Do We Need Zinc to Think?

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(Published 13 May 2003)

Zinc (Zn^{2+}) is found in every cell in our bodies. Most is tightly bound to proteins, but certain neurons in our brains contain a relatively large pool of free Zn^{2+} sequestered in vesicles in their terminals. These neurons, which use glutamate as a transmitter, are not uniformly distributed, but are concentrated in certain forebrain regions, including the hippocampus, amygdala, and neocortex (*1*). What possible function could this chelatable Zn^{2+} have?

It turns out that it could have many functions. We are just beginning to learn which actions of Zn^{2+} operate in situ, and how they might interact under normal physiological conditions to affect how we think. Zn^{2+} appears not only to be released as a neurotransmitter, but also to behave as a second messenger in the neurons that receive these glutamate signals. Additionally, Zn^{2+} can modulate the activity of various ion channels and neurotransmitter receptors, including the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptor. A recent paper by Ueno and colleagues (*2*) demonstrates that synaptically released Zn^{2+} can modulate NMDA receptor-mediated responses in regions neighboring the active synapses. Combining Zn^{2+} imaging with electrophysiological recording during electrical stimulation of the CA3 region of rat hippocampus slices, the authors confirmed release of Zn^{2+} from mossy fiber terminals in one synaptic region of the dendrites that bear few NMDA receptors. They went on to demonstrate extracellular diffusion (or "spillover") of Zn^{2+} from mossy fiber terminals into a neighboring synaptic region, the stratum radiatum, where the dendrites express abundant NMDA receptors. There it alters the response of NMDA receptors to glutamate released from nerve terminals in the stratum radiatum. NMDA receptors are involved in pathological conditions, as well as normal processes such as learning and memory. Thus, Zn^{2+} spillover provides a mechanism for heterosynaptic modulation of receptor activity that could modulate cognitive processes.

Zn^{2+} Modulation of Synaptic Transmission

Some of the brain's most remarkable feats, such as learning and memory, are thought to emerge from the elementary properties of chemical synapses. A distinctive feature of synapses is that action potentials in the presynaptic terminals elicit the release of chemical transmitters. It has been surmised for some time that Zn^{2+} is released from synaptic terminals during neuronal activity. This conclusion was based largely on the localization of vesicular Zn^{2+} with glutamate in nerve terminals and on indirect observations that Zn^{2+} was lost from synaptic terminals after various depolarizing stimuli (*3-6*).

Probably the most widely accepted proposal about the function of vesicular Zn^{2+} is that it acts as a modulator of synaptic transmission. In particular, a number of studies in cultured neurons have demonstrated modulatory effects of physiologically relevant concentrations of Zn2+ on various agonist-gated and voltage-gated ion channels. These channels include receptors for NMDA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), γ-aminobutyric acid type A (GABAA), glycine, and ATP; voltage-dependent Ca2+ channels (VDCCs); and the Kv4-type channel that mediates the transient outward K^+ current IA (*7-14*). Cultured neurons, however, do not sequester vesicular Zn^{2+}, nor do they establish the same complex neural network that occurs in our brains. We are just beginning to understand the effects of Zn^{2+} released during synaptic transmission in vivo.

The abundance of Zn^{2+}-containing terminals in the hippocampal mossy fiber pathway make it an attractive system for characterizing synaptically released Zn^{2+} in the brain (*15*). Using the Zn^{2+} fluorophore Newport Green (K_d ~ 1 μM), our group has directly demonstrated the release of Zn^{2+} from synaptic terminals during neuronal activity in the same fashion as a neurotransmitter (*16*). Thompson *et al*. (*17*), using a highly sensitive carbonic anhydrase-based Zn^{2+}-selective fluorophore, anticipated our study. These conclusions are echoed in the report by Ueno and colleagues (*2*), who took advantage of the high selectivity and sensitivity of the new fluorescent indicator ZnAF-2 (K_d = 2.7 nM) developed by their group. To examine the spatiotemporal dynamics of extracellular Zn^{2+} concentration, or $[Zn^{2+}]_o$, after synaptic activity, Ueno and colleagues (*2*) applied electrical stimulation to the mossy fiber pathway in stratum lucidum of living hippocampal slices with ZnAF-2 in the extracellular space. Zn^{2+} release under this treatment was abolished by the Na^+ channel blocker tetrodotoxin and by the removal of extracellular Ca^{2+}, supporting previous observations that the release of Zn^{2+} depends on neural activity and is Ca^{2+}-dependent.

