— **Review** —

Analysis of Brain Function and Prevention of Brain Diseases: the Action of Trace Metals

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Trace metals such as zinc, manganese, and iron are necessary for the growth and function of the brain. The transport of trace metals into the brain is strictly regulated by the brain barrier system, *i.e.*, the blood-brain and blood-cerebrospinal fluid barriers. The alteration of homeostasis of trace metals in the brain is associated with brain diseases. Trace metals usually serve the function of metalloproteins in neurons and glial cells, while a portion of trace metals exists in the presynaptic vesicles and may be released with neurotransmitters into the synaptic cleft. Zinc and manganese influence the concentration of neurotransmitters in the synaptic cleft, probably *via* the action against neurotransmitter receptors and transporters and ion channels. Zinc may be an inhibitory neuromodulator of glutamate release in the hippocampus, while neuromodulation by manganese might have both functional and toxic aspects in the synapse. Dietary zinc deficiency affects zinc homeostasis in the brain, followed by an enhanced excitotoxicity of glutamate in the hippocampus. Transferrin may be involved in the physiologic transport of iron and manganese into the brain and their utilization there.

Key words —— zinc, manganese, neuromodulator, hippocampus, zinc deficiency, iron

INTRODUCTION

Trace elements, *i.e.*, zinc, iron, manganese, copper, selenium, iodine, molybdenum, chromium, and cobalt, are essential for humans and animals. Other trace elements such as arsenic, nickel, and silicon are also known to be essential in animals.[1)] Although not all trace elements that are essential for humans and animals have known functions for neural activity, several trace elements such as zinc, manganese, and iron are transported into the brain, probably as required components for neural function.[2)] However, their transports are strictly regulated by the brain barrier system, *i.e.*, the blood-brain and blood-cerebrospinal fluid (CSF) barriers,[2)] and the transport of cadmium, which is probably nonessential for the brain, is blocked by the brain barrier system.[3,4)] On the other hand, the alteration of homeostasis of trace metals in the brain is associated with not only acute brain diseases such as stroke/ischemia and temporal lobe epilepsy, but also chronic brain diseases such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis.

The present paper deals with the movement and action of zinc, manganese, and iron in the brain to understand brain function and also to prevent brain diseases.

ZINC

Zinc is the second most abundant trace element in the body and powerfully influences cell division and differentiation.[5)] 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. Zinc is necessary for brain maturation and function.[6–8)] Approximately 90% of the total brain zinc is in zinc metalloproteins. Approximately 10% is in the presynaptic vesicles and is histochemically reactive (as revealed by Timm's sulfide-silver stain-

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ing method).[9] The presence of zinc-containing neurons that sequester zinc in the presynaptic vesicles has been demonstrated in the brain, especially in the telencephalon. Vesicular zinc is released in a calcium- and impulse-dependent manner. 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-isoxazolepropionate (AMPA)/kainate, *N*-methyl-D-aspartate (NMDA), and γ-amino butyric acid (GABA) receptors.[2,10]

The hippocampal and amygdalar regions may possess zinc-containing glutamatergic neuron terminals in high densities.[9] 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.[2] Lu *et al*. demonstrated that endogeneous zinc is required for the induction of long-term potentiation (LTP) in hippocampal mossy fiber synapses.[11] Li *et al*. demonstrated that the induction of LTP in hippocampal mossy fiber synapses requires translocation of synaptically released zinc.[12] However, the impairment of spatial learning, memory, or sensorimotor functions was not observed in zinc transporter-3-null mice, which lack the histochemically reactive zinc in synaptic vesicles,[13] although zinc transporter-3-null mice are more sensitive than control mice to seizures induced with kainate.[14] There is also some evidence that zinc has no role in the CA3 mossy fiber LTP.[15] Thus the physiologic significance of zinc released into the synaptic cleft is still poorly understood.[16]

On the other hand, approximately 50% of the world population does not get adequate zinc.[17] Dietary zinc deficiency not only retards the growth of humans and animals, but also affects brain maturation.[6,8,16] Marginal zinc deficiency could cause malformations during early brain development and microscopic abnormalities and dysfunction later. However, the molecular mechanism of the brain dysfunction in zinc deficiency is poorly understood.

The following section describes the movement and action of zinc in the brain and altered brain function due to dietary zinc deficiency. Brain tumor imaging with radioactive zinc is also described.

Zinc Transport into the Brain

Zinc-binding affinity for ligands in the plasma is important to understand the mechanism of zinc transport into the brain through the brain barrier system.[2] Plasma zinc (approximately 15 μM) is partitioned between high molecular-weight and low molecular-weight fractions. 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.

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 ^{65}Zn distribution in the brain is similar to that in normal rats and that albumin is not essential for zinc transport into the brain.[18] However, plasma albumin appears to participate in zinc transport as a large pool of exchangeable zinc in normal animals (Fig. 1). 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.

