New Insights into Neurozinc and Metallothioneins

Review

Is Vulnerability of the Dentate Gyrus to Aging and Amyloid- β_{1-42} Neurotoxicity Linked with Modified Extracellular Zn^{2+} Dynamics?

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The basal levels of extracellular Zn^{2+} are in the range of low nanomolar concentrations in the hippocampus and perhaps increase age-dependently. Extracellular Zn^{2+} dynamics is critical for cognitive activity and excess influx of extracellular Zn^{2+} into hippocampal neurons is a known cause of cognitive decline. The dentate gyrus is vulnerable to aging in the hippocampus and affected in the early stage of Alzheimer's disease (AD). The reasons remain unclear. Neurogenesis-related apoptosis may induce non-specific neuronal depolarization by efflux of intracellular K^+ in the dentate gyrus and be markedly increased along with aging. Extracellular Zn^{2+} influx into dentate granule cells *via* high K^+ -induced perforant pathway excitation leads to cognitive decline. Modified extracellular Zn^{2+} dynamics in the dentate gyrus of aged rats is linked with vulnerability to cognitive decline. Amyloid- β_{1-42} ($\text{A}\beta_{1-42}$) is a causative candidate for AD pathogenesis. When $\text{A}\beta_{1-42}$ concentration reaches picomolar in the extracellular compartment in the dentate gyrus, $\text{Zn-A}\beta_{1-42}$ is formed in the extracellular compartment and rapidly taken up into dentate granule cells, followed by $\text{A}\beta_{1-42}$ -induced cognitive decline that is due to Zn^{2+} released from $\text{A}\beta_{1-42}$, suggesting that dentate granule cells are sensitive to extracellular Zn^{2+} -dependent $\text{A}\beta_{1-42}$ toxicity. This paper deals with proposed vulnerability of the dentate gyrus to aging and $\text{A}\beta_{1-42}$ neurotoxicity.

Key words zinc; amyloid- β ; dentate gyrus; aging

1. INTRODUCTION

Hippocampal formation is a key area for memory and recognition of novelty and is composed of the entorhinal cortex, the dentate gyrus, the CA3 and the CA1 subfields, and the subiculum. Information on memory from the entorhinal cortex is input to the dentate gyrus and processed by an unidirectional circuit, *i.e.*, the dentate gyrus–CA3–CA1 trisynaptic circuits and the subiculum.^{1,2)} The entorhinal cortex is also directly connected with the CA3 and the CA1 subfields, in addition to the dentate gyrus.³⁾ These main neural circuits are glutamatergic and glutamate is a key neurotransmitter for memory.

Changes in both presynaptic and postsynaptic strength, *i.e.*, synaptic plasticity such as long-term potentiation (LTP) and long-term depression (LTD), have been extensively studied in glutamatergic synapses of the hippocampus.^{4,5)} Ca^{2+} -permeable α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors are dynamically linked with the influx of extracellular Zn^{2+} and intracellular Zn^{2+} signaling plays a key role for cognitive performance and memory as well as intracellular Ca^{2+} signaling.^{6,7)} However, glutamate receptor activation by excess extracellular glutamate induces glutamate excitotoxicity^{8,9)} and leads to a common pathway for neuronal death.^{10,11)} Hippocampal CA1 pyramidal cells are the most susceptible to neuronal death in the hippocampus after stroke/ischemia. CA1 pyramidal cell death is due to extracellular Zn^{2+} influx into postsynaptic neurons through Ca^{2+} -permeable AMPA receptors, which is dynamically linked with Zn^{2+} release from zincergic Shaffer collaterals, a subclass of glu-

tamatergic fibers, which concentrates zinc in the presynaptic vesicles.^{12–16)} Approximately 50% of the Shaffer collaterals are zincergic. On the other hand, it has been reported that the dentate gyrus is vulnerable to aging in the hippocampus.²⁾

Alzheimer's disease (AD), a progressive neurological disease, is the most common cause of dementia in elderly people.¹⁷⁾ In AD, pathogenic progression may last for 20–30 years before clinical onset. Individuals with mild cognitive impairment (MCI) have approximately 30% fewer neurons in the entorhinal cortex and show synaptic loss to the dentate gyrus, which correlates with cognitive impairment.^{18–20)} This cellular disconnection in the dentate gyrus suggests that this region is one of the earliest sites to show synaptic dysfunction.²¹⁾ In postmortem studies, it is reported that the hippocampus and entorhinal cortex may be the first brain regions to be affected in AD.²²⁾ Amyloid- β ($\text{A}\beta$) is the major component of neuritic senile plaques in AD brain and is a causative candidate for AD pathogenesis.

