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# Zinc and Disease of the Brain

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## Abstract

Zinc is one of the most abundant transition metals in the brain. A substantial fraction (10–15%) of brain zinc is located inside presynaptic vesicles of certain glutamatergic terminals in a free or loosely bound state. This vesicle zinc is released with neuronal activity or depolarization, probably serving physiologic functions. However, with excess release, as may occur in a variety of pathologic conditions, zinc may translocate to and accumulate in postsynaptic neurons, events which may contribute to selective neuronal cell death. Intracellular mechanisms of zinc neurotoxicity may include disturbances in energy metabolism, increases in oxidative stress, and activation of apoptosis cascades. Zinc inhibits glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and depletes nicotinamide adenine dinucleotide ($NAD^+$) and adenosine triphosphate (ATP). On the other hand, zinc activates protein kinase C (PKC) and extracellular signal-regulated kinase (Erk-1/2), and induces NADPH oxidase; these events result in oxidative neuronal injury. Zinc can also trigger caspase activation and apoptosis via the $p75^{NTR}$ pathway. Interestingly, the converse—depletion of intracellular zinc—also induces neuronal death, but in this case, exclusively via classical apoptosis. In addition to the neurotoxic effect, zinc may contribute to the pathogenesis of chronic neurodegenerative disease. For example, in Alzheimer's disease (AD), mature amyloid plaques, but not preamyloid deposits, are found to contain high levels of zinc, suggesting the role of zinc in the process of plaque maturation. Further insights into roles of zinc in brain diseases may help set a new direction toward the development of effective treatments.

**Index Entries:** GAPDH; ATP; NADPH oxidase; PKC; Erk; Egr-1; PARP; $p75^{NTR}$; NADE.

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## Introduction

Zinc is a transition metal abundantly present in all animal tissues. Diverse classes of proteins require bound zinc for their normal functions. First, there are zinc metalloenzymes, several hundred of which are known to exist *(1–4).* Well-known examples are carbonic anhydrase, superoxide dismutase-1 (SOD-1), and matrix metalloproteases (MMPs). In most of these enzymes, zinc binds to the catalytic sites and helps enzyme reactions by facilitating the formation of hydroxide ions or Lewis acids. Second, there are transcription factors contain zinc-binding motifs such as zinc finger, zinc twist, zinc cluster, and RING finger *(5).* Binding of zinc to these transcription factors provides correct conformation for their binding to DNA grooves. Examples of zinc transcription factors are transcription factor IIIA, egr-1, steroid receptors, p53, and GAL4, just to name a few *(6).* Third, there are proteins in which zinc binding serves signaling functions. For example, protein kinase C (PKC) contains zinc-finger domains, which may be necessary for the binding of lipid activators and for the translocation to the membrane *(7).* Similarly, porcine diacylglycerol kinase has zinc-finger motifs *(8).* Other such examples include calmodulin and S-100, which have separate zinc-binding sites from calcium-binding ones *(9,10).* Although precise roles for zinc in these signaling molecules are yet to be further characterized, zinc binding is likely to affect their signaling activity. Finally, proteins with multiple zinc-binding sites such as metallothioneins may buffer cytosolic zinc *(11,12).*

Of organs, the brain probably contains highest levels of zinc in the body, with a possible exception of pancreatic β islets *(13,14).* The gray matter of the forebrain, especially has high levels of zinc, reaching 60–90 ppm. The white matter contains slightly lower levels of zinc (26–40 ppm), but it may be an underestimation because of lower water contents in the white matter *(14).* Most of the zinc in the brain, as in other tissues, is tightly bound to proteins *(15),* and hence cannot be visualized with simple histochemical techniques such as neo-Timms and *N*-(6-methyl-8-quinolye)-*para*-tolurnesulfonamide (TSQ) fluorescence stainings *(16,17).* One interesting aspect of the brain zinc is that a substantial fraction of it *(10–15%)* is localized in synaptic vesicles of certain glutamatergic neuron terminals *(18).*

Particularly high levels of synaptic zinc are present in the limbic systems including the mossy-fiber terminals of hippocampal dentate granule neurons (total zinc concentration, 136–145 ppm). In other brain regions, neocortical layers II–III and V, striatum, and thalamic dorsomedial and reticular nuclei, are also enriched with zinc-containing terminals *(14,19).* By comparison, cerebellum, brain stem, and spinal cord are low in zinc-containing terminals *(14).*

The synaptic vesicle zinc is not tightly bound to proteins, but exists in a free or loosely bound state. Thus the vesicle zinc can be easily visualized with the aforementioned histochemical methods. Whereas both extracellular and intracellular free zinc concentrations are quite low (nM range) *(14,20),* zinc concentration in vesicles may reach m*M* range *(14).* This huge concentration gradient calls for the presence of a special zinc transporter. Recently, zinc transporter 3 (ZnT3) was discovered as the transporter responsible for zinc accumulation in vesicles *(18).* However, little is known about its kinetics and mode of action. Moreover it is unknown whether vesicle zinc accumulation occurs distally in axon terminals or more proximally in the cell body.