The next largest component of exchangeable zinc in the plasma is amino acids, *i.e*., histidine and cysteine. Aiken *et al*. reported that the ratio of ^{65}Zn concentration in the brain, as well as in other tissues, to plasma ^{65}Zn concentration is enhanced by L-histidine infusion.[19] Brain distribution of ^{65}Zn-His is consistent with the data of an L-histidine infusion experiment.[20] It is possible that L-histidine is involved in zinc transport into the brain parenchyma through the brain barrier system (Fig. 1).[21] A rat brain peptide/histidine transporter (PHT1) has been cloned.[22] PHT1 mRNA is intensely expressed in the choroid plexus. However, it is unknown 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.[23] Alternatively, it is possible that histidine serves 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.[24]

Zinc is transported into the brain across the blood-CSF barrier, in addition to the blood-brain barrier (Fig. 1), the main supply path of zinc; the choroid plexus might participate in slow supply of zinc to the brain. ^{65}Zn was highly distributed in the choroid plexus of mice and rats 1 hr after intravenous injection of $^{65}ZnCl_2$ and then distributed in the brain parenchyma with a decrease in choroidal ^{65}Zn.[25] The maximum concentrations of ^{65}Zn in the

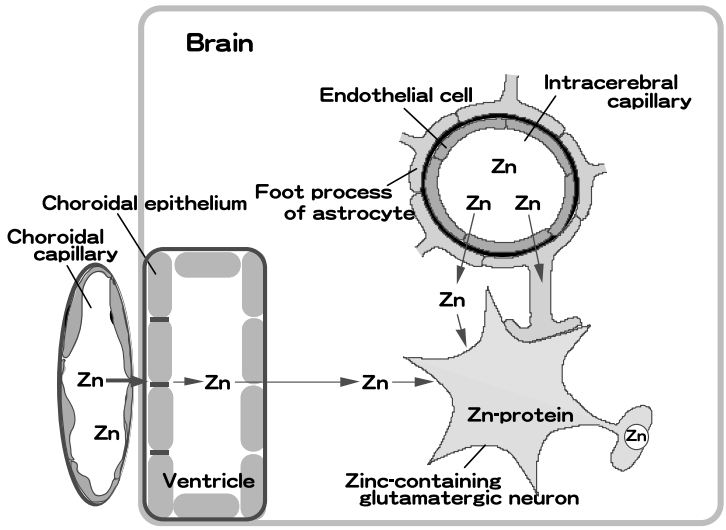

Fig. 1. Zinc Transport into the Brain

Zinc bound to albumin and amino acids, *i.e.*, histidine and cysteine, serves as the exchangeable zinc pool in the plasma. Zinc may be involved in zinc transport into the brain *via* the blood-brain and the blood-CSF barriers. Some transporters such as DMT1, ZIP2, and PHT1 might be involved in zinc transport. A large portion of zinc functions as zinc metalloproteins. A portion of zinc is sequestered in the synaptic vesicles of zinc-containing glutamatergic neurons and released from the neuron terminals.

rat brain were observed 6–10 days after the injection.[26] In the brain, ^{65}Zn was concentrated in the hippocampus, especially the hippocampal CA3 and dentate gyrus, and also in the amygdala, especially the amygdalopiriform transition and the amygdalohippocampal transition areas. The average zinc concentration in the brain is approximately 150 μM.[27] The zinc concentration is also high in the hippocampus and amygdala.

The zinc concentration in the CSF is approximately 0.15 μM. To study zinc uptake by the brain parenchyma cells *via* the CSF, $^{65}ZnCl_2$ or ^{65}Zn-His were injected intracerebroventricularly in rats.[28,29] ^{65}Zn was distributed in the brain parenchyma, *i.e.*, the hippocampus and hypothalamus, after intracerebroventricular injection of $^{65}ZnCl_2$, while the radioactivity from ^{65}Zn-His was distributed extensively in the brain compared with that from $^{65}ZnCl_2$. PHT1 mRNA is widely expressed in the brain.[22] Especially intense hybridization signals are found in the hippocampus, cerebellum, and pontine nucleus. It is possible that PHT1 is 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 in neuron terminals.[30] Because the mechanism of cellular zinc uptake is poorly understood, further investigation is necessary.

The half-time for elimination of ^{65}Zn from the rat brain was in the range of 16–43 days; the longest was observed in the amygdala, followed by those in the piriform cortex and perirhinal cortex.[26]

Movement of Zinc Associated with Neuronal Activity and its Functional Significance

Zinc taken up by neurons is transported anterogradely and retrogradely *via* the axonal transport system.[31,32] In zinc-containing glutamatergic neurons, the zinc appears to be transported into synaptic vesicles (Fig. 1).[16] Zinc concentration in the synaptic vesicles of hippocampal mossy fibers is estimated to be higher than that in the cell body. Zinc sequestered in the synaptic vesicles is released with glutamate into the synaptic cleft.[33] However, the physiologic significance of zinc is still poorly understood. To examine zinc action in presynatic glutamate release, *in vivo* microdialysis experiments were carried out in the rat hippocampus.[34,35]