Interestingly, extracellular Zn^{2+} influx into hippocampal neurons is increased by not only glutamatergic synapse excitation^{23,24)} but also extracellular $\text{A}\beta_{1-42}$.²⁵⁾ Glutamatergic synapse excitation rapidly increases intracellular Zn^{2+} through Ca^{2+} -permeable AMPA receptors followed by cognitive decline. The formation of $\text{Zn-A}\beta_{1-42}$ in the extracellular compartment also rapidly increases intracellular Zn^{2+} followed by $\text{A}\beta_{1-42}$ -induced cognitive decline. The evidence indicates that modification of extracellular Zn^{2+} dynamics, which is linked with age-related changes, is critical for cognitive decline. It is estimated that the dentate gyrus is vulnerable to this modifica-

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tion. The present paper deals with proposed vulnerability of the dentate gyrus to aging and $A\beta_{1-42}$ neurotoxicity.

2. PHYSIOLOGY OF HIPPOCAMPAL Zn^{2+} IN SYNAPTIC PLASTICITY

Zn^{2+} concentration in the brain extracellular fluid, which is estimated to be approximately 10 nM in adults,²⁶⁾ is much lower than Ca^{2+} concentration (*ca.* 1.3 mM). Thus much less attention has been paid to essentiality of Zn^{2+} in brain extracellular fluid for synaptic neurotransmission. Artificial cerebrospinal fluid (ACSF), *i.e.*, brain extracellular medium, without Zn^{2+} has been widely used for *in vitro* and *in vivo* experiments. However, synaptic neurotransmission including not only neuronal excitation but also synaptic plasticity such as LTP is modified in brain slices bathed in ACSF without Zn^{2+} .²⁷⁾ Extracellular Zn^{2+} in the range of physiological concentrations is important to understand synaptic function precisely. Low nanomolar concentrations of Zn^{2+} are physiologically relevant, compared with micromolar concentrations of Zn^{2+} widely used, which are often neurotoxic.²⁸⁾

Spontaneous presynaptic activity in the stratum lucidum where zincergic mossy fibers are contained is suppressed in brain slices from young rats bathed in ACSF containing 10 nM Zn^{2+} , indicating that extracellular Zn^{2+} suppressively modulates hippocampal presynaptic activity.²⁹⁾ Micromolar Zn^{2+} also suppresses hippocampal mossy fiber exocytosis.³⁰⁾ It is likely that Zn^{2+} dose-dependently suppresses presynaptic activity in the hippocampus. To examine the *in vivo* action of 10 nM Zn^{2+} on LTP, furthermore, the recording region is perfused using a recording electrode attached to a microdialysis probe. The magnitude of LTP at medial perforant pathway-dentate granule cell synapses is not significantly modified in young rats under perfusion with ACSF containing 10 nM $ZnCl_2$, compared to perfusion with ACSF without Zn^{2+} . Interestingly, the magnitude of LTP is attenuated in young rats under perfusion with ACSF containing 100 nM $ZnCl_2$, whereas it is not modified in aged rats. In aged rats, the magnitude of LTP is enhanced under perfusion with ACSF containing 10 mM Ca-ethylenediaminetetraacetic acid (CaEDTA), an extracellular Zn^{2+} chelator. The findings suggest that the basal levels of extracellular Zn^{2+} , which are the range of low nanomolar concentrations (*ca.* 10 nM), are age-dependently increased in the aged dentate gyrus and negatively modulate LTP.²⁹⁾ The finding that extracellular zinc concentration in the hippocampus determined by hippocampal perfusion using the microdialysis technique is increased age-dependently supports this idea.³¹⁾ It is likely that the basal level of extracellular Zn^{2+} physiologically reaches approximately 100 nM in aged rats.

It is reported that zinc concentration in CSF is in the range 150–380 nM.^{32–34)} If it is the same as zinc concentration in the brain extracellular fluid, a large portion of zinc is not free ion in the brain extracellular fluid under the basal (static) condition. At zincergic synapses such as Shaffer collateral and mossy fiber synapses, extracellular Zn^{2+} levels are dynamically modified by the degree of activity-dependent Zn^{2+} release, which is required for learning and memory *via* synaptic plasticity as described below.^{6,7)} ZnAF-2DA, a membrane-permeable Zn^{2+} indicator, is a useful tool for examining the direct involvement of synaptic Zn^{2+} in cognitive function. ZnAF-2DA is taken up into cells through the cell membrane

and is hydrolyzed by esterase in the cytosol followed by the production of ZnAF-2, which cannot permeate the cell membrane.^{35,36)} When ZnAF-2DA is locally injected into the hippocampus, ZnAF-2 (K_d , 2.7 nM for Zn^{2+}) taken up into neurons blocks cellular transient changes in Zn^{2+} concentration, *i.e.*, intracellular Zn^{2+} signaling only in the injected area.