Previous studies on mossy fiber Zn^{2+}, including ours (*18*), have focused mainly on its homosynaptic action. Ueno and colleagues (*2*) reported that Zn^{2+} influenced NMDA receptor function at neighboring synapses in stratum radiatum as well. They observed a gradual increase of $[Zn^{2+}]_o$ in the stratum radiatum adjacent to stratum lucidum. This increase was not due to diffusion of Zn^{2+}-fluorophore complexes, but reflected the distribution of Zn^{2+} itself. Because there was no apparent recovery of fluorescent signal after photobleaching of the proximal area of stratum radiatum in the continuous presence of exogenous Zn^{2+}, it is unlikely that unbleached fluorophore diffused from stratum lucidum into the adjacent stratum radiatum. To address the functional significance of Zn^{2+} spillover from mossy fiber terminals, they recorded the NMDA receptor-mediated excitatory postsynaptic potential (EPSP) in stratum radiatum, where the associational-commissural pathway forms synapses with CA3 pyramidal cells. The NMDA receptor-mediated EPSP in the proximal, but not the distal, stratum radiatum declined transiently in response to mossy fiber stimulation. This depression was relieved after bath application of a selective Zn^{2+} chelator. The results from this study support the observation by Vogt *et al*. (*19*) that Zn^{2+} modulates NMDA receptor function in hippocampal CA3. Ueno and colleagues also provide evidence for

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a heterosynaptic action of Zn^{2+} on NMDA receptors (2).

The heterosynaptic effects of Zn^{2+} are especially interesting in light of possible Zn^{2+} involvement in the pathological physiology of epilepsy (*20*). Whereas Zn^{2+} is released only from glutamatergic terminals, spillover of Zn^{2+} resulting from a high level of Zn^{2+} release might lead to inhibition of nearby GABAA receptors, as has been hypothesized to occur after aberrant mossy fiber sprouting in the kindling model of epilepsy (*12*). The work of Ueno *et al.* (*2*) suggests that this is possible.

Zn^{2+} as a Trans-Synaptic Second Messenger

In addition to being stored in presynaptic vesicles and released as a neurotransmitter or neuromodulator upon synaptic activation, Zn^{2+} also enters postsynaptic neurons, producing transient increases in $[Zn^{2+}]_i$ (*16*, *18*, *21*). Presynaptic Zn^{2+} is co-released with glutamate from excitatory terminals and enters postsynaptic neurons, so it is reasonable to expect that Zn^{2+} might permeate ionotropic glutamate receptors and VDCCs. Indeed, Zn^{2+} can cross the plasma membrane by the same routes used by Ca^{2+}, which is always present in the extracellular space; these routes include VDCCs, Ca^{2+}-permeable AMPA or kainate channels, and the NMDA receptor channel (*22*). Activation of AMPA receptors depolarizes the plasma membrane and allows entry of Zn^{2+} through VDCCs.

Once Zn^{2+} enters neurons, it can directly interact with many cytosolic proteins (Fig. 1). Several studies demonstrate that Zn^{2+} regulates components in various intracellular signaling pathways such as the kinases [for example, protein kinase C (PKC), calcium/calmodulin-dependent protein kinase II (CaMKII), mitogen-activated protein kinase (MAPK), and possibly adenosine 3′,5′-

