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Contributions of Ca^{2+} and Zn^{2+} to spreading depression-like events and neuronal injury

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Abstract

The phenomenon of spreading depression (SD) involves waves of profound neuronal and glial depolarization that spread throughout brain tissue. Under many conditions, tissue recovers full function after SD has occurred, but SD-like events are also associated with spread of injury following ischemia or trauma. Initial large cytosolic Ca^{2+} increases accompany all forms of SD, but persistently elevated Ca^{2+} loading is likely responsible for neuronal injury following SD in tissues where metabolic capacity is insufficient to restore ionic gradients. Ca^{2+} channels are also involved in the propagation of SD, but the channel subtypes and cation fluxes differ significantly when SD is triggered by different types of stimuli. Ca^{2+} influx via P/Q type channels is important for SD generated by localized application of high K^{+} solutions. In contrast SD-like events recorded in *in vitro* ischemia models are not usually prevented by Ca^{2+} removal, but under some conditions, Zn^{2+} influx via L-type channels contributes to SD initiation. This review addresses different roles of Ca^{2+} in the initiation and consequences of SD, and discusses recent evidence that selective chelation of Zn^{2+} can be sufficient to prevent SD under circumstances that may have relevance for ischemic injury.

1. Cortical Spreading Depression

The phenomenon of spreading depression (SD) has intrigued neurophysiologists since its first description in the 1940s by Aristides Leão (Leao 1944). Leão made electrocorticographic recordings from the surface of the cat cortex, and found that strong stimulation could lead to a transient suppression of ongoing electrical activity. The depression of activity spread in an organized manner for considerable distances from the stimulation site and despite complete silencing of synaptic function, functional recovery was apparently complete after a period of 5–10 minutes (Leao 1944). Previous reviews have addressed in detail mechanisms of SD initiation and propagation (Somjen 2001, Smith *et al.* 2006, Martins-Ferreira *et al.* 2000, Hansen 1985), and discussed the basis of large ionic shifts that occur when neurons and astrocytes become fully depolarized during SD. When SD involves healthy brain tissue, neuronal damage is not generally observed, as the tissue restores normal ionic gradients fairly quickly following the passage of SD, and full synaptic function recovers (Buresova & Bures 1969, Nedergaard & Hansen 1988). It is not clear whether this type of fully-recoverable SD may have roles in normal brain function, however there is strong evidence that cortical SD can contribute to the phenomenon of migraine aura (Lauritzen 2001, Moskowitz 2007, Lauritzen 1994).

2. Spreading Depression-Like Events and Brain Injury

Leão also reported that a spreading depression-like event could be generated by carotid artery occlusion and raised the possibility that such events could contribute to ischemic injury (Leao 1947). A recent 2 photon imaging study has shown a wave-like loss of dendritic spines that is likely to underlie loss of synaptic activity during brief carotid occlusions (Murphy *et al.* 2008) and a large body of work has examined the potential roles of SD-like events in ischemic and traumatic brain injury (Strong *et al.* 2007, Gorji 2001, Hossmann 1996, Siesjö & Bengtsson 1989). Repetitive SD-like events often originate from the edge of infarcted areas (giving rise to the term “peri-infarct depolarizations”) and importantly, these events appear to contribute to injury progression, rather than simply being a secondary consequence of ischemic cell death (Hossmann 1996, Hartings *et al.* 2003, Strong *et al.* 2000, Nedergaard & Astrup 1986, Busch *et al.* 1996).

The relevance of SD-like events recorded in animal models to human ischemic injury has been questioned, partly because it has been difficult to make appropriate recordings to detect SD-like events in human subjects. However, a number of recent multi-center studies have established that such events are indeed very common in patients following ischemic or hemorrhagic stroke (Dohmen *et al.* 2008, Fabricius *et al.* 2008). SD-like events are also generated following traumatic injury to brain slices (Church & Andrew 2005) and while SD involvement in the progression of injury in an *in vivo* rat model has been questioned (von Baumgarten *et al.* 2008), SD-like events have been implicated in human traumatic brain injury (Strong *et al.* 2007, Fabricius *et al.* 2008).

To evaluate mechanisms that may be related to ischemic injury, much progress has been made from brain slice studies where SD-like events are generated by removal of oxygen from the superfusate (often termed hypoxic SD; HSD (Somjen 2001)). Fewer studies have examined responses produced by reducing both oxygen and glucose (usually termed oxygen-glucose deprivation; OGD), but SD-like events are also reliably produced with OGD. Once initiated, SD-like events triggered by hypoxia or OGD are remarkably similar to those produced by high K⁺ applications. Thus extracellular potential recordings report very similar electrical responses, as the responses sweep through the recording area with velocities similar to events recorded *in vivo* (~3–5mm/min). Despite the great similarity in the propagation of events, the consequences of SD-like events generated by hypoxia or OGD are substantially different to high K⁺-induced SD (Somjen 2001, Aitken *et al.* 1998, Dietz *et al.* 2008b). Responses to sustained hypoxia or OGD can result in irreversible neuronal injury, identified by severe beading of neuronal dendrites and similar responses are produced by bath application of the Na⁺/K⁺/ATPase inhibitor ouabain (Basarsky *et al.* 1999, Obeidat & Andrew 1998, Dietz *et al.* 2008b, Jarvis *et al.* 2001, Balestrino *et al.* 1999). In contrast, if SD is generated by localized high K⁺ applications, multiple events can be generated repetitively in the same tissues, if these brief stimuli are applied to healthy tissue with sufficient intervals (~10s of minutes) for full recovery of function between trials.