The concurrent tests of *in vivo* LTP and learning behavior in separated experiments using ZnAF-2DA indicates essentiality of synaptic Zn^{2+} for cognition; the influx of extracellular Zn^{2+} into CA1 pyramidal cells, which is linked to Zn^{2+} release from the Schaffer collaterals, is required for object recognition memory *via in vivo* Schaffer collateral LTP.⁶⁾ On the other hand, glutamatergic input to CA1 pyramidal cells *via* the medial perforant pathway (the temporoammonic pathway) from the entorhinal cortex facilitates memory consolidation and is required for temporal association memory and spatial working memory.^{37–39)} Although the medial perforant pathway from the entorhinal cortex is non-zincergic,⁴⁰⁾ intracellular Zn^{2+} signaling, which originates in internal stores/proteins, is involved in LTP at medial perforant pathway-CA1 pyramidal cell synapses.⁷⁾ Cellular transient changes in Zn^{2+} concentration from both extracellular and intracellular compartments function in CA1 pyramidal cells.

In dentate granule cells, which are innervated by the non-zincergic medial perforant pathway, intracellular Zn^{2+} signaling, which also originates in internal stores/proteins, is involved in object recognition memory *via* LTP at medial perforant pathway-dentate granule cells synapses.⁴¹⁾ LTP maintenance underlies memory retention. The mechanism maintaining medial perforant pathway LTP also requires intracellular Zn^{2+} signaling in dentate granule cells, which is involved in the formation of F-actin, and retains space recognition memory.⁴²⁾ It is reported that the basal concentration of intracellular (cytosol) Zn^{2+} is less than 1 nM.^{43,44)} Cellular transient changes in Zn^{2+} concentration are used as a marker for plastic changes in synapse function and structure induced by learning and cognitive performance. Intracellular Zn^{2+} levels can transiently reach a few nanomolar concentrations after LTP induction, while the changes in intracellular Zn^{2+} level remain unclear in the process of LTP maintenance. LTP maintenance at medial perforant pathway-dentate granule cell synapses is affected by blocking intracellular Zn^{2+} signaling with ZnAF-2DA.^{42,45)} Zn^{2+} seems to be required for enlarging synapse structure and also maintaining the enlargement.⁴⁶⁾

3. MODIFICATION OF EXTRACELLULAR Zn^{2+} DYNAMICS AND ITS RELATIONSHIP TO $A\beta$ NEUROTOXICITY

New neurons are generated from neural stem/progenitor cells in the subgranular zone of the dentate gyrus throughout life. As aging progresses, the rate of neurogenesis decreases exponentially, which might be responsible for age-related cognitive decline in humans and animals.^{47,48)} Because neurogenesis-related apoptosis also occurs in the subgranular zone, the apoptosis is likely to be significantly increased along with aging. It is possible that the apoptosis is involved in the vulnerability of the dentate gyrus to aging, which might be linked with cognitive decline.⁴⁹⁾ The apoptosis locally elevates extracellular K^+ concentration in the subgranular zone and the elevation is due to the efflux of intracellular K^+ (ap-

Fig. 1. A Model for Neuronal Intoxication by Zn- $A\beta_{1-42}$ Complexes

Dentate granule neurons only take up $A\beta_{1-42}$ when the peptide is bound to Zn^{2+} , whereupon Zn- $A\beta_{1-42}$ complexes enter dentate granule neurons and either the complexes or the liberated Zn^{2+} induces cognitive decline. Cd^{2+} can displace Zn^{2+} from $A\beta_{1-42}$ and prevent it from being taken up into dentate granule neurons, neutralizing its neurotoxicity. Zn^{2+} is withdrawn into peptide complexes more readily by oligomers. $A\beta_{1-42}$ more rapidly forms oligomers and fosters neuronal uptake more readily than $A\beta_{1-40}$.⁶²⁾ (Color figure can be accessed in the online version.)

proximately 140 mM) by disruption of cell membranes. The elevation of extracellular K^+ concentration can non-specifically excite granule cells, which exist nearby in the dentate gyrus, followed by rapid increase in extracellular Zn^{2+} influx via excess glutamate signaling. Both memory acquisition via LTP induction and memory retention via LTP maintenance are affected after local injection of high K^+ into the dentate gyrus, which rapidly increases intracellular Zn^{2+} in dentate granule cells through Ca^{2+} -permeable AMPA receptors.^{24,50)} Although high K^+ -induced Zn^{2+} influx also affects memory acquisition via LTP induction in the hippocampal CA1,²³⁾ neurogenesis-related apoptosis, which can be a cause of cognitive decline, may be involved in age-related modification of dentate gyrus function, in addition to the decreased neurogenesis.

