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Review

Zinc homeostasis and functions of zinc in the brain

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Abstract

The brain barrier system, i.e., the blood-brain and blood-cerebrospinal fluid barriers, is important for zinc homeostasis in the brain. Zinc is supplied to the brain via both barriers. A large portion of zinc serves as zinc metalloproteins in neurons and glial cells. Approximately 10% of the total zinc in the brain, probably ionic zinc, exists in the synaptic vesicles, and may serve as an endogenous neuromodulator in synaptic neurotransmission. The turnover of zinc in the brain is much slower than in peripheral tissues such as the liver. However, dietary zinc deprivation affects zinc homeostasis in the brain. Vesicular zinc-enriched regions, e.g., the hippocampus, are responsive to dietary zinc deprivation, which causes brain dysfunctions such as learning impairment and olfactory dysfunction. Olfactory recognition is reversibly disturbed by the chelation of zinc released from amygdalar neuron terminals. On the other hand, the susceptibility to epileptic seizures, which may decrease vesicular zinc, is also enhanced by zinc deficiency. Therefore, zinc homeostasis in the brain is closely related to neuronal activity. Even in adult animals and probably adult humans, adequate zinc supply is important for brain functions and prevention of neurological diseases.

Introduction

Zinc, an essential nutrient, is the second most abundant trace element in the body and powerfully influences cell division and differentiation (Vallee & Falchuk 1981; Coleman 1992). In microorganisms, plants and animals, over 300 enzymes require zinc for their functions. Zinc has three functions in zinc enzymes: catalytic, coactive (or cocatalytic) and structural (Vallee & Auld 1992; Vallee & Falchuk 1993).

In the brain, zinc is necessary for the maturation and function. Approximately 90% of the total brain zinc is bound in zincproteins (Frederickson 1989). The rest is in the presynaptic vesicles and histochemically reactive (as revealed by Timm's sulfide-silver staining method) (Haug 1973; Frederickson 1989; Howell & Frederickson 1989). Vesicular zinc, probably ionic zinc, may play a role in synaptic neurotransmission in the mammalian brain and serve as an endogenous neuromodulator of several important receptors including the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptor, N-methyl-D-aspartate (NMDA) and γ-aminobutyric acid (GABA) receptors (Harrison *et al.* 1993; Smart *et al.* 1994; Huang 1997).

The presence of zinc-containing 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 the telencephalon (Assaf & Chung 1984; Howell *et al.* 1984; Perez-Clausell & Danscher 1985; Frederickson & Danscher 1990). The hippocampal and amygdalar regions may possess zinc-containing neuron terminals in high densities (Christensen & Frederickson 1998; Frederickson *et al.* 2000). All zinc-containing neurons reported so far are considered to be glutamatergic (Crawford & Connor 1973). However, not all glutamatergic neurons are zinc-containing (Frederickson & Moncrieff 1994). Neural circuits of the zinc-containing glutamatergic neurons are considered

to be associated with the episodic memory function and are important for behavior, emotional expression and cognitive-mnemonic operations (Frederickson & Danscher 1990; Takeda *et al.* 1995). Thus, zinc serves not only intraneuronal and intraglial functions but also in synaptic neurotransmission.

Zinc concentration in the brain increases with growth after birth (Sawashita *et al.* 1997) and is maintained constant in the adult brain (Markesbery *et al.* 1984). Zinc is also supplied to the adult brain, probably as a required component for neural functions (Pullen *et al.* 1991; Takeda *et al.* 1994a). The turnover of zinc in the brain is slower than in peripheral tissues such as the liver (Kasarskis 1984; Takeda *et al.* 1995). The slow turnover of zinc is due to the presence of the brain barrier system, i.e., the blood-brain and the blood-cerebrospinal fluid (CSF) barriers.

The brain barrier system is important for zinc homeostasis in the brain, and its alteration may be associated with brain dysfunctions and neurological diseases (Takeda 2000). Dietary zinc deprivation causes alteration of zinc homeostasis in the brain (Takeda *et al.* 2000d; Takeda *et al.* 2001) and brain dysfunctions, e.g., mental disorders (Dreosti 1983; Golub *et al.* 1995).

This review summarizes that brain zinc homeostasis is closely related to neural functions.