In the hippocampal CA3 region innervated by mossy fibers, the glutamate concentration in the extracellular fluid was decreased by perfusion with $ZnCl_2$ 10–300 μM.[34] Chelation of endogeneous zinc with CaEDTA 1 mM increased the glutamate concentration in the extracellular fluid, suggesting that the endogeneous zinc released into the synaptic cleft attenuates glutamate release in the CA3 region. The GABA concentration in the extracellular fluid was increased by the addition of zinc, while it was decreased by perfusion with CaEDTA, suggesting that

endogeneous zinc enhances GABA release in the CA3 region. The AMPA/kainate receptors are abundantly expressed on hippocampal interneurons. GABA release is enhanced by activation of the AMPA/kainate receptors and is critical to regulate the excitation of glutamatergic neurons in the hippocampus.[36,37] Because zinc potentiates AMPA/kainate receptor-mediated excitation,[38] it is possible that this potentiation by zinc on interneurons is involved in GABA release. The increase in GABA concentration in the presence of zinc 30 μM was inhibited by the addition of 2,3-dioxo-6-nitro-1,2.3,4-tetrahydrobenzo[f]quinoxaline-7-sulphonamide (NBQX), an antagonist of AMPA/kainate receptors, suggesting that GABA release by zinc is due to excitation of GABAergic interneurons *via* potentiation of the AMPA/kainate receptors. On the other hand, no effect of MK801 and verapamil on GABA release by zinc 30 μM suggests that GABA release by zinc is independent of the NMDA receptors and voltage-dependent calcium channels. Furthermore, the decrease in glutamate concentration in the presence of zinc 30 μM was suppressed by the addition of NBQX, suggesting that GABA release by zinc may attenuate presynaptic glutamate release in the CA3 region (Fig. 2).[39]

The hippocampal CA1 is connected with CA3 pyramidal cells *via* Schaffer collaterals, which are zinc-containing glutamatergic fibers. The selenium retrograde method labels CA1 pyramidal cells more intensely than CA3 pyramidal cells, indicating that CA1 pyramidal cells are extensively innervated by zinc-containing glutamatergic neurons.[40] Because the extracellular glutamate concentration was decreased by perfusion with zinc in the CA1 region, zinc action in the postsynaptic response (glutamate release) was studied using the CA1-entorhinal connection.[41] Perfusion of the CA1 with tetrodotoxin 1 μM, a sodium channel blocker, significantly decreased the extracellular glutamate concentration in the entorhinal cortex, while perfusion of the CA1 with glutamate 50 μM significantly increased it, indicating that the response of postsynaptic CA1 pyramidal cells can be monitored by the level of glutamate released from the neuron terminals in the entorhinal cortex. When the CA1 region was perfused with zinc 50 μM, the glutamate concentration was decreased not only in the CA1 but also in the entorhinal cortex. Furthermore, the increase in glutamate concentration in the entorhinal cortex during perfusion of the CA1 with glutamate 50 μM was completely suppressed by the addition of zinc

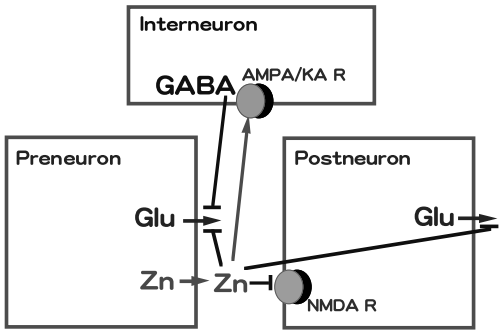

Fig. 2. Inhibitory Zinc Action against Presynaptic and Postsynaptic Glutamate Release in the Hippocampus

In the hippocampus, zinc may attenuate presynaptic glutamate release. GABA release from interneurons by zinc may be involved in this attenuation, followed by the attenuation of postsynaptic glutamate release. KA, kainate; R, receptor.

50 μM to the CA1. Zinc could potentiate AMPA/kainate receptors on CA1 pyramidal cells. However, in addition to the attenuation of glutamate release from presynaptic neurons, the inhibition of NMDA receptors on the CA1 pyramidal cells and the enhancement of GABA release from interneurons, which are both mediated by zinc, appear to attenuate the effect of the above potentiation, resulting in the attenuation of glutamate release from the postsynaptic neuron terminals (Fig. 2). Zinc appears to be an inhibitory neuromodulator of glutamate release in the hippocampus.

Amygdalar zinc is released with glutamate from the neuron terminals, as well as hippocampal zinc.[42,43] Zinc appears to be involved in amygdalar function such as emotional behavior. When the amygdala was perfused with CaEDTA 100 μM to chelate extracellular zinc, the extracellular glutamate concentration decreased significantly, whereas it tended to be increased by perfusion with ZnEDTA 100 μM as a control.[44] The effect of CaEDTA on extracellular glutamate levels was different between the amygdala and hippocampus, implying that modulation of glutamate signaling by zinc differs between them.[43,44] To evaluate the effects of the chelation of zinc on rat behavior, moreover, perfusion of the amygdala with CaEDTA was started 40 min before a behavioral test for passive avoidance.[44] The behavior for passive avoidance was impaired during perfusion with CaEDTA. On the other hand, the behavior during perfusion with ZnEDTA developed more rapidly than that with vehicle alone. These results suggest that amygdalar vesicular zinc is involved in the behavior for passive avoidance. The amygdala was also perfused with diethyldithio-

carbamate 10 μM, a membrane-permeable zinc chelator, during a behavioral test for odor recognition.[42] The recognition of aversive odor was reversibly disturbed by the perfusion. Amygdalar vesicular zinc may be also associated with olfactory appreciation.

