Review Article

Insight into Glutamate Excitotoxicity from Synaptic Zinc Homeostasis

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Zinc is released from glutamatergic (zincergic) neuron terminals in the hippocampus, followed by the increase in Zn^{2+} concentration in the intracellular (cytosol) compartment, as well as that in the extracellular compartment. The increase in Zn^{2+} concentration in the intracellular compartment during synaptic excitation is mainly due to Zn^{2+} influx through calcium-permeable channels and serves as Zn^{2+} signaling as well as the case in the extracellular compartment. Synaptic Zn^{2+} homeostasis is important for glutamate signaling and altered under numerous pathological processes such as Alzheimer’s disease. Synaptic Zn^{2+} homeostasis might be altered in old age, and this alteration might be involved in the pathogenesis and progression of Alzheimer’s disease; Zinc may play as a key-mediating factor in the pathophysiology of Alzheimer’s disease. This paper summarizes the role of Zn^{2+} signaling in glutamate excitotoxicity, which is involved in Alzheimer’s disease, to understand the significance of synaptic Zn^{2+} homeostasis in the pathophysiology of Alzheimer’s disease.

1. Introduction

Over 300 proteins require zinc for their functions in microorganisms, plants, and animals. Zinc powerfully influences cell division and differentiation [1]. Zinc is essential for brain growth and its function [2, 3]. Zinc concentration in the adult brain reaches approximately 200 μM [4]. Extracellular zinc concentration in the adult brain is estimated to be less than 1 μM [5]. Zinc concentration in the cerebrospinal fluid (CSF) is approximately 0.15 μM [6], while that in the plasma is approximately 15 μM. Zinc transport from the plasma to the cerebrospinal fluid is strictly regulated by the blood-brain barrier system, that is, the blood-CSF barrier. The blood-CSF barrier, in addition to the blood-brain barrier, is involved in zinc homeostasis in the brain [7, 8]. Zinc is relatively concentrated in the hippocampus and amygdala [9, 10]. The biological half-life of zinc is relatively long in these two areas (hippocampus, 28 days; amygdala, 42 days). Zinc homeostasis in the brain is closely associated with neurological diseases including Alzheimer’s disease [11–13] and may be spatiotemporally altered in their pathogenesis and progression.

Approximately 90% of the total brain zinc exists as zinc metalloproteins. The rest mainly exists in the presynaptic vesicles and is histochemically reactive as revealed by Timm’s sulfide-silver staining method [14]. Histochemically reactive zinc is released along with neuronal activity; there is a large number of evidence on zincergic neurons that sequester zinc in the presynaptic vesicles and release it in a calcium- and impulse-dependent manner [15–18]. In the rat brain, Timm’s stain is hardly observed just after the birth, and its intensity increases with brain development [19, 20], indicating that histochemically reactive zinc is involved in not only brain growth but also brain function. However, impairment of spatial learning, memory, or sensorimotor functions is not observed in zinc transporter-3-null mice, which lack the histochemically reactive zinc in synaptic vesicles [21]. Zinc transporter-3 is involved in zinc transport into synaptic vesicles. Therefore, physiological significance of histochemically reactive zinc in neuronal activity is still poorly understood.

The hippocampus plays an important role in learning, memory, and recognition of novelty [22]. The hippocampus
receives major input from the entorhinal cortex via the perforant pathway, the dentate granule cells project to the CA3 pyramidal cells via the mossy fibers, and the CA3 pyramidal cells project to the CA1 pyramidal cells via the Schaffer collaterals. The three pathways are glutamatergic (zincergic), and terminals of them are stained by Timm’s method [23]. Zinc concentration in the presynaptic vesicles is the highest in the giant boutons of hippocampal mossy fibers. All giant boutons of mossy fibers contain zinc in the presynaptic vesicles, while approximately 45% of Schaffer collateral/commissural pathway is zinc-positive [24]. It has been reported that histochemically reactive zinc serves 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) receptors, and γ-aminobutyric acid (GABA) receptors [25, 26]. The zinc may participate in synaptic plasticity such as long-term potentiation (LTP) and long-term depression (LTD) that is believed as the mechanism of learning and memory [27–29].

