Current Topics

The Target Factor of Heavy Metal Toxicity and Its Molecular Mechanism

Review

Age-Dependent Modification of Intracellular Zn\(^{2+}\) Buffering in the Hippocampus and Its Impact

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The basal concentrations of extracellular Zn\(^{2+}\) and intracellular Zn\(^{2+}\), which are approximately 10 nM and 100 pM, respectively, in the brain, are markedly lower than those of extracellular Ca\(^{2+}\) (1.3 mM) and intracellular Ca\(^{2+}\) (100 nM), respectively, resulting in much less attention paid to Zn\(^{2+}\) than to Ca\(^{2+}\). However, intracellular Zn\(^{2+}\) dysregulation, which is closely linked with glutamate- and amyloid \(\beta\)-mediated extracellular Zn\(^{2+}\) influx, is more critical for cognitive decline and neurodegeneration than intracellular Ca\(^{2+}\) dysregulation. It is estimated that the age-dependent increase in the basal concentration of extracellular Zn\(^{2+}\) in the hippocampus plays a key role in cognitive decline and neurodegeneration. The characteristics of extracellular Zn\(^{2+}\) influx in the hippocampus may be modified age-dependently, probably followed by modification of intracellular Zn\(^{2+}\) buffering that is closely linked with age-related cognitive decline and neurodegeneration. Reducing intracellular Zn\(^{2+}\)-buffering capacity may be linked with the pathophysiology of progressive neurodegeneration such as Alzheimer’s disease. This paper deals with age-dependent modification of intracellular Zn\(^{2+}\) buffering in the hippocampus and its impact. On the basis of the estimated impact, we propose a potential defense strategy against Zn\(^{2+}\)-mediated neurodegeneration, i.e., metallothionein induction in the hippocampus.

Key words Zn\(^{2+}\); metallothionein; ZnAF-2DA; Zn\(^{2+}\) buffering; hippocampus; aging

1. INTRODUCTION

Zrt-Irt-like proteins (ZIPs), which are involved in the transport of Zn\(^{2+}\) into the cytoplasm, and the zinc transporter (ZnT) family, which is involved in the transport of Zn\(^{2+}\) out of the cytoplasm, serve to maintain Zn\(^{2+}\) homeostasis in the living body including the brain.\(^\text{1–3}\) The zinc concentration in the brain increases in the process of development (infant brain, 8.2 ± 0.8 \(\mu\)g/g wet weight) and reaches 13.3 ± 0.3 \(\mu\)g/g wet weight in the human adult brain.\(^\text{4}\) Zinc homeostasis in the brain is strictly regulated through the brain barrier system, i.e., the blood–brain and blood–cerebrospinal fluid (CSF) barriers.\(^\text{5,6}\) The cells constructing the brain barrier system, which express ZIPs and ZnT, regulate Zn\(^{2+}\) transport into the brain parenchyma, i.e., neurons and glial cells.\(^\text{7}\) Zn\(^{2+}\) is very slowly transported through the brain barrier system, especially the blood–CSF barrier.\(^\text{8,9}\)

Approximately 80% of brain zinc is zinc metalloproteins, while approximately 20%, which is histochemically reactive with ZIPs and ZnT, regulates Zn\(^{2+}\) transport into the brain parenchyma, i.e., neurons and glial cells.\(^\text{7}\) Zn\(^{2+}\) is very slowly transported through the brain barrier system, especially the blood–CSF barrier.\(^\text{8,9}\)

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the brain and is approximately 1.3 mM in the CSF and brain extracellular fluid in adult rats. Artificial cerebrospinal fluid (ACSF) used for in vivo and in vitro experiments contains approximately 2 mM Ca$^{2+}$, based on the essentiality of intracellular Ca$^{2+}$ signaling for neurons and glial cells. However, excess influx of extracellular Ca$^{2+}$ into neurons, which is induced by glutamate excitotoxicity, is involved in the pathological process of neuronal death.

