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Possible involvement of plasma histidine in differential brain permeability to zinc and cadmium

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Abstract

Zinc gets into the brain parenchyma across the blood-brain and the blood-cerebrospinal fluid barriers, while cadmium hardly gets into the brain parenchyma. Because histidine may be involved in zinc transport across the brain barrier systems, the binding to histidine was compared between zinc and cadmium to understand the difference in brain permeability to both metals. Sephadex G-10 gel filtration indicated that ^{109}Cd, unlike ^{65}Zn, does not bind to histidine. When the plasma incubated with ^{65}Zn or ^{109}Cd was dialyzed in physiological saline containing histidine (0-10 mM), ^{65}Zn concentration in the dialysate was increased with the increase of the histidine concentration, suggesting the transfer of zinc from plasma proteins to histidine. The low affinity of zinc to plasma proteins may be important for brain permeability to this metal. On the other hand, ^{109}Cd was not detected in the dialysate in the presence of 0.1 mM histidine, which is equal to the concentration in the plasma, suggesting no transfer of cadmium from plasma proteins to histidine. These results suggest that the avid binding of cadmium to plasma proteins is related to brain impermeability to this metal.

Introduction

Zinc, an essential trace metal, is transported into the brain parenchyma across the blood-cerebrospinal fluid (CSF) barrier, in addition to the blood-brain barrier (Takeda *et al.* 1994a,b, Takeda *et al.* 2000b) as a required component for neural functions (Takeda 2000, Takeda *et al.* 2001). However, the mechanism on zinc transport into the brain is poorly understood.

Cadmium, a toxic metal (Minami *et al.* 2001), is hardly taken up by the brain parenchyma because of the protection by the brain barrier systems (Arvidson & Tjälve 1986; Takeda *et al.* 1999). This metal can compete with zinc for binding to zinc-binding proteins (Palmiter 1994). Gaither & Eide (2000) reported the human zinc transporter, hZIP2, which is involved in cellular zinc uptake. Zinc uptake via hZIP2 is inhibited by cadmium in vitro, although the expression of hZIP2 is not reported yet in the brain.

The affinity of zinc to proteins and amino acids in the plasma may be different from that of cadmium (Frazier 1980). The transfer of plasma proteins from plasma to brain extracellular fluid is strictly limited by the brain barrier systems. Thus, there is the possibility that the affinity of zinc and cadmium to plasma ligands is critical in brain permeability to both metals. The present paper describes that the transfer from plasma proteins to histidine is different between zinc and cadmium.

Materials and methods

Animals

Male rats (Wistar strain, Japan SLC Inc) were housed under standard laboratory conditions (23 ± 1 °C, 55 ± 5% humidity). Rats had access to tap water and were fed a conventional mouse chow diet (Oriental Yeast

