Mini-Symposium

The Neurophysiology and Pathology of Brain Zinc

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Our understanding of the roles played by zinc in the physiological and pathological functioning of the brain is rapidly expanding. The increased availability of genetically modified animal models, selective zinc-sensitive fluorescent probes, and novel chelators is producing a remarkable body of exciting new data that clearly establishes this metal ion as a key modulator of intracellular and intercellular neuronal signaling. In this Mini-Symposium, we will review and discuss the most recent findings that link zinc to synaptic function as well as the injurious effects of zinc dyshomeostasis within the context of neuronal death associated with major human neurological disorders, including stroke, epilepsy, and Alzheimer's disease.

Introduction

Brain zinc, in its free ionic form (Zn^{2+}), is present within synaptic vesicles at glutamatergic nerve terminals and is synaptically released during neuronal activity. Zn^{2+} is also bound to metalloproteins and intracellularly mobilized upon oxidative stress. A growing and exciting body of evidence indicates that Zn^{2+} plays a dynamic role in both the physiology and pathophysiology of brain function.

Synaptic activation releases vesicular Zn^{2+}, bringing its concentrations in the synaptic cleft to transiently rise. The exact amount of such release is controversial, but many laboratories have indicated (with the limitation of the current imaging techniques) that transient Zn^{2+} increases may reach 1–100 μM (Vogt et al., 2000; Qian and Noebels, 2005; Frederickson et al., 2006). Others have indications for lower (submicromolar) concentrations (Komatsu et al., 2005). Fuelling the controversy is the fact that measurement of actual Zn^{2+} levels within the synaptic cleft is technically challenging, given the short time in which the free ion is present in the synapse (Hurst et al., 2010). Some authors have alternatively suggested that the ion does not diffuse in the cleft and is actually only externalized following exocytosis. In this view, Zn^{2+} remains bound to the plasma membrane, forming a "veneer" on presynaptic terminals (Kay, 2003; Kay and Tóth, 2008). While this is an intriguing hypothesis, the interpretation of these results needs to take into account the variability in preexisting vesicular Zn^{2+} levels, as these are known to be affected by changes in previous synaptic activity (i.e., sensory experience), the animal age, and the methods used in the preparation of brain slices (Frederickson et al., 2006; Nakashima and Dyck, 2009). Future studies combining electrophysiology with state-of-the-art synaptic Zn^{2+} imaging are likely to give a more accurate description of the precise dynamics and concentrations of the ion during activity-dependent synaptic activity.

Exogenously applied Zn^{2+} profoundly affects the activity of glutamate, $GABA_A$, and glycine ionotropic receptors. Extracellular Zn^{2+} therefore is likely to be intimately linked to the balance of excitation and inhibition in the brain. Indeed following stimulation of Zn^{2+}-containing fibers, endogenous Zn^{2+} has been shown to block postsynaptic NMDA (Vogt et al., 2000; Molnár and Nadler, 2001a) and $GABA_A$ (Ruiz et al., 2004) receptors. However, the modulation of postsynaptic receptors by Zn^{2+} is likely complex, as other investigators have failed to find effects of vesicular Zn^{2+} on $GABA_A$ receptors (Molnár and Nadler, 2001b) and neuronal excitability (Lopantsev et al., 2003; Lavoie et al., 2007).

More recent findings also indicate that synaptically released Zn^{2+} activates a specific metabotropic Zn^{2+}-sensing receptor (Fig. 1*B*) (Besser et al., 2009; Chorin et al., 2011). Zn^{2+} can flux into neurons and be taken up in organelles such as mitochondria (Sensi et al., 2000; Caporale et al., 2009; Dittmer et al., 2009), and recent evidence in non-neuronal cells indicates that some uptake might occur in the endoplasmic reticulum and the Golgi apparatus (Qin et al., 2011) as well. Neurons also keep cytosolic $[Zn^{2+}]_i$ levels very low by using several ZnT transporters (the SLC30 family), ZIP transporters (the SLC39 family), and Zn^{2+} buffering metallothioneins (MTs) as well as other transporters such as a putative Na^+/Zn^{2+} exchanger (Sensi et al., 2009).

On the dark side, Zn^{2+} is also a potent neurotoxin (Fig. 2) involved in variety of conditions that have been associated with