3. Relationship Between Neuronal Ca²⁺ Accumulation and Injury Following Different Forms of SD

From numerous studies since the mid 1980s, excessive Ca²⁺ accumulation has emerged as a central feature of many hypotheses of acute neuronal degeneration, and Ca²⁺ overload following SD is a likely contributor to injury following some forms of SD. The importance of Ca²⁺ influx has been demonstrated in studies of HSD, where selective removal of extracellular Ca²⁺ does not prevent the onset of SD, but does prevent subsequent neuronal injury (Balestrino & Somjen 1986, Roberts & Sick 1988, Tanaka *et al.* 1999, Yamamoto *et al.* 1997). If oxygen is restored quickly enough after the onset of HSD, neuronal viability

can be recovered, even if Ca^{2+} is present throughout (Somjen 2001, Tanaka et al. 1999), suggesting that lack of metabolic capacity to restore cytosolic Ca^{2+} levels could be responsible for injury.

Fluorescence measurements of intracellular Ca^{2+} levels support the idea that all forms of SD produce large initial Ca^{2+} increases, but injury only ensues when neuronal Ca^{2+} levels remain persistently elevated after the passage of SD. Single-cell recordings have been reported using the high-affinity Ca^{2+} indicator Fura-2 injected into single CA3 pyramidal neurons in hippocampal slice cultures. SD was generated by strong synaptic stimulation combined with brief reduction of extracellular Cl^- (by substitution of sodium chloride with sodium acetate) to reduce inhibitory currents. Very large Ca^{2+} increases were seen in apical dendrites and somata, but these responses recovered quite quickly and SD could be generated again in the same preparations (Kunkler & Kraig 2004). We used the lower affinity indicator Fura-6F to compare Ca^{2+} increases generated by high K^+ , OGD and ouabain in CA1 neurons. All these stimuli produced initial Ca^{2+} elevations of similar magnitude, reaching levels of tens of micromolar after the onset of SD-like events. However, responses to high K^+ then recovered quickly to baseline, while intracellular Ca^{2+} increases remained elevated throughout neurons following OGD-SD, or ouabain-SD, and were accompanied by rapid loss of indicator, suggesting membrane damage (Dietz et al. 2008b).

These observations support the idea that it is not the absolute amplitude of Ca^{2+} elevations that hold the key to degeneration, but rather the net Ca^{2+} flux that determines injury following SD (Somjen 2001). A similar situation has been described for excitotoxic injury in CA1 pyramidal cell dendrites, where neurons recover Ca^{2+} homeostasis after brief excursions of dendritic Ca^{2+} into the micromolar range, but are irreversibly damaged and lead to neuronal death when similar levels are maintained for periods of minutes (Shuttleworth & Connor 2001, Connor & Shuttleworth 2001).

The extended time course of Ca^{2+} loading following some forms of SD may be due to progressive inhibition of metabolic function during the time period before the SD arrives at a region of tissue. In the cases of SD triggered by localized high K^+ or brief trains of electrical stimulation, the onset of SD is virtually instantaneous and the response sweeps through regions of tissue that have had no prior metabolic challenge. In contrast, responses produced by hypoxia, OGD or ouabain are preceded by relatively long periods of time. When the SD event finally arrives at a location, a large Ca^{2+} overload occurs in the cytosol of neurons that have already been subjected to metabolic challenge, and/or other consequences of sustained depolarization. It is not yet known whether metabolic compromise leads to greater Ca^{2+} flux under these conditions, or alternatively whether decreased buffering and clearance of similar initial Ca^{2+} loads may be more important. Intracellular Na^+ loading appears to be a critical upstream requirement that contributes to loss of effective Ca^{2+} homeostasis during excitotoxic challenge (Vander Jagt *et al.* 2008) and Na^+ loading prior to SD could potentially underlie subsequent loss of Ca^{2+} homeostasis. As discussed below, progressive compromise of mitochondrial function prior to SD may also contribute to inability to restore neuronal Ca^{2+} homeostasis. Because of these observations, understanding events prior to the onset of SD could be helpful for understanding both the ultimate fate of neurons after different types of SD, as well as initiation of the events themselves.

4. Ca^{2+} influx is required for some forms of SD

SD that is triggered by either localized high K^+ applications or brief electrical stimulation appears to rely critically on Ca^{2+} influx for propagation of the event. SD produced by

localized high K^+ exposures in rat neocortical slices was completely prevented by pre-exposure to Ca^{2+} -free media (Footitt & Newberry 1998, Peters *et al.* 2003). Similarly, the non-selective Ca^{2+} channel blockers Co^{2+} and Ni^{2+} completely abolished the propagation of SD in hippocampal slices following localized high K^+ applications (Jing *et al.* 1993). Interestingly, initial voltage and extracellular Ca^{2+} shifts characteristic of SD were still recorded at the site of K^+ application (although amplitudes were substantially reduced), suggesting that Ca^{2+} channels were required for propagation, rather than initial depolarization (Jing *et al.* 1993). Ca^{2+} dependence of high K^+ -induced SD was recently confirmed in murine hippocampal slices, where we used autofluorescence imaging to track the initiation and progression of the event. Consistent with prior reports, when slices were pre-exposed to Ca^{2+} -free media, there was still evidence of a strong depolarization at the site of K^+ application, but no propagation of the event (Dietz *et al.* 2008b).

