Influence of Histidine on Zinc Transport into Rat Brain

Atsushi Takeda,* Mai Suzuki, Shoji Okada, and Naoto Oku

Department of Radiobiochemistry, School of Pharmaceutical Sciences, University of Shizuoka, 52–1 Yada, Shizuoka 422–8526, Japan

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The brain of rats injected intravenously with ^{65}Zn-His or $^{65}ZnCl_2$ was subjected to autoradiography to study the role of histidine on zinc transport into the brain. One hour after injection, the radioactivity from ^{65}Zn-His was largely concentrated in the choroid plexus in the ventricles. Six days after injection, the radioactivity from ^{65}Zn-His was relatively concentrated in the hippocampal CA3 and dentate gyrus and the amygdala. The relative distribution of ^{65}Zn-His in the brain was similar to that of $^{65}ZnCl_2$ group at both 1 h and 6 days, suggesting that histidine may participate in zinc uptake in the brain. On the other hand, the clearance of the ^{65}Zn-His group from the blood was higher than that of the $^{65}ZnCl_2$ group. Brain uptake of the former was lower than that of the latter both 1 h and 6 days after injection. These results suggest that zinc uptake in the brain is influenced by histidine levels in the bloodstream.

Key words —— zinc, histidine, brain, blood-brain barrier, blood-cerebrospinal fluid barrier

INTRODUCTION

The roles of essential trace metals in brain function are not well understood. Of essential trace metals, zinc is the second most abundant trace element in the adult brain (approximately 10—20 μg/g wet weight). Zinc has a special function as a neuromodulator, in addition to its known role in a number of zinc metalloenzymes, in the brain.[1,2] Zinc concentration in the brain increases with growth after birth and is maintained constantly in the adult brain.[3] On the other hand, the brain functions are affected by dietary zinc deprivation.[4–6] Therefore, a proper zinc supply to the brain may be important for brain functions as well as for brain development.[7]

Some molecules involved in zinc uptake have been cloned from yeast.[8,9] In mammalian cells, some zinc transporters have also been cloned: for example, ZnT-1 mediates zinc efflux,[10] ZnT-2 facilitates zinc accumulation in the endosomal vesicles,[11] and ZnT-3 sequesters zinc in the synaptic vesicles.[12,13] However, the molecule involved in zinc uptake across plasma membranes has not been cloned. *In vitro* experiments using rat liver cells suggest that zinc ion passes across the plasma membranes.[14] Gunshin *et al.*[15] reported DMT1, a divalent metal transporter, which serves for a number of trace elements including zinc. On the other hand, other data suggests that histidine-bound forms actually pass across plasma membranes, as observed *in vitro* experiments using rat erythrocytes[16,17] and rabbit kidney proximal cells.[18] Thus, the mechanism of zinc uptake across the plasma membranes is still controversial.

Brain autoradiography with $^{65}ZnCl_2$ demonstrates that zinc may be taken up in the brain *via* the blood-cerebrospinal fluid (CSF) barrier, in addition to the blood-brain barrier.[19,20] In the present paper, to evaluate the role of histidine on zinc transport into the brain across the blood-brain and the blood-CSF barriers, brain autoradiography was performed after intravenous (i.v.) injection of ^{65}Zn-His or $^{65}ZnCl_2$.

MATERIALS AND METHODS

Animals —— Male ddY mice (6-week-old) were purchased from Japan SLC Inc., Hamamatsu, Japan and housed under the standard laboratory conditions with

*To whom correspondence should be addressed: Department of Radiobiochemistry, School of Pharmaceutical Sciences, University of Shizuoka, 52–1 Yada, Shizuoka 422–8526, Japan. Tel.: +81-54-264-5700; Fax: +81-54-264-5705; E-mail: takedaa@u-shizuoka-ken.ac.jp

food (MF, Oriental Yeast, Hujinomiya, Japan) and water ad libitum. The lights were automatically turned on at 8 : 00 and off at 20 : 00.