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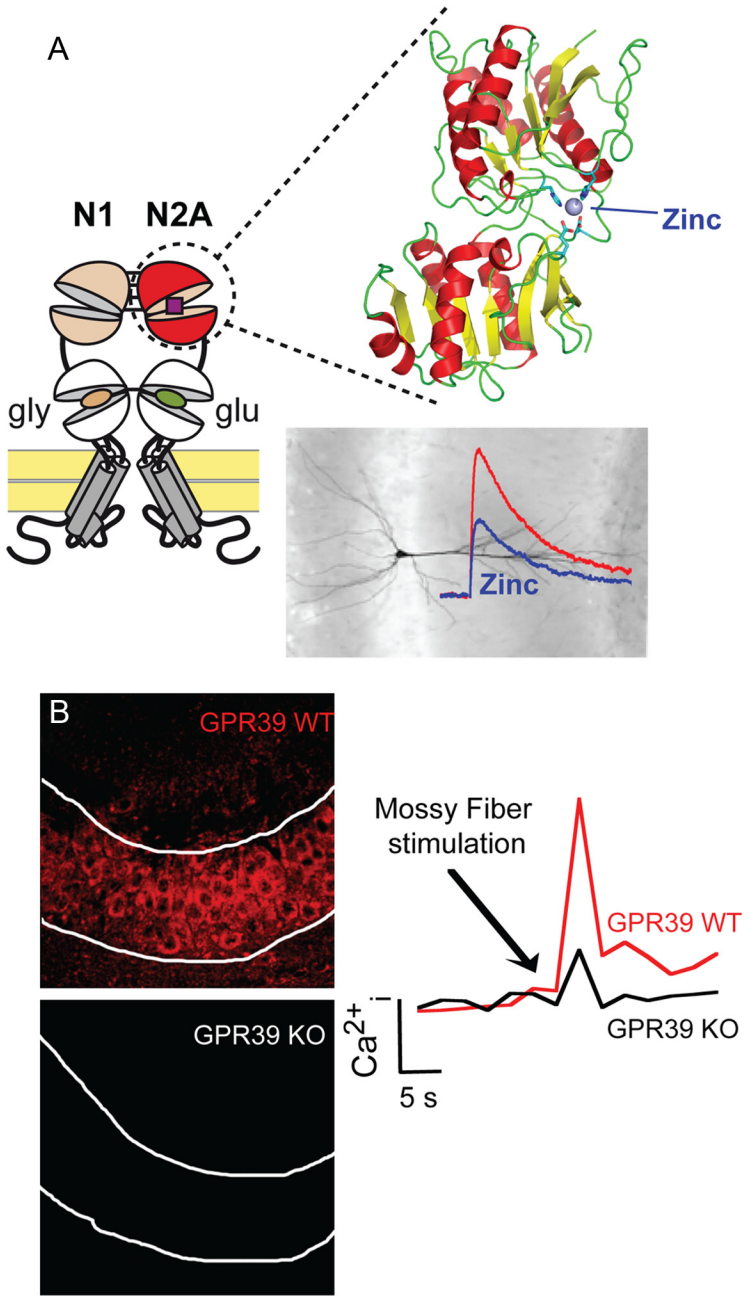

Figure 1. Zn^{2+} and synaptic function. ***A***, A high-affinity "Zn^{2+} sensor" in NMDA receptors. NMDAR subunits contain in their extracellular region a tandem of clamshell-like domains, the N-terminal domain (NTD) and the agonist-binding domain, which is directly connected to the transmembrane pore region. In the GluN2A subunit, the NTD forms a discrete high-affinity Zn^{2+} binding site that underlies allosteric inhibition of NMDAR-mediated synaptic currents by nanomolar Zn^{2+} concentrations. ***B***, Synaptic Zn^{2+} activates a specific mZnR. Synaptic Zn^{2+} released from the mossy fibers activates metabotropic Ca^{2+} release via the ZnR. The expression of GPR39 (left panel) and synaptic Zn^{2+}-dependent Ca^{2+} release (right panel) are eliminated in the CA3 pyramidal cell layer in GPR39 KO mice. The activity of the mZnR triggers phosphorylation of ERK1/2 and regulation of Cl^- transport, which lead to increased inhibitory drive.

excitotoxicity, including ischemia, epilepsy, and brain trauma (Sensi et al., 2009). Zn^{2+} promotes both neuronal and glial death *in vitro* and *in vivo* (Choi et al., 1988). Landmark *in vivo* studies have shown that the transsynaptic movement of Zn^{2+}, a process also called "Zn^{2+} translocation," plays a role in neuronal death associated with transient global ischemia (TGI) (Tønder et al., 1990; Koh et al., 1996). However, more recent evidence indicates that Zn^{2+} mobilization from intracellular pools is also a crucial contributor to neuronal injury (Aizenman et al., 2000; Lee et al., 2000, 2003; Hwang et al., 2008).

Finally, Zn^{2+} can play an important role in the development of Alzheimer's disease (AD). The cation is a key component of amyloid plaques, and it is now suggested that Zn^{2+} deregulation in the brain facilitates the synaptic deficits and cognitive decline observed in AD, while restoring brain Zn^{2+} homeostasis may represent an important and novel therapeutic avenue (Corona et al., 2010) (Fig. 3*A*).

In this Mini-Symposium, we review novel, exciting, and sometimes controversial, findings that substantiate a major role for Zn^{2+} in the physiological and pathological functioning of the brain.

Zn^{2+} and synaptic function

Free or "chelatable" Zn^{2+} is concentrated within synaptic vesicles at glutamatergic terminals through the activity of the specific transporter ZnT3 (Paoletti et al., 2009). The role of synaptic Zn^{2+} in regulating plasticity has been addressed either using extracellular metal chelators or in animal models lacking synaptic Zn^{2+} (ZnT3 KO and the Mocha mutation), but the precise roles this ion plays in synaptic function is still controversial. While some studies suggest that long-term potentiation (LTP) and synaptic excitability in CA3 hippocampal neurons are unaffected by Zn^{2+} under physiological conditions (Vogt et al., 2000; Lopantsev et al., 2003), others reports have shown the opposite (Li et al., 2001; Huang et al., 2008). In CA3 hippocampal neurons, Zn^{2+} can directly promote the transactivation of the BDNF-related TrkB pathway (Huang et al., 2008); however, TrkB signaling can also be activated by extracellular Zn^{2+} in a metalloproteinase-dependent manner by releasing pro-BDNF and converting it to mature BDNF (Hwang et al., 2005). Paralleling these *in vitro* Zn^{2+}-mediated neurotrophic effects, chronic dietary treatment with Zn^{2+} has been found to induce an increase of brain levels of BDNF (Nowak et al., 2004; Corona et al., 2010) (Fig. 3*B*). It should be noted that in CA3 neurons, BDNF has been shown to activate a Zn^{2+}-independent Ca^{2+} current that is mediated by TRPC3 channels (Li et al., 2010).