Synaptic activity dynamically regulates extracellular $A\beta$ levels in the brain. Extracellular $A\beta$ levels are directly linked with synaptic vesicle exocytosis. $A\beta$ release from synaptic vesicle is linked with dynamic changes in extracellular $A\beta$ levels, which are independent of changes in APP processing.⁵¹⁾ In normal young animal experiments, endogenous $A\beta$ is involved in learning and memory and endogenous $A\beta_{1-42}$ supports LTP expression.^{52,53)} It is likely that $A\beta_{1-42}$ supports LTP and memory at picomolar concentrations under physiological conditions, while it affects them at pathological nanomolar concentrations.^{54,55)} Interestingly, intracellular Zn^{2+} dysregulation, which is a cause of cognitive decline, is induced by extracellular $A\beta_{1-42}$, as well as excess extracellular glutamate. Because Zn^{2+} readily induces toxic $A\beta$ oligomerization, this metal ion has been implicated in AD pathogenesis.⁵⁶⁻⁵⁸⁾ $A\beta$ is bound to Zn^{2+} via histidine residues and the K_d values of Zn^{2+} to $A\beta_{1-40}$ are in the range 0.1–60 μM .⁵⁹⁾ However, the K_d value of Zn^{2+} to $A\beta_{1-42}$ is unreported. It is estimated that, as is the case with Cu^{2+} binding to $A\beta$,⁶⁰⁾ the apparent K_d of metal binding to $A\beta_{1-42}$ is higher in affinity than that to $A\beta_{1-40}$ probably due to the disturbed equilibrium caused by the enhanced self-assembly of $A\beta_{1-42}$ oligomers.

Short-term memory loss emerges in normal elderly people and pathologically increases in the pre-dementia stage of AD.

In young rats, *in vivo* perforant pathway LTP is unaffected even under perfusion with 1000 nM $A\beta_{1-42}$ in ACSF without Zn^{2+} , while it is attenuated under pre-perfusion with 5 nM $A\beta_{1-42}$ in ACSF containing 10 nM Zn^{2+} , suggesting that $A\beta_{1-42}$ -induced attenuation of LTP is extracellular Zn^{2+} -dependent.⁶¹⁾ When extracellular $A\beta_{1-42}$ concentration reaches 500 pM in the dentate gyrus, Zn- $A\beta_{1-42}$ is rapidly formed in the extracellular compartment and taken up into dentate granule cells (Fig. 1). The rapid uptake of both $A\beta_{1-42}$ and Zn^{2+} is blocked by CaEDTA and by Cd^{2+} , a metal that displaces Zn^{2+} for $A\beta_{1-42}$ -binding. Local injection of $A\beta_{1-42}$ into the dentate granule cell layer of young rats induces rapid memory disturbance that is rescued by co-injection of Zn^{2+} chelators and $CdCl_2$.^{25,61)} Therefore once ferried by $A\beta_{1-42}$ into dentate granule cells, the Zn^{2+} cargo is released from $A\beta_{1-42}$ in dentate granule cells. $A\beta_{1-42}$ -mediated cognitive decline may be induced by the intrusion of $A\beta$, Zn^{2+} , or both together into the normal dentate granule cells⁶¹⁾ (Fig. 1). The *in vivo* rapid action of picomolar $A\beta_{1-42}$ with extracellular Zn^{2+} in the dentate gyrus suggests that Zn^{2+} has a high affinity for $A\beta_{1-42}$ (*in vivo* K_d value of Zn^{2+} to $A\beta_{1-42}$, approximately 3–30 nM) and that dentate granule cells are vulnerable to Zn- $A\beta_{1-42}$ neurotoxicity. On the other hand, $A\beta_{1-40}$ and Zn^{2+} are not taken up into dentate granule cells, consistent with lower affinity of $A\beta_{1-40}$ for Zn^{2+} than $A\beta_{1-42}$.⁶¹⁾ Blocking the formation of Zn- $A\beta_{1-42}$ in the extracellular compartment may be effective for preventing $A\beta_{1-42}$ -mediated cognitive decline (Fig. 1).