The basal concentrations of extracellular Zn$^{2+}$ and intracellular Zn$^{2+}$, which are approximately 10 nM and 100 pM, respectively, in the brain, are markedly lower than those of extracellular Ca$^{2+}$ (1.3 mM) and intracellular Ca$^{2+}$ (100 mM), respectively, resulting in much less attention paid to Zn$^{2+}$ than to Ca$^{2+}$. For studying synaptic function, no attention has been paid to Zn$^{2+}$. Zn$^{2+}$ is not included in ACSF, i.e., the brain extracellular medium widely used for in vitro and in vivo experiments. Spontaneous presynaptic activity in the stratum lucidum where mossy fibers are contained, which is determined with FM4-64, an indicator of exocytosis (presynaptic activity), is suppressed in brain slices from young rats bathed in ACSF containing 10 nM Zn$^{2+}$, indicating that glutamatergic presynaptic activity is enhanced in brain slices bathed in ACSF without Zn$^{2+}$. We reported that not only neuronal excitation but also synaptic plasticity such as long-term potentiation (LTP) and intracellular Ca$^{2+}$ buffering, which is involved in the pathological process of neuronal death.

The action of extracellular Zn$^{2+}$ at physiological concentrations is important to understand synaptic function precisely as well as to understand bidirectional Zn$^{2+}$ actions under physiological and pathological conditions. It has been recognized that low nanomolar concentrations of Zn$^{2+}$ are more physiologically relevant than its micromolar concentrations, which have been widely used and are often neurotoxic. On the other hand, the role of endogenous Zn$^{2+}$ released from zincergic neurons has been studied in acute brain slice preparations using ACSF without Zn$^{2+}$. Because zinc concentrations in the presynaptic vesicles are reduced in the process of slice preparation, it is estimated that in vitro Zn$^{2+}$ release is reduced to approximately 25% as compared with in vivo Zn$^{2+}$ release. Interestingly, the extracellular zinc concentration, which was determined by in vivo microdialysis, increases age-dependently in the hippocampus, suggesting that the extracellular Zn$^{2+}$ concentration is also physiologically increased in the hippocampus with aging.

3. MODIFICATION OF INTRACELLULAR ZN$^{2+}$ BUFFERING WITH AGING

It was reported that vulnerability to Ca$^{2+}$ dysregulation is facilitated in the process of brain aging. Ca$^{2+}$ dysregulation is not ubiquitous in the brain but has been observed in specific cell populations and areas. For example, the expression of L-type Ca$^{2+}$ channels is age-dependently elevated in hippocampal pyramidal cells. N-Methyl-D-aspartate (NMDA) receptor function is age-dependently reduced in the hippocampus, suggesting that a compensatory mechanism is induced in the process of brain aging to maintain the availability of intracellular Ca$^{2+}$ signaling. On the other hand, intracellular Ca$^{2+}$ buffering, which is involved not only in cognitive function but also in neurodegeneration, is weakened during brain aging.

To maintain the availability of intracellular Zn$^{2+}$ signaling, it is likely that a compensatory mechanism is also induced in the process of brain aging. The ZnT-3-dependent zinc concentration in presynaptic vesicles is decreased with aging, while the extracellular zinc concentration is age-dependently increased in the hippocampus. Intracellular Zn$^{2+}$ buffering is critical not only for cognitive function but also for neurodegeneration via regulating the availability of intracellular Zn$^{2+}$ signaling. However, the Zn$^{2+}$-buffering system is more poorly understood than the Ca$^{2+}$-buffering system. It was reported that weakened intracellular Ca$^{2+}$ buffering, with a net decrease in the Ca$^{2+}$-buffering capacity, is linked with both normal aging and neurological disorders such as AD.