Zn^{2+} also modulates LTP in the amygdala through regulation of feedforward GABAergic inhibition (Kodirov et al., 2006). A direct role for synaptic Zn^{2+} in learning and memory has been only recently uncovered. ZnT3 KO mice exhibit clear cognitive deficits, but only in animals aged beyond 6 months (Adlard et al., 2010). In other studies, 3- to 4-month-old ZnT3 KO animals show impaired contextual discrimination, spatial working mem-

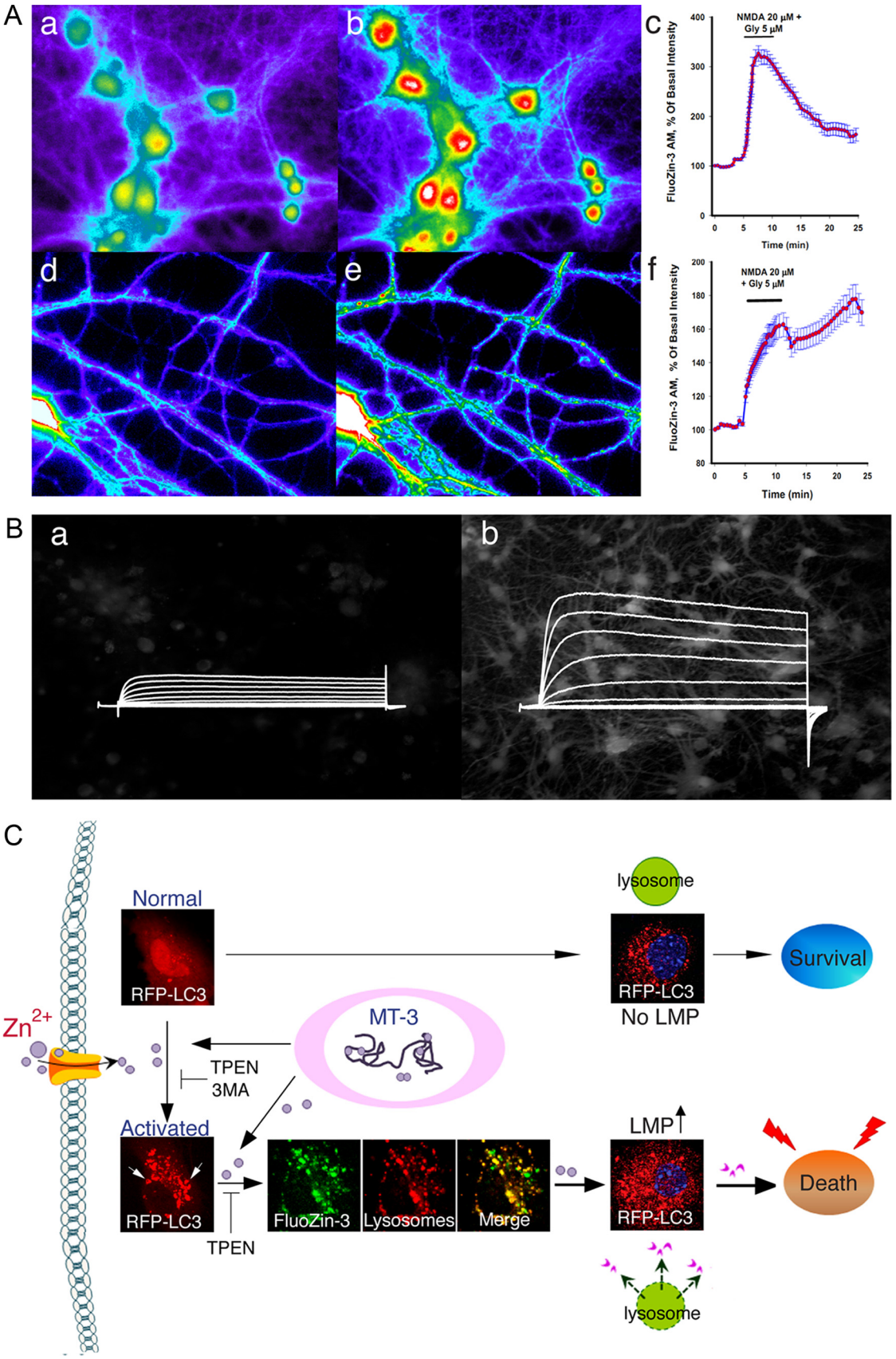

Figure 2. Zn^{2+}-mediated neuronal death and dysfunction. ***A***, NMDAR activation induces $[Zn^{2+}]_i$ mobilization in neuronal soma and dendrites. Neurons loaded with the Zn^{2+}-selective probe FluoZin3-AM (***a***, ***d***) were exposed to NMDA (20 μM) for 5 min. NMDAR activation induces $[Zn^{2+}]_i$ rises (***b***, ***e***) that show a different profile when analyzing changes in neuronal somata (*Figure legend continues*.)

ory, or learned fear extinction (Martel et al., 2010; Martel et al., 2011; Sindreu et al., 2011).