With neuronal activity or membrane depolarization, zinc is released into the synaptic cleft *(21,22),* probably along with its co-transmitter, glutamate. At the peak of intensive release, extracellular zinc concentration may reach several hundred μ*M (22).* Considering that the extracellular zinc concentration at rest may be under 1 μ*M (14),* this dramatic change in zinc concentration suggests roles of zinc in synapse physiology. In fact, zinc has diverse effects on glutamatergic and GABAergic transmissions *(23).*

## Zinc: An Ionic Mediator of Neuronal Death in Acute Conditions

Tonder and colleagues first reported the accumulation of zinc in degenerating hippocampal hilar neurons following transient global ischemia *(24).* Such zinc accumulation in dying or dead neurons is not limited to the hippocampal hilar area, but occurs also in all the brain regions damaged in global ischemia, such as hippocampal CA1, neocortex, thalamus, and striatum *(25).* Indicating that zinc accumulation is a critical event for the neuronal death, CaEDTA, a zinc-chelating agent, markedly protects neurons against transient cerebral ischemia *(25).* In addition to global ischemia, Lee and colleagues reported intraneuronal zinc accumulation in the infracted area of middle cerebral artery (MCA)-occluded rats *(26).* This finding suggests that zinc-mediated neuronal injury may contribute also to focal ischemia-associated brain injury. Since presynaptic zinc is the only known pool of releasable zinc, and since presynaptic zinc depletion occurs concurrently with postsynaptic zinc accumulation, it has been proposed that presynaptic zinc translocates to postsynaptic neurons *(27).* While this hypothesis is still likely to be true in most cases, studies done in ZnT3 null mice *(28)* where synaptic zinc is absent *(29)* or those in cerebellum where zinc terminal is lacking (Suh and Frederickson, personal communication), suggests the existence of alternative or additional sources of toxic zinc accumulation. It seems possible that zinc released from zinc-containing proteins such as metallothioneins *(30,31)* may contribute to zinc accumulation in certain cases.

The zinc-based mechanism of cell death is not unique to ischemic brain injury. In fact, the phenomenon was first discovered in a seizure model. Frederciskon and colleagues demonstrated that there was a near-perfect correlation between zinc accumulation and neuronal cell death following kainate-induced seizures *(26).* Following this pioneer study, it was found that zinc chelation treatment is markedly protective *(32,33).* Moreover, injury-related events such as heat-shock protein (HSP) 70 induction was also correlated with zinc accumulation *(33).* Physical injury also can trigger zinc release and cell death in the brain. In a percussion model of brain trauma in rats, zinc release from presynaptic terminals of hippocampal hilus has been observed *(34).* Again in this model, there were perfect correlation between zinc accumulation in neuronal cell bodies and protective effects of CaEDTA.

Earlier culture studies showed that zinc entry into neurons is an essential event for the zinc-induced cell death. The next question is which downstream events mediate the cell death (Fig. 1). Sheline and colleagues have shown that zinc neurotoxicity involves the inhibition of glycolysis and depletion of ATP *(35).* Although the detailed mechanism is still being investigated, an alternative fuel pyruvate, markedly protect against zinc toxicity, indicating that the aberrancy in energy metabolism is a critical event for zinc toxicity. Besides this, zinc injury involves the activation of a variety of signaling enzymes such as PKC and extracellular signal-regulated kinase (Erk-1/2) *(36,37).* Erk-1/2 may induce egr-1 *(37),* a zinc-finger transcription factor. All these events seem to be involved in zinc toxicity. Although there exists a pathway linking PKC activation to Erk activation, the precise relationship between PKC and Erk-1/2 in zinc toxicity needs further investigation. At any rate, all these events seem to be linked to increased oxidative stress in cells *(38).* Although effectors generating free radicals in zinc toxicity could be diverse, we have found that reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, a well-known free radical-generating enzyme, is induced and activated in zinc-exposed corticall cells in a PKC-dependent manner *(39).* However, it is unknown which transcription factors are involved in the NADPH oxidase induction by zinc. Regardless of the precise mechanism of induction, inhibitors of NADPH oxidase attenuated zinc toxicity *(39).* Hence, NADPH oxidase may contribute to zinc-induced oxidative