Preparation of ^{65}Zn-His —— $^{65}ZnCl_2$ [0.5 ml (230μCi), 85.1 MBq (2.30 mCi)/mg, Du Pont/NEN Research Products, Boston, MA] was incubated with 0.5 ml of 200 mM L-histidine (Wako Pure Chemical Industries Ltd., Osaka, Japan) for 1 h at 37℃. The mixture was applied to a Sephadex G-10 gel column [Pharmacia Biotech, Uppsala, Sweden; eluent, 50 mM Tris-HCl buffer (pH 7.4)]. The eluates obtained were counted for radioactivity in a γ-counter (Packard 5530, Packard Instrument Co., Meriden CT) and assayed for the amino acid with 0.2% ninhydrin in ethanol. The concentration of histidine in the ^{65}Zn-His solution obtained was approximately 10 mM (labeling efficiency, approximately 100%).

Brain Autoradiography and γRay Counting —— $^{65}ZnCl_2$ or ^{65}Zn-His [185kBq (5 μCi)/0.2 ml/30 g body weight] was injected into the tail vein of mice (28—32 g, n=4). One hour and 6 days after injection, blood was collected from the mice under ether anesthesia and the brain was excised. The blood was weighed and counted for radioactivity in a γ-counter. The brain was frozen immediately, fixed quickly with ice-cold 4% sodium carboxymethyl cellulose, and then kept frozen at −20℃. The brain thus treated was sliced at 300 μm thickness at −20℃ with a microtome (Cryostat HM505E, MICROM Laborgerte GmbH, Heidelberg, Germany). The distribution of radioactivity in each area in the slices was determined by autoradiography (Bio-imaging Analyzer BAS 2000, Fuji Photo Film Co. Ltd., Tokyo, Japan) after exposure to the imaging plates for approximately 8 d. 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.

To convert the radioactivity (% dose/g wet weight) in the blood counted with a γ-counter into PSL/mm^2, aliquots of the blood were also frozen immediately and subjected to autoradiography as described above.