Among the targets of synaptic Zn^{2+}, NMDA receptors (NMDARs) are rather unique as they display very high sensitivity to extracellular Zn^{2+} (Paoletti et al., 1997; Traynelis et al., 1998). At low nanomolar concentrations, Zn^{2+} allosterically inhibits the activity of NMDARs containing the GluN2A subunit, a subunit that is widespread in the adult CNS. The cation acts on a discrete Zn^{2+}-binding site located in the large bilobate N-terminal domain of the GluN2A subunit (Fig. 1*A*) (Paoletti et al., 2000). The Zn^{2+}–GluN2A interaction likely mediates tonic inhibition of NMDARs by ambient Zn^{2+} levels. At higher Zn^{2+} concentrations (micromolar range), such as may occur during phasic synaptic release, Zn^{2+} binds to the N-terminal domain of the GluN2B subunit, thereby inhibiting GluN2B-containing receptors (Rachline et al., 2005). The *in vivo* relevance of the high-affinity Zn^{2+} inhibition of NMDARs has been recently addressed using a knock-in (KI) mouse line in which the GluN2A Zn^{2+} site has been specifically eliminated. GluN2A-KI mice display a pronounced pain phenotype, showing both hypersensitivity to acute thermal and chemical nociception and enhanced allodynia in models of inflammatory and neuropathic pain (Nozaki et al., 2011). Moreover, in the KI animals, analgesia produced by exogenous Zn^{2+} administrations is completely suppressed, revealing an essential role of the Zn^{2+}–GluN2A interaction in the pain-relieving effects of the cation (Nozaki et al., 2011).

Synaptic Zn^{2+} also interacts with a selective metabotropic receptor, the mZnR, which has been recently identified as the previously orphan G-protein-linked receptor GPR39 (Fig. 1*B*) (Besser et al., 2009). Zn^{2+} released from mossy fibers directly and specifically activates the mZnR in CA3 hippocampal neurons. The mZnR response is mediated by a G_q-coupled pathway that triggers IP3 production, followed by Ca^{2+} release from thapsigargin-sensitive stores. Subsequent activation of MAPK and CAMKII-dependent pathways has also been demonstrated. Employment of mice lacking GPR39 revealed that the ZnR-dependent Ca^{2+} response requires this protein. The mZnR is likely to modulate neuronal excitability as its activation enhances KCC2 function and Cl^- efflux in postsynaptic neurons, thereby inducing a pronounced hyperpolarizing shift in the $GABA_A$ reversal potential (Chorin et al., 2011). Notably, this effect is absent in GPR39 KO or ZnT3 KO mice lacking mZnR or synaptic Zn^{2+}, respectively. Thus, synaptic Zn^{2+}-dependent enhancement of KCC2 function, via the mZnR, alters the Cl^- gradient and may potentiate $GABA_A$ receptor-mediated inhibition. Synaptic Zn^{2+} deficiency, induced by decreased dietary intake, acute chelation, or genetic manipulations, leads to enhanced susceptibility to seizures (Fukahori and Itoh, 1990; Cole et al., 2000; Blasco-Ibáñez et al., 2004). Based on these results and the documented role of KCC2 in epilepsy, we suggest that the mZnR may provide a crucial link between activity-dependent release of synaptic Zn^{2+} and modulation of neuronal inhibition.

Zn^{2+}-dependent injury: a death by multiple cuts

Zn^{2+} promotes neuronal death by affecting multiple systems. Zn^{2+} can induce profound mitochondrial dysfunction by being sequestered in that organelle (for review, see Sensi et al., 2009). Zn^{2+} can trigger mitochondrial depolarization and the generation of reactive oxygen species (ROS) (Sensi et al., 1999, 2000; Dineley et al., 2005). These events, once investigated only in the neuronal somata, are now also identified in dendrites where they show a specific temporal pattern that differs from that found in the soma. This phenomenon may serve as *primum movens* for neuronal deafferentation and subsequent death (Medvedeva et al., 2009; Frazzini et al., 2011) (Fig. 2*A*).

Furthermore, Zn^{2+} induces a multiconductance cation channel activity in the inner mitochondrial membrane that is consistent with the activation of the mitochondrial permeability transition pore (mPTP; Jiang et al., 2001; Sensi et al., 2003; Gazaryan et al., 2007), thereby leading to release of pro-apoptotic mitochondrial proteins such as cytochrome C (Cyt-C) and apoptosis-inducing factor (AIF) (Jiang et al., 2001). Zn^{2+} can also profoundly affect mitochondrial trafficking and morphology (Malaiyandi et al., 2005).

Zn^{2+} deregulation favors neuronal death by increasing cytosolic oxidative stress through PKC (protein kinase C)-dependent activation of NADPH oxidase (Kim et al., 1999; Noh et al., 1999) as well as by activating the neuronal isoform of nitric oxide synthase (Kim et al., 1999; Kim and Koh, 2002). Moreover, Zn^{2+} triggers a lethal depletion of neuronal ATP by inhibiting the GAPDH (glyceraldehyde-3-phosphate dehydrogenase) enzyme, a process mediated by the reduction of cytosolic NAD^+ (Sheline et al., 2000) and reverted by pyruvate (Sheline et al., 2000). Evidence also indicates that Zn^{2+}-mediated NAD^+ depletion requires the activation of the sirtuin pathway as SIRT proteins are NAD^+-catabolic protein deacetylases and sirtuin inhibitors are neuroprotective against both acute and chronic Zn^{2+}-dependent toxicity, while, on the contrary, sirtuin activators promote NAD^+ depletion and neuronal death (Cai et al., 2006).