Altered Brain Zinc Homeostasis and Enhanced Excitotoxicity of Glutamate due to Zinc Deficiency

Inadequate dietary zinc causes changes in behavior such as reduced activity and responsiveness.[6] Periods of rapid growth such as pregnancy and infancy are particularly susceptible to dietary zinc deficiency. In dietary zinc deficiency in infancy, the learning behavior of passive avoidance was impaired in zinc-deficient diet-treated rats.[45] Even in adult animals, the learning behavior was impaired by zinc deficiency.

Dietary zinc deficiency causes a decrease in the plasma zinc concentration,[46] probably in the exchangeable zinc pool, followed by a decrease in the zinc concentration in the liver and femur. On the other hand, several researchers failed to find any change in the brain zinc concentration due to dietary zinc deficiency.[6] They emphasized that the zinc concentration is strictly regulated in the brain even under conditions of zinc deficiency. However, dietary zinc deficiency was found to affect zinc homeostasis in the brain; the zinc concentration in the hippocampus was significantly decreased in rats fed a zinc-deficient diet for 12 weeks.[27] The attenuation of N-[6-methoxy-8-quinolyl]-P-toluenesulfonamide (TSQ) staining and Timm's staining, with which histochemically reactive zinc in the presynaptic vesicles is detected, was observed in the hippocampal mossy fiber of rats and mice fed a zinc-deficient diet.[11,47,48] Attenuation of Timm's staining was also extensively observed in the telencephalon. These results suggest that the zinc concentration in synaptic vesicles is responsive to dietary zinc. It is possible that zinc levels in synaptic vesicles influence the degree and balance of inhibition-excitation in synapses because zinc may be an inhibitory neuromodulator of glutamate release.

The zinc concentration in the hippocampal extracellular fluid was significantly decreased in zinc deficiency.[47] Zinc is important for the function of many enzymes and other proteins, including some unique to the brain and important in neurotransmission. It is also possible that zinc-requiring proteins, which are unique to the brain and important to neurotransmission, are responsive to dietary zinc and influence the degree and balance of inhibition-excitation in synapses.

Alteration of zinc homeostasis in the brain may be associated with the etiology and manifestation of epileptic seizures.[49–52] Susceptibility to kainate-induced seizures was enhanced in mice and rats fed a zinc-deficient diet for 4 weeks.[48] In zinc-deficient rats, the zinc concentration in hippocampal extracellular fluid, which was less than 50% of that in control rats, was increased by treatment with kainate, although its increased concentrations were lower than the basal concentration in control rats. Glutamate concentrations in hippocampal extracellular fluid were increased more by treatment with kainate than those in the control rats, whereas GABA concentrations did not increase in zinc-deficient rats, unlike those in the control rats. The function of zinc-requiring proteins in the brain appears to be impaired by zinc deficiency, resulting in severe changes in the metabolic function of neurons and glial cells, which may be associated with excessive release of glutamate and the lack of enhanced release of GABA in zinc-deficient rats.[47] This imbalance of inhibition-excitation is a possible mechanism for the increased seizure susceptibility in zinc deficiency.[48]

Glutamate can be released from the cells by two mechanisms: either by calcium-dependent vesicular release or, in pathologic conditions, by reversed operation of the plasma membrane glutamate uptake carrier.[53,54] The increase in the extracellular potassium concentration, which is observed in neurologic disorders such as ischemia and epilepsy, induces glutamate release from glial cells *via* the reversed operation, in addition to the increase in calcium-dependent vesicular glutamate release, resulting in an excess of the extracellular glutamate concentration. In the case of the perfusion of the hippocampus with KCl 100 mM, the glutamate concentration in the hippocampal extracellular fluid was increased more in zinc-deficient rats than in control rats, suggesting that susceptibility to glutamate excitotoxicity is enhanced in the hippocampus in zinc deficiency (Fig. 3).[47] An excess of the extracellular glutamate concentration induces neurotoxicity associated with numerous pathologic processes such as Alzheimer's disease and amyotrophic lateral sclerosis, in addition to stroke/ischemia and temporal lobe epilepsy.[55,56] Therefore brain zinc homeostasis appears important to prevent neurodegeneration due to glutamate excitotoxicity.

^{65}Zn Imaging of Rat Brain Tumors

Zinc uptake is critical for cell proliferation.[57–59]

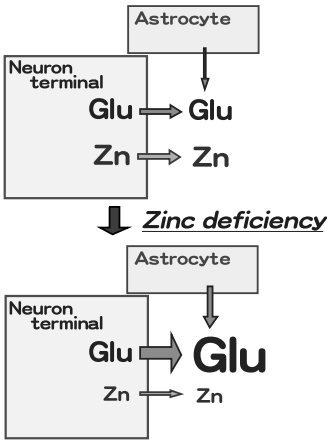

Fig. 3. Enhanced Susceptibility to Glutamate Excitotoxicity in Zinc Deficiency

The extracellular glutamate concentration in the hippocampus during excessive stimulation is increased more in zinc deficiency. This increase might be due to enhanced glutamate release from neurons and astrocytes. The zinc concentration in synaptic vesicles is decreased in zinc deficiency and the amount of zinc released from neuron terminals is also decreased.