The subtype(s) of Ca^{2+} channels involved in SD were investigated in hippocampal slice cultures, following electrical stimulation coupled with transient exposure to NaAc-based superfusate. Like high K^+ -SD, this event appears fully recoverable and was prevented by non-selective Ca^{2+} channel blockers Ni^{2+} and Cd^{2+} and also selective blockers of P/Q type Ca^{2+} channels (ω -conotoxin-IVA) were very effective (Kunkler & Kraig 2004). P/Q channels are important throughout the brain for Ca^{2+} -dependent transmitter release, and impairment of depolarization-induced glutamate release may account substantially for the effectiveness of Ca^{2+} removal under these conditions.

Work with Ca^{2+} channel mutants strongly suggests that P/Q type Ca^{2+} channels may be responsible for propagation of cortical SD *in vivo*, and provide an additional strong link between SD and some forms of migraine. Mutations in the gene encoding the pore-forming subunit of P/Q type Ca^{2+} channels (the CACN1A gene) have been identified in two mutant mouse strains (tottering and leaner), and both these strains show higher thresholds for initiation of SD following localized K^+ applications *in vivo*. Once initiated, SD progressed substantially slower in these animals, consistent with a role of P/Q channels in cortical SD propagation (Ayata *et al.* 2000). In addition, gain-of-function knockin mutations of the CACN1A gene produced increased susceptibility to spreading depression following electrical stimulation to the cortical surface. Once initiated, SD events propagated at more than double the control rate, again suggesting an important role for P/Q channels in SD propagation (van den Maagdenberg *et al.* 2004). The mutation used in the latter study is also found in approximately 50% of cases of familial hemiplegic migraine, suggesting that this mutant mouse could be a useful model for studies of migraine therapies (van den Maagdenberg *et al.* 2004).

5. Ca^{2+} accumulation is not required for some forms of SD

In studies of hippocampal slices, it is conspicuous that Ca^{2+} removal does not prevent SD generated by either hypoxia, OGD or ouabain (Young & Somjen 1992, Rader & Lanthorn 1989, Basarsky *et al.* 1998, Dietz *et al.* 2008b, Bahar *et al.* 2000). In fact, it has been noted that Ca^{2+} removal may cause some enhancement in HSD onset (Somjen 2001). It has become recognized that substantial extracellular Ca^{2+} reductions can lead to activation of a non-selective cation current in hippocampal neurons (Chinopoulos *et al.* 2007, Xiong *et al.* 1997), and it seems possible that consequent Na^+ influx could facilitate reaching the threshold for SD initiation a little earlier when slices are challenged in Ca^{2+} -free media.

Despite the lack of effect of Ca^{2+} removal, it has been reported that non-specific Ca^{2+} channel blockers can impair the progression of HSD. When slices were pre-exposed to high concentrations of Ni^{2+} or Co^{2+} (2mM), HSD was prevented approximately 50% of the time, and when HSD did occur, the responses had longer latencies, and smaller ionic and voltage

shifts during the events (Jing et al. 1993). The mismatch between the lack of effect of Ca^{2+} removal (described above) and the effects of these channel blockers may be explained by non-specific effects of the blockers used (Somjen 2001). However, we recently observed a similar mismatch between the effects of Ca^{2+} removal and L-type channel blockers (nimodipine and nifedipine), when tested on ouabain-SD in hippocampal slices. Removal of extracellular Ca^{2+} did not prevent ouabain-SD under any recording conditions we examined. However, under a specific set of recording conditions (including activation of A1 subtype of adenosine receptors, 30 μM ouabain), L-type Ca^{2+} channel blockers always prevented the onset of ouabain-SD. Since these blockers are reportedly quite selective for L-type channels, we considered the possibility that effects of the blockers may be explained by flux of a different cation via L-type channels that contributes to ouabain-SD initiation. Zn^{2+} emerged as a candidate for this response since it permeates neuronal L-type channels (Kerchner et al. 2000, Weiss et al. 1993) and (as discussed below) accumulation of endogenous Zn^{2+} has become established as a likely contributor to some other forms of neurodegeneration.

It is important to note that a critical role for L-type channels was only found under some experimental conditions. Thus when A1 receptors were blocked, nimodipine did not prevent SD (Dietz et al. 2008b) and likewise when SD is generated by a higher ouabain concentration (100 μM) nimodipine does not prevent SD (Dietz and Shuttleworth, unpublished observations). L-type flux appears to act together with NMDA receptor activation to generate ouabain-SD and blocking L-type flux is not sufficient to block SD when glutamate receptor activation is enhanced.

6. Extracellular Zn^{2+} increases associated with SD

Zn^{2+} dynamics can be investigated by using high-affinity fluorescent indicators. FluoZin-3 has proven particularly useful, as this indicator has no demonstrable affinity for Ca^{2+} in most studies and membrane-permeable forms have been used to assess Zn^{2+} accumulation in neuronal cultures and brain slices (Martin et al. 2006, Devinney et al. 2005, Gee et al. 2002, Zhao et al. 2008). The membrane-impermeant form of FluoZin-3 has been used to load the extracellular space in brain slices, so that synaptic release of Zn^{2+} can be assessed by localized fluorescence increases (Qian & Noebels 2005, Qian & Noebels 2006, Datki et al. 2007). We used a similar approach to examine extracellular Zn^{2+} dynamics associated with SD, and then in separate experiments loaded membrane-impermeant FluoZin-3 into single CA1 neurons, in order to examine intracellular Zn^{2+} dynamics.

With all forms of SD we have examined so far (ouabain (Dietz et al. 2008b) OGD, high K^{+} SD (Dietz et al. 2008a)), large extracellular FluoZin-3 (FluoZin-3_{Ext}) increases were observed coincident with the onset of SD. Changes in tissue autofluorescence or contamination of Zn^{2+} from exogenous sources (Kay 2004) did not contribute to these signals. Since there do not appear to be major differences in extracellular Zn^{2+} response between SD stimuli tested, at this point it appears that the large spreading wave of neuronal and astrocytic depolarization may be sufficient to release Zn^{2+} from intra (or possibly extracellular) binding sites.

