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# Elimination of zinc-65 from the brain under kainate-induced seizures

Atsushi Takeda*, Maki Hirate & 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)*

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**Abstract**

On the basis of the previous evidence that $^{65}Zn$ concentrations in the brain of EL (epilepsy) mice was affected by induction of seizures, $^{65}Zn$ movement in the brain was quantitatively evaluated in ddY mice treated with kainate. Six days after intravenous injection of $^{65}ZnCl_2$, mice were intraperitoneally injected with kainate (10 mg/kg $\times$6 times in 2 weeks). Myoclonic jerks were observed during treatment with kainate. Twenty days after $^{65}Zn$ injection, $^{65}Zn$ distribution in the brain was compared between the kainite-treated and control mice. $^{65}Zn$ distribution in the brain of the kainate-treated mice was overall lower than in the control mice. $^{65}Zn$ concentration was significantly decreased in the frontal cortex, hippocampal CA1, thalamus and hypothalamus by treatment with kainate. These results demonstrate that kainate-induced seizures are linked to decreased zinc concentrations in the brain.

## Introduction

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 alteration of zinc homeostasis in the brain may be associated with the etiology and manifestation of epileptic seizures (Sterman *et al.* 1988; Buhl *et al.* 1996; Takeda 2000, 2001). Zinc concentration in the hippocampal dentate area of seized EL (epilepsy) mice is significantly lower than that of control mice (Fukahori *et al.* 1988). The movement of $^{65}Zn$ in the brain of EL mice was studied by brain autoradiography. $^{65}Zn$ concentrations in the brain of seized EL mice were almost the same as those of untreated EL mice and ddY mice 6 days after injection of $^{65}ZnCl_2$, while $^{65}Zn$ concentrations in the brain of seized EL mice were decreased 20 days after the injection (Takeda *et al.* 1999). In the recent paper using the multitracer technique, on the other hand, 24 h after injection of the multitracer, $^{65}Zn$ concentrations in the brain of seized EL mice were lower than in untreated EL mice (Hirate *et al.* 2002). There is the possibility that zinc concentrations in the brain are responsive to seizures and that both the uptake of zinc by the brain and the elimination of zinc from the brain are affected by epileptic seizures.

The kainate-treated mice and rats are an experimental model of temporal lobe epilepsy (Ben-Ari 1985). They may be useful 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). The decrease may be associated with the release of zinc in the synaptic vesicles in the hippocampus. However, zinc movement in other brain regions was not studied in the kainate-treated rats. The present study deals with the elimination of $^{65}Zn$ from the brain under seizures induced with kainate, an agonist of glutamate receptors.

## Materials and methods

### *Experimental animals*

Male ddY mice (5 weeks old) were purchased from Japan SLC (Hamamatsu, Japan). They were housed under the standard laboratory conditions (23 $\pm$ 1 °C,

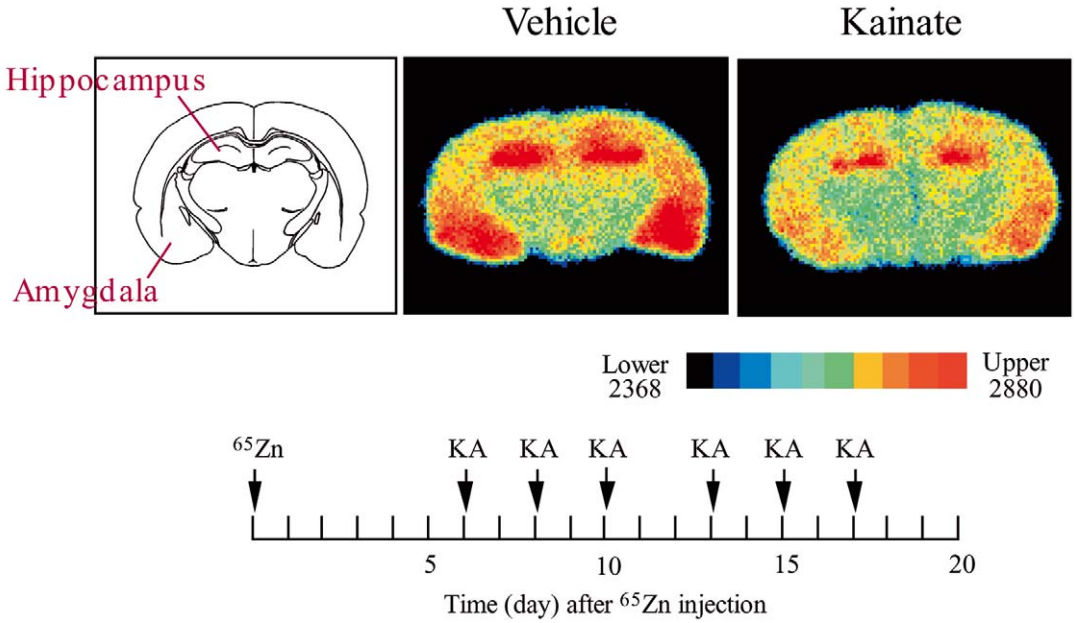


*Fig. 1.* $^{65}$Zn-imaging of the brain of kainate-treated mice. Radioimaging was performed on a selected coronal slice of vehicle-treated and kainate-treated mice 20 days after injection of $^{65}ZnCl_2$ ($n = 5$). The scheme (left-hand side) shows a map of the rat brain. The time sequence of injections of $^{65}ZnCl_2$ and kainate (KA) is shown under the autoradiograms.