Based on the idea that zinc uptake can be an index of viability in proliferating cells (Fig. 4),[60] tumor imaging with ^{65}Zn was performed using autoradiography.[61] Following subcutaneous implantation of ascites hepatoma (AH7974F) cells into the dorsum, 1 hr after intravenous injection of $^{65}ZnCl_2$, ^{65}Zn uptake in the tumor was higher than in the brain tissue, but lower than in the liver, suggesting that brain tumors can be positively imaged with ^{65}Zn. Following implantation of AH7974F cells into the periaqueductal gray, 1 hr after intravenous injection of $^{65}ZnCl_2$, ^{65}Zn uptake in the tumor was approximately 10 times higher than in other brain regions. Following implantation of C6 glioma cells into the hippocampus, ^{65}Zn uptake in the tumor was also much higher than in other brain regions (Fig. 4). These findings demonstrate that brain tumors can be imaged with radioactive zinc. To compare brain tumor imaging with ^{65}Zn with that of ^{18}F-fluorodeoxyglucose (FDG), which is widely used for the diagnosis of brain tumors, ^{14}C-FDG imaging of the C6 glioma was performed in the same manner. ^{14}C-FDG uptake in the tumor was approximately 1.5 times higher than in the contralateral region in which ^{14}C-FDG uptake was relatively high to utilize glucose as the main energy source. It is likely that zinc uptake is more specific for brain tumors than FDG uptake, suggesting that there is great potential for the use of ^{69m}Zn, a short half-life gamma emitter, in the diagnosis of brain tumors.[62–64]

MANGANESE

Manganese has been found as Mn^{2+}, Mn^{3+}, and Mn^{4+} in both animals and humans.[65] However, the function of manganese is poorly understood, in spite of it being an essential requirement for humans and animals.[1] This is due to the low concentration of manganese in living tissues. In many human and animal tissues, manganese concentrations are less than 1 μg/g wet weight.[66] Manganese is involved in the metabolism of protein, lipid, and carbohydrate and serves as a cofactor for enzymes such as decarboxylase, hydrolase, and kinase.[1,67,68] However, manganese action is not manganese specific. Because Mn^{2+} resembles Mg^{2+} in some physicochemical properties, a number of enzymes can substitute magnesium for manganese in their activation *in vitro*. On the other hand, mitochondrial superoxide dismutase is a known manganese metalloprotein and exists ubiquitously. Glutamine synthetase is a glia-specific manganese metalloprotein in the brain.

Manganese concentrations in the human brain are higher in adults (approximately 0.25 μg/g wet weight) than in infants (0–1 year old),[69] suggesting that manganese is required for brain function.[70] Dietary manganese deficiency might affect manganese homeostasis in the brain, as evidenced by increased susceptibility of manganese-deprived rats to convulsions.[71] Carl *et al*. indicated that manganese concentrations in tissues including the brain are increased after epileptic seizures.[72] Blood manganese levels in patients with idiopathic epilepsy are lower than in normal populations.[73] It is possible that the movement of manganese is associated with neuronal activity in the brain.[67,68] Manganese also acts as a toxicant to the brain. This metal is abnormally concentrated in the brain, especially in the basal ganglia, resulting in neurologic disorders similar to Parkinson's disease.[66] This next section summarizes the movement and action of manganese in the brain to understand the functional and toxic actions of manganese in neuronal activity.

Manganese Transport into the Brain

Dietary manganese is transported to the liver after absorption from the gut. After transport into the liver *via* the portal vein, divalent manganese can be oxidized to trivalent manganese, probably by ceruloplasmin.[74] Alternatively, it is possible that divalent manganese absorbed from the gut is oxidized by ceruloplasmin in the plasma. The liver may be important as a depot for manganese, with hepatic