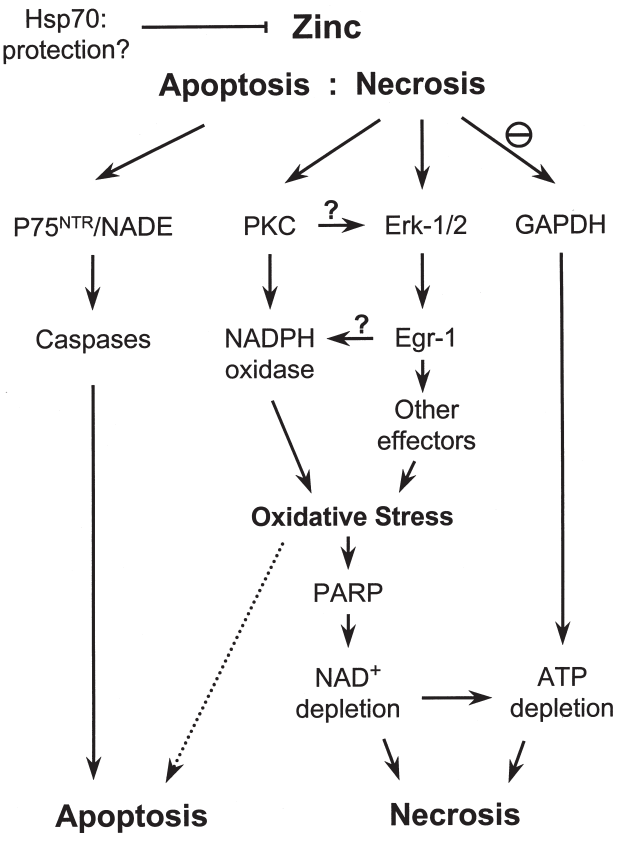


Fig. 1. A diagram for mechanisms of zinc neurotoxicity. The HSP-70 induction by low-intensity zinc exposure *(27)* may be a protective event. Zinc toxicity can induce both apoptosis and necrosis. Zinc inhibits glycolysis and depletes ATP *(35),* which disrupts ionic homeostasis and causes necrosis. In addition, zinc activates PKC and Erk-1/2, which, via induction of Egr-1 and induction/activation of NADPH oxidase, leads to increased oxidative stress *(36–38).* The oxidative injury may activate PARP or related enzymes *(40),* which in turn deplete $NAD^+$ and ATP in cells. These events may lead to necrosis *(61).* Another pathway of zinc toxicity is the activation of $p75^{NTR}$ and NADE *(41).* By activating caspases, this pathway induces apoptosis. Although oxidative stress may lead to apoptosis, this possibility in zinc toxicity in cortical culture has not been directly tested.

injury to cortical cells. Interestingly, zinc toxicity is accompanied by marked depletion of $NAD^+$ *(35).* Inhibitors of $NAD^+$ catabolism almost completely block zinc toxicity, indication $NAD^+$ catabolism is a crucial event. Pending future confirmation, poly(ADP-ribose) polymerase (PARP) activation triggered by oxidative injury *(40)* may be involved in $NAD^+$ depletion in zinc toxicity *(35).*

In parallel to the fulminant oxidative injury, which causes mainly necrosis, we have found that zinc, triggers apoptosis at least in part by activating the $p75^{NTR}$ system *(41).* Zinc exposure induced not only NGF and $p75^{NTR}$ in cortical neurons, but also the newly discovered death mediator, NADE *(41)* [$p75^{N}$TR-*A*ssociated *D*eath *E*xecutor *(42)*]. These results suggest that specific apoptosis cascade is activated in zinc neurotoxicity.

## Protective Measures Against Zinc-Induced Neuronal Death

Even though the understanding of the mechanism of zinc-induced neuronal death is not yet complete, various neuroprotective measures against this have appeared in literature. The first class are zinc-chelating agents. Indeed, a cell membrane-impermeant chelator, CaEDTA, has been shown to protect against zinc toxicity in culture, as well as ischemic and seizure-induced neuronal death in vivo *(25,32,33).* However, it may not be easy to apply CaEDTA in humans, since CaEDTA may not cross the blood-brain barrier (BBB). More lipid-soluble and potent chelators, on the other hand, may severely deplete intracellular zinc, which itself can lead to cell death *(43).* The second class is the blockers of zinc entry into neurons. To this class belong various blockers of calcium-permeable channels, such as MK-801, nimodipine, and NBQX *(23,44).* The third class of protective agents against zinc toxicity includes pyruvate and PARP inhibitors *(35).* These may normalize disturbances of energy metabolism induced by zinc. The fourth class includes inhibitors of downstream cascades of zinc death, for example, PKC and Erk *(36,37).* The fifth one includes various anti-oxidative measures including NADPH oxidase inhibitors *(38,39).* The sixth class includes anti-apoptosis

measures including those targeting the $p75^{NTR}$ system *(41).* Finally, measures increasing zinc efflux may be used to curb zinc toxicity. For example, overexpressed zinc transporter 1 (ZnT1) attenuates zinc toxicity in cultured cells *(45).* In addition, we have recently found that tPA and HGF activates zinc efflux from cells via $Na^+/Ca^{2+}$ exchangers in an EGF receptor-dependent manner *(46,47).*