Zinc transport across the brain barrier system

^{65}Zn is concentrated in the choroid plexus of young adult mice and rats 1 h after intravenous injection of $^{65}ZnCl_2$ and then concentrated in the brain parenchyma with decrease in choroidal ^{65}Zn (Takeda *et al.* 1994a, 2000c). The maximum uptake of ^{65}Zn is probably 6–10 days after parenteral injection to rats (Kasarskis 1984; Takeda *et al.* 1994a). In the brain of adult rats, ^{65}Zn is concentrated and retained in the hippocampal CA3 and dentate gyrus. ^{65}Zn is also concentrated and retained in the amygdala, especially the amygdalopiriform transition and the amygdalo-hippocampal transition areas (Takeda *et al.* 1995). In the case of intracerebroventricular injection of $^{65}ZnCl_2$, ^{65}Zn is concentrated in the brain parenchyma, e.g., the hippocampus and hypothalamus, in young adult rats (Takeda *et al.* 1994b). Zinc is transported into the brain across the blood-CSF barrier, in addition to the blood-brain barrier (Pullen *et al.* 1990; Franklin *et al.* 1992) (Figure 1). The main supply path of zinc to the brain is the blood-brain barrier.

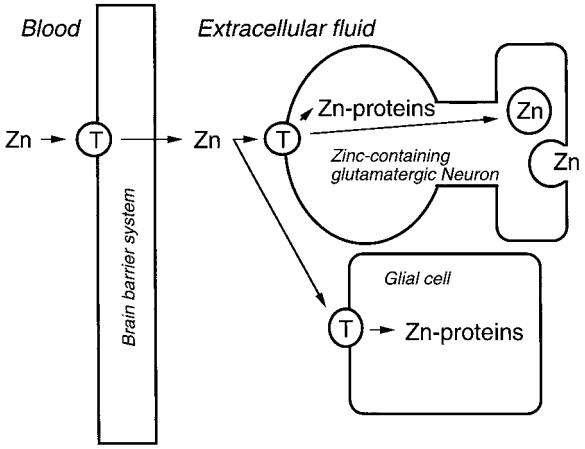

Fig. 1. Zinc transport into the brain. Zinc bound to histidine (and to albumin), which serve as the exchangeable zinc pool in the plasma, may be involved in zinc transport into the brain via transporters on the blood-brain and the blood-CSF barriers. Some transporters such as DMT1, ZIP2 and PHT1 are candidates for zinc transport. They might be also involved in zinc uptake in neurons and glial cells. A large portion of zinc functions as zinc metalloproteins. A portion of zinc is sequestered in the synaptic vesicles and functions as a neuromodulator. T; transporter.

The choroid plexus may participate in slow supply of some trace metals such as zinc and manganese to the brain (Takeda *et al.* 1994a, b, 2000a).

Zinc-binding affinity for ligands in the plasma is important for understanding the mechanism of zinc transport into the brain across the brain barrier system. Plasma zinc (approximately 15 μM) 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. A brain autoradiogram with ^{65}Zn in 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.* 1997a). However, plasma albumin may participate in zinc transport as a large pool of exchangeable zinc in normal animals. Zinc is also known to bind to other plasma proteins such as transferrin and α_2–macroglobulin. Although zinc firmly binds to α_2-macroglobulin, its functional significance is unknown (Giroux & Henkin 1972).

The next largest components of exchangeable zinc in the plasma are complexes of 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 L-histidine infusion. Buxani-Rice *et al.* (1994) report that ^{65}Zn transport into the brain during a short cerebrovascular perfusion is enhanced by addition of 100 μM L-histidine. Brain distribution of ^{65}Zn-His is consistent with the data of the L-histidine infusion experiment (Takeda *et al.* 2000c). On the other hand, supplementation with L-histidine during dietary zinc repletion improves short-term memory in zinc-restricted young adult male mice (Keller *et al.* 2000). L-histidine is probably involved in zinc transport into the brain across the brain barrier system. A rat brain peptide/histidine transporter (PHT1) has been cloned (Yamashita *et al.* 1997). PHT1 mRNA is intensely expressed in the choroid plexus. However, it is obscure whether histidine-bound forms actually pass across the plasma membranes of the choroidal epithelial cells (and brain capillary endothelial cells). On the other hand, DMT1, a divalent metal transporter, is expressed in brain capillary endothelial cells and choroidal epithelial cells (Gunshin *et al.* 1997). Histidine might serve to transfer zinc to DMT1. There is also the possibility that other zinc transporters, e.g., hZIP (ZRT1, IRT1-like protein), are involved in zinc transport across the brain barrier system (Grotz *et al.* 1998; Gaither & Eide, 2000).