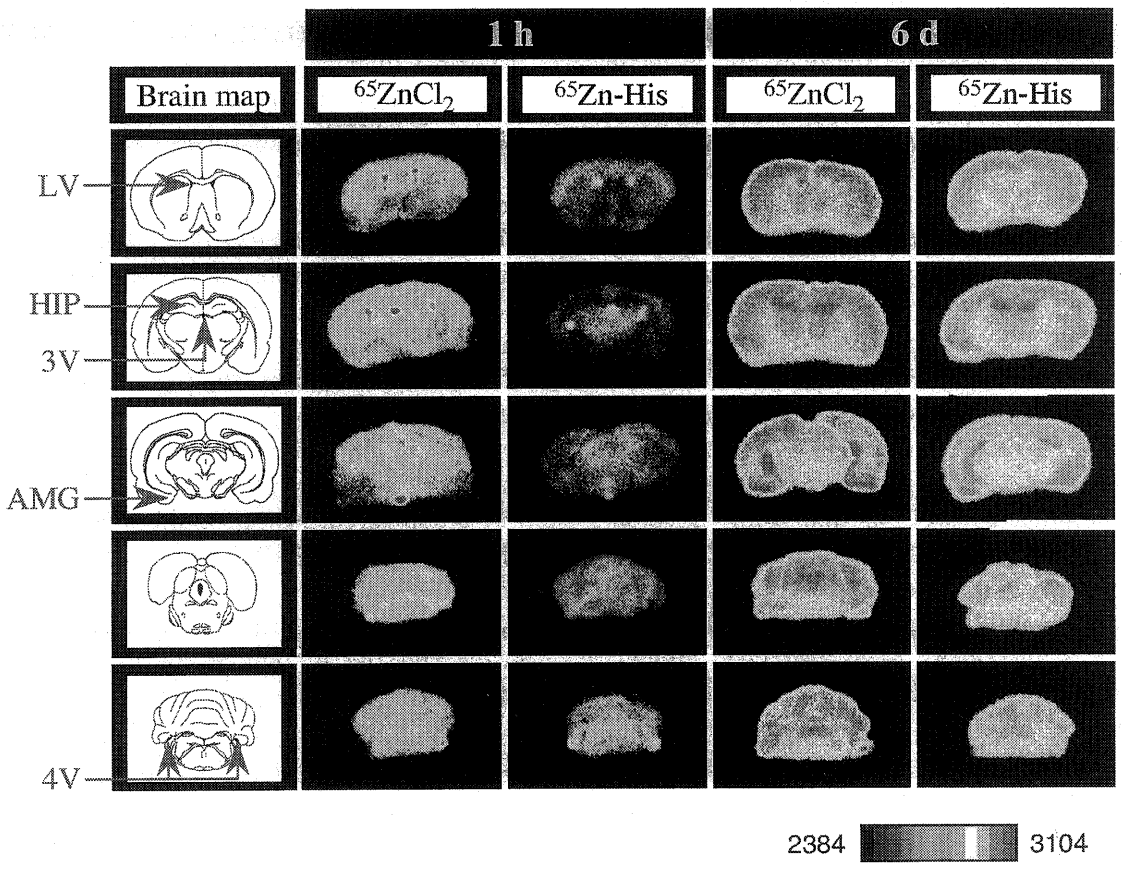

Fig. 1. ^{65}Zn-Imaging of Mouse Brain after i.v. Injection

The radioimaging 1 h and 6 d after i.v. injection of ^{65}Zn-His or $^{65}ZnCl_2$ was performed on selected coronal slices of mouse brain (n=4). The schemes (left-hand side) show maps of the rat brain. LV, lateral ventricule; HIP, hippocampus; 3V, third ventricle, AMG, amygdala; 4V, fourth ventricle.

RESULTS

The brain of rats injected i.v. with ^{65}Zn-His or $^{65}ZnCl_2$ was subjected to autoradiography. One hour after injection, the radioactivity from ^{65}Zn-His is largely concentrated in the lateral, the third and the fourth ventricles, including the choroid plexus, with only small amounts in the cerebral aqueduct (Fig. 1). The relative distribution of the ^{65}Zn-His group in the brain was similar to that of $^{65}ZnCl_2$. However, the radioactivity from ^{65}Zn-His in the choroid plexus was a little lower than that from $^{65}ZnCl_2$ and the radioactivity from ^{65}Zn-His in the major part of the brain parenchyma was significantly lower than that from $^{65}ZnCl_2$ (Fig. 2).

