiments were performed in accordance with the Japanese Pharmacological Society guide for the care and use of laboratory animals.

Timm's sulfide–silver staining

Rats were deeply anesthetized with chloral hydrate, and then perfused transcardially with 0.1% Na_2S in phosphate buffer (pH 7.4). The brains were excised and immersed in 4% (w/v) paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4) for 24 h and then in 10–30% sucrose for 72 h. Coronal 30 μm sections were prepared in a cryostat at −20 °C. Timm's staining was performed according to the procedure described previously (Danscher 1981).

In vivo *microdialysis*

Thirteen days after the start of feeding the control or zinc-deficient diet, a guide tube was surgically implanted into the hippocampal CA3 subregion (stereotaxic coordinates: AP = −5.6 mm, ML = −4.6 mm, DV = + 5.0 mm) of chloral hydrate-anesthetized rats. Forty-eight hours after implantation of the guide tube, the hippocampus was perfused with ACSF by using a microdialysis probe (3-mm membrane CMA12 probe, CMA Microdialysis, Solna, Sweden) at a flow rate of 5.0 μl/min for 120 min under anesthesia and then 100 mM KCl for 40 min. The perfusate samples were collected for intervals of every 20 min.

Zinc concentration in the extracellular fluid

The perfusate samples (50 μl) were diluted with 2% nitric acid (100 μl). Analysis of the samples in triplicate was conducted using a flameless atomic absorption spectrophotometer (Shimadzu AA6800F, Kyoto, Japan). The accuracy was checked by analysis of the samples in the presence of a standard solution of zinc.

Histological analysis

Rats were deeply anesthetized with chloral hydrate and perfused intracardially with saline, followed by perfusion with 4% (w/v) paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4). The brains were removed quickly, further fixed with the same fixation solution at 4 °C overnight and processed for cryo-sectioning by incubation in a series of 10, 20 and 30% sucrose at 4 °C for 3 days. Afterwards, brains were frozen in ethanol (−80 °C) and kept at −80 °C. Ten micrometer coronal sections were cut on a cryostat and immediately collected on glass slides. For assessment of neuronal damage in the hippocampus, sections were stained with 0.15% (w/v) cresyl violet.

Hippocampal slice preparation

The brains were quickly removed from anesthetized rats and immersed in ice-cold choline-Ringer's solution (pH. 7.3) (127 mM choline, 2.5 mM KCl, 0.5 mM $CaCl_2$, 2.5 mM $MgCl_2$, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$ and 10 mM D-glucose) continuously bubbled with 95% O_2 and 5% CO_2. The brain was mounted on a super microslicer ZERO-1 (Dosaka, Kyoto, Japan) in a chamber filled with ice-cold choline-Ringer's solution continuously bubbled with 95% O_2 and 5% CO_2. Horizontal slices were cut at 400-μm thickness and put in ACSF at 25 °C for 30 min.

Intracellular calcium imaging

Slices were put in ACSF (2 ml) containing 10 μM fura-2 AM, a membrane-permeable calcium indicator, in the dark for 45 min at 25 °C. To remove unincorporated fura-2 AM from the extracellular fluid, the slices were put in ACSF (100 ml) in the dark at 25 °C, transferred to a chamber for observation filled with ACSF (2 ml) and mounted on the stage of an inverted microscope (Diaphot TMD 300, Nikon, Tokyo, Japan). Fluorescent intensity was measured in the stratum lucidum, in which mossy fiber terminals from dentate granule cells exist, and the CA3 pyramidal cell layer with an Argus-50/CA system (Hamamatsu Photonics, Hamamatsu, Japan; excitation, 340 nm; dichroic beam splitter, 505 nm) at 25 °C. The dentate granular cell layer was stimulated with 1 μl of 1 mM kainate. The stimulant was loaded via a microdialysis probe without membrane at a flow rate of 1 μl/s by using a microinjection pump (CMA/100, CMA Microdialysis), at a given time after the start of measuring fluorescent intensity.

ACSF is continuously bubbled with 95% O_2 and 5% CO_2 in each step till the start of measuring fluorescent intensity.

Statistical analysis

Student's *t*-test was used for comparison of the means of unpaired data. For multiple comparison ANOVA followed by Fisher's Protected Least Significant Difference (PLSD) was performed.

