Kalzium Ist Nicht Alles

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Elevation of cerebral Mg^{2+} with a novel orally delivered ionophore, magnesium threonate, enhances cognition in young and old rats over a 12-24 day treatment interval, as outlined in a paper by Slutsky et al. in this issue of *Neuron*. Despite both Mg^{2+} and Zn^{2+} blocking the NMDA receptor channel, sustained extracellular Mg^{2+} elevation mimics sustained synaptic Zn^{2+} concentrations by increasing hippocampal NR2B expression and bouton density.

Since Otto Loewy remarked "Ja Kalzium, das ist alles!" in 1959, calcium developed an almost unassailable status as the predominant inorganic metal ion for its diverse roles in biochemistry and, of course, neurochemistry (Carafoli, 2002). But in the last 10 years a steadily increasing amount of research has highlighted the importance of the other bioinorganic metal ions in functional neurochemistry. In this issue of *Neuron*, Slutsky et al. (2010) present compelling data that an oral agent that delivers magnesium (Mg) to the brain improves cognition in young and old rats. These findings carry several important implications, not the least of which is that oral agents that target the inorganic milieu of the brain can markedly impact cognitive function and its biochemical substrates.

Mg supplementation has been previously reported to enhance cognitive performance in young and old rats (Landfield and Morgan, 1984), but, as Slutsky et al. point out, oral Mg supplementation is not efficiently bioavailable, and the large doses of Mg^{2+} required to benefit the animals lead to gastrointestinal side effects that limit the translatability of the benefits. Slutsky et al. successfully overcame this problem by identifying a Mg^{2+} ionophore, threonate, that forms a stable complex with Mg (MgT) with much better orally bioavailability than Mg^{2+} salts. The blood-brain barrier (BBB) is remarkably impervious to plasma metal ion fluctuations so that brain tissue is protected from the dramatic changes in metal ion levels that occur with prandial status. The authors point out that even a sustained elevation in plasma Mg^{2+} by infusion with $MgSO_4$ causes only a modest elevation in CSF Mg in humans, and no change in brain Mg in rats. Therefore, they searched for a chaperone or ionophore to try to overcome the BBB impermeability. Following oral treatment with Mg-equivalent doses of MgT, $MgCl_2$, and magnesium-gluconate, only MgT induced an elevation in CSF Mg levels after 24 days. Even then the increase in CSF Mg was only modest (15%), but there was no increase at all with the other supplements. The elevation of CSF Mg induced by MgT may actually reflect a much larger Mg increase in the cortical parenchyma or synapses, because Mg^{2+} may enter the CSF from the brain tissue (Hallak et al., 1992).

Because Mg^{2+} blocks the NMDA receptor (NMDAR) channel opening, elevating extracellular Mg^{2+} might have been expected to inhibit cognition. However, the authors had built their hypothesis also upon their previous observations that increasing extracellular Mg^{2+} concentrations 50% within the physiological range for protracted (4 hr) periods in primary neuronal cultures causes permanent enhancement of synaptic plasticity despite blocking Ca^{2+} flux through the NMDAR channel (Slutsky et al., 2004). In the current manuscript, the group extends their in vitro examination of the sustained elevation of extracellular Mg^{2+} by extensive electrophysiological studies in primary neuronal cells and hippocampal slices. The slices were treated for 5 hr with 50% elevated Mg^{2+} (1.2 mM), which, the authors show, leads to an increase in the number of functional presynaptic release sites with lower release probability. To summarize the in vitro studies, sustained elevation of extracellular Mg^{2+} does not merely block the NMDAR, but acts also as a first messenger to induce adaptation of the synapse in a manner that facilitates LTP: despite the block, an apparent upregulation of the NMDAR leads to NMDAR background currents remaining constant, but with enhanced current during bursting activity.

The authors argue that that this mechanism could underlie the impressive array of cognitive benefits that follow treatment with MgT for 12–30 days. Young (2-month-old) and old (18- to 22- month-old) MgT-treated rats demonstrated significantly improved performance in this time frame in the novel object recognition test (short-term memory), the T-maze test (spatial working memory), and the Morris water maze (spatial long-term memory, and memory recall). Curiously, while the improvement in T-maze performance was sustained in the young rats for 5 weeks after MgT treatment, the performance of old treated rats rapidly deteriorated but could be revived with a second treatment. This could reflect an age-dependent inability to maintain a reservoir of brain Mg. While this storage lesion is still unknown, its importance is underscored by the prevalence of Mg deficiency in old age that the authors note.

Consistent with the increase in functional release sites found in neurons and slices treated with sustained elevations of extracellular Mg^{2+}, MgT-treated rats had significantly increased densities of hippocampal presynaptic boutons, and the increase correlated with improved memory performance. The authors link this to a 60% elevation in hippocampal NR2B (but not NR2A or NR1) and BDNF (+36%) levels in MgT-treated animals. This is reminiscent of our own recent findings that hippocampal NR2B levels also

depend on the presence of sustained extracellular Zn^{2+}, the other extracellular metal ion that blocks the NMDAR channel. ZnT3 knockout mice, which lose the release of presynaptic Zn^{2+} upon activation at glutamatergic synapses, exhibit decreases in NR2B (−50%), pro-BDNF (−30%), and the density of hippocampal boutons (Adlard et al., 2010). Therefore, NR2B expression may be regulated by factors that block the channel. Notably, however, Mg^{2+} and Zn^{2+} bind at different sites of the channel, but the physiological significance is unclear (Mony et al., 2009).

Like any good paper, this report opens many questions. The caveat acknowledged by the authors is the proposed mechanism of action of MgT. While the in vitro data on the proposed adaptive mechanism is attractive, its premise is that extracellular Mg^{2+} elevations are sustained, and there is no direct evidence of that, only the modest rise (7%–15%) in CSF Mg, which does not reach the 50% level of the in vitro studies. In vivo microdialysis measurements may help defend the proposed model of action. Also, there is an intracellular Mg^{2+} store that seems to fatigue with aging, yet it could also represent a site of action of MgT that cannot be excluded from the data.

Considering that intravenous Mg^{2+} has been in existence as a treatment for preeclamptic seizures for decades (Euser and Cipolla, 2009), and that evidence supports its use in preventing damage of the BBB (e.g., in stroke), it is remarkable how little is known about the mechanism of Mg^{2+} transport across the BBB. Potential mechanisms of action of Mg^{2+} in these clinical situations include stabilization of the BBB and increased perfusion, which may also contribute to the benefits of MgT in the current report. Yet, Mg