The exact chemical form of histochemically reactive zinc is unknown. The zinc released in the extracellular space is estimated to serve in free form (Zn²⁺) [30]. The basal Zn²⁺ concentrations are extremely low in both the extracellular (∼10⁻⁸ M) and intracellular (cytosol) (<10⁻⁹ M) compartments [31, 32]. Zn²⁺ concentration increases in both compartments by excitation of zincergic neurons [33] and serves for signaling [34, 35]. However, the extracellular and intracellular concentrations of Zn²⁺ reached after synaptic excitation are obscure. Other organelles such as the mitochondria and the endoplasmic reticulum including the cytoplasm may participate in the increase in cytosolic Zn²⁺ [36–38]. The mechanisms on Zn²⁺ homeostasis in both compartments remain to be clarified [39, 40].

Zn²⁺ signaling is required for brain function, while alteration of Zn²⁺ homeostasis may modify glutamate excitotoxicity, which is involved in Alzheimer’s disease. This paper summarizes the role of Zn²⁺ signaling in glutamate excitotoxicity to understand the significance of zinc as a key-mediating factor in the pathophysiology of Alzheimer’s disease.

2. Modulation of Glutamate Signaling by Zinc

ZnAF-2 is a membrane-impermeable zinc indicator and has a low Kd value of 2.7 nM for zinc, and its fluorescence is minimally changed in the presence of calcium, magnesium, cadmium, nickel, or other heavy metals [41]. ZnAF-2 DA, a diacetylated form of ZnAF-2, is taken up by cells and hydrolyzed to ZnAF-2, which cannot permeate the cell membrane. These two indicators make possible an observation of Zn²⁺ dynamics in extracellular and intracellular compartments. Zn²⁺ released from zincergic neuron terminals is immediately retaken up by the same terminals during tetanic stimulation and also taken up into postsynaptic neurons [33, 35]. Calcium cannels such as calcium-permeable AMPA/kainate receptors are involved in Zn²⁺ influx during synaptic excitation [5, 31, 33, 35, 42] (Figure 1). Because kainate receptors are abundantly expressed in mossy fibers, they might be involved in zinc influx into mossy fiber terminals [43].

Quinta-Ferreira and Matias [44, 45] report that Ca²⁺ influx into mossy fibers by tetanic stimulation is inhibited by endogenous zinc. In the CA3 and CA1, furthermore, Zn²⁺ released from zincergic neuron terminals suppresses the increase in Ca²⁺ influx into the presynaptic terminals after tetanic stimulation, followed by negative modulation of the presynaptic activity (exocytosis) (Figure 2) [33, 35]. In an experiment using synaptosomal fraction from rat hippocampal CA3, Zn²⁺ inhibits glutamate release via activation of presynaptic ATP-dependent potassium (Kₘ₃₈) channels [46]. Zn²⁺ released from zincergic neuron terminals may serve for negative feedback mechanisms against glutamate release in both the extracellular and intracellular compartments (Figure 2).

3. Crosstalk of Zn²⁺ Signaling to Ca²⁺ Signaling in Glutamate Excitotoxicity

In both the extracellular and the intracellular compartments, it is possible that zinc signaling plays a neuroprotective role against glutamate-induced excitotoxicity [46, 47]. Activation of presynaptic kainate receptors is involved in the release of zinc and glutamate from mossy fibers [48, 49], and astrocytes also release glutamate [50]. Loss of astrocyte glutamate homeostasis is a prerequisite for the excitotoxic cascade, a phenomenon that is becoming recognized in an increasing number of neurological disorders [51]. The significance of zinc release in excess excitation of mossy fibers is examined by regional delivery of glutamate (1 mM) to the stratum lucidum, in which mossy fibers exist. Zn²⁺ may negatively modulate Ca²⁺ mobilization in CA3 pyramidal cells under the delivery [52]. Intracellular Ca²⁺ mobilization via group I metabotropic glutamate receptor activation can be also negatively modulated by Zn²⁺ signaling in CA3 pyramidal cells [34]. These findings suggest that Zn²⁺ can protectively act on glutamate excitotoxicity via crosstalk to Ca²⁺ signaling.