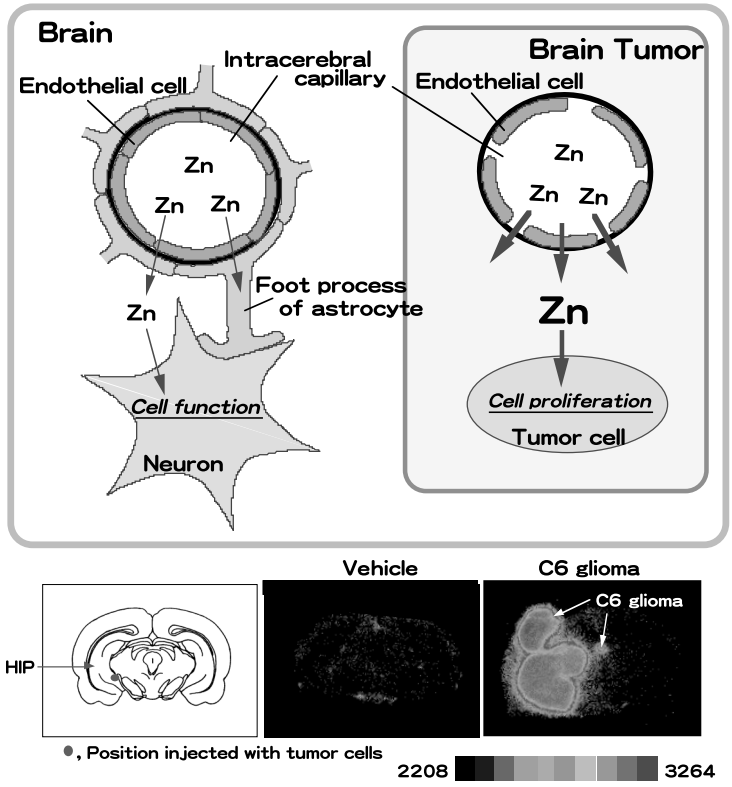

Fig. 4. Zinc Transport and Utilization in Brain Tumors

Zinc is easily transported into the extracellular fluid in brain tumor tissue, because the blood-brain barrier does not exist there. Zinc may be utilized for cell proliferation. As shown in the lower panels, C6 glioma, which was transplanted into the hippocampus, was positively imaged with ^{65}Zn. The middle and right panels are brain autoradiograms 1 hr after intravenous injection of $^{65}ZnCl_2$.

manganese later delivered to the brain.[75] Transferrin is a plasma carrier protein for trivalent manganese, and transferrin-bound manganese is mainly detected in the plasma after oral administration of manganese.[76]

Manganese is rapidly removed from the blood after intravenous injection and a portion of the manganese is transported into the brain for a short period.[66,75] This may be due to the presence of nontransferrin-mediated manganese uptake mechanisms in the brain barrier system. In an experiment using $^{54}MnCl_2$, ^{54}Mn was widely distributed in the choroid plexus 1 hr after intravenous injection and then in the brain parenchyma.[25] Maximum concentrations of ^{54}Mn in rat brain were observed 6–15 days after intravenous injection of $^{54}MnCl_2$.[26] It is likely that manganese, as well as zinc, is transported into the brain across the blood-CSF barrier in addition to the blood-brain barrier.

To examine the role of transferrin in manganese distribution in the brain, brain distribution after intravenous injection of $^{54}MnCl_2$ was compared with that after intravenous injection of pH 8.6 buffer-treated $^{54}MnCl_2$, which is estimated to be trivalent, and that after intravenous injection of transferrin-bound ^{54}Mn.[77] One hour after intravenous injection of $^{54}MnCl_2$ or pH 8.6 buffer-treated $^{54}MnCl_2$, both the radioactivities were distributed in the brain to almost the same extent and were widely distributed in the choroid plexus. Blood clearance of ^{54}Mn was high in both cases. Nonprotein-bound divalent ^{54}Mn and trivalent ^{54}Mn might be readily taken up by the choroid plexus and brain parenchyma. Rabin *et al.* indicated that approximately 30% of divalent ^{54}Mn injected is nonprotein bound in the plasma and that ^{54}Mn is readily transported into the brain, most likely as free ion.[78] ^{54}Mn was concentrated in the superior olivary complex, inferior colliculi, and red nuclei 6 days after intravenous injection of $^{54}MnCl_2$, pH 8.6 buffer-treated $^{54}MnCl_2$, and transferrin-bound ^{54}Mn.[70,77] The radioactivity from transferrin-bound ^{54}Mn in the brain was the lowest of all three substrates. Transferrin receptors are present on the surface of brain capillary endothelial cells. The transport of transferrin-bound manganese into the brain is less and manganese might be released from trans-

ferrin during the transport process. However, transferrin may be involved in the physiologic transport of manganese into the brain across the blood-brain barrier *via* receptor-mediated endocytosis.[68]

To examine manganese transport into the brain parenchyma *via* the CSF, brain autoradiography was performed after intracerebroventricular injection of $^{54}MnCl_2$.[28] ^{54}Mn distribution in the brain was similar to that after intravenous injection. These results strongly suggest that manganese is transported into the brain across the blood-CSF barrier. On the other hand, ^{54}Mn was distributed only around the ipsilateral lateral ventricle after intracerebroventricular injection of transferrin-bound ^{54}Mn, suggesting that transferrin-bound manganese in the CSF is not readily transported to the brain parenchymal cells.[77] The transfer rate of ^{125}I-transferrin from the CSF to the blood is relatively high after intracerebroventricular injection.[79] Transferrin secreted from the choroidal epithelial cells appears to sequester manganese in the CSF.

Manganese Movement and Action in the Synapse

To study manganese transport in the neural circuit of rat brain, brain autoradiography was performed after injection of $^{54}MnCl_2$ into the brain parenchyma. ^{54}Mn was detected in the substantia nigra and striatum after intrastriatal and intranigral injection of $^{54}MnCl_2$, respectively.[80] When $^{54}MnCl_2$ was bilaterally injected into the striata after unilateral injection of colchicine, an inhibitor of axonal transport, into the medial forebrain bundle, ^{54}Mn transport to the substantia nigra was suppressed. Manganese is subjected to axonal transport in the GABAergic striatonigral and/or dopaminergic nigrostriatal pathways, suggesting that manganese is associated with neuronal activity.

