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Commentary

Metal ions and synaptic transmission: Think zinc

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Zinc is abundant in the brain, having, after iron, the highest concentration among all transition metals. Most of this brain zinc (≈90%) is bound up in metal–protein complexes, such as zinc-finger gene regulatory proteins, in which the zinc is used for enzymatic catalysis or for structural stability, and this tightly complexed portion of the zinc pool is essentially invisible to histochemical stains specific for metal ions. Many neurons in the brain, however, contain in addition a significant amount of histochemically reactive, ionic zinc, which is classically detected by Timm's sulfide-silver staining method. Such histochemical stains reveal that large amounts of chelatable zinc are concentrated in the cerebral cortex and the limbic region, notably in the hippocampal formation (1).

Ultrastructural studies show most of the ionic zinc detected by histochemical stains in the above-mentioned areas is localized within synaptic vesicles of glutamatergic neurons (1), and researchers have thus suspected for many years that zinc acts as a neurotransmitter or neuromodulator at excitatory synapses. Staining for synaptic zinc is especially intense in hippocampal mossy fibers (2), which project from granule cells in the dentate gyrus to the CA3 region of the hippocampus, and stimulation of these fibers causes the release of zinc from synaptic boutons (3). The zinc concentration in the synaptic cleft is estimated to reach nearly 300 μM during strong stimulation (4), certainly enough to have profound effects. Despite this accumulation of data, however, determining the true function of vesicular zinc has been problematic, in part because of the lack of any specific method to inhibit synaptic zinc function and because of ignorance about interactions between zinc and glutamate transmitter. Wenzel *et al.* (5) have taken a step toward amending these difficulties by showing that a putative zinc transporter (ZnT-3) is specifically localized to the surfaces of glutamate-containing synaptic vesicles in mossy fiber terminals. Using a combination of electron microscopy, immunocytochemistry, and histochemical methods, they demonstrated that all such vesicles express ZnT-3, and up to 80% contain zinc. Taken together, these results confirm that synapses in the mossy fiber system of the hippocampus store and thus corelease zinc with glutamate transmitter.

Although proof that ZnT-3 is capable of transporting zinc awaits demonstration of its functional properties, this gene is classed as a zinc transporter based on its homology with ZnT-1 and Znt-2, members of a defined family of mammalian zinc transporters (6); ZnT-1 and ZnT-2, respectively, mediate zinc efflux from cells and zinc accumulation in endosomal vesicles. This family in turn belongs to a larger family of heavy metal ion transporters, all characterized by possessing six putative transmembrane domains with a conserved signature sequence between domains I and II (7). ZnT-3, the latest mammalian addition to this family, is abundantly expressed in hippocampus and cerebral cortex, in an overall pattern that resembles Timm's stain for zinc (6). By extrapolating from the current results of Wenzel *et al*., it thus seems probable that all synaptic zinc is colocalized in vesicles with glutamate transmitter and that its vesicular uptake is mediated by ZnT-3, or a transporter complex containing ZnT-3.

As noted above, the localization of zinc to synaptic vesicles has led to the speculation that it has an important role in neurotransmission. Aside from prompting wonder on the cellular effort expended to sequester synaptic zinc, the present results leave open the main question of its endogenous function. Any answer to this question must certainly take into account the high estimated vesicular concentrations of zinc and its specific colocalization with glutamate transmitter. One interesting proposal is that zinc acts as a cofactor to stabilize the storage of vesicular molecules (such as glutamate), as it does in non-neural cells. For example, zinc ions are necessary to maintain the crystalline structure of the insulin precipitate in secretory granules of pancreatic β cells (1). Another idea is that zinc, like glutamate, acts directly as a neurotransmitter. Possible receptors for zinc transmission, however, are unknown.

Probably the most widely accepted proposal regarding the function of synaptic zinc is that it acts as a modulator of synaptic transmission. In particular, a number of studies have addressed the issue of how zinc affects glutamate receptors (8–10). At glutamatergic synapses in the central nervous system, several types of glutamate receptor channels on the postsynaptic membrane mediate glutamate transmission. Broadly, these receptors are divided into *N*-methyl-D-aspartate (NMDA) receptors and non-NMDA receptors, both of which pass cationic current in response to glutamate binding but which differ in several important respects. Specifically, NMDA receptors are distinguishable from non-NMDA receptors by their permeability to calcium ions and the fact that they open chiefly under conditions of strong synaptic stimulation. Calcium influx through NMDA receptor-channels is critical for inducing increases in synaptic strength (termed long-term potentiation, or LTP) believed to underlie the formation of memories. Also, NMDA receptors are thought to mediate a form of neurotoxicity caused by excessive glutamate excitation (glutamate excitotoxicity), which is implicated in neuronal death following anoxic events or seizures, as well as in neurodegenerative disorders such as Alzheimer's disease (11).