Zn^{2+} also triggers autophagic neuronal death (Hwang et al., 2008). Lysosomes are the organelles in which autophagic degradation occurs. Lysosomal hydrolases released into the cytosol promote cell death by breaking down cellular components as well as by activating death inducers such as BID through a process termed lysosomal membrane permeabilization (LMP). Recently, evidence has indicated that LMP is a key contributing mechanism in oxidative- and Zn^{2+}-induced hippocampal neuronal death (Hwang et al., 2008). Following exposure to H_2O_2 or toxic levels of Zn^{2+}, Zn^{2+} rapidly accumulates in lysosomes and Zn^{2+}-laden lysosomes undergo membrane disintegration releasing the toxic enzyme cathepsin. Exposure to the cell-permeable Zn^{2+} chelator tetrakis(2-pyridylmethyl)ethylenediamine (TPEN) not only completely blocks lysosomal Zn^{2+} rises but also inhibits LMP. Thus, Zn^{2+} deregulation may function as a linker between oxidative stress and LMP; however, what favors Zn^{2+} accumulation in lysosomes under oxidative conditions is still completely unknown. Zn^{2+} may enter lysosomes through Zn^{2+} transporters, Zn^{2+}-permeable channels, or exchangers. Alternatively, Zn^{2+} may be released inside lysosomes from proteins, but the precise inter-organelle dynamics of Zn^{2+} inside cells warrant further investigations.

←

(*Figure legend continued.*) (***c***) compared to dendrites (***f***). Time course graphs show changes in fluorescence levels in neuronal somata and dendrites before and after the NMDA challenge, respectively. Note how the dendritic $[Zn^{2+}]_i$ rises are long lasting, while in the soma, $[Zn^{2+}]_i$ levels rapidly recover to baseline. ***B***, A Zn^{2+}–K^+ continuum in neuronal apoptosis. Oxidant exposure in neurons (***a***) results in the liberation of Zn^{2+} from intracellular metal binding proteins (***b***). This Zn^{2+} activates a signaling cascade that ultimately produces a robust enhancement of voltage-activated delayed rectifier K^+ currents (***b***). This, in turn, leads to the loss of intracellular K^+, creating a permissive environment for the completion of apoptotic programs. ***C***, Zn^{2+} influx or intracellular Zn^{2+} release from metallothionein 3 (MT-3) activates autophagy and causes accumulation of Zn^{2+} in autophagosomes and autolysosomes. Under physiological conditions, activated autophagy serves beneficial functions by removing abnormal proteins and organelles. However, when in excess, it leads to LMP and neuronal death.

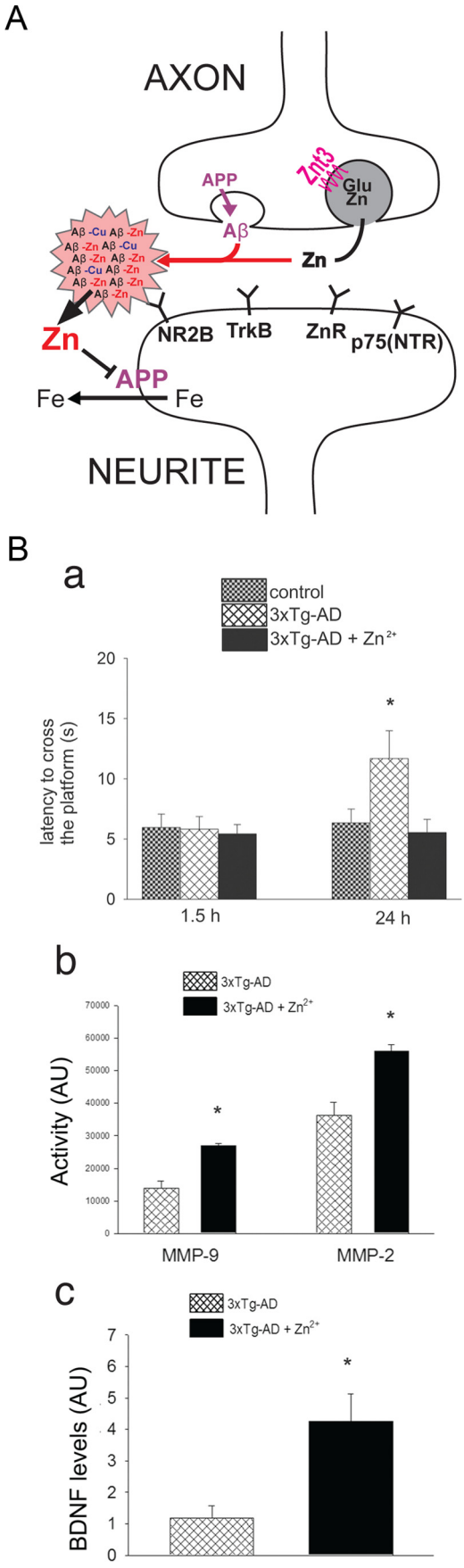

Figure 3. Zn^{2+} in Alzheimer's disease. ***A***, Zn^{2+} released during neurotransmission is trapped by amyloid, depriving targets essential for LTP. In addition, the Zn^{2+} transfers inappropriately to APP and inhibits its ferroxidase activity and ability to facilitate iron release from neurons, leading to pro-oxidant intraneuronal iron accumulation as a downstream consequence of extracellular Zn^{2+} accumulation. ***B***, Zn^{2+} supplementation is beneficial in an animal model of AD. 3xTg-AD mice chronically fed (11–13 months) with water containing 30 ppm of $ZnSO_4$ are protected from the appearance at 12–14 months of age of hippocampus-dependent memory deficits (as assessed with the Morris water maze test). Mice were tested when the platform was removed 1.5 h (***a***, left panel; to investigate short-term memory) and 24 h (***a***, right panel; to investigate long-term memory) after the last training trial. Zn^{2+}-fed 3xTg-AD mice exhibited a marked recovery in their long-term memory as indicated by the decreased time (latency) they used to reach the point where the platform used to be. ***b***, Zn^{2+} supplementation promotes metalloproteinase (MMPs) activation in 3xTg-AD mice as shown by gelatin zymography indicating a significant increase of MMP-2 and MMP-9 induction in 3xTg-AD mice brains. ***c***, BDNF immunoblotting reveals that Zn^{2+}-fed 3xTg-AD mice showed a fourfold increase in BDNF levels compared to untreated mice (modified from Corona et al., 2010). Error bars indicate mean values ± SEM. * indicates $p < 0.05$ in ***a*** and ***c*** and $p < 0.01$ in ***b***.