Results

Synaptic zinc levels in zinc deficiency

The mean body weight of 6-week-old rats, which were fed the zinc-deficient diet for 2 weeks, was 85.2 ± 2.0 g and that of the control rats was 132.4 ± 3.4 g (102 ± 1.3 g and 201 ± 2.8 g in the case after 4-week zinc deprivation). Timm's

staining was performed to estimate zinc concentration in the presynaptic vesicle. The stain was not appreciably attenuated in mossy fibers and other areas in the hippocampus after 2-week zinc deprivation (Figure 1). On the other hand, the stain of the hippocampus, especially mossy fibers, was attenuated after 4-week zinc deprivation.

The hippocampus of the control and zinc-deficient rats was perfused with ACSF and then stimulated with high K^+ in ACSF to check zinc release from neuron terminals by depolarization. The basal zinc concentration in the perfusate was not decreased after 2-week zinc deprivation, unlike the case of 4-week zinc deprivation (Figure 2A and B). Extracellular zinc concentration during stimulation with high K^+ was approximately 15 times higher than the basal concentration after 2-week zinc deprivation (Figure 2A). This increase of zinc-deficient group was significantly more than in the control group.

Susceptibility to kainate-induced seizures

Kainate (5 mg/kg body weight) was injected into the control and zinc-deficient rats. The latency in wet dog shakes and myoclonic jerks of zinc-deficient rats was significantly shorter than in the control rats, while the latency in clonic convulsions was not different between the two groups (Figure 3). Maximum seizure score was not significantly different between them.

Hippocampal cell death was analyzed by cresyl violet staining 3 days after treatment with kainate. There was no appreciable difference in cresyl violet stain between kainate-treated control and zinc-deficient rats (Figure 4). TUNEL labeling with counterstaining with 4′,6-diamidino-2-phenylindole, dihydrochloride (DAPI) was also performed in the hippocampus. TUNEL-positive cells were minimally observed in both kainate-treated control and zinc-deficient rats (data not shown).

Intracellular calcium imaging

To evaluate the excitability of dentate granule cells and CA3 neurons in zinc deficiency, intracellular calcium signal was monitored in the hippocampal CA3 by using membrane-permeable fura-2 AM after delivery of kainate (1 mM, 1 μl) to the dentate granule cell layer (Figure 5). Fura-2 signal was immediately increased in the CA3 areas of both groups after stimulation with kainate. The increase

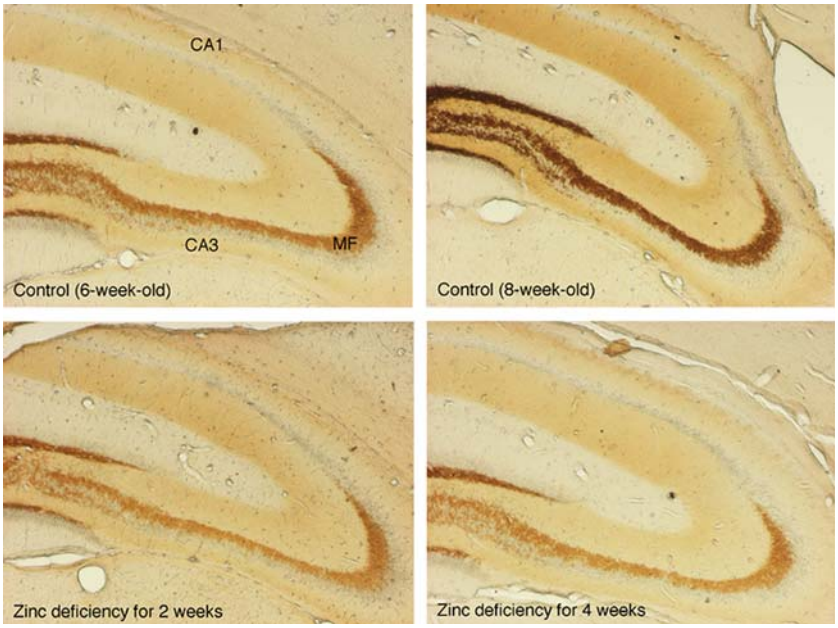

Figure 1. Timm's stain in the hippocampus in zinc deficiency. Coronal slices for Timm's staining were prepared from rats fed a control or zinc-deficient diet for 2 or 4 weeks (n = 4). Note that the stain was not appreciably attenuated in the hippocampus after 2-week zinc deprivation, unlike the case of 4-week zinc deprivation.