Researchers have shown that physiological concentrations of zinc significantly inhibit NMDA receptor function while slightly potentiating the activity of non-NMDA receptors (9). The precise mechanism by which zinc interferes with NMDA receptor function is not known, but it appears to act as a noncompetitive antagonist whose major site of action lies outside of the channel pore (12). This action contrasts with the action of other divalent cations, notably Mg^{2+}, which binds within the pore to block ion permeation. Given that zinc potently inhibits NMDA receptor activity, one would certainly expect its corelease with glutamate to modulate transmission at glutamatergic synapses. Of particular relevance in this regard is whether zinc affects different subtypes of NMDA receptors differently. One recent report (13) demonstrates that the magnitude of NMDA receptor inhibition by zinc depends on the molecular subunit composition of the receptor. This result raises the possibility that glutamatergic synapses may have differential sensitivities to zinc inhibition and might even

be capable of modulating their zinc sensitivity by altering NMDA receptor subunit composition.

In any case, zinc blockade of NMDA receptor activity might have several functional consequences. Zinc may act to block NMDA receptor-mediated LTP at specific synapses and may inhibit NMDA receptor-mediated excitotoxicity, as discussed above. Interestingly, mossy fiber synapses, which possess very large amounts of synaptic zinc, express a special form of LTP that does not depend on NMDA receptor activity, although these synapses do have NMDA receptors (14). Zinc is found, however, in other hippocampal synapses that do express NMDA receptor-dependent LTP, so it should not be inferred that the presence of synaptic zinc is incompatible with this form of LTP. Finally, studies examining the effect of zinc on neurotoxicity have shown that micromolar concentrations of zinc protect cortical neurons from the toxic effects of excessive glutamate exposure (15). This result suggests that by modulating NMDA receptor activity, synaptically released zinc may have neuroprotective effects.

Aside from its effects on glutamate receptors, zinc has also been shown to block certain types of γ-aminobutyric acid (GABA) receptor channels, which mediate inhibitory synaptic transmission in the central nervous system. Although the relevance of this block to normal synaptic behavior is not well understood, it is of special interest in light of possible zinc involvement in the pathological physiology of epilepsy. Among the disease features of human temporal lobe epilepsy are hyperexcitability and seizure activity, death of hippocampal neurons, and aberrant sprouting of hippocampal mossy fibers so that they form recurrent synapses onto dentate gyrus granule cells. Several lines of evidence point to the involvement of zinc in these alterations. The most general observations are that synaptic zinc is most abundant in brain areas particularly prone to seizures (i.e., the limbic regions) and that zinc distribution in the brain alters significantly as a result of prolonged seizure activity (1). Furthermore, a recently proposed model of temporal lobe epilepsy postulated that zinc released from aberrantly sprouted mossy fibers contributes to the ultimate collapse of GABA-mediated inhibitory drive in the dentate gyrus (16). In support of this idea, researchers have demonstrated that GABA receptor channels on dentate granule cells from rats with experimentally induced epilepsy are sensitive to zinc blockade (16). Collapse of GABA inhibition may be a key feature of epilepsy, leading to chronic excitability and the spread of seizure activity. However, zinc also has antiexcitatory, neuroprotective properties, as the above discussion on zinc modulation of glutamate receptors demonstrates, and some researchers have proposed that synaptically released zinc may actually palliate epileptic symptoms. Clearly, considerable further work must be done to clarify the role of zinc in synaptic pathologies.

The same, in fact, can be said about the role of zinc in normal synaptic transmission. Despite what is already known, it would not be surprising to find that there are still missing pieces to the puzzle of synaptic zinc function. For instance, the estimated concentrations of zinc reached in the synaptic cleft appear to exceed that required to modulate glutamate and GABA receptors (4, 9). Thus, the identification of ZnT-3 as the synaptic vesicle zinc transporter by Wenzel *et al*. may be exactly the advance needed to bring this field into its own. Construction of mutant mice with targeted deletions of ZnT-3 will be an excellent step toward elucidating the endogenous function of synaptic zinc (as well as its role in disease states), and it may be especially desirable to create mice with mutations restricted to specific regions of the forebrain, such as has been done for the NMDA receptor (17). Then, truly might we begin to understand how zinc helps us to think.

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