Interestingly, Zn^{2+} deprivation can also be a trigger for neuronal death and a converging set of evidence indicates that, most likely, neurons possess a finely tuned "Zn^{2+} set-point." In this respect, even though chelation of deregulated $[Zn^{2+}]_i$ is neuroprotective, excessive depletion of $[Zn^{2+}]_i$ by high-affinity cell-permeable Zn^{2+} chelators can be lethal (Lee et al., 2008). Zn^{2+} depletion can enhance endonuclease activity (Vincent and Maiese, 1999; Vincent et al., 1999) as well as counteract neuronal apoptosis by inhibiting Bax and Bak activation (Ganju and Eastman, 2003). In addition Zn^{2+} chelation by TPEN promotes neuronal apoptosis by inducing caspase-11 and caspase-3 activation (Lee et al., 2008). TPEN-induced Zn^{2+} depletion also favors a "dying-back" pattern of axon and dendrite degeneration due to Zn^{2+}-mediated ATP depletion and autophagy (Yang et al., 2007).

The possibility of neuronal injury triggered by Zn^{2+} depletion should be considered in light of the fact that Zn^{2+}-chelating strategies have been proposed as therapeutic measures in the aftermath of an ischemic insult as well as for the treatment of AD (Corona et al., 2011). The idea of a Zn^{2+} set-point is further substantiated within the context of AD as recent findings indicate that, in an AD mouse model, dietary supplementation of the cation starting as early as 1-month-old animals largely prevents the development of age-dependent mitochondrial dysfunction and hippocampal-dependent cognitive deficits and induces a potent increase in BDNF levels (Fig. 3*B*) (Corona et al., 2010).

Zn^{2+} dyshomeostasis and ischemic neuronal injury

A series of recent studies has promoted a reevaluation of the "calcium-centric" hypothesis that has dominated the field of ischemic neuronal death in the past three decades (Choi, 1988). Real-time "single-cell" ionic imaging techniques have in fact shown that Ca^{2+} works in synergy with Zn^{2+} to promote ischemic death. Ca^{2+} imaging in acute hippocampal slices undergoing oxygen and glucose deprivation (OGD; an *in vitro* model of brain ischemia) using a low-affinity Ca^{2+}-sensitive probe has shown that the OGD-driven increase in the probe fluorescence is substantially stunted (by 70%) by TPEN, indicating that, at least in some models, ischemia promotes a parallel, and possibly interdependent, surge of $[Ca^{2+}]_i$ and $[Zn^{2+}]_i$ (Stork and Li, 2006).

Indeed, analyzing the ionic changes of CA1 pyramidal neurons exposed to OGD, a more recent study has dissected the interplay between the two cations. The study showed that, within few minutes after OGD induction, neurons undergo Ca^{2+} deregulation and irreversible alteration of plasma membrane permeability (Medvedeva et al., 2009). Surprisingly, both processes are

preceded by elevations in $[Zn^{2+}]_i$ and associated with mitochondrial Zn^{2+} uptake as well as with depolarization and $[Zn^{2+}]_i$ chelation with TPEN results in delaying the phenomena (Medvedeva et al., 2009). A novel link explaining the synergistic deregulation of the two cations is offered by the downstream effects produced by glutamate and Ca^{2+} influx on the acidification of the neuronal cytosol. Acidosis is a potent trigger for Zn^{2+} mobilization from MTs and a recent study indicates that glutamate/Ca^{2+} influx leads to acidification of the neuronal cytosol, and that this is key to promote neuronal $[Zn^{2+}]_i$ rises (Kiedrowski, 2011). Synaptically released Zn^{2+} can promote ischemic neuronal death (especially in TGI) by entering postsynaptic neurons through routes that are used by Ca^{2+}, such as NMDARs and voltage-sensitive Ca^{2+} channels (VSCC), however most Zn^{2+} preferentially fluxes through Ca^{2+}-and Zn^{2+}-permeable GluA2-lacking AMPA receptors (Ca/ARs; for review, see Sensi et al., 2009). Ca/ARs are highly expressed and dynamically upregulated after TGI (Pellegrini-Giampietro et al., 1997) on postsynaptic membranes in the dendritic tree of TGI-vulnerable neurons and their pharmacological inhibition prevents Zn^{2+} influx and is highly neuroprotective in brain slices undergoing OGD or in animals exposed to TGI (Yin et al., 2002; Noh et al., 2005).

TGI-related apoptosis is also modulated by Zn^{2+} as the cation induces mPTP opening in isolated postischemic mitochondria extracted immediately after TGI and these intramitochondrial Zn^{2+} increases are linked to increased proteolytic cleavage of BCL-xL and the accumulation of the pro-apoptotic byproduct, deltaN-BCL-xL (Bonanni et al., 2006). Furthermore, the extracellular Zn^{2+} chelator, clioquinol (CQ), is neuroprotective and decreases the expression levels of caspase-3 and -9 and AIF in the hippocampus of CQ-treated gerbils undergoing ischemia (Wang et al., 2010).