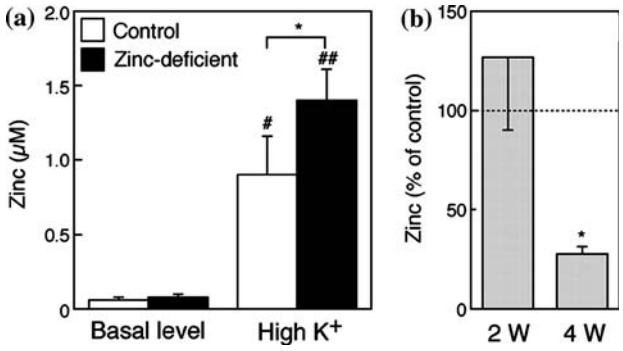

Figure 2. Zinc concentrations in the hippocampal extracellular fluid. The hippocampus of rats fed a zinc-deficient diet for 2 or 4 weeks was perfused with ACSF for 120 min and 100 mM KCl in ACSF for 40 min. The perfusate samples were collected for intervals of every 20 min. (A) The data represent zinc concentration in the perfusate after 2-week zinc deprivation. The basal level represents the mean of three samples before stimulation with 100 mM KCl. The high K^+ represents the mean of two samples during stimulation with 100 mM KCl. Note that zinc concentration in the hippocampal extracellular fluid was almost the same between the control and zinc-deficient rats. (B) The data represent the ratio (%) of the basal concentration in the perfusate after 2 or 4-week zinc deprivation to the basal concentration in the perfusate of each control. 2 W, 2-week zinc deprivation; 4 W, 4-week zinc deprivation. Note that zinc concentration in the hippocampal extracellular fluid was decreased after 4-week zinc deprivation, unlike 2-week zinc deprivation. Each bar and line represent the mean ± s.e.m. (n = 6). *, $P < 0.05$, vs. the control; #, $P < 0.05$, ##, $P < 0.01$, vs. the basal level.

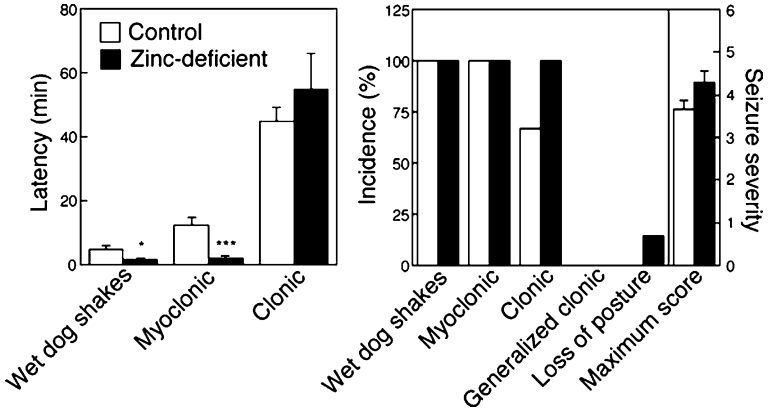

Figure 3. Susceptibility to kainate-induced seizures. Rats were fed a control or zinc-deficient diet for 2 weeks and then intraperitoneally injected with 5 mg/kg kainate. The latency represents the period of the first development of wet dog shakes, myoclonic jerks and clonic convulsions. The incidence represents the rate of seized mice to the total mice. Seizure severity represents the maximum seizure score, which was observed for 3 h after treatment with kainate. Seizure scores (the score 2, wet dog shakes; 3, myoclonic jerks; 4, clonic convulsions; 6, loss of posture, 8, status epilepticus) were taken according to the procedure reported previously (Racine 1972; Sperk *et al.*, 1985) [19,23]. Each bar and line represent the mean ± s.e.m (n = 6–7). *, $P < 0.05$; ***, $P < 0.001$, vs. the control.

in Fura-2 signal was more sustained in zinc-deficient group and the signal was significantly higher in the CA3 areas of zinc-deficient group than in the control group 4 s after treatment with kainate.