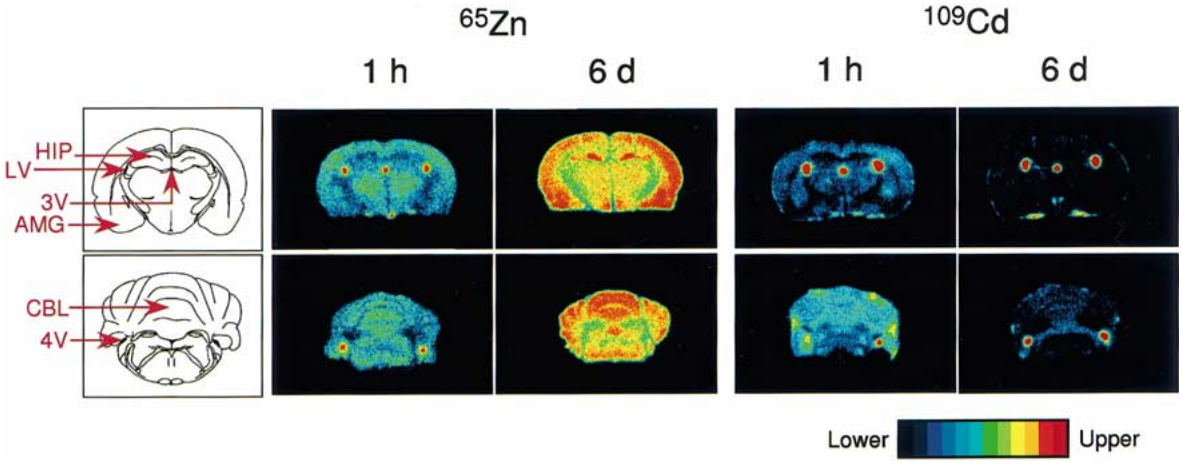

Fig. 1. Brain imaging with ^{65}Zn or ^{109}Cd. The radioimaging 1 h and 6 days after intraveous injection of $^{65}ZnCl_2$ or $^{109}CdCl_2$ was performed on selected coronal slices of rat brain ($n = 4$). Each experiment was performed four times and the autoradiograms obtained were almost identical. The schemes (left-hand side) show maps of the rat brain. LV, lateral ventricle; 3V, 3rd ventricle; HIP, hippocampus; AMG, amygdala; CBL, cerebellar lobules; 4V, 4th ventricle.

Co., Ltd) *ad libitum*. The lights were automatically turned on at 8:00 and off at 20:00. All experiments were carried out in accordance with the Principles of laboratory animal care of the NIH and the University of Shizuoka.

Brain autoradiography

Male rats (110–140 g, $n = 4$) were intravenously injected with $^{65}ZnCl_2$ [85.1 MBq (2.30 mCi)/mg, Du Pont/NEN Research Products, 740 kBq (20 μCi)/0.2 ml/rat] or $^{109}CdCl_2$ [36.25 GBq (975 mCi)/mg, Amersham Pharmacia Biotech, 950 kBq (25.7 μCi)/0.2 ml/rat]. One hour and 6 days after injection, the brains were excised from the rats under ether anesthesia. The brains were frozen immediately, fixed quickly with ice-cold 4% sodium carboxymethyl cellulose, and sliced at 300 μm thickness at -20 °C with a microtome (Cryostat HM505E, Microm Laborgerate GmbH). The distribution of radioactivity in each area of the slices was measured by autoradiography (Bio-imaging Analyzer BAS2000, Fuji Photo Film Co. Ltd.) after exposure to the imaging plate for approximately 7 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.

Sephadex G-10 gel filtration

Ten microlitter of $^{65}ZnCl_2$ or $^{109}CdCl_2$ [3.7 kBq (0.1 μCi)/μl] was added to 10 μl of 200 mM L-histidine, immediately diluted with 180 μl of water, and incubated at 37 °C for 30 min. Aliquot (100 μl) of the samples was analyzed by Sephadex G-10 [Amersham Pharmacia Biotech; eluent, 50 mM Tris-HCl buffer (pH 7.4)]. The radioactivity of each fraction was measured by γ-counter. The ninhydrin reaction was also performed for the detection of L-histidine in each faction. The experiment was done in duplicate.

Dialysis

Ten μl of $^{65}ZnCl_2$ or $^{109}CdCl_2$ [3.7 kBq (0.1μCi)/μl] was incubated with 90 μl of plasma obtained from rats at 37 °C for 30 min. As a control, to prepare ^{65}Zn-histidine, ten microlitter of $^{65}ZnCl_2$ [3.7 kBq (0.1 μCi)/μl] was incubated with 90 μl of 10 mM L-histidine at 37 °C for 30 min. Each sample was transferred in a cellulose tube (Visking Co.) and dialyzed in physiological saline containing L-histidine (0–10 mM) at 37 °C for 1 h. The radioactivity in aliquot of the dialysate were measured by γ-counter.