The mechanism of zinc secretion from brain capillary endothelial cells and choroidal epithelial cells to the brain extracellular fluid and the CSF, respectively, is unknown.

The half-time for elimination of ^{65}Zn from the rat brain is in the range of 16–43 days (Takeda *et al.* 1995). There are considerable differences in elimination of ^{65}Zn between the brain regions. Zinc might be eliminated from the arachnoid villi and/or arachnoid granulations via the CSF.

Uptake and release of zinc in neurons and glial cells

Four putative zinc transporters, known as ZnT-1 through ZnT-4, have been cloned (McMahon and Cousins 1998). ZnT-1 is ubiquitously expressed and associated with zinc efflux (Tsuda *et al.* 1997; McMahon & Cousins 1998). The mechanism by which zinc is taken up in neurons and glial cells is poorly understood (Figure 1). DMT1 is present in the hippocampal pyramidal and granule cells, cerebellar granule cells, the preoptic nucleus and pyramidal cells of the piriform cortex in high densities (Gunshin *et al.* 1997). This transporter appears to be involved in zinc uptake into neurons (Colvin *et al.* 2000).

CSF zinc is approximately 0.15 μM and zinc concentrations into the brain parenchyma cells are estimated to be approximately 150 μM, judging from the average total brain zinc concentration (Frederickson 1989). To study zinc uptake into the brain parenchyma cells via CSF, ^{65}Zn-His and $^{65}ZnCl_2$ are injected intracerebroventricularly to rats. The radioactivity from ^{65}Zn-His is distributed extensively in the brain compared to that from $^{65}ZnCl_2$ (Takeda *et al.* 2000b). PHT1 mRNA is widely expressed in the brain (Yamashita *et al.* 1997). Especially, the intense hybridization signals are found in the hippocampus, cerebellum and pontine nucleus. There is the possibility that PHT1 are involved in zinc uptake in neurons and glial cells. On the other hand, the finding that histidine decreases ^{65}Zn uptake in the synaptosomal preparation suggests that histidine does not participate in a carrier-mediated uptake by neuron terminals (Wensink *et al.* 1988). There might be differences in the mechanism of zinc uptake between neuron terminal and the cell body. Moreover, a unique mechanism of zinc uptake might exist in zinc-containing glutamatergic neuron terminals, where zinc is concentrated in the synaptic vesicles via ZnT-3, which transports cytosolic zinc into the synaptic vesicles (Palmiter *et al.* 1996; Wenzel *et al.* 1997; Cole *et al.* 1999).

Zinc taken up by neurons is transported anterogradely and retrogradely via the axonal transport system (Takeda *et al.* 1997b, 1998). In zinc-containing glutamatergic neurons, zinc is transported to synaptic vesicles (Figure 1). Zinc concentration in the vesicles in the giant boutons of hippocampal mossy fibers is estimated to be 300–350 μM (Frederickson *et al.* 1983), and is higher than in the cell body. Zinc sequestered in the synaptic vesicles is released with glutamate, and may modulate excitatory neurotransmission via glutamate (Spiridon *et al.* 1998; Traynelis *et al.* 1998; Vogt *et al.* 2000). The glutamate released into the synaptic clefts is primarily removed by transport into glial cells via a glutamate transporter (Zerangue & Kavanaugh 1996). In the case of zinc released into the synaptic cleft, a portion of the zinc may be taken up by postsynaptic neurons, in addition to presynaptic neurons (Takeda *et al.* 1997b). Especially, zinc uptake in postsynaptic neurons is observed after excessive excitation of zinc-containing glutamatergic neurons, followed by

degeneration of postsynaptic neurons (Sloviter 1985; Choi *et al.* 1988; Tonder *et al.* 1990; Koh *et al.* 1996; Choi & Koh 1998). Zinc may be taken up in neurons by mechanisms via the voltage-gated calcium channel (Wang & Quastel 1990; Weiss *et al.* 1993), NMDA receptor (Koh & Choi, 1994; Koh *et al.* 1996) and calcium-permeable AMPA/kainate receptor (Yin & Weiss 1995; Sensi *et al.* 1997; Yin *et al.* 1998).