Neurons can be also killed by intraneuronal mobilization of the metal. Studies using ZnT3KO mice have, in fact, shown that glutamate-driven $[Zn^{2+}]_i$ accumulation can result from Zn^{2+} released from sources such as MTs, mitochondria, and lysosomes (for review, see Sensi et al., 2009).

MTs are key players in excitotoxic and ischemic injury as they release Zn^{2+} upon oxidative stress, a phenomenon occurring in neurons and glia (Aizenman et al., 2000; Malaiyandi et al., 2001, 2004) that can lead to both caspase-dependent and caspase-independent forms of cell death (Aizenman et al., 2000; McLaughlin et al., 2001; Du et al., 2002). In the case of caspase-dependent cell death, the liberated Zn^{2+} triggers a signaling cascade that creates a permissive environment for the effective activation of proteases and nucleases. K^+ is a key modulator of this process as cells undergoing caspase-mediated death develop an early, robust drop of intracellular K^+ levels (Yu et al., 1997; Hughes and Cidlowski, 1999) reaching, in some cases, a final concentration of 50 mM (Hughes et al., 1997). This loss of $[K^+]_i$ favors the activation of caspases, including caspase 3, while the process and the subsequent neuronal death is inhibited when $[K^+]_i$ is maintained at physiological levels (Bortner et al., 1997; Hughes et al., 1997; Yu et al., 1997). In cortical and midbrain neurons, K^+ efflux is facilitated by a dramatic enhancement of delayed rectifier, voltage-activated K^+ currents mediated by Kv2.1-encoded channels (Fig. 2*B*) (McLaughlin et al., 2001; Pal et al., 2003; Redman et al., 2006). This K^+ current surge results from the following series of events: (1) Zn^{2+}-dependent activation of Src kinase in parallel to Zn^{2+}-induced inhibition of protein tyrosine phosphatase ε (PTPε), ensuring phosphorylation of Kv2.1 N-terminal tyrosine residue Y124 (Redman et al., 2009); (2) Zn^{2+}-triggered activation of p38 MAPK via the MAP3K apoptosis signaling kinase-1 (ASK-1; McLaughlin et al., 2001; Aras and Aizenman, 2005), resulting in the phosphorylation of Kv2.1 C-terminal serine residue S800 (Redman et al., 2007, 2009), and (3) exocytotic, SNARE-dependent membrane insertion of the Y124/S800 dual-phosphorylated Kv2.1 channel, leading to enhanced K^+ current densities (Pal et al., 2003, 2006). Preventing the rise of intracellular Zn^{2+} (McLaughlin et al., 2001), blocking any of the signaling steps along the pathway (McLaughlin et al., 2001; Pal et al., 2004, 2006; Aras and Aizenman, 2011), or interfering with the functional expression or membrane insertion of Kv2.1 (Pal et al., 2003, 2006) is sufficient to prevent neuronal death following oxidative, nitrosative, or chemical injury. Indeed, targeting the K^+ current surge-signaling pathway might provide novel therapeutic strategies in neuroprotection (Aras and Aizenman, 2011).

Zn^{2+} can also affect Cl^- homeostasis as a recent study reported that $[Zn^{2+}]_i$ rises inhibit the activity of the K^+/Cl^- cotransporter-2 (KCC2), the major Cl^- outward transporter in neurons and thereby a key determinant of GABAergic neurotransmission (Hershfinkel et al., 2009). OGD-triggered $[Zn^{2+}]_i$ rises are followed by a profound KCC2 inhibition and a depolarizing shift in the $GABA_A$ reversal potential, a process reversed by intraneuronal Zn^{2+} chelation. This process again indicates that $[Zn^{2+}]_i$ dyshomeostasis is an early and critical component of ischemic injury.

Adding a new angle to Zn^{2+}-dependent ischemic neuronal loss, evidence indicates that the cation also promotes injury by inhibiting the ubiquitin–proteasome system (Chen et al., 2009). Recent evidence in brain slices undergoing OGD also indicates that Zn^{2+} favors ischemic spreading depression, a wave of neuronal and glial depolarization that is thought to be a contributing factor in the enlargement of the infarct area (Carter et al., 2011).

Parenchymal acidosis is also a key modulator of Zn^{2+} dyshomeostasis upon cerebral ischemia. Ischemic acidosis can increase Zn^{2+} influx through VSCC and Ca/ARs and promote Zn^{2+} release from MTs, thereby favoring an overall neurotoxic increase in $[Zn^{2+}]_i$ levels (Jiang et al., 2000; Sensi et al., 2003; Frazzini et al., 2007). As protons also block NMDARs, ischemic acidosis can therefore serve as a switch to decrease NMDAR-mediated neuronal death while potentiating injury triggered by the activation of VSCC and AMPARs. Data from cultured neurons indicate that, in fact, AMPAR activation promotes ROS-mediated $[Zn^{2+}]_i$ rises that are enhanced by mild acidosis (Frazzini et al., 2007). Interestingly, Zn^{2+} can itself disrupt the neuronal acid–base equilibrium by blocking the Na^+/H^+ exchanger, thereby creating a feedforward loop as the cation promotes intracellular acidification and also delays recovery from intracellular acidification (Dineley et al., 2002).