Zn^{2+} is present at high concentrations in the brain and concentrated within synaptic vesicles (Frederickson et al. 2005). In the CA1 region of the hippocampus, there is evidence that Schaffer collateral stimulation increases release of Zn^{2+} into the extracellular space, where it was detected with FluoZin-3. These signals were abolished in mice lacking the ZnT3 transporter, and since these animals lack Zn^{2+} in synaptic vesicles, this argues for a synaptic source of Zn^{2+} in the CA1 region (Qian & Noebels 2006). It is possible that this same source of Zn^{2+} is responsible for liberating large amounts of Zn^{2+} that are detected with

extracellular FluoZin-3 during the progression of SD (regardless of the initiating stimulus), as the terminals depolarize and release Zn^{2+} together with glutamate and other neurotransmitters.

A conspicuous difference in FluoZin-3_{Ext} dynamics was noted prior to the onset of SD. With both ouabain- and OGD-SD, significant FluoZin-3_{Ext} decreases occurred for approximately 5 min prior to the onset of SD. This signal may be due to movement of Zn^{2+} from the extracellular space via L-type channels, since nimodipine abolished initial FluoZin-3_{Ext} decreases when tested with ouabain-SD.

7. Intracellular Zn^{2+} increases and generation of SD-like events

Recordings from single CA1 neurons injected with FluoZin-3 support the suggestion of transmembrane Zn^{2+} flux, since significant intracellular FluoZin-3 (FluoZin-3_{Int}) increases were detected with a very similar time course (approximately 5 min) prior to the onset of SD, and these increases were also abolished by L-type channel block (Dietz et al. 2008b). The initial Zn^{2+} increases via L-type channels appear sufficient to explain the inhibitory effects of L-type channels on ouabain-SD under the recording conditions described above (30 μM ouabain, with an A1 agonist). Thus selective Zn^{2+} binding with the membrane permeable chelator TPEN abolished initial FluoZin-3 increases and also prevented the initiation of ouabain-SD under the same conditions where nimodipine was effective. Under some conditions Zn^{2+} can contribute significantly to SD triggered by OGD. Thus if synaptic transmission is reduced by adenosine A1 receptor activation, chelation of Zn^{2+} alone can be sufficient to prevent the onset of OGD-SD (Dietz et al. 2008b).

The source(s) of Zn^{2+} responsible for FluoZin-3_{Int} increases and SD initiation are not yet known, but as mentioned above, release from synaptic sources is a possibility. If this is the case, then it is noteworthy that the levels of synaptic Zn^{2+} are strongly developmentally regulated and mature levels of hippocampal Zn^{2+} do not accumulate until rodents mature past at least 2–3 weeks of age (Frederickson *et al.* 2006, Slomianka & Geneser 1997). This developmental difference could contribute to differences in apparent roles of Zn^{2+} under different experimental models and it is not yet known whether differences in Zn^{2+} availability may be relevant to the known increase in HSD sensitivity as animals develop. Postnatal development in rats is associated with a decreased latency to HSD onset, and the threshold extracellular K^{+} required for initiation of the event is also reduced (reviewed in Somjen 2001). While factors such as maturation of GABA and glutamatergic transmission and also reduction in extracellular volume are likely to contribute to this developmental change, increasing availability of Zn^{2+} could be an additional factor.

8. Zn^{2+} chelation alone is not sufficient to prevent some forms of SD

It is important to emphasize that there are many conditions where chelation of Zn^{2+} with TPEN is not sufficient to prevent SD. Thus we have not observed any demonstrable changes in FluoZin-3_{Ext} prior to the onset of high K^{+} -SD, and high K^{+} -SD was unaffected by Zn^{2+} chelation. These slice studies suggest little role for Zn^{2+} in non-injurious forms of cortical SD that are modeled by brief high K^{+} exposures. We have identified recording conditions where a critical role for Zn^{2+} can be demonstrated for OGD and ouabain-SD, but if synaptic release mechanisms are intentionally enhanced (by block of A1 receptors), the sensitivity of OGD and ouabain-SD to TPEN decreases. Block of NMDA receptors with MK801 remains fully effective under all recording conditions tested, so it appears that Zn^{2+} -dependent mechanisms supplement NMDA receptor activation to reach SD threshold. When synaptic activity is suppressed, the relative contribution of Zn^{2+} dependent mechanisms can be large enough that there is block of SD by TPEN, but with strong contributions from synaptic release, NMDA receptor activation alone can be sufficient to reach SD threshold. Adenosine

accumulation and suppression of transmission is a consequence of ischemic insults *in vivo*, suggesting that the contribution of Zn^{2+} to postischemic SD-like events could be significant, by increasing the relative contribution of Zn^{2+} mechanisms to SD threshold.

9. Relationship between Ca^{2+} and Zn^{2+} signals

Co-loading single hippocampal CA1 neurons with Fura-6F and FluoZin-3 was used to compare Ca^{2+} and Zn^{2+} signals associated with ouabain-SD. Fura indicators can be very sensitive to Zn^{2+} when measured in free solution, but when recordings are made in neurons with physiological Ca^{2+} concentrations and high indicator concentrations, interactions of Zn^{2+} with Fura can become greatly reduced (Devinney et al. 2005). Consistent with this view, FluoZin-3_{Int} increases observed prior to ouabain-SD were not matched by Fura-6F (or Fura-2) ratio increases (Dietz et al. 2008b).