Results and discussion

To compare brain permeability to zinc and cadmium, brain autoradiography was performed after injection of $^{65}ZnCl_2$ or $^{109}CdCl_2$ into the tail vein of rats. Both

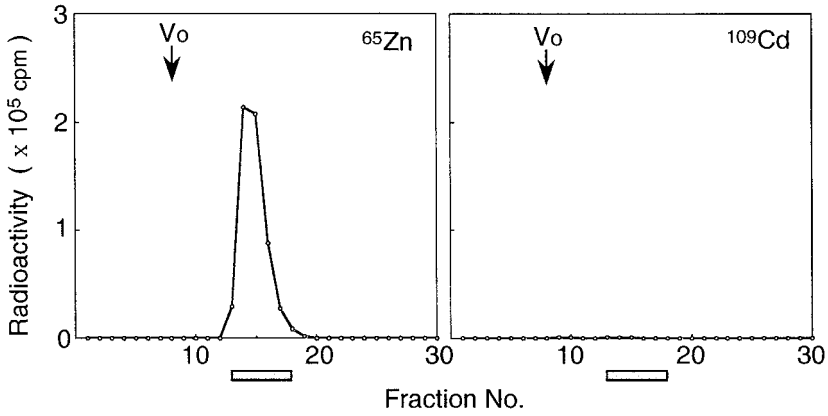

Fig. 2. In vitro binding of ^{65}Zn and ^{109}Cd to histidine. $^{65}ZnCl_2$ or $^{109}CdCl_2$ was incubated with histidine. Aliquot of the samples was analyzed using Sephadex G-10. The experiment was done in duplicate. Arrows (Vo) and shaded bars indicate the void volume and ninhydrin-positive fractions, respectively.

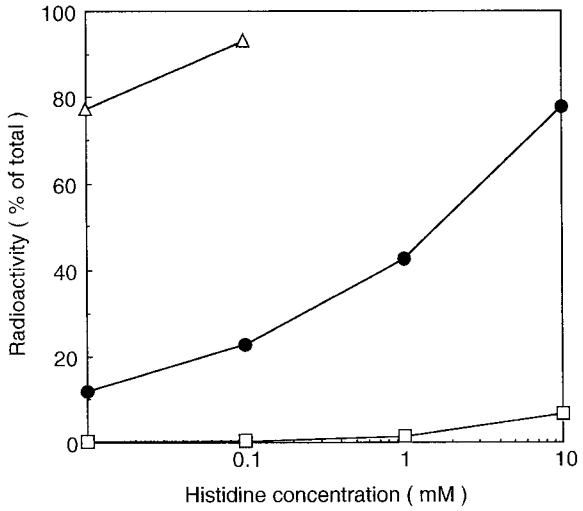

Fig. 3. Influence of histidine in binding of ^{65}Zn and ^{109}Cd to plasma proteins. Rat plasma was incubated with $^{65}ZnCl_2$ or $^{109}CdCl_2$ and then dialyzed in physiological saline containing 0, 0.1, 1 and 10 mM histidine. ^{65}Zn-histidine was prepared as described previously (Takeda *et al.* 2000a,b). The radioactivity in aliquot of the dialysate were measured by γ-counter. Triangle, ^{65}Zn-histidine (control); closed circle, ^{65}Zn; square, ^{109}Cd.

^{65}Zn and ^{109}Cd were largely concentrated in the lateral, the third and the fourth ventricles, including the choroid plexus 1 h after injection (Figure 1). Six days after injection, ^{65}Zn was concentrated in the brain parenchyma, e.g., the hippocampal CA3 region, dentate gyrus and amygdala, whereas ^{109}Cd was retained in the choroid plexus and hardly taken up by the brain parenchyma in agreement with the previous data (Takeda *et al.* 1994a, 1999).

The binding of zinc to plasma ligands is important to understand the mechanism of zinc transport into the brain parenchyma across the brain barrier systems (Takeda 2001). Plasma zinc (approximately 15 mM) is partitioned between high molecular weight and low molecular weight fractions (Prasad & Oberleas 1970; Henkin 1979). The former is a protein-bound form (98%) and the latter is a low molecular weight ligand-bound form (1–2%) and ionic zinc, which is estimated to be as low as 10^{-9}–10^{-10} M (Magneson *et al.* 1987).