Brain zinc homeostasis

Zinc homeostasis in the brain is very complex. Metallothioneins, known as MT-I through MT-IV, are metal-binding proteins and may be involved in intracellular zinc homeostasis (Ebadi *et al.* 1995; Vallee 1995). However, the function of MTs in zinc homeostasis in the brain is poorly understood. MT-I and MT-II are ubiquitously expressed. In the brain, MT-I and MT-II in astrocytes are induced by heavy metals such as zinc and copper, glucocorticoid and interleukin-1 (Ebadi *et al.* 1995; Hidalgo & Carrasco 1998). MT-III is abundantly expressed in the brain and suppresses neurite formation (Uchida *et al.* 1991). Although the induction of MT-III is not noticeable in comparison with MT-I and MT-II, MT-III is highly expressed in zinc-containing glutamatergic neurons (Palmiter *et al.* 1992; Masters *et al.* 1994). This metalloprotein may be an important regulator of neuromodulatory zinc in the brain (Erickson *et al.* 1997). Uchida *et al.* (1991) and Tsuji *et al.* (1992) reported that MT-III is deficient in Alzheimer's disease brain. On the other hand, there are some reports that the regulation of MT-III is not impaired in Alzheimer's disease brain (Erickson *et al.* 1994; Amoureux *et al.* 1997). The implication of MT-III in the development of Alzheimer's disease is controversial.

Alteration of zinc homeostasis in the brain may be involved in neurological diseases such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, in which oxidative stress has been implicated as a cause (Cuajungco & Lees, 1997a, b). Zinc can reduce oxidative stress by binding to thiol groups, decreasing their oxidation. MT is a good scavenger of free radicals, resulting in zinc release from MT by disulfides formed under oxidative stress (Sato & Bremner 1993; Maret 1994, 2000; Maret & Vallee 1998). Zinc is also a structural component of superoxide dismutase (SOD), and copper, zinc-SOD is a cytosolic antioxidant. Mutations in SOD cause one form of familiar amyotrophic lateral sclerosis (Rosen *et al.* 1993).

Alteration of brain zinc homeostasis by zinc deficiency

Zinc concentration in the extracellular fluid in the brain is estimated to be almost the same as that in the CSF (Hershey *et al.* 1983; Palm *et al.* 1986). There is a 100-fold difference in zinc concentration between the extracellular fluid and plasma. Dietary zinc deprivation causes a decrease in plasma zinc concentration (Prohaska *et al.* 1974), probably in the exchangeable zinc pool, resulting in a decrease of zinc in peripheral tissues such as the liver. On the other hand, several researchers failed to find any change in brain zinc concentration during dietary zinc deprivation. They demonstrated that zinc concentration is tightly regulated in the brain (Prohaska *et al.* 1974; O'Dell *et al.* 1976; Szerdahelyi *et al.* 1982; Wallwork *et al.* 1983; Prohaska 1987; Golub *et al.* 1986, 1995). However, inadequate dietary zinc supplies cause changes in behavior such as reduced activity and responsiveness (Shagal 1980; Dreosti 1983; Golub *et al.* 1995). In the case of dietary zinc deprivation in infancy, the improvement of learning behavior is not observed in zinc-deficient diet-treated rats (Takeda *et al.* 2000d). Especially, periods of rapid growth such as pregnancy and infancy are susceptible to dietary zinc deprivation (Favier 1992; Sandstead *et al.* 2000). Even in adult animals, learning behavior is impaired by dietary zinc deprivation (Takeda *et al.* 2000d). Thus, it is likely that dietary zinc deprivation affects zinc homeostasis in the brain.

Exogeneous zinc uptake in the brain under zinc deficiency is an index of endogeneous zinc status in the brain. Kasarskis (1984) reported a insufficient gain in brain weight and a increase of ^{65}Zn uptake in the brain of rats, which were fed a zinc-deficient diet for 45 days after wean, suggesting that brain development is suppressed by dietary zinc deprivation. Zinc concentration and ^{65}Zn uptake in the brain were also examined using adult rats fed a halfzinc-deficient diet for 12 weeks (Takeda *et al.* 2000d). Zinc concentrations in the brain, except for the hippocampal formation, did not decrease significantly in zinc-deficient rats, although zinc concentration in the liver of the zinc-deficient rats was approximately one of that of control rats (Figure 2). In an experiment of brain autoradiography with ^{65}Zn, ^{65}Zn concentration in any brain