Following ouabain-SD, FluoZin-3_{Int} increases initially tracked well with large Fura-6F ratio increases, but then decreased while Fura-6F ratio remained at high levels (Dietz et al. 2008b). It is likely that the loss of FluoZin-3 signal is an artifact due to loss of indicator from the neuron following SD and similar loss is seen in single wavelength Fura measurements. Ratiometric Zn^{2+} indicators with appropriate affinities will be very useful for addressing the relationship between Zn^{2+} accumulation and injury following SD, since it might be expected that Zn^{2+} levels would continue to rise following SD-induced neuronal injury. For example Zn^{2+} can be released from intracellular binding proteins, during excitotoxic or oxidative challenges (Aizenman *et al.* 2000, Bossy-Wetzel *et al.* 2004) and Zn^{2+} has been suggested to contribute to neuronal injury following OGD in brain slices (Stork & Li 2006). However, it appears likely that sustained very high Ca^{2+} loads (rather than Zn^{2+} increases) are responsible for the initial disruption of the plasma membrane and loss of fluorescent indicators from CA1 neurons during NMDA or OGD exposures in slice (Vander Jagt et al. 2008, Medvedeva, Shuttleworth & Weiss, unpublished)

The initial sites of Zn^{2+} accumulation have been difficult to assess with current imaging methods. While FluoZin-3 loading has proven adequate for measurements within neuronal somata, the degree of loading of fine dendritic processes is generally inferior to that observed with Fura-6F or related indicators and it is not yet known whether Zn^{2+} increases may originate in dendritic compartments.

10. Contributions of Ca^{2+} and Zn^{2+} to mitochondrial depolarization associated with SD

The involvement of Ca^{2+} -dependent mitochondrial depolarization in some forms of SD has been examined in brain slice studies using the potential-sensitive indicator Rhodamine 123 (Rh123). When HSD was examined in rat hippocampal slices, a slow ramp-like depolarization was observed prior to the onset of SD, followed by a much larger depolarization that occurred coincident with the onset of the SD event (Bahar et al. 2000). Removal of extracellular Ca^{2+} from the superfusate had no effect on these responses and it was suggested that Ca^{2+} release from intracellular stores could be involved, or alternatively other consequences of hypoxia could lead to the mitochondrial depolarization, and be more significant contributors than Ca^{2+} accumulation. We have observed very similar Rh123 signals when examining SD generated by ouabain in murine hippocampal slices. Thus a slow mitochondrial depolarization preceded SD, and a much larger rapid mitochondrial depolarization occurred coincident with the SD event. Like the previous HSD work, Ca^{2+} removal did not prevent the initial mitochondrial depolarization, but the responses were sensitive to block of plasma membrane Ca^{2+} channels (with nimodipine). Furthermore, this

effect can be explained in part by influx of Zn^{2+} , since TPEN significantly reduced initial Rh123 increases (Dietz et al. 2008b).

These data raise the possibility that deleterious effects of Zn^{2+} on mitochondrial function (Sensi *et al.* 1999) might contribute to the onset of ouabain-SD and OGD-SD. Previous work showing that inhibitors of mitochondrial function decrease the latency before HSD onset would be consistent with this possibility (Gerich *et al.* 2006), however it is also possible that that mitochondrial depolarization is an additional consequence of Zn^{2+} accumulation and not directly involved in the triggering the response. For example, it has been shown that Zn^{2+} accumulation can inhibit mitochondrial motility and cause neuronal injury at concentrations that do not impair the bioenergetic function of mitochondria (Malaiyandi *et al.* 2005). Additional targets that could contribute to SD include inhibition of glycolysis (Sheline *et al.* 2000) and possibly potentiation of NMDA receptor function (Kim *et al.* 2002).

Mitochondrial dysfunction due to Zn^{2+} has been implicated in neuronal injury following *in vivo* ischemia, and chelation of Zn^{2+} is effective even when administered well after the end of the ischemic episode (Calderone *et al.* 2004, Bonanni *et al.* 2006). It is currently unknown whether the beneficial effects of late Zn^{2+} chelation may include prevention of SD-like events, in addition to mitigation of other injury cascades.

11. Conclusions

All forms of SD involve large accumulations of Ca^{2+} and probably Zn^{2+} following the SD event, but an inability to restore ionic gradients likely underlies catastrophic Ca^{2+} overload and rapid injury following some forms of SD. From brain slice studies, it appears that Zn^{2+} accumulation plays little role in non-injurious forms of SD, and Zn^{2+} -dependent mechanisms would therefore seem unlikely to contribute to conditions such as migraine. In contrast, Zn^{2+} can contribute to generation of irrecoverable forms of SD, and this raises the possibility that Zn^{2+} accumulation could contribute to SD-like events following *in vivo* ischemia.