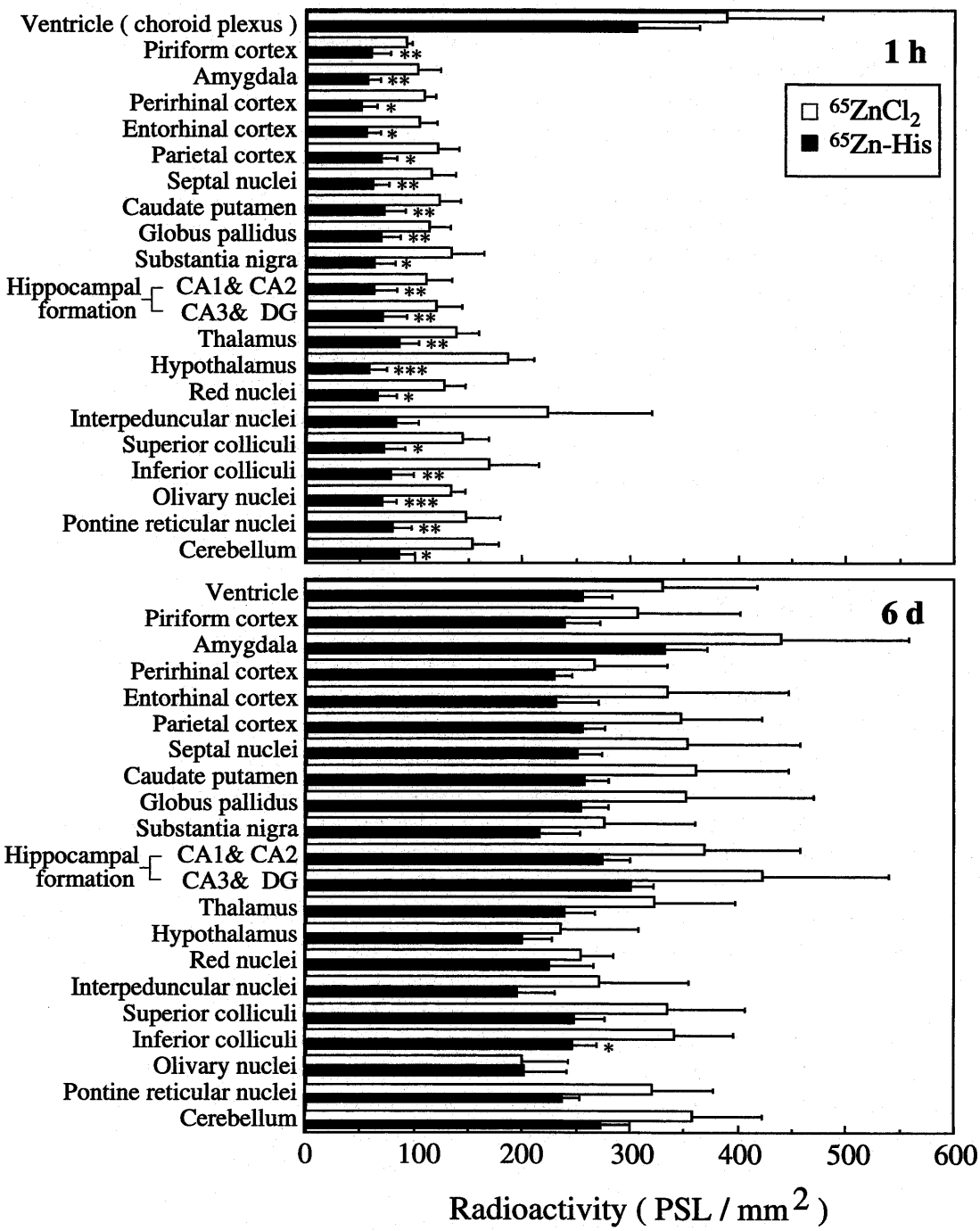

Fig. 2. ^{65}Zn Distribution in the Brain after i.v. Injection

Each value (mean±S.D.), which was measured with a Bio-imaging Analyzer, represents the radioactivity (PSL/mm²) in brain autoradiograms obtained in Fig. 1. Asterisks indicate significant difference (*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$) from $^{65}ZnCl_2$ group.

Six days after injection, the radioactivity from ^{65}Zn-His was relatively concentrated in the hippocampal CA3 and dentate gyrus and the amygdala, similar to the radioactivity from $^{65}ZnCl_2$ (Fig. 1). However, the radioactivity of the former in the brain was also lower than that of the latter (Fig. 2).

The radioactivity of the ^{65}Zn-His group (0.49 ± 0.06% dose/g) in the blood was significantly lower than that of the $^{65}ZnCl_2$ group (1.36 ± 0.16% dose/g) 1 h after injection. The radioactivity of the former (0.21 ± 0.003% dose/g) in the blood was also lower than that of the latter (0.47 ± 0.07% dose/g) 6 d after injection.

To examine the stability of ^{65}Zn-His in the serum, 10 μl of ^{65}Zn-His used for the injection was incubated with 90μl of mouse serum for 30 min at 37℃. The mixture was subjected to Sephadex G-10 gel filtration. Approximately 90% and 10% of total radioactivity were detected in the void volume fraction, at which serum proteins were eluted, and the histidine-eluted fraction, respectively (data not shown). On the other hand, in the case of incubation of $^{65}ZnCl_2$ in mouse serum, most of the radioactivity was detected in the void volume fraction.

DISCUSSION

Serum zinc (approximately 15 μM) is partitioned among three fractions; protein-bound form, low molecular weight ligand-bound form and free Zn^{2+}. The zinc bound to serum proteins and low molecular weight ligands is approximately 98% and 1—2%, respectively, of total serum zinc.[21,22] On the other hand, the concentration of free Zn^{2+} in mammalian serum has been estimated to be as low as 10^{-9}—10^{-10} M[23]; free Zn^{2+} is a very small fraction of the exchangeable zinc in the serum.