The largest component of exchangeable zinc in the plasma is albumin. When $^{65}ZnCl_2$ was incubated with rat plasma, a large portion of ^{65}Zn was detected at the retention time of mercaptalbumin on HPLC (data not shown). However, brain autoradiography using the Nagase analbuminemic rat, which has a genetic mutation affecting albumin mRNA processing and lacks plasma albumin, demonstrated that albumin is not essential for zinc transport into the brain (Takeda *et al.* 1997). It is likely that mercaptalbumin may participate in zinc transport as a large pool of exchangeable zinc in the plasma of normal animals. On the other hand, zinc is reported to bind to a_2-macroglobulin firmly (Giroux & Henkin 1972). Its functional significance is unknown.

The next largest component of exchangeable zinc in the plasma is amino acids, i.e., histidine and cysteine (Hallman *et al.* 1971; Harris & Keen 1989). Aiken *et al.* (1992) report that ^{65}Zn uptake in the brain, as well as in other tissues, expressed relative to plasma ^{65}Zn level is enhanced by histidine infusion. Buxani-Rice *et al.* (1994) report that ^{65}Zn transport into the brain during a short cerebrovascular perfu-

sion is enhanced by addition of 100 mM histidine. Moreover, ^{65}Zn was also concentrated in the brain parenchyma, e.g., the hippocampal CA3 region, dentate gyrus and amygdala, after intravenous injection of ^{65}Zn-histidine (Takeda *et al.* 2000a). A supplementation with histidine during dietary zinc repletion improves short-term memory in zinc-restricted young adult male mice (Keller *et al.* 2000). It is likely that histidine is involved in zinc transport into the brain parenchyma across the brain barrier systems.

Thus, the binding of cadmium to histidine was compared with that of zinc. When $^{65}ZnCl_2$ or $^{109}CdCl_2$ were incubated with 10 mM histidine (Figure 2), Sepadex G-10 elution profile indicated that ^{109}Cd, unlike ^{65}Zn, is not detected in any fraction. ^{109}Cd was retained in the Sephadex G-10 gel column. These results suggest that cadmium cannot bind to histidine and that cadmium ion non-specifically binds to the Sephadex gel containing carboxyl group.

To examine the influence of histidine in the binding of zinc and cadmium to plasma proteins, rat plasma was incubated with $^{65}ZnCl_2$ or $^{109}CdCl_2$ and then dialyzed in physiological saline containing histidine (Figure 3). A large potion of ^{65}Zn-histidine, as a control, was dialyzed in physiological saline. When plasma incubated with ^{65}Zn were dialyzed in physiological saline, 10% of total radioactivity was detected in the dialysate. The radioactivity in the dialyzate was increased with the increase of the histidine concentration. These results indicate low affinity of zinc to plasma proteins. The transfer of zinc from plasma proteins, especially mercaptalbumin, to histidine may be important for brain permeability to zinc. A rat brain peptide/histidine transporter (PHT1) is cloned by Yamashita *et al.* (1997). PHT1 mRNA is intensely expressed in the choroid plexus. There is the possibility that histidine-bound forms pass across the plasma membranes of the choroidal epithelial cells (and brain capillary endothelial cells). Alternatively, histidine might serve to transfer zinc to unidentified transporters on the brain barrier systems.

In the case of plasma incubated with ^{109}Cd, the radioactivity was not detected in the dialysate containing 0.1 mM histidine, which is equal to the concentration in the plasma (Figure 3). In the plasma, major cadmium-binding proteins are estimated to be albumin and/or α_2-macrogloblin (Watkins *et al.* 1977; Carson 1984). Suzuki *et al.* (1986) demonstrated that mercaptalbumin may be a selective cadmium-binding protein in rat serum. The present dialysis experiment suggests that the binding of cadmium to plasma proteins is more avid than that of zinc. Judging from brain impermeability to plasma proteins such as albumin, the avid binding of cadmium to plasma proteins may be related to brain impermeability to this metal.

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