In the case of injection of $^{54}MnCl_2$ into the mitral cells and external plexiform layers of the olfactory bulb, ^{54}Mn taken up by the soma of secondary olfactory neurons in these layers was transported to the entorhinal area, the secondary olfactory cortex, along the olfactory tract.[80] It is possible that manganese is released from mitral cell terminals in the piriform cortex and/or amygdala. When the release of ^{54}Mn previously taken up in the amygdala was examined by stimulation with KCl 100 mM, ^{54}Mn concentration in amygdalar extracellular fluid was increased,[81] and this increase was inhibited by the addition of tetrodotoxin. These results suggest that manganese is dynamically linked to neuronal signaling processes in the amygdala (Fig. 5).[68,82] Enhanced ^{54}Mn release by stimulation with high K^+ concentrations was also observed in the hippocampus and striatum, but not in the substantia nigra. The increment in the extracellular glutamate concentration during stimulation with high K^+ concentrations was highly correlated with that in the extracellular ^{54}Mn level, suggesting that manganese is released with glutamate from neuron terminals (Fig. 5).

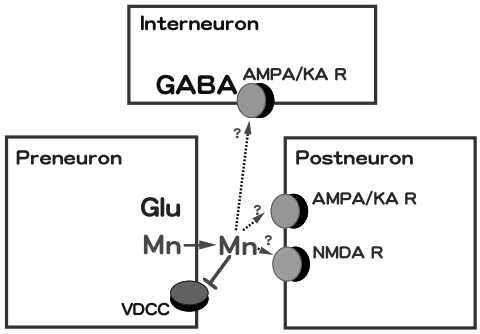

Fig. 5. Manganese Action in the Glutamatergic Synapse

Manganese may be released with glutamate from neuron terminals. Manganese may influence neurotransmitter concentrations in the synaptic cleft, possibly *via* the action against neurotransmitter receptors and transporters and channels, *e.g.*, voltage-dependent calcium channels (VDCC).

Activation of kainate receptors has been shown to be involved in the inhibition of glutamate release from hippocampal neuron terminals, probably because of the inhibition of calcium influx *via* presynaptic kainate receptor activation.[83,84] On the other hand, Rodriguez-Moreno *et al*. reported two populations of kainate receptors with separate signaling mechanisms in hippocampal interneurons.[85] The presynaptic activation of kainate receptors weakens the synaptic inhibition induced by GABA, while it induces membrane depolarization. Thus it is considered that the release of excitatory and inhibitory neurotransmitters from neuron terminals can be differentially modulated by kainate. In the case of perfusion with kainate 30 μM in the hippocampus, the ^{54}Mn concentration, as well as the concentrations of glutamate and aspartate, in the perfusate was decreased by kainate, whereas the concentrations of glycine and GABA were increased by kainate.[82] Furthermore, membrane depolarization of excitatory neurons was elicited by stimulation with KCl 100 mM in the presence of kainate 30 μM. The concentrations of glutamate and aspartate, as well as the ^{54}Mn concentration, increased more during stimulation with high K^+ concentrations in the presence of kainate than during stimulation with kainate alone. These results strongly suggest that manganese is

released with glutamate from neuron terminals.

Manganese action in the synapse was examined by perfusion with $MnCl_2$ 20 or 200 nM. The manganese concentrations used were fairly low, judging from the manganese concentration in the CSF (0.83–1.50 μg/l).[86] Interestingly, the concentrations of glutamate, aspartate, and GABA in the perfusate were remarkably decreased by the addition of manganese in the hippocampus.[82] In the striatum, the GABA concentration was markedly decreased by the addition.[87] Divalent cations such as zinc, cadmium, and cobalt act as voltage-dependent calcium-channel blockers.[2] Manganese also blocks voltage-dependent calcium channels and nerve-evoked neurotransmitter release *in vitro*, although the effects of manganese on the voltage-dependent calcium channels and the release of neurotransmitters are controversial.[68] These results suggest that manganese could modulate the release of neurotransmitters such as glutamate, aspartate, and GABA (Fig. 5).

Manganese may have a role in the pathogenesis of chronic hepatic encephalopathy.[88] In patients receiving long-term parenteral nutrition, some brain disorders have been ascribed to manganese toxicity.[89] An abnormal deposit of manganese in the basal ganglia was observed in those patients using magnetic resonance imaging, which might be due to increased levels of manganese in the blood.[66] The deposition of manganese can cause an irreversible neurologic syndrome similar to Parkinson's disease.[90] Experimental observations suggest that secondary excitotoxic mechanisms play a crucial role in the development of manganese-induced neurodegeneration in the striatum. Centonze *et al*. demonstrated that the abnormal excitation of striatal neurons during manganese intoxication may be due to hyperactivity of corticostriatal neurons.[91] Therefore manganese action in the synapse may also be important to clarify manganese neurotoxicity.