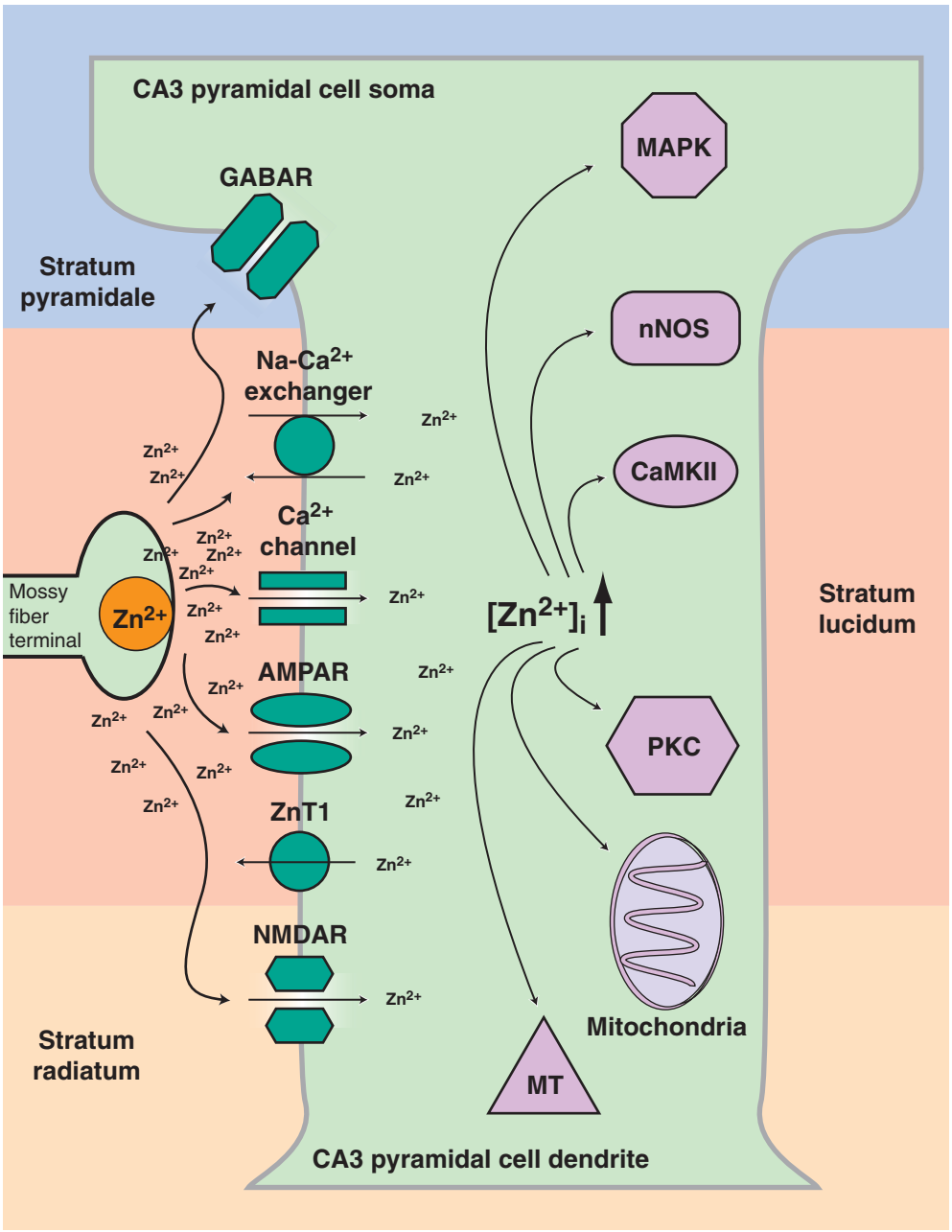

Fig. 1. Schematic illustration of a Zn^{2+}-containing nerve terminal and a postsynaptic neuron, indicating Zn^{2+} movements after its release. In the presynaptic terminal, Zn^{2+} is transported into vesicles of glutamate-containing neurons by the Zn^{2+} transporter ZnT3, and is co-released with glutamate into the synaptic space upon presynaptic activation. Released Zn^{2+} can interact with several inhibitory and excitatory receptors (for example, NMDA, AMPA, and $GABA_A$ receptors), neurotransmitter uptake proteins (for instance, glutamate transporter), and ion channels (for instance, VDCCs and K^+ channels). There are several routes through which Zn^{2+} might move across the plasma membrane: VDCC, Ca^{2+}-permeable AMPA or kainate channels, NMDA receptor (NMDAR) and the Na^+-Ca^{2+} exchanger. The Zn^{2+} transporter, ZnT1, extrudes Zn^{2+} from neurons and helps to maintain low cytosolic levels. Metallothioneins (MT) are important buffers of intracellular Zn^{2+}. Following its entry, Zn^{2+} can interact with proteins in signaling pathways such as PKC, CaMKII, PKA, neuronal nitric oxide synthase (nNOS), Na^+- and K^+-dependent ATPase (Na,K^+-ATPase) and can even affect gene expression, such as the induction of the transcription factor EGR1. AMPAR, AMPA receptor.; GABAR, $GABA_A$ receptor.