Discussion

Zinc deficiency in children is a nutritional and health problem in both developing and developed countries (Prasad 1983; Gibson 1998; Sandstead 1995; Hambidge 2000; Penland 2000). The evidence from experimental animals indicates that zinc deprivation during periods of rapid development impairs behavior and brain function in addition to brain development (Halas *et al.* 1983, 1986; Golub *et al.* 1995). However, the mechanism of brain dysfunctions in zinc deficiency is poorly understood (Sandstead *et al.* 2000). Zinc deficiency affects zinc homeostasis in the brain (Takeda 2001). The alteration of zinc homeostasis in the brain is associated with the etiology and

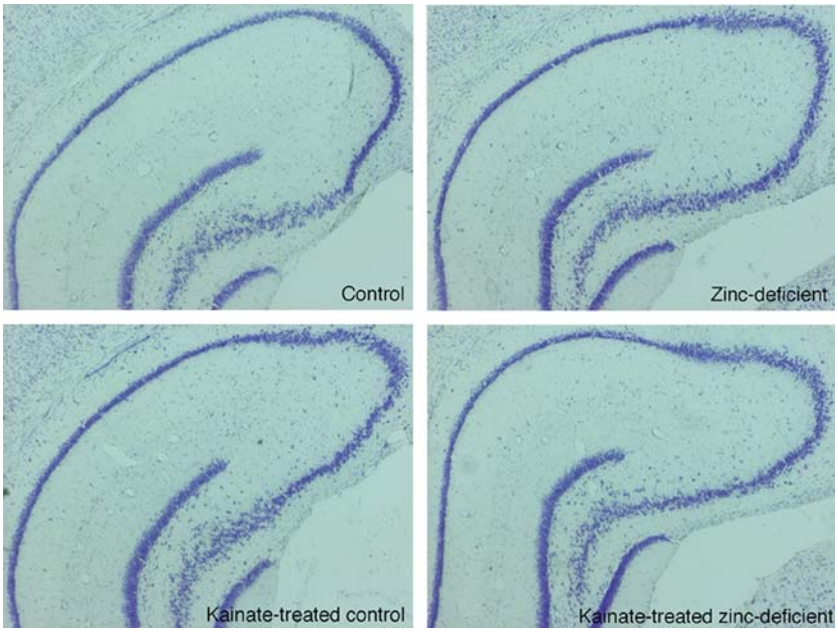

Figure 4. Hippocampal staining with cresyl violet after treatment with kainate. Rats were fed a control or zinc-deficient diet for 2 weeks and then intraperitoneally injected with 5 mg/kg kainate. The brains were quickly removed from the control and zinc-deficient rats 3 days after treatment with kainate, followed by preparation of coronal sections. The sections from each group of six rats were stained with cresyl violet to observe surviving cells in the hippocampus.