4. WEAKENED INTRACELLULAR Zn^{2+} -BUFFERING IN THE DENTATE GYRUS

It has been reported that weakened intracellular Ca^{2+} -buffering, a net decrease in the Ca^{2+} -buffering capacity is linked with not only aging but also neurological diseases such as AD.^{62,63)} Judging from the estimated elevation of extracellular Zn^{2+} concentration and enhancement of extracellular Zn^{2+} influx in aged rats,^{29,31)} it is possible that intracellular Zn^{2+} -buffering is modified in aged rats. High K^+ -induced

Fig. 2. Intracellular Zn^{2+} -Buffering Is Weakened in the Aged Dentate Gyrus

Intracellular Zn^{2+} -buffering is weakened at medial perforant pathway (MPP)-dentate granule cell synapses of the aged dentate gyrus⁴⁶⁾ and may be linked with vulnerability to cognitive decline. Zn - $\text{A}\beta_{1-42}$ -induced cognitive decline might more readily emerge because of weakened intracellular Zn^{2+} -buffering in the normal aged dentate gyrus. CC, Ca^{2+} channel; ZIP, zinc import protein. (Color figure can be accessed in the online version.)

increase in extracellular Zn^{2+} influx into dentate granule cells is enhanced in aged rats, followed by both attenuations of LTP induction and maintained LTP at medial perforant pathway-dentate granule cell synapses, suggesting that the aged dentate gyrus is vulnerable to cognitive decline associated with intracellular Zn^{2+} dysregulation.^{31,45)} Glutamate receptor 2 (GluR2)-lacking Ca^{2+} -permeable AMPA receptors play a key role for not only Zn^{2+} -mediated neuronal death in the hippocampal CA1,^{64,65)} but also Zn^{2+} -mediated cognitive decline.^{31,45)} In the hippocampus, levels of mRNA encoding GluR1 and GluR2 are highest in the dentate gyrus and the GluR1/GluR2 mRNA ratios are increased along with aging.⁶⁶⁾ Influx of extracellular Zn^{2+} through Ca^{2+} -permeable AMPA receptors may be involved in age-related cognitive decline associated with intracellular Zn^{2+} dysregulation^{31,45)} (Fig. 2).

When ZnAF-2DA is locally injected into the dentate gyrus, interestingly, the Zn^{2+} -binding capacity of intracellular ZnAF-2, which is assessed by changes in fluorescence intensity, is more rapidly lost in the aged dentate molecular layer where medial perforant pathway-dentate granule cell synapses are contained than in the young dentate molecular layer. The data suggest that intracellular Zn^{2+} -buffering capacity is decreased in the aged dentate gyrus⁴⁵⁾ (Fig. 2). Characteristics (easiness) of extracellular Zn^{2+} influx may be linked with decreased intracellular Zn^{2+} -buffering capacity in the aged dentate gyrus.⁴⁵⁾ The intracellular Zn^{2+} -buffering system remains unclear and Ca^{2+} -permeable channels, Zn^{2+} transporters (ZIP and ZnT), Zn^{2+} -binding proteins such as metallothioneins, and internal stores containing Zn^{2+} may construct the Zn^{2+} -buffering system. The vulnerability of the dentate gyrus to aging and $\text{A}\beta_{1-42}$ neurotoxicity seems to be linked with age-related modification of extracellular Zn^{2+} dynamics, which is induced with glutamate and $\text{A}\beta_{1-42}$ in the extracellular compartment (Fig. 2).

5. PERSPECTIVE

Intracellular Zn^{2+} -buffering is weakened in the dentate gyrus along with aging, probably due to easiness of extracellular Zn^{2+} influx into dentate granule cells (Fig. 2), which increases vulnerability to cognitive decline. $\text{A}\beta_{1-42}$ -induced cognitive decline, which is extracellular Zn^{2+} -dependent, is due to increase in Zn^{2+} released from $\text{A}\beta_{1-42}$ in dentate granule cells (Fig. 1). Intracellular Zn^{2+} -buffering may be also weakened by formation of Zn - $\text{A}\beta_{1-42}$ in the extracellular compartment (Fig. 2). To find out a strategy for preventing $\text{A}\beta_{1-42}$ -mediated cognitive decline, therefore, it is important to understand the relation between age-related modifications of extracellular Zn^{2+} dynamics and the Zn^{2+} -buffering system.

Conflict of Interest The authors declare no conflict of interest.

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