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References

- Aitken PG, Tombaugh GC, Turner DA, Somjen GG. Similar propagation of SD and hypoxic SD-like depolarization in rat hippocampus recorded optically and electrically. *J Neurophysiol.* 1998; 80:1514–1521. [PubMed: 9744955]
- Aizenman E, Stout AK, Hartnett KA, Dineley KE, McLaughlin B, Reynolds IJ. Induction of neuronal apoptosis by thiol oxidation: putative role of intracellular zinc release. *J Neurochem.* 2000; 75:1878–1888. [PubMed: 11032877]
- Ayata C, Shimizu-Sasamata M, Lo EH, Noebels JL, Moskowitz MA. Impaired neurotransmitter release and elevated threshold for cortical spreading depression in mice with mutations in the $\alpha 1A$ subunit of P/Q type calcium channels. *Neuroscience.* 2000; 95:639–645. [PubMed: 10670432]
- Bahar S, Fayuk D, Somjen GG, Aitken PG, Turner DA. Mitochondrial and intrinsic optical signals imaged during hypoxia and spreading depression in rat hippocampal slices. *J Neurophysiol.* 2000; 84:311–324. [PubMed: 10899206]
- Balestrino M, Somjen GG. Chlorpromazine protects brain tissue in hypoxia by delaying spreading depression-mediated calcium influx. *Brain Res.* 1986; 385:219–226. [PubMed: 3022872]

- Balestrino M, Young J, Aitken P. Block of (Na⁺,K⁺)ATPase with ouabain induces spreading depression-like depolarization in hippocampal slices. *Brain Res.* 1999; 838:37–44. [PubMed: 10446314]
- Basarsky TA, Duffy SN, Andrew RD, MacVicar BA. Imaging spreading depression and associated intracellular calcium waves in brain slices. *J Neurosci.* 1998; 18:7189–7199. [PubMed: 9736642]
- Basarsky TA, Feighan D, MacVicar BA. Glutamate release through volume-activated channels during spreading depression. *J Neurosci.* 1999; 19:6439–6445. [PubMed: 10414972]
- Bonanni L, Chachar M, Jover-Mengual T, et al. Zinc-dependent multi-conductance channel activity in mitochondria isolated from ischemic brain. *J Neurosci.* 2006; 26:6851–6862. [PubMed: 16793892]
- Bossy-Wetzel E, Talantova MV, Lee WD, et al. Crosstalk between nitric oxide and zinc pathways to neuronal cell death involving mitochondrial dysfunction and p38-activated K⁺ channels. *Neuron.* 2004; 41:351–365. [PubMed: 14766175]
- Buresova O, Bures J. The effect of prolonged cortical spreading depression on learning and memory in rats. *Journal of neurobiology.* 1969; 1:135–146. [PubMed: 5407041]
- Busch E, Gyngell ML, Eis M, Hoehn-Berlage M, Hossmann KA. Potassium-induced cortical spreading depressions during focal cerebral ischemia in rats: contribution to lesion growth assessed by diffusion-weighted NMR and biochemical imaging. *J Cereb Blood Flow Metab.* 1996; 16:1090–1099. [PubMed: 8898680]
- Calderone A, Jover T, Mashiko T, Noh KM, Tanaka H, Bennett MV, Zukin RS. Late calcium EDTA rescues hippocampal CA1 neurons from global ischemia-induced death. *J Neurosci.* 2004; 24:9903–9913. [PubMed: 15525775]
- Chinopoulos C, Connor JA, Shuttleworth CW. Emergence of a spermine-sensitive, non-inactivating conductance in mature hippocampal CA1 pyramidal neurons upon reduction of extracellular Ca²⁺: dependence on intracellular Mg²⁺ and ATP. *Neurochem Int.* 2007; 50:148–158. [PubMed: 16962211]
- Church AJ, Andrew RD. Spreading depression expands traumatic injury in neocortical brain slices. *J Neurotrauma.* 2005; 22:277–290. [PubMed: 15716633]
- Connor, JA.; Shuttleworth, CW. Intracellular Ca²⁺ signals underlying rapid and delayed neuronal death in mature CNS neurons. In: Lin, RCS., editor. *New concepts in cerebral ischemia.* CRC Press; 2001. p. 113-134.
- Datki ZL, Hunya A, Penke B. A novel and simple fluorescence method for the measurement of presynaptic vesicular zinc release in acute hippocampal slices with a fluorescence plate reader. *Brain research bulletin.* 2007; 74:183–187. [PubMed: 17683806]
- Devinney MJ 2nd, Reynolds IJ, Dineley KE. Simultaneous detection of intracellular free calcium and zinc using fura-2FF and FluoZin-3. *Cell Calcium.* 2005; 37:225–232. [PubMed: 15670869]
- Dietz, RM.; Weiss, JH.; Shuttleworth, CW. Contributions of Ca²⁺ and Zn²⁺ to spreading depression in hippocampal slices. 2008 Neuroscience Meeting Planner. Society for Neuroscience; 2008; 2008a. Online
- Dietz RM, Weiss JH, Shuttleworth CW. Zn²⁺ influx is critical for some forms of spreading depression in brain slices. *J Neurosci.* 2008b; 28:8014–8024. [PubMed: 18685026]
- Dohmen C, Sakowitz OW, Fabricius M, et al. Spreading depolarizations occur in human ischemic stroke with high incidence. *Ann Neurol.* 2008; 63:720–728. [PubMed: 18496842]
- Fabricius M, Fuhr S, Willumsen L, et al. Association of seizures with cortical spreading depression and peri-infarct depolarisations in the acutely injured human brain. *Clin Neurophysiol.* 2008; 119:1973–1984. [PubMed: 18621582]
- Footitt DR, Newberry NR. Cortical spreading depression induces an LTP-like effect in rat neocortex in vitro. *Brain Res.* 1998; 781:339–342. [PubMed: 9507183]
- Frederickson CJ, Giblin LJ 3rd, Balaji RV, et al. Synaptic release of zinc from brain slices: factors governing release, imaging, and accurate calculation of concentration. *J Neurosci Methods.* 2006; 154:19–29. [PubMed: 16460810]
- Frederickson CJ, Koh JY, Bush AI. The neurobiology of zinc in health and disease. *Nature reviews.* 2005; 6:449–462.