The Zn$^{2+}$-buffering system is composed of Zn$^{2+}$ transporters (ZIPs and ZnT), Zn$^{2+}$-binding proteins such as metallothioneins (MTs), internal stores containing Zn$^{2+}$, and Ca$^{2+}$-permeable channels, which is dynamically linked with synaptic activity. Judging from the increased extracellular Zn$^{2+}$ concentration and extracellular Zn$^{2+}$ influx in the aged rat hippocampus, it is estimated that intracellular Zn$^{2+}$ buffering is modified in the dentate gyrus of aged rats.
Glutamate excitotoxicity is a common pathway to neurodegeneration, and extracellular glutamate accumulation causes neurodegeneration via $\text{Ca}^{2+}$ influx through calcium channels such as NMDA receptors. On the other hand, $\text{Zn}^{2+}$ can pass through calcium channels and preferentially passes through GluR2-lacking $\text{Ca}^{2+}$-permeable $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors. It was reported that glutamate excitotoxicity, which is involved in the pathology of most neurodegenerative disorders including ischemia, epilepsy, and AD, is due to $\text{Zn}^{2+}$ neurotoxicity rather than to $\text{Ca}^{2+}$ neurotoxicity. This may be due to the extremely low concentration (approx. 100pM) of basal intracellular $\text{Zn}^{2+}$, which reflects the necessity of strict $\text{Zn}^{2+}$ regulation in the intracellular (cytosolic) compartment to maintain the intracellular environment.

Aging is a major risk factor for the onset of AD. The first step in pathological alteration in the AD brain is the abnormal processing of $\text{A}\beta$ peptides and their accumulation, which is initiated more than two decades before the onset of AD. $\text{A}\beta$ is secreted into the extracellular space after sequential cleavage of the amyloid precursor protein (APP). $\text{A}\beta$ has self-aggregation ability and $\text{A}\beta$ fibrils are the main components of $\text{A}\beta$ plaques, a pathological hallmark of AD. $\text{A}\beta$ is bound to $\text{Zn}^{2+}$ via histidine residues, and the $K_d$ values of $\text{Zn}^{2+}$ to $\text{A}\beta_{1-42}$ are in the range of 0.1–60µM. On the other hand, it was reported that the formation and propagation of misfolded aggregates of $\text{A}\beta_{1-42}$ rather than of $\text{A}\beta_{1-40}$ contribute to AD pathogenesis. However, the structural details of misfolded $\text{A}\beta_{1-42}$ are poorly understood.

The C-terminal carboxylate anion of $\text{A}\beta_{1-42}$ constructs the C-terminal hydrophobic core, which accelerates neurotoxic oligomerization. C-terminal Ala42 that is absent in $\text{A}\beta_{1-40}$ constrains a salt bridge with Lys28 to create a self-recognition molecular switch, which is the $\text{A}\beta_{1-42}$-selective self-replicating amyloid-propagation machinery. The aggregating property of $\text{A}\beta_{1-42}$ is rapidly promoted with $\text{Zn}^{2+}$, resulting in much higher affinity of $\text{A}\beta_{1-42}$ to $\text{Zn}^{2+}$ than that of $\text{A}\beta_{1-40}$, and the $K_d$ values of $\text{Zn}^{2+}$ to $\text{A}\beta_{1-42}$ are in the range of 3–30nM. Unlike $\text{A}\beta_{1-40}$, captures extracellular $\text{Zn}^{2+}$ at high picomolar levels, and the formation of $\text{Zn-}\text{A}\beta_{1-42}$ in the extracellular compartment is essential for $\text{A}\beta_{1-42}$ uptake into dentate granule cells, followed by cognitive decline (Fig. 2). Soluble $\text{A}\beta_{1-42}$ oligomers that are strong synaptotoxic molecules in AD induce synaptic dysfunction and cognitive decline.