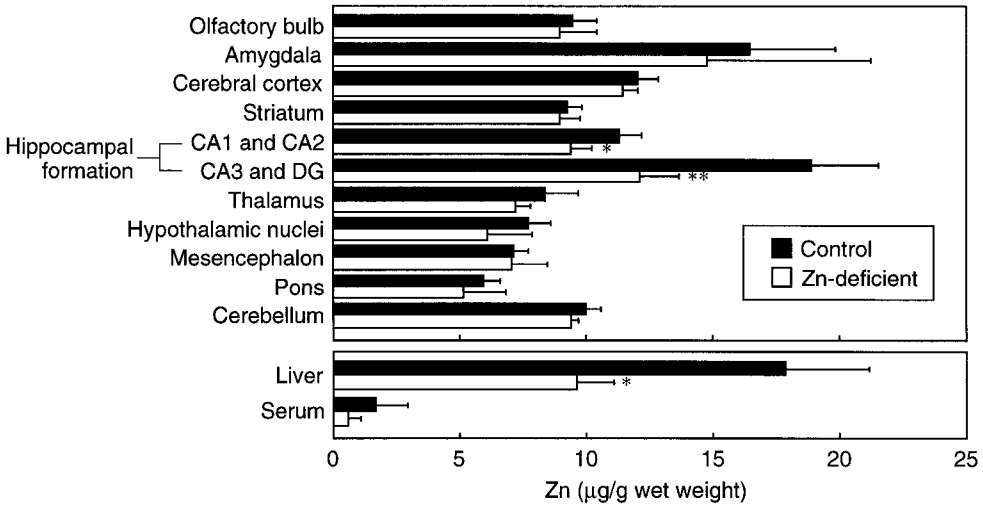

Fig. 2. Zinc concentrations in rats fed zinc-deficient diet. Rats were fed control or zinc-deficient diet for 12 weeks. Each value represents the mean ± SD ($n = 5$). Asterisks indicate significant difference ($^{*}P < 0.05$; $^{**}P < 0.01$; t-test) from control. In the brain, zinc concentration in the hippocampal formation was significantly decreased by dietary zinc deprivation, implying that dietary zinc deprivation affects vesicular zinc levels. [Reprinted with permission from Takeda *et al.* 2001.]

region of the zinc-deficient rats is significantly higher than in control rats 6 days after intravenous injection of $^{65}ZnCl_2$ (Takeda *et al.* 2001). The increase rate of ^{65}Zn concentration in the brain by the zinc deprivation is approximately 150%. Interestingly, the increase rate is similar to that in the liver. The increase rate of the serum is also approximately 150%. Zinc concentration in the brain, unlike in the liver, is not increased after parenteral injection of zinc (Ebadi 1986), indicating that zinc concentration in the brain is tightly regulated by the brain barrier system in the case of an increase of zinc in the serum. The ^{65}Zn supply to the brain of zinc-deficient rats reflects a requirement for brain functions. On the other hand, the half-time for elimination of ^{65}Zn from the rat brain is in the range of 16–43 days (Takeda *et al.* 1995). Thus, dietary zinc deprivation can cause a scarcity of zinc in the brain. Even in adult rats and probably in adult humans, adequate zinc supply is important for brain functions and also for prevention of neurological diseases.

Hippocampal functions under zinc deficiency

In the brain of zinc-deficient rats, the increase rate of ^{65}Zn concentration in the hippocampal CA3 and the dentate gyrus seems to be the lowest in the brain in spite of the significant decrease of zinc concentration in the hippocampal CA3 and the dentate gyrus (Takeda *et al.* 2001). Dietary zinc deprivation for 12 weeks in rats also demonstrated a 30% maximum reduction of zinc concentration in hippocampal mossy fibers (Wensink *et al.* 1987). The hippocampal formation, especially hippocampal mossy fibers, may be the most responsive to dietary zinc deprivation in the brain (Figure 2). The hippocampal mossy neuropil is a region with the highest density of zinc-containing neuron terminals (Frederickson & Danscher 1990). It is considered that vesicular zinc is responsive to dietary zinc deprivation. If zinc replenishment to the synaptic vesicles of zinc-containing glutamatergic neurons depends on a supply from the plasma zinc pool in addition to reutilization of zinc released into synaptic clefts, vesicular zinc could be decreased by dietary zinc deprivation (Dreosti *et al.* 1981; Lu *et al.* 2000).