- Gee KR, Zhou ZL, Ton-That D, Sensi SL, Weiss JH. Measuring zinc in living cells. A new generation of sensitive and selective fluorescent probes. *Cell Calcium*. 2002; 31:245–251. [PubMed: 12098227]
- Gerich FJ, Hepp S, Probst I, Muller M. Mitochondrial inhibition prior to oxygen-withdrawal facilitates the occurrence of hypoxia-induced spreading depression in rat hippocampal slices. *J Neurophysiol*. 2006; 96:492–504. [PubMed: 16611842]
- Gorji A. Spreading depression: a review of the clinical relevance. *Brain Res Brain Res Rev*. 2001; 38:33–60. [PubMed: 11750926]
- Hansen AJ. Effect of anoxia on ion distribution in the brain. *Physiol Rev*. 1985; 65:101–148. [PubMed: 3880896]
- Hartings JA, Rolli ML, Lu XC, Tortella FC. Delayed secondary phase of peri-infarct depolarizations after focal cerebral ischemia: relation to infarct growth and neuroprotection. *J Neurosci*. 2003; 23:11602–11610. [PubMed: 14684862]
- Hossmann KA. Periinfarct depolarizations. *Cerebrovascular and brain metabolism reviews*. 1996; 8:195–208. [PubMed: 8870974]
- Jarvis CR, Anderson TR, Andrew RD. Anoxic depolarization mediates acute damage independent of glutamate in neocortical brain slices. *Cereb Cortex*. 2001; 11:249–259. [PubMed: 11230096]
- Jing J, Aitken PG, Somjen GG. Role of calcium channels in spreading depression in rat hippocampal slices. *Brain Res*. 1993; 604:251–259. [PubMed: 8384511]
- Kay AR. Detecting and minimizing zinc contamination in physiological solutions. *BMC Physiol*. 2004; 4:4. [PubMed: 15113426]
- Kerchner GA, Canzoniero LM, Yu SP, Ling C, Choi DW. Zn²⁺ current is mediated by voltage-gated Ca²⁺ channels and enhanced by extracellular acidity in mouse cortical neurones. *J Physiol*. 2000; 528(Pt 1):39–52. [PubMed: 11018104]
- Kim TY, Hwang JJ, Yun SH, Jung MW, Koh JY. Augmentation by zinc of NMDA receptor-mediated synaptic responses in CA1 of rat hippocampal slices: mediation by Src family tyrosine kinases. *Synapse*. 2002; 46:49–56. [PubMed: 12211081]
- Kunkler PE, Kraig RP. P/Q Ca²⁺ channel blockade stops spreading depression and related pyramidal neuronal Ca²⁺ rise in hippocampal organ culture. *Hippocampus*. 2004; 14:356–367. [PubMed: 15132435]
- Lauritzen M. Pathophysiology of the migraine aura. The spreading depression theory. *Brain*. 1994; 117 (Pt 1):199–210. [PubMed: 7908596]
- Lauritzen M. Cortical spreading depression in migraine. *Cephalalgia*. 2001; 21:757–760. [PubMed: 11595007]
- Leao AAP. Spreading depression of activity in the cerebral cortex. *J Neurophysiol*. 1944; 7:359–390.
- Leao AAP. Further observations on the spreading depression of activity in the cerebral cortex. *J Neurophysiol*. 1947; 10:409–414. [PubMed: 20268874]
- Malaiyandi LM, Honick AS, Rintoul GL, Wang QJ, Reynolds IJ. Zn²⁺ inhibits mitochondrial movement in neurons by phosphatidylinositol 3-kinase activation. *J Neurosci*. 2005; 25:9507–9514. [PubMed: 16221861]
- Martin JL, Stork CJ, Li YV. Determining zinc with commonly used calcium and zinc fluorescent indicators, a question on calcium signals. *Cell Calcium*. 2006; 40:393–402. [PubMed: 16764924]
- Martins-Ferreira H, Nedergaard M, Nicholson C. Perspectives on spreading depression. *Brain Res Brain Res Rev*. 2000; 32:215–234. [PubMed: 10751672]
- Moskowitz MA. Genes, proteases, cortical spreading depression and migraine: impact on pathophysiology and treatment. *Functional neurology*. 2007; 22:133–136. [PubMed: 17925161]
- Murphy TH, Li P, Betts K, Liu R. Two-photon imaging of stroke onset in vivo reveals that NMDA-receptor independent ischemic depolarization is the major cause of rapid reversible damage to dendrites and spines. *J Neurosci*. 2008; 28:1756–1772. [PubMed: 18272696]
- Nedergaard M, Astrup J. Infarct rim: effect of hyperglycemia on direct current potential and [14C]2-deoxyglucose phosphorylation. *J Cereb Blood Flow Metab*. 1986; 6:607–615. [PubMed: 3760045]
- Nedergaard M, Hansen AJ. Spreading depression is not associated with neuronal injury in the normal brain. *Brain Res*. 1988; 449:395–398. [PubMed: 3395856]