IRON

Iron concentrations in the human brain are approximately five times higher in adults (approximately 65 μg/g wet weight) than in infants less than 1 year of age,[69] suggesting that iron is a required component for brain maturation and function. In the brain, iron is found in oligodendrocytes in high density and is required for myelin production.[92] Iron uptake is the highest during postnatal development at a time that coincides with peaks in brain growth and myelin production,[93] and an insufficient iron supply to the brain results in hypomyelination.[94] Thus an adequate supply of iron is important for brain development.

On the other hand, iron is a toxicant in excessive amounts; free iron can be cytotoxic because it catalyzes the production of hydroxyl radicals from hydrogen peroxide.[95] Abnormalities in brain iron metabolism have been described in several neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and Huntington's disease. Transferrin is thought to be important for brain iron metabolism. However, brain transferrin levels decrease with age and its decrease is dramatic when Alzheimer's disease or Parkinson's disease is superimposed on the aging process. Transferrin is synthesized primarily in the liver, while a significant amount is also produced in the brain.[96] In the brain, transferrin mRNA exists in oligodendrocytes and also in the choroid plexus in concentrations equal to that found in the liver. Recent evidence has suggested a differential function between transferrin synthesized in the brain and in other tissues such as the liver and a specific role of transferrin in oligodendrocyte maturation and in myelinogenesis.[97] This following section deals with the role of transferrin in iron transport into the brain and its utilization there.

Iron Transport and Utilization in the Brain

Craven *et al*. reported the tissue distribution of nontranferrin-bound ^{59}Fe in the case of transient saturation of plasma transferrin by intravenous injection of ferric citrate.[98] Plasma clearance of nontransferrin-bound ^{59}Fe is very high after intravenous injection of ^{59}Fe in citric acid. In iron-loaded rats, > 80% of the injected radioactivity is eliminated from the plasma by 30 sec, probably owing to rapid uptake of nontransferrin-bound ^{59}Fe by the liver. On the basis of the evidence that the tissue iron distribution is changed by iron saturation of plasma transferrin, the influence of transient saturation of transferrin in iron transport into the brain was studied using brain autoradiography.[99] Twenty-four hours after intravenous injection of $^{59}FeCl_3$ into iron-loaded mice, ^{59}Fe distribution in the brain was different between control and iron-loaded mice. ^{59}Fe concentrations in the brain of iron-loaded mice were approximately 40–50% of those of control mice in all brain regions tested except the choroid plexus, in which the ^{59}Fe concentration was equal. Transferrin-bound iron may be responsible for the fraction of iron in

circulation that enters the brain.

In hereditary hemochromatosis, which is characterized by the triad of increased iron absorption by gastrointestinal cells, high or total iron saturation of plasma transferrin, and abnormal iron deposition in the tissues, especially in the liver, iron deposition in the brain has not been described as a pathologic phenomenon and this disease is not usually associated with neurologic symptoms.[100] Sotogaku *et al*. demonstrated that the iron concentration in the brain, unlike that in the liver, scarcely increases after persistent iron overloading.[66] Therefore it is likely that nontransferrin-bound iron present in the circulation of hemochromatosis patients is of little significance as a cause of pathologic iron accumulation in the brain.

Hypotransferrinemic (HP) mice have a point mutation or small deletion in the transferrin gene and produce < 1% of the normal circulating level of plasma transferrin.[101] The affected animals are small, pale, and severely anemic at birth and require weekly injections of serum or purified transferrin for survival. The role of transferrin in iron utilization in the brain was studied using 7-day-old HP mice without administration of transferrin.[101–103] Twenty-four hours after injection of $^{59}FeCl_3$ into HP mice, ^{59}Fe was widely distributed in the choroid plexus. ^{59}Fe concentrations in brain parenchyma were lower than in nonmutant mice. ^{59}Fe distribution in the brain of HP mice was similar to the case of transient saturation of transferrin by iron loading,[24] strongly suggesting that plasma transferrin is involved in physiologic iron delivery to the brain. In the brain of adult HP mice, the cellular and regional distributions of iron, transferrin, transferrin receptor, and ferritin are similar to those in normal mice, although transferrin in the brain is of exogeneous origin.[104] Interestingly, the iron concentration in the brain of 7-day-old HP mice was approximately three-fold higher than that in nonmutant mice.[102] The circulation of iron in the brain extracellular fluid might be impaired by the lack of transferrin, resulting in abnormal iron accumulation in the brain. It is likely that the management of iron is affected in the brain of HP mice. Brain transferrin may be involved in the management of iron in the brain.[100]

PERSPECTIVES ON THE FUTURE

The action of zinc and manganese in the synapse must be analyzed from both the functional and toxic aspects. Furthermore, it is possible that trace metals such as copper and iron act in the synapse, because copper and iron exist in the synaptic vesicles.[105] Brain function strictly requires not only homeostasis of these trace metals in neurons and glial cells but also in the synapse. However, the mechanism of cellular and synaptic homeostasis of trace metals in the brain is poorly understood. Further investigation on the precise movement and action of trace metals in the brain, especially in the synapse, is important to understand brain function and to prevent brain diseases.

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