Long-term potentiation (LTP), which is involved in learning and memory function, has been observed in zinc-containing glutamatergic neuron-rich areas such as the hippocampus and amygdala. Hesse (1979) demonstrated abnormal hippocampal mossy fiber synaptic responses during low-frequency stimulation in zinc-deficient rats. Vesicular zinc might be involved in the induction of LTP (Weiss *et al.* 1989) and dietary zinc deprivation might affect the development of LTP. Learning behavior is reversibly impaired by chelation of zinc in the synapses; Frederickson *et al.* (1990) demonstrated disturbance of hippocampal-dependent spatial-working memory function by diethyldithiocarbamate infusion into the hippocampus.

Amygdalar functions under zinc deficiency

Dietary zinc deprivation can decrease zinc concentration in the amygdala, in addition to the hippocampus (unpublished data), although zinc concentration in the amygdala is not decreased appreciably in Figure 2. Amygdalar functions seem to be affected by dietary zinc deprivation, which disturbs olfactory appreciation in humans and animals (Henkin *et al.* 1975; Mackay-Sim & Dreosti 1989). The amygdala is a region with high densities of zinc-containing neuron terminals and is a part of the olfactory cortices. Vesicular zinc-enriched neuron terminals also exist in the olfactory bulb (Jo *et al.* 2000). There is the possibility that dietary zinc deprivation decreases vesicular zinc in zinc-containing neurons in the olfactory areas and that its decrease is involved in olfactory dysfunction. When the amygdalae are perfused with diethyldithiocarbamate during behavioral test for odor recognition, the recognition of aversive odor is reversibly disturbed by the perfusion (Takeda *et al.* 1999b). Therefore, vesicular zinc is probably involved in amygdalar functions e.g., olfactory appreciation. The relationship between vesicular zinc and functions in the limbic system indicates that zinc homeostasis in zinc-containing glutamatergic synapses is important for the excitatory neurotransmission via glutamate (Takeda 2000). On the other hand, because olfactory epithelium (receptor cell) is differentiated from the basal cell, the regrowth of olfactory epithelium might be inhibited by zinc deficiency (Shigihara *et al.* 1987).

Zinc and epilepsy

Zinc has been reported to act either as an anticonvulsant (Williamson and Spencer, 1995) or a proconvulsant (Pei *et al.* 1983). Alteration of zinc homeostasis in the brain may be associated with the etiology and manifestation of epileptic seizures (Sterman *et al.* 1988). Elimination of zinc from the brain of El (epilepsy) mice is facilitated during induction of seizures (Takeda *et al.* 1999b). Seizure susceptibility of El mice is decreased by dietary zinc loading, while it is increased by dietary zinc deficiency (Fukahori & Itoh 1990). Zinc concentration in the hippocampal dentate area of seized El mice is significantly lower than that of control mice (Fukahori *et al.* 1988), suggesting that the decrease of the hippocampal zinc is involved in the pathophysiology of convulsive seizures in the El mice.

A selective loss of Timm's stain in the hippocampal mossy fiber was observed after electrical stimulation of the perforant path, which evoked hippocampal granule spikes and epileptiform discharges (Sloviter 1985). The loss of zinc stain with N-(6-methoxy-8-quinolyl)-para-toluenesulfonamide (TSQ) in the mossy fibers was also caused by administration of kainate, a seizure-inducing agent (Frederickson *et al.* 1988, 1989). Zinc homeostasis in zinc-containing glutamatergic synapses is altered by the excess excitation (Frederickson *et al.* 2000; Takeda 2000). This alteration may influence the degree and balance of inhibition-excitation (Xie & Smart 1991) and cause an increase in susceptibility to epileptic seizures (Takeda *et al.* 1999b). Actually, ZnT-3-null mice, which lack histochemically reactive zinc in synaptic vesicles, are more sensitive than control mice to seizures induced by kainate (Cole *et al.* 2000). Vesicular zinc may be important for the regulation of excitatory neurotransmission via glutamate.

Perspective on the future

Zinc homeostasis in the brain is closely related to brain functions. The alteration of zinc homeostasis in the brain seems to be a cause of brain dysfunctions and some neurological diseases. However, the mechanism of the alteration is poorly understood. The decrease of zinc metalloproteins affects gene expression and enzyme activity, followed by the alteration of various physiological responses. The decrease of vesicular zinc may affect the balance of inhibition-excitation in zinc-containing glutamatergic synapses. The clarification of neuronal and glial zinc homeostasis is important for understanding zinc functions in the brain.

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