The cytosolic $\text{Zn}^{2+}$ concentration in dentate granule cells is much lower than the extracellular $\text{Zn}^{2+}$ concentration. When $\text{Zn-}\text{A}\beta_{1-42}$ forms, is taken up into dentate granule cells, $\text{Zn}^{2+}$ can be released from $\text{A}\beta_{1-42}$ resulting in an increase in neurotoxic $\text{Zn}^{2+}$ (Fig. 2), which is assessed based on the increase in intracellular $\text{ZnAF-2}$ fluorescence. $\text{Zn-}\text{A}\beta_{1-42}$ uptake is increased in the aged dentate gyrus compared with the young dentate gyrus, consistent with the estimated increase in extracellular $\text{Zn}^{2+}$ in the hippocampus with aging. It is likely that $\text{Zn}^{2+}$ neurotoxicity, which originates in $\text{A}\beta_{1-42}$, is readily induced with aging. On the other hand, weakened intracellular $\text{Zn}^{2+}$ buffering is observed in the aged dentate gyrus. Although aging is a major risk factor for AD, the
characteristic age-related Zn-Aβ1–42 formation in the extracellular compartment and weakened intracellular Zn²⁺ buffering may be linked with the major risk.

It is possible that the Aβ1–42-mediated increase in intracellular Zn²⁺ facilitates hyperphosphorylation of the microtubule-associated protein tau, which is a main pathological hallmark as well as hippocampus and affects axonal transport, subsequently leading to neurodegeneration. Neuronal loss, tau hyperphosphorylation, and hippocampus-dependent cognitive decline are observed in mice subjected to repeated injection of Aβ1–42 oligomers into the hippocampus.⁵³) Tau hyperphosphorylation in AD is linked with a reduction in protein phosphatase 2A (PP2A) activity.⁶⁴,⁶⁵) PP2A regulates tau phosphorylation at multiple sites in the normal human brain. Zn²⁺ promotes tau hyperphosphorylation by inactivating PP2A.⁶⁶,⁶⁷) Zn²⁺ binds PP2A and directly inhibits its activity at a low micromolar concentration of Zn²⁺ (10 μM) in vitro.⁶⁸) Furthermore, Zn²⁺ 0.25 μM changes the conformation of tau, and Zn²⁺ 5 μM promotes tau aggregation in vitro.⁶⁹) Although the Zn²⁺ concentrations used in in vitro experiments is extremely high compared with physiologically estimated intracellular Zn²⁺ in the in vivo picomolar range, it is possible that a local increase in intracellular Zn²⁺ induced by Aβ1–42 is linked with tau hyperphosphorylation via PP2A inactivation and tau aggregation (Fig. 2).

5. PERSPECTIVE ON A DEFENSE STRATEGY AGAINST NEURODEGENERATION INDUCED BY ZN-Aβ1–42

The blockage of Aβ1–42 binding to extracellular Zn²⁺ and capturing Zn²⁺ from Zn-Aβ1–42 in the intracellular compartment, which are performed using extracellular Zn²⁺ and intracellular Zn²⁺ chelators, respectively, may be an effective strategy for preventing Aβ1–42-mediated cognitive decline and neurodegeneration.⁷⁰) The basal level of MTs in the hippocampus is approximately 65 nM in young rats, as determined in the silver-binding assay.⁴⁷) MTs can bind seven micromolar concentration of Zn²⁺ (10⁻⁸ to 10⁻¹² M), and cytosolic MTs exist mostly in the form of Zn₀MT under physiological (static) conditions, which has the capacity to capture two more free Zn²⁺.⁷ⁱ) Thus, it is estimated that hippocampal MTs can buffer 130 nM of free Zn²⁺ in the cytosol of young rats. Even if the cytosolic Zn²⁺ concentration reaches the extracellular concentration (approx. 10 nM) via Aβ1–42-mediated Zn²⁺ influx, induced MTs capture Zn²⁺ released from Zn-Aβ1–42. On the other hand, the basal level of MTs in the hippocampus is slightly higher in aged than in young rats. When the increase in intracellular Zn²⁺-buffering capacity is compared between the young and aged hippocampus, as determined based on the reduction in intracellular ZnAF-2 fluorescence, after the systemic administration of dexamethasone, there is no appreciable different between them.⁷²) Therefore, a blood–brain barrier-permeable MT-inducing agent may be promising for preventing neurodegenerative disorders such as AD linked with intracellular Zn²⁺ toxicity⁷²) (Fig. 2).

Conflict of Interest

The authors declare no conflict of interest.

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