Zn^{2+} in Alzheimer's disease

Aβ accumulation in the neocortex in AD is pathognomonic of AD, yet the mere production of this ubiquitously expressed 39–43 residue peptide does not offer explanations for why amyloid only forms in the neocortex, why mice and rats do not develop amyloid pathology with age, or why women and APP transgenic mice have accelerated amyloid formation. The exceptional colocalization of Aβ and Zn^{2+} in the glutamatergic synapses of the neocortex offers plausible explanations (Fig. 3*A*). Zn^{2+} induces the rapid, but reversible, aggregation of Aβ into amyloid precipitates (Bush et al., 1994; Cherny et al., 1999), the pathological hallmark of AD. The rat/mouse Aβ possesses three amino acid substitutions that attenuate the interaction of Zn^{2+} and prevent Zn^{2+}-induced precipitation (Bush et al., 1994). As

described earlier, Zn^{2+} is released in a dissociable form by glutamatergic fibers in the cortex and hippocampus, and ZnT3 loads Zn^{2+} into these synaptic vesicles. The distribution of ZnT3 expression closely approximates with the anatomical sites of Aβ deposition. ZnT3 is not appreciably expressed outside of the brain, and therefore the synaptic release of Zn^{2+} in the neocortex is a cogent explanation for why Aβ, which is released in the same vicinity, is liable to precipitate only in the brain. While several reports have found Zn^{2+} to be enriched in extracellular amyloid deposits (Lovell et al., 1998; Lee et al., 1999; Miller et al., 2006; Adlard et al., 2008), this represents only a small fraction of the total cortical volume, and the tissue total Zn^{2+} concentrations only rise during advanced pathology (Religa et al., 2006).

Genetic ablation of ZnT3 abolishes interstitial (Lee et al., 2002) and vessel-wall (Friedlich et al., 2004) amyloid pathology in transgenic mice overexpressing human Aβ. The increase in the levels of soluble Aβ in the brains of the APP transgenic $\times$ ZnT3 KO mice (Lee et al., 2002) confirmed that Zn^{2+} holds the amyloid mass in a dissociable equilibrium (Huang et al., 1997). Ablation of ZnT3 also abolished the difference in genders for this mouse model in amyloid burden. Female mice have greater levels of dissociable Zn^{2+} in this system (Lee et al., 2002), and ovariectomy raises hippocampal synaptic vesicle Zn^{2+} levels further, whereas estrogen replacement opposed this rise (Lee et al., 2004).

As mentioned above, Zn^{2+} may be a key modulator of synaptic activity and substrate for LTP. This may explain why ZnT3 KO mice develop a cognitive and memory loss by the age of 6 months, becoming a phenocopy for the cognitive loss seen in the AD model transgenic Aβ overexpressors (Adlard et al., 2010). Therefore, by trapping extracellular Zn^{2+}, amyloid pathology may deprive these targets of physiological Zn^{2+} and so contribute to downstream cognitive loss through a variety of mechanisms. At the same time, Zn^{2+} flux through the NMDAR promotes the attachment of Aβ oligomers to the NR2B subunit, which may also impair LTP, but can be reversed by treatment with the Zn^{2+} ionophore, CQ (Deshpande et al., 2009). This ionophoric mechanism that liberates Zn^{2+} from Aβ oligomers, returning Zn^{2+} to the relatively deficient neighboring cells, may explain the rapid benefits of PBT2 (an analog of CQ) on cognition and neurite outgrowth in AD animal and cell culture models (Adlard et al., 2008, 2011), as well as the rapid efficacy of the drug candidate in a phase 2 clinical trial of AD patients (Lannfelt et al., 2008; Faux et al., 2010).

The trapping of Zn^{2+} by extracellular amyloid also impacts upon neuronal iron homeostasis. The amyloid protein precursor (APP) is a ferroxidase that catalytically loads Fe^{3+} into transferrin, and is required for optimal iron export from neurons (Duce et al., 2010). Brain neuronal iron levels are increased in APP knock-out mice, as well as in AD, which provokes oxidative damage (Smith and Goldin, 1997; Duce et al., 2010). APP ferroxidase activity is 75% decreased in AD cortical tissue, caused by dissociation of Zn^{2+} from amyloid, and not caused by a decrease in APP levels (Duce et al., 2010). Abnormal iron homeostasis can also have broad sequelae on heme synthesis, and is another of the downstream ramifications of Zn^{2+} trapping by amyloid.

One major question to be answered is why extracellular Zn^{2+} begins to react with soluble Aβ with advanced aging. Extracellular Aβ concentrations are elevated in uncommon familial AD mutations, but there is no evidence of an elevation with age in sporadic cases. The prediction is that extracellular Zn^{2+} levels may rise with age. Zn^{2+} coreleased with glutamate in the synapse must be, like glutamate, taken back into the cells by a very rapid transport with a pattern of Zn^{2+} levels in the synaptic cleft that is likely to not be steady, but rather rapidly sinusoidal. There is no evidence for increased synaptic Zn^{2+} in AD, but it is possible that Zn^{2+} reuptake, which is energy dependent, may be fatigued with aging. Recent data have implicated the presenilins (PSs), whose mutations cause familial AD, in Zn^{2+} uptake (Greenough et al., 2011). Together, these data indicate that PS may be able to influence Aβ aggregation by metal ion clearance in the extra-neuronal vicinity, which is currently being studied further.

Conclusions

Critical new findings have begun to uncover the many physiological roles for synaptically released Zn^{2+}, as well as for intracellularly mobilized Zn^{2+}, acting as an important player in the modulation of neuronal excitability and survival. New territories, however, need to be explored. For instance, the physiopathological activity of Zn^{2+} in glial cells and how this is factored within the context of neuron–glia interaction requires further investigation. A more detailed road map of the regulatory processes that affect Zn^{2+} homeostasis and Zn^{2+}-dependent signaling is also needed. All these steps are crucial to find better pharmacological tools able to modulate cellular Zn^{2+}. These drugs are urgently needed as they are likely to have an important impact in the management of major neurological conditions like AD, epilepsy, and stroke.

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