- Obeidat AS, Andrew RD. Spreading depression determines acute cellular damage in the hippocampal slice during oxygen/glucose deprivation. *Eur J Neurosci.* 1998; 10:3451–3461. [PubMed: 9824458]
- Peters O, Schipke CG, Hashimoto Y, Kettenmann H. Different mechanisms promote astrocyte Ca^{2+} waves and spreading depression in the mouse neocortex. *J Neurosci.* 2003; 23:9888–9896. [PubMed: 14586018]
- Qian J, Noebels JL. Visualization of transmitter release with zinc fluorescence detection at the mouse hippocampal mossy fibre synapse. *J Physiol.* 2005; 566:747–758. [PubMed: 15919713]
- Qian J, Noebels JL. Exocytosis of vesicular zinc reveals persistent depression of neurotransmitter release during metabotropic glutamate receptor long-term depression at the hippocampal CA3-CA1 synapse. *J Neurosci.* 2006; 26:6089–6095. [PubMed: 16738253]
- Rader RK, Lanthorn TH. Experimental ischemia induces a persistent depolarization blocked by decreased calcium and NMDA antagonists. *Neurosci Lett.* 1989; 99:125–130. [PubMed: 2546106]
- Roberts EL Jr, Sick TJ. Calcium-sensitive recovery of extracellular potassium and synaptic transmission in rat hippocampal slices exposed to brief anoxia. *Brain Res.* 1988; 456:113–119. [PubMed: 3409029]
- Sensi SL, Yin HZ, Carriedo SG, Rao SS, Weiss JH. Preferential Zn^{2+} influx through Ca^{2+} -permeable AMPA/kainate channels triggers prolonged mitochondrial superoxide production. *Proc Natl Acad Sci U S A.* 1999; 96:2414–2419. [PubMed: 10051656]
- Sheline CT, Behrens MM, Choi DW. Zinc-induced cortical neuronal death: contribution of energy failure attributable to loss of NAD(+) and inhibition of glycolysis. *J Neurosci.* 2000; 20:3139–3146. [PubMed: 10777777]
- Shuttleworth CW, Connor JA. Strain-dependent differences in calcium signaling predict excitotoxicity in murine hippocampal neurons. *J Neurosci.* 2001; 21:4225–4236. [PubMed: 11404408]
- Siesjo BK, Bengtsson F. Calcium fluxes, calcium antagonists, and calcium-related pathology in brain ischemia, hypoglycemia, and spreading depression: a unifying hypothesis. *J Cereb Blood Flow Metab.* 1989; 9:127–140. [PubMed: 2537841]
- Slomianka L, Geneser FA. Postnatal development of zinc-containing cells and neuropil in the hippocampal region of the mouse. *Hippocampus.* 1997; 7:321–340. [PubMed: 9228529]
- Smith JM, Bradley DP, James MF, Huang CL. Physiological studies of cortical spreading depression. *Biological reviews of the Cambridge Philosophical Society.* 2006; 81:457–481. [PubMed: 16848916]
- Somjen GG. Mechanisms of spreading depression and hypoxic spreading depression-like depolarization. *Physiol Rev.* 2001; 81:1065–1096. [PubMed: 11427692]
- Stork CJ, Li YV. Intracellular zinc elevation measured with a “calcium-specific” indicator during ischemia and reperfusion in rat hippocampus: a question on calcium overload. *J Neurosci.* 2006; 26:10430–10437. [PubMed: 17035527]
- Strong AJ, Hartings JA, Dreier JP. Cortical spreading depression: an adverse but treatable factor in intensive care? *Current opinion in critical care.* 2007; 13:126–133. [PubMed: 17327732]
- Strong AJ, Smith SE, Whittington DJ, Meldrum BS, Parsons AA, Krupinski J, Hunter AJ, Patel S, Robertson C. Factors influencing the frequency of fluorescence transients as markers of peri-infarct depolarizations in focal cerebral ischemia. *Stroke.* 2000; 31:214–222. [PubMed: 10625740]
- Tanaka E, Yamamoto S, Inokuchi H, Isagai T, Higashi H. Membrane dysfunction induced by in vitro ischemia in rat hippocampal CA1 pyramidal neurons. *J Neurophysiol.* 1999; 81:1872–1880. [PubMed: 10200222]
- van den Maagdenberg AM, Pietrobon D, Pizzorusso T, et al. A *Cacna1a* knockin migraine mouse model with increased susceptibility to cortical spreading depression. *Neuron.* 2004; 41:701–710. [PubMed: 15003170]
- Vander Jagt TA, Connor JA, Shuttleworth CW. Localized loss of Ca^{2+} homeostasis in neuronal dendrites is a downstream consequence of metabolic compromise during extended NMDA exposures. *J Neurosci.* 2008; 28:5029–5039. [PubMed: 18463256]
- von Baumgarten L, Trabold R, Thal S, Back T, Plesnila N. Role of cortical spreading depressions for secondary brain damage after traumatic brain injury in mice. *J Cereb Blood Flow Metab.* 2008; 28:1353–1360. [PubMed: 18414497]

- Weiss JH, Hartley DM, Koh JY, Choi DW. AMPA receptor activation potentiates zinc neurotoxicity. *Neuron*. 1993; 10:43–49. [PubMed: 7678965]
- Xiong Z, Lu W, MacDonald JF. Extracellular calcium sensed by a novel cation channel in hippocampal neurons. *Proc Natl Acad Sci U S A*. 1997; 94:7012–7017. [PubMed: 9192683]
- Yamamoto S, Tanaka E, Shoji Y, Kudo Y, Inokuchi H, Higashi H. Factors that reverse the persistent depolarization produced by deprivation of oxygen and glucose in rat hippocampal CA1 neurons in vitro. *J Neurophysiol*. 1997; 78:903–911. [PubMed: 9307123]
- Young JN, Somjen GG. Suppression of presynaptic calcium currents by hypoxia in hippocampal tissue slices. *Brain Res*. 1992; 573:70–76. [PubMed: 1315607]
- Zhao J, Bertoglio BA, Gee KR, Kay AR. The zinc indicator FluoZin-3 is not perturbed significantly by physiological levels of calcium or magnesium. *Cell Calcium*. 2008; 44:422–426. [PubMed: 18353435]