In contrast, excess of intracellular Zn²⁺ is potentially neurotoxic as well as excess of intracellular Ca²⁺ [53–60] (Figure 2). The origin of the toxic zinc is a matter of debate and seems to be not only the extracellular compartment but also the intracellular compartment [61]. The exact borderline of intracellular Zn²⁺ level between physiological regulation and pathological effects remains poorly defined as discussed later. Côté et al. [62] report that the neurotoxic and neuroprotective actions of Zn²⁺ depend on its concentration and that this dual action is cell type specific. Lavoie et al. [63] report that intracellular zinc chelator influences hippocampal neuronal excitability in rats. Furthermore, chelation of endogenous zinc by CaEDTA causes a significant increase in ischemic cell death in hippocampal slice cultures [46]. In an in vivo microdialysis experiment, the increase in extracellular glutamate concentration induced with high 100 mM KCl was significantly enhanced in the presence of 1 mM CaEDTA in both the control and zinc-deficient rats [64]. These findings indicate that Zn²⁺ released from zincergic neurons may...
reduce glutamate release under pathological condition and protect hippocampal cells from the excitotoxicity (Figure 2).

4. Dietary Zinc Deficiency and Glutamate Excitotoxicity

Extracellular glutamate concentration is estimated to be around 2 μM in the brain, while glutamate concentration in the synaptic vesicles is markedly high (~100 mM) [65]. Excessive activation of glutamate receptors by excess of extracellular glutamate leads to a number of deleterious consequences, including impairment of calcium buffering, generation of free radicals, activation of the mitochondrial permeability transition, and secondary excitotoxicity [66, 67]. Glutamate excitotoxicity, a final common pathway for neuronal death, is observed in numerous pathological processes such as stroke/ischemia, temporal lobe epilepsy, Alzheimer’s disease, and amyotrophic lateral sclerosis [68–70]. The hippocampus is susceptible to glutamate excitotoxicity, is enriched with glucocorticoid receptors [71], and is a major target of glucocorticoids. Glucocorticoids may potentiate glutamate excitotoxicity, followed by the increase in neuronal death [72].

Dietary zinc deficiency readily decreases serum zinc level in mice and rats, while it increases serum corticosterone level through the increased hypothalamic-pituitary-adrenal (HPA) axis activity [73]. Brain zinc concentration is hardly decreased by zinc deficiency, while both histochemically reactive zinc and extracellular zinc in the brain are susceptible to chronic zinc deficiency [64, 74–76] (Figure 3). Excitability of zincergic neurons is potentially changed in cooperation with corticosterone under zinc deficiency [27]. Thus, the increased secretion of corticosterone might be
associated with the decrease in histochemically reactive zinc and extracellular zinc under zinc deficiency. The increase in extracellular glutamate induced by 100 mM KCl is potentiated under zinc deficiency [64, 76]. Kainate and NMDA-induced seizures are potentiated in young mice and rats after 4-week zinc deprivation, which decreases histochemically reactive zinc [74, 77], and hippocampal cell death, which is induced by treatment with kainate, is increased under zinc deficiency [78]. These findings suggest that endogenous zinc, especially histochemically reactive zinc, has a protective action against glutamate excitotoxicity. The neurological symptoms associated with glutamate excitotoxicity may be aggravated by zinc deficiency.

Neuritic plaques, a pathological hallmark of Alzheimer’s disease, are composed of β-amyloid that is precipitated by zinc released from zincergic neurons [79–81]. Glutamate excitotoxicity is associated with pathophysiology of Alzheimer’s disease [67]. Glutamatergic signaling is compromised by β-amyloid-induced modulation of synaptic glutamate receptors in specific brain regions, paralleling early cognitive deficits [82]. Dietary zinc deficiency significantly increases total plaque volume in APP/PS1 mice, a transgenic mouse model of Alzheimer’s disease, suggesting that zinc deficiency is a risk factor for Alzheimer’s disease [83]. Interestingly, no obvious changes in histochemically reactive zinc levels are observed in zinc-deficient APP/PS1 mice. It is possible that the HPA axis activity in APP/PS1 mice is potentiated by zinc deficiency, like the case of normal mice and rats. Serum glucocorticoids are associated with the clearance of amyloid-beta peptide [84]. Thus, it seems to be important to study the participation of glucocorticoids in the β-amyloid plaque formation and degradation.