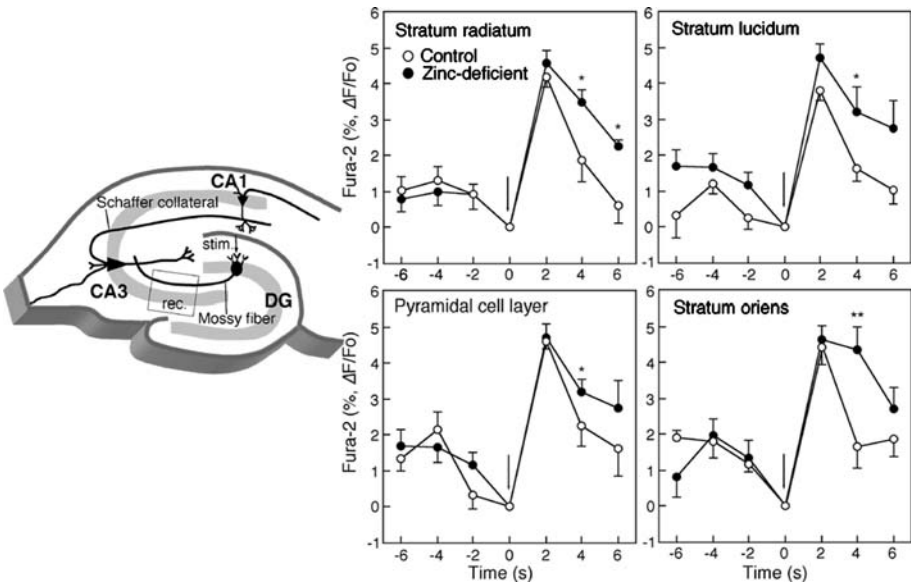

Figure 5. Imaging of intracellular calcium with fura-2 in the hippocampal CA3. Fura-2 AM was applied to hippocampal slices of rats, which were fed a control or zinc-deficient diet for 2 weeks (n = 5). The dentate granule cell layer was stimulated with 1 μl kainate (1 mM) for 1 s, as indicated by the arrow (stim.) in the illustration. The change of fura-2 signal was monitored in the stratum radiatum, CA3 pyramidal cell layer, stratum lucidum and stratum oriens. The data represent the ratio (%) of fluorescent intensity of each time to a basal fluorescent intensity before the stimulation indicated by the arrow. Each point and line represent the mean ± s.e.m. of 11 slices. *, $P < 0.05$; **, $P < 0.01$, vs. the control.

manifestation of epileptic seizures (Sterman *et al.* 1988; Fukahori & Itoh 1990). Susceptibility to epileptic seizures is decreased by dietary zinc loading, while this susceptibility in cats is increased by zinc deprivation (Sterman *et al.* 1986). The alteration of susceptibility to epileptic seizures in zinc deficiency is an important issue, because approximately 50% of the world population does not get adequate zinc (Brown *et al.* 2001).

To evaluate the involvement of vesicular zinc in epileptic seizures, in the present study, zinc concentration in the presynaptic vesicles and zinc release from neuron terminals were checked in the hippocampus in zinc deficiency. Timm's stain was not attenuated in mossy fibers and other areas in the hippocampus after 2-week zinc deprivation, whereas the attenuation was observed after 4-week zinc deprivation. Extracellular zinc concentration was not decreased after 2-week zinc deprivation, unlike the case after 4-week zinc deprivation. Because zinc concentration in the hippocampal extracellular fluid seems to be the most responsive to dietary zinc deficiency (Takeda *et al.* 2001), zinc homeostasis in the brain may be minimally affected in young rats by 2-week zinc deprivation. To check the capacity for zinc release from neuron terminals after 2-week zinc deprivation, the hippocampus was excessively stimulated with 100 mM KCl. The increase in extracellular zinc concentration of zinc-deficient group was significantly more than that of control group. These results suggest that zinc release from hippocampal neuron terminals is not affected by 2-week zinc deprivation. Judging from the excessive zinc release after 2-week zinc deprivation, it is possible that glutamate release from neuron terminals during stimulation with high K^+ is also more enhanced in the zinc-deficient young rats than in the control rats, as well as the case of young rats after 4-week zinc deprivation (Takeda *et al.* 2003c). This idea led an experiment examining whether glutamate excitotoxicity is altered in the hippocampus after 2-week zinc deprivation.

Susceptibility to kainate-induced seizures was examined in young rats fed the zinc-deficient diet for 2 weeks. The latency in myoclonic jerks of zinc-deficient rats was significantly shorter than in the control rats. However, the latency in clonic convulsions was not different between the two groups and maximum seizure score was not more increased by 2-week zinc deprivation. The current dose (5 mg/kg) of kainate minimally caused hippocampal cell death in both groups. The early stage of epileptic seizures might be facilitated in young rats by 2-week zinc deprivation. In young rats fed the zinc-deficient diet for 4 weeks, the latency in clonic convulsions of zinc-deficient group is significantly shorter than in the control group after treatment with kainate (5 mg/kg) and maximum seizure score of zinc-deficient group is significantly higher than that of control group (unpublished data). It is possible that kainate-induced seizures in young rats are aggravated in association with the decrease in vesicular zinc after 4-week zinc deprivation.