The largest component of exchangeable zinc in the serum is albumin. The bovine albumin molecule is estimated to have two zinc-binding sites of equal high affinity.[24] Brain autoradiograms of the Nagase analbuminemic rat, which has a genetic mutation affecting albumin mRNA processing and lacks serum albumin, after i.v. injection of $^{65}ZnCl_2$, demonstrate that albumin is not essential for zinc transport into the brain,[25] although serum albumin may participate in zinc uptake as a large pool of exchangeable zinc in normal animals.

The second largest component of exchangeable zinc in the serum is amino acids, *i.e.*, histidine and cysteine.[26,27] There are some reports that L-histidine enhances zinc uptake *in vivo*.[28,29] In the case of the brain, Aiken *et al.*[28] reported that ^{65}Zn uptake in the brain expressed relative to plasma ^{65}Zn level is enhanced by L-histidine infusion. Buxani-Rice *et al.*[29] reported that ^{65}Zn transport into the brain during short cerebrovascular perfusion is enhanced by addition of 100 μM L-histidine.

To evaluate the role of L-histidine on zinc transport into the brain across the blood-brain and the blood-CSF barriers, the brain of rats injected i.v. with prepared ^{65}Zn-His was subjected to autoradiography. One hour after injection, the radioactivity from ^{65}Zn-His was largely concentrated in the choroid plexus in the ventricles, similarly to that from $^{65}ZnCl_2$. Six days after injection, the radioactivity from ^{65}Zn-His was relatively concentrated in the hippocampal CA3 and dentate gyrus and the amygdala. The relative distribution of the ^{65}Zn-His group in the brain was similar to that of the $^{65}ZnCl_2$ group both at 1 h and 6 d, suggesting that histidine may participate in zinc uptake in the brain.

On the other hand, brain uptake of the ^{65}Zn-His group was lower than that of the $^{65}ZnCl_2$ group both 1 h and 6 d after injection. Because the clearance of the ^{65}Zn-His group from the blood was higher than that of the $^{65}ZnCl_2$ group, uptake ratios expressed relative to blood radioactivity of ^{65}Zn-His or $^{65}ZnCl_2$ were calculated to correct the brain uptake for changes in blood radioactivity. Brain uptake ratios of the ^{65}Zn-His group were higher than those of the $^{65}ZnCl_2$ group both 1 h and 6 d after injection (data not shown), in agreement with data from the histidine infusion experiment of Aiken *et al.*[28] These results suggest zinc transport into the brain across the blood-brain and the blood-CSF barriers is enhanced by histidine.

At serum pH, zinc may be present as $Zn(His)_2$ and $Zn(His)^+$, and other complexes such as $Zn(Cys)(His)^-$ may also be present.[30] Histidine concentration in the serum of mice is around 50 μM. In the present study, histidine concentration in the serum is increased immediately after injection of ^{65}Zn-His. However, it is considered that the ^{65}Zn-His injected is not stable in the serum. Thus, 30 min after incubation of ^{65}Zn-His in

mouse serum, approximately 90% of total radioactivity was detected in the protein-eluted fraction by Sephadex G-10 gel filtration. These results suggest that ^{65}Zn is readily released from histidine in the bloodstream in the case of injection of ^{65}Zn-His. Judging from higher clearance of the radioactivity of the ^{65}Zn-His group from the blood, there is the possibility that histidine-bound forms or zinc ion released from histidine may be involved in zinc uptake in the brain.

The possibility of carriage of a zinc-histidine complex on either the L- or Y^+-transporters, which are involved in L-histidine uptake, was studied by looking for inhibition of zinc transport in the presence of 100 μM histidine by either 500 μM L-phenylalanine (L-transporter) or 500 μM L-arginine (Y^+-transporter).[29] Zinc transport into the brain was inhibited by neither amino acid. Therefore, it is likely that zinc ion may be involved in zinc transport into the brain across the blood-brain and the blood-CSF barriers. Histidine might serve to transfer zinc to the plasma membrane proteins such as DMT1 and/or unidentified zinc transporters on the brain capillary endothelial cells and choroidal epithelial cells.

In conclusion, histidine may participate in zinc uptake in the brain. Zinc uptake in the brain is influenced by histidine level in the bloodstream.

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