5. Zinc Homeostasis and Glutamate Excitotoxicity in Old Age

Zinc concentration in the brain remains constant in aged animals [85] and humans [4], whereas serum zinc level is significantly lower in aged animals than in young animals [86] and decreases with age in humans [87]. Histochemically reactive zinc levels are also lower in aged animals than in adult animals [88, 89]. Zinc transporter-3 expression, which is correlated with histochemically reactive zinc levels, is decreased with aging [90]. Thus, it is possible that histochemically reactive zinc levels are reduced in normal aging in humans [12, 90] (Figure 3). On the other hand, serum glucocorticoid concentration is significantly higher in aged animals [91]. The selective increase in the nocturnal levels of cortisol is observed in aged humans [92]. The increase in serum glucocorticoid level elicits some common changes in both aging and zinc deficiency. In addition to the decrease in serum zinc, the increase in the basal levels of intracellular Ca2+ and modification of Ca2+ signaling is observed in both aged [93, 94] and zinc-deficient [73, 77, 95] animals. It is likely that glucocorticoids influence the dynamics of both zinc signal and calcium signal and that the increased glucocorticoid secretion is associated with dysfunctions in zinc deficiency and aging that may increase the risk of diseases [28]. Aged animals and human might be more susceptible to glutamate excitotoxicity that is potentiated in zinc-deficient animals.

Insulin-degrading enzyme is a candidate protease in the clearance of amyloid-beta peptide from the brain and its levels are decreased in Alzheimer’s disease. Insulin-degrading enzyme activity is known to be inhibited by glucocorticoid. Serum cortisol is associated with the clearance of amyloid-beta peptide [81] and the progression in subjects with Alzheimer-type dementia [96, 97]. Correlations have been reported between increases in HPA system activity and dementia severity or hippocampal volume loss in individuals with probable Alzheimer’s disease [96]. On the other hand, serum zinc is decreased in progression of Alzheimer’s disease [98]. Because zinc participates in amyloid-beta plaque deposition [79–81, 99], this metal may play as a key-mediating factor in the pathophysiology of Alzheimer’s disease [100, 101]. Adlard et al. [90] report that cognitive loss is observed in 6-month-old zinc transporter-3-null mice, but not in 3-month-old zinc transporter-3-null mice. Cognitive impairment is age-dependent in zinc transporter-3-null mice, suggesting that long-term lack of synaptic zinc is implicated in the pathology leading to Alzheimer’s disease.
Because zinc transporter-3 expression is reduced in the brain with Alzheimer’s disease [90], it is possible that histochemical reactive zinc level is reduced in progression of Alzheimer’s disease and that this reduction participates in its pathophysiology. In contrast, histochemically reactive zinc levels are not significantly changed in zinc-deficient APP/PS1 mice as described above [83]. Cognitive loss is potentially observed prior to the decrease in histochemically reactive zinc in zinc-deficient rats [102]. Judging from these data, it is likely that the increase in HPA axis activity participates in the pathogenesis and progression of Alzheimer’s disease (Figure 3). This increase might be associated with the decrease in histochemically reactive zinc levels.

The basal (resting) level of histochemical reactive zinc/Zn$^{2+}$ is estimated to be pico- to nanomolar in the cytosolic compartment ($8.1 < \log[Zn^{2+}]_{\text{free}} < 10$) [103–105]. The synaptic vesicles serve as a large pool of histochemical reactive zinc in zincergic neurons. Other organelles such as the mitochondria and the endoplasmic reticulum might generally serve as the pool of histochemical reactive zinc in neurons and glia cells [36, 106]. Metallothioneins are also pools of Zn$^{2+}$ [37, 38, 107]. On the other hand, extracellular zinc concentration after tetanic stimulation is estimated to range between 10 and 100$\mu$M, because the low-affinity site (IC$_{50} \approx 20\mu$M at $-40$mV) of NMDA receptors is bound by zinc as an NMDA receptor blocker [108]. Hippocampal LTP is multifunctionally modulated in the presence of $5\mu$M ZnCl$_2$ [43, 109–111], suggesting that the concentration of endogenous zinc reaches very low micromolar concentrations in the extracellular compartment during the LTP induction. Judging from this estimation, it is possible that zinc signal transiently increases to more than 100 times of the basal level in the cytosolic compartment. Zn$^{2+}$ might potentially reach submicromolar concentrations ($-\log[Zn^{2+}]_{\text{free}} < 6$) under pathological conditions [105].

In conclusion, the analysis on the relationship between Zn$^{2+}$ dynamics and glutamatergic (zincergic) neuron activity in the brain in process of aging may be useful to find out the strategy to prevent neurodegenerative disorders such as Alzheimer’s disease [112].

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