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 Principles of Laboratory Animal Care of the National Institute of Health and the University of Shizuoka.

*Injection of $^{65}ZnCl_2$ and seizure induction*

$^{65}ZnCl_2$ (85.1 MBq (2.30 mCi)/mg, Du Pont/NEN Research Products, Wilmington, 185 kBq (5 $\mu$Ci)/2.17 $\mu$g Zn/0.2 ml/35 g body weight) diluted with 0.1 M acetate buffer (pH 4.0) was injected into the tail vein of mice (6 weeks old) ($n = 5$). Because the maximum levels of $^{65}$Zn in the brain were observed around 6 days after $^{65}$Zn injection (Takeda *et al.* 1995), the treatment with kainite was begun 6 days after $^{65}$Zn injection according to the time sequence (Figure 1). The mice were intraperitoneally injected with vehicle (0.9% NaCl) or kainate (10 mg/kg body weight) six times in two weeks. The behavior of mice was recorded with a video camera.

*Brain autoradiography*

Twenty days after injection of $^{65}ZnCl_2$, the brains were excised from the mice under ether anesthesia, frozen immediately, fixed quickly with ice-cold 4% sodium carboxymethyl cellulose, and then kept frozen at −20 °C. The brains thus treated were sliced in 300 $\mu$m thickness at −20 °C with a microtome (Cryostat HM505E, MICROM Laborgeräte GmbH, Heidelberg). The distribution of radioactivity in each area of the slices was determined by autoradiography (Bio-imaging Analyzer BAS 2000, Fuji Photo Film Co. Ltd., Tokyo) after exposure to the imaging plates for approximately 15 days. The exact time of exposure was determined by taking account of the physical decay. Radioactivity (photo-stimulated luminescence (PSL)/mm$^2$) in each area from the autoradiograms was measured quantitatively with a Bio-imaging Analyzer, and corrected according to PSL/mm$^2$ of internal standards in each autoradiogram.

## Results and discussion

To evaluate zinc movement in the brain after seizure induction, the treatment with kainate was performed according to the time sequence shown in Figure 1. The mice exhibited staring posture, ear and facial twitching, and myoclonic jerks after injection of kainate. $^{65}$Zn concentrations in the brain of the kainate-treated and control mice were compared 20 days after injec-