Geng *et al.* (2002) indicate that calcium concentration in rat hippocampal cells, which is determined by fura-2, is increased by zinc deficiency. Intracellular calcium signaling is linked with neuronal excitability and the cross talk between zinc and calcium signals is performed via proteins such as calcium channels (Minami *et al.* 2006). To evaluate the excitability of dentate granule cells and CA3 neurons in zinc deficiency, hippocampal slices were prepared from young rats fed the zinc-deficient diet for 2 weeks. When fura-2 signal was monitored in the CA3 areas after application of 1 μl kainate (1 mM) to the dentate granule cell layer, the increase in fura-2 signal was more sustained in zinc-deficient group and the signal was significantly higher in the CA3 areas of zinc-deficient group than in the control group 4 s after treatment with kainate. The excitability of hippocampal neurons might be enhanced in association with alteration of intracellular calcium signaling in zinc deficiency (Takeda *et al.* 2005). It is likely that intracellular calcium signaling in the hippocampus in zinc deficiency is altered before the decrease in extracellular zinc concentration in the brain, which is linked with zinc homeostasis in the brain. This alteration seems to be involved in susceptibility to kainate-induced seizures in zinc-deficient young rats.

In conclusion, susceptibility to kainate-induced seizures may be altered prior to the decrease in extracellular zinc concentration and zinc release from neuron terminals in the brain of zinc-deficient young rats. Further investigation on intracellular calcium signaling in zinc deficiency is necessary to understand the alteration of neuronal excitability in the hippocampus.

References

Acsady L, Kamondi A, Sik A, Freund T, Buzsaki G. 1998 GABAergic cells are the major postsynaptic targets of mossy fibers in the rat hippocampus. *J Neurosci* **18**, 3386–3403.

Assaf SY, Chung S-H. 1984 Release of endogeneous Zn^{2+} from brain tissue during activity. *Nature* **308**, 734–735.

Ben-Ari Y. 1985 Limbic seizure and brain damage produced by kainic acid: mechanisms and relevance to human temporal lobe epilepsy. *Neuroscience* **14**, 375–403.

Brown KH, Wuehler SE, Peerson JM. 2001 The importance of zinc in human nutrition and estimation of the blobal prevalence of zinc deficiency. *Food Nutr Bull* **22**, 113–125.

Buhl EH, Otis TS, Mody I. 1996 Zinc-induced collapse of augmented inhibition by GABA in a temporal lobe epilepsy model. *Science* **271**, 369–373.

Chicurel ME, Harris KM. 1992 Three-dimensional analysis of the structure and composition of CA3 branched dendritic spines and their synaptic relationship with mossy fiber boutons in the rat hippocampus. *J Com Neurol* **325**, 169–182.

Cohen-Kfir E, Lee W, Eskandari S, Nelson N. 2005 Zinc inhibition of r-aminobutyric acid transporter 4 (GAT4) reveals a link between excitatory and inhibitory neurotransmission. *Proc Natl Acad Sci USA* **102**, 6154–6159.

Cole TB, Robbins CA, Wenzel HJ, Schwartzkroin PA, Palmiter RD. 2000 Seizures and neuronal damage in mice lacking vesicular zinc. *Epilepsy Res* **39**, 153–169.

Danscher G. 1981 Histochemical demonstration of heavy metals. A revised version of the sulphide silver method suitable for both light and electronmicroscopy. *Histochemistry* **71**, 1–16.

Frederickson CJ. 1989 Neurobiology of zinc and zinc-containing neurons. *Int Rev Neurobiol* **31**, 145–238.

Frederickson CJ, Danscher G. 1990 Zinc-containing neurons in hippocampus and related CNS structures. *Prog Brain Res* **83**, 71–84.

Fukahori M, Itoh M. 1990 Effects of dietary zinc status on seizure susceptibility and hippocampal zinc content in the El (epilepsy) mouse. *Brain Res* **529**, 16–22.

Geng Z, Hong Y, Li S, Wang D. 2002 Effect of zinc deficiency on the behavior and calcium status of hippocampal neurons in rats. *Wei Sheng Yan Jin* **31**, 90–92.

Gibson RS. 1998 Zinc: a critical nutrient in growth and development. *N Z Med J* **111**, 63–64.

Golub MS, Keen CL, Gershwin ME, Hendrickx AG. 1995 Developmental zinc deficiency and behavior. *J Nutr* **125**, 2263–2271.

Halas ES, Eberhardt MJ, Diers MA, Sandstead HH. 1983 Learning and memory impairment in adult rats due to severe zinc deficiency during lactation. *Physiol Behav* **30**, 371–381.