## The Other Side of the Coin: Zinc Depletion-Induced Apoptosis

As briefly discussed earlier, not only excessive but also too little zinc can cause cell death. Whereas zinc-induced neuronal death exhibits features both of apoptosis and necrosis *(38,40),* zinc-depletion death is almost purely apoptosis *(43,48,49).* The most important issue is whether zinc-depletion death has any relevance to brain diseases. To this question, no definite answer is yet available. However, it was suggested that the decrease of cellular zinc levels in hippocampus contributes to the pathogenesis of AD *(50).* It is known that zinc directly inhibits caspases *(51).* Hence, intracellular zinc depletion may render cells more vulnerable to apoptosis-triggering insults, such as β amyloid (Aβ) toxicity. Another disease possibly related to zinc-depletion injury, although not that of the brain, is age-related macular degeneration *(52).* Here, ocular zinc is reportedly reduced, and zinc supplementation seems to ameliorate the disease progression. We have found that both retinal neurons and retinal pigment epithelial cells in culture undergo caspase-dependent apoptosis upon intracellular zinc depletion *(48,49).*

## Possible Role of Zinc in the Plaque Formation in Alzheimer's Disease

Recent studies indicate that the total levels of zinc in AD brains, especially in the plaque and the neuropil, are rather increased (about 2.5–4-folds) *(53).* In addition, other endogenous heavy metals known to promote aggregation of Aβ—iron and copper—are also increased *(53).* More recently, using TSQ fluorescence, Suh and colleagues have demonstrated chelatable zinc in autopsied human AD brains *(54).* In addition, they showed that zinc is also present in association with neurofibrillary tangles. Hence, it appears that the distribution of chelatable zinc in the brain is markedly altered in AD brains, since in normal situation all the histochemically visible zinc is associated with synaptic vesicles. We confirmed the presence of zinc in all the mature congophilic plaques but not in pre-amyloid deposits of Swedish mutant APP transgenic mouse brain, and in addition, demonstrated that zinc may bind not only to Aβ but also to heparan sulfate proteoglycan *(55).*

Then, what is the meaning of zinc accumulation in mature plaques? Bush and colleagues have shown that metals including zinc facilitate the aggregation of Aβ *(56).* Our study using human Swedish mutant APP transgenic (Tg2576) mice *(57)* suggests that diffuse preamyloid deposits may precede zinc accumulation in mature plaques *(55).* The endogenous zinc, possibly the presynaptic zinc, may promote aggregation of Aβ in preamyloid deposits turning it into dense congophilic plaques in AD brains. As aggregation of Aβ may be critical for its cytotoxicity *(58),* this aggregation process may increase the neurotoxic action of Aβ in AD brains. Alternatively, zinc binding may rather decrease the oxidative potential of Aβ, whereas copper (+) and iron (+) Aβ do the opposite; in this scheme, zinc (+) Aβ may be a harmless destination of the Aβ lifecycle in AD *(59).* Regardless whether the metal critical for the neurotoxicity of Aβ in AD is zinc, iron, or copper, chelators are being considered as the potential therapeutic regimen in AD *(60).*

Finally, at quick glance, it seems contradictory that zinc depletion-induced apoptosis and zinc-triggered Aβ plaque formation can occur concurrently in AD. However, this scenario is not completely implausible since substantial amount of zinc is bound up in plaques and thus may not be accessible for re-use, which may ren-

der neurons effectually deficient in intracellular free zinc in spite of excess total zinc. This possibility should be carefully examined in the future.

## Summary and Conclusion

A growing body of evidence now suggests that endogenous zinc, particularly the chelatable zinc packed in synaptic vesicles, is a critical player in neuronal cell death in acute brain injury. In animal models of ischemia, seizures, and trauma, zinc is found to accumulate in all degenerating neuronal cell bodies, and blockade of zinc accumulation with zinc chelators markedly attenuate the cell death. Zinc neurotoxicity begins with zinc entry into neurons via various calcium-permeable channels. Inside neurons, excess zinc inhibits GAPDH, depletes $NAD^+$ and ATP, activates PKC, Erk-1/2 and egr-1, and induces HSP-70, NADPH oxidase, and $p75^{NTR}$/NADE. The induction of NADPH oxidase may contribute to oxidative injury, whereas that of $p75^{NTR}$ and NADE may contribute to caspase activation and apoptosis. Interestingly, zinc depletion also induces neuronal death, but in this case the death occurs exclusively by apoptosis. In addition to inducing neuronal cell death, zinc may contribute to the maturation of Aβ plaques in AD. Considering that the research on the brain zinc has just begun to take off, it is likely to be found that the zinc's role in brain disease is far broader than what we currently envision.

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