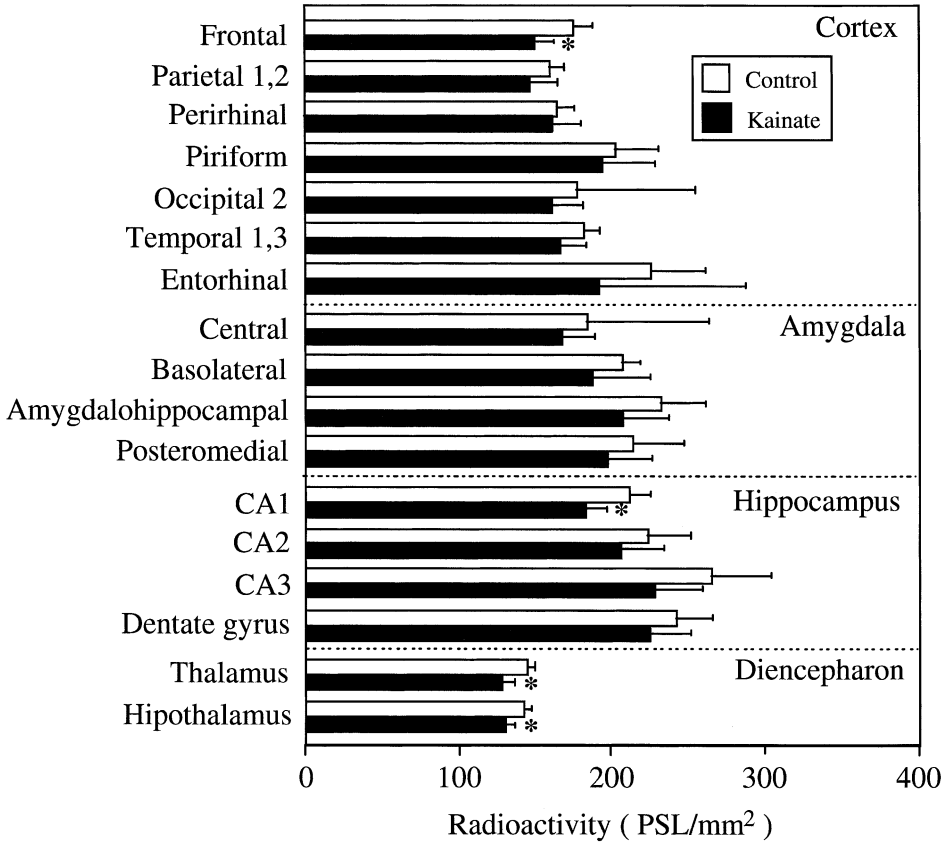


*Fig. 2.* $^{65}$Zn distribution in the brain of kainate-treated mice The radioactivity of each area in the autoradiograms from five vehicle-treated and five kainate-treated mice obtained in Figure 1 was measured using a Bio-imaging Analyzer. Each bar and line represent the mean ± SD ($n = 5$). Asterisks indicate significant difference (*, $P < 0.05$; $t$-test) from the control (vehicle).

tion of $^{65}ZnCl_2$ (Figure 1). $^{65}$Zn concentrations in the brain of the kainate-treated mice were overall lower than those of control mice. The decrease in $^{65}$Zn concentrations in the brain were also observed in seized EL mice 20 days after injection of $^{65}ZnCl_2$ (Takeda *et al.* 1999), although the data was not analyzed quantitatively. In the present study, $^{65}$Zn concentration in each area in the brain was quantitatively analyzed by using a Bio-imaging Analyzer. $^{65}$Zn concentrations in the frontal cortex, hippocampal CA1, thalamus and hypothalamus were significantly decreased by treatment with kainate (Figure 2). These results suggest that the elimination of $^{65}$Zn from the mouse brain is enhanced by kainate-induced seizures.

Approximately 10% of the total brain zinc exists in the presynaptic vesicles and is histochemically reactive (as revealed by Timm's sulfide-silver staining method) (Frederickson 1989). The presence of zinc-containing glutamatergic neurons that sequester zinc in the presynaptic vesicles and release it in a calcium- and impulse-dependent manner has been demonstrated in the brain, especially in the telencepharon (Assaf & Chung, 1984; Howell *et al.* 1984). Zinc-containing glutamatergic neuron terminals exist in the frontal cortex and hippocampus (Frederickson 1989). There is the possibility that zinc and glutamate are excessively released from the zinc-containing glutamatergic neuron terminals by treatment with kainate, followed by the loss of extracellular zinc to the leaky blood vessels. The decrease in hippocampal zinc is reported in kainate-treated rats (Assaf & Chung 1984). Moreover, the loss of zinc stain with N-[6-methoxy-8-quinolyl]-P-toluenesulfonamide (TSQ), by which histochemically reactive zinc in the synaptic vesicles is detected, is observed in the hippocampal mossy fibers after administration of kainate (Frederickson *et al.* 1988). The loss of Timm's stain is also observed in the hippocampal mossy fibers after electrical stimulation of the perforant path, which evoked hippocampal granule spikes and epileptiform discharges (Sloviter 1985).

Therefore, it is likely that zinc concentrations in the synaptic vesicles in zinc-containing glutamatergic neuron-rich regions, e.g., the hippocampus and frontal cortex, are decreased by kainate-induced seizures. The enhanced elimination of $^{65}Zn$ from the brain may reflect the decrease of the zinc concentrations. Recently, it is found that zinc concentrations in the amygdala and cerebral cortex, as well as the hippocampus, are significantly decreased by treatment with kainate, while that in the cerebellum is not decreased (Takeda *et al.* 2003). The decrease in vesicular zinc might be involved in an imbalance of inhibition-excitation in synapse, which is observed in temporal lobe epilepsy (Ben-Ari 1985), because Zinc transporter-3-null mice, which lack histochemically reactive zinc in synaptic vesicles, are more sensitive than the control mice to kainate-induced seizures (Cole *et al.* 2000).

Approximately 90% of the total brain zinc is in zinc metalloproteins. There is the possibility that zinc homeostasis in zinc metalloproteins is also affected by kainate-induced seizures. Zinc is important for the function of many enzymes and other proteins, including some unique to the brain and important to neurotransmission (Ebadi *et al.* 1984; Prohaska 1987; Golub *et al.* 1995; Sandstead *et al.* 2000). Seizure susceptibility of EL mice is decreased by dietary zinc loading, while it is increased by dietary zinc deficiency (Fukahori & Itoh 1990). Susceptibility to kindled seizures is also decreased by dietary zinc loading, while this susceptibility in cats is increased by zinc deprivation (Sterman *et al.* 1986). Thus, zinc homeostasis in the brain appears to be involved in the prevention of seizure development. In conclusion, the present $^{65}Zn$ imaging of brain demonstrates that kainate-induced seizures are linked to decreased zinc concentrations in the brain.

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