Halas ES, Hunt CD, Eberhardt MJ. 1986 Learning and memory disabilities in young adult rats from mildly zinc deficient dams. *Physiol Behav* **37**, 451–458.

Hambidge M. 2000 Human zinc deficiency. *J Nutr* **130**, 1344S–1349S.

Minami A, Sakurada N, Fuke S, Kikuchi K, Nagano T, Oku N, Takeda A. 2006 Inhibition of presynaptic activity by zinc released from mossy fiber terminals during tetanic stimulation. *J Neurosci Res* **83**, 167–176.

Ojemann GA. 1987 Surgical therapy for medically intractable epilepsy. *J Neurosurg* **66**, 489–499.

Penland JG. 2000 Behavioral data and methodology issues in studies of zinc nutrition in humans. *J Nutr* **130**, 361S–364S.

Prasad AS. 1983 Clinical srectrum and diagnostic aspects of human zinc deficiency. In: Prasad AS, eds., *Essential and Toxic Trace Elements in Human Health and Disease*. New York: Liss, pp. 3–53.

Racine RJ. 1972 Modification of seizure activity by electrical stimulation. II. Motor seizure. Electroencephalogr. *Clin Neurophysiol* **32**, 281–294.

Sandstead HH. 1995 Is zinc deficiency a public health problem? *Nutrition* **11**, 87–92.

Sandstead HH, Frederickson CJ, Penland JG. 2000 History of zinc as related to brain function. *J Nutr* **130**, 496S–502S.

Sindreu CB, Varoqui H, Erickson JD, Perez-Clausell J. 2003 Boutons containing vesicular zinc define a subpopulation of synapses with low AMPAR content in rat hippocampus. *Cerebral Cortex* **13**, 823–829.

Sperk G, Lassmann H, Baran H, Kish SJ, Seitelberger F, Hornykiewiez O. 1985 Kainic acid-induced seizures: dose-relationship of behavioral, neurochemical and histopathological changes. *Brain Res* **338**, 289–295.

Sterman MB, Shouse MN, Fairchild MD, Belsito O. 1986 Kindled seizure induction alters and is altered by zinc absorption. *Brain Res* **383**, 382–386.

Sterman MB, Shouse MN, Fairchild MD. 1988 Zinc and seizure mechanisms. In: Morley JE, Sterman MB, Walsh JH, eds. *Nutritional Modulation of Neural Function*. San Diego: Academic Press, pp. 307–319.

Takeda A. 2001 Zinc homeostasis and functions of zinc in the brain. *BioMetals* **14**, 343–352.

Takeda A, Sawashita J, Okada S. 1995 Biological half-lives of zinc and manganese in rat brain. *Brain Res* **695**, 53–58.

Takeda A, Minami A, Takefuta S, Tochigi M, Oku N. 2001 Zinc homeostasis in the brain of adult rats fed zinc-deficient diet. *J Neurosci Res* **63**, 447–452.

Takeda A, Minami A, Seki Y, Oku N. 2003a Inhibitory function of zinc against excitation of hippocampal glutamatergic neurons. *Epilepsy Res* **57**, 169–174.

Takeda A, Hirate M, Tamano H, Oku N. 2003b Zinc movement in the brain under kainate-induced seizures. *Epilepsy Res* **54**, 123–129.

Takeda A, Hirate M, Tamano H, Oku N. 2003c Release of glutamate and GABA in the hippocampus under zinc deficiency. *J Neurosci Res* **72**, 537–542.

Takeda A, Hirate M, Tamano H, Nishibaba D, Oku N. 2003d Susceptibility to kainate-induced seizures under dietary zinc deficiency. *J Neurochem* **85**, 1575–1580.

Takeda A, Minami A, Seki Y, Oku N. 2004 Differential effects of zinc on glutamatergic and GABAergic neurotransmitter systems in the hippocampus. *J Neurosci Res* **75**, 225–229.

Takeda A, Yamada K, Minami A, Nagano T, Oku N. 2005 Enhanced excitability of hippocampal Mossy Fibers and CA3 neurons under dietary zinc deficiency. *Epilepsy Res* **63**, 77–84.