monophosphate (cAMP)-dependent protein kinase (PKA)]. They also indicate that Zn^{2+} interferes with cellular metabolism (*23-29*). The induction of long-term potentiation (LTP), a form of synaptic plasticity implicated as a cellular mechanism of learning (*30*), critically depends in many neurons on an initial postsynaptic rise in $[Ca^{2+}]_i$. One hippocampal synapse that seems to be an exception to this rule is the mossy fiber input to CA3 pyramidal neurons. Our recent findings suggest that the translocation of Zn^{2+} into CA3 pyramidal neurons is required for LTP induction (*18*). This function would establish synaptically released Zn^{2+} as an essential element in modulating neuronal transmission and synaptic plasticity. Depletion of presynaptic Zn^{2+}, either by chronic dietary deficiency or by acute depletion with a membrane-permeable Zn^{2+} chelator, also impairs LTP at mossy fiber-CA3 synapses (*31*). To our knowledge, Zn^{2+} is the only messenger substance that is released presynaptically and moves relatively freely into postsynaptic neurons.

Conclusions

This is an interesting time in the Zn^{2+} neurobiology field. Yet our understanding of the functional significance of synaptically released Zn^{2+} on various receptor-gated and voltage-gated channels is still at an early stage. One reason for this slow progress is that most studies have been conducted in cultured neurons that lack presynaptic vesicular Zn^{2+}. Another problem has been the lack of specific Zn^{2+}-sensitive fluorophores that do not cross cell membranes. Although Fura-2 and Mag-fura-2, for example, are sensitive to low concentrations of Zn^{2+}, they also fluoresce when bound to physiological concentrations of Ca^{2+}, and are therefore best known as Ca^{2+} fluorophores. The development of new Zn^{2+}-selective fluorescent dyes that can be restricted to intracellular or extracellular compartments has greatly aided recent studies (*2*, *16*, *18*) and can be expected to increase our knowledge of the possible functions of Zn^{2+}. A Perspective on the current status of Zn^{2+} imaging—and of the development of new Zn^{2+}-sensitive probes—appears in this issue of *Science's STKE* (*32*).

Efforts to sequester synaptically released Zn^{2+} with chelators have also been frustrated by the rapid, nonequilibrium conditions of synaptic Zn^{2+} release and the relatively slow kinetics of Zn^{2+} binding to chelator. In particular, as demonstrated and modeled by our group (*18*), concentrations of calcium-ethylenediaminetetraacetic acid (CaEDTA) that are sufficient to chelate synaptic Zn^{2+} at equilibrium do not effectively chelate Zn^{2+} within the tens to hundreds of microseconds it takes Zn^{2+} to cross the synapse and interact with various postsynaptic sites (*19*). In contrast, a higher concentration of CaEDTA markedly reduces the Zn^{2+} signal (*18*). The development of new, more rapid Zn^{2+} chelators should shed additional light on the functions of synaptically released Zn^{2+}.

Results from several laboratories point to a role for Zn^{2+} in synaptic transmission. The work of Ueno *et al*. (*2*) establishes that Zn^{2+} is an activity-dependent, spatiotemporal modulator of NMDA receptors, and provides new insights into information processing in the hippocampus. Emerging data suggest that both Zn^{2+} and Ca^{2+} are involved in neurotransmission, synaptic plasticity, and neuropathologies, such as global ischemia. Therefore, it is critical to understand the interaction of Zn^{2+} and Ca^{2+} with proteins important in signal transduction (*33*). Zn^{2+} may need to enter cells to carry out many of its biological activities, such as in the induction of LTP in the mossy fiber-CA3 synapse (*18*). A perplexing question is how timing and specificity within a neuron can be achieved when synaptically released Zn^{2+} directly interacts, through translocation into neurons, with proteins in intracellular signaling pathways. Ueno and colleagues' demonstration of heterosynaptic modulation of activity by Zn^{2+} is also certain to spur further work in many laboratories. As new tools are developed and interest in Zn^{2+} grows, we can expect further breakthroughs in this exciting field.

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Citation: Y. V. Li, C. J. Hough, J. M. Sarvey, Do we need zinc to think? *Sci. STKE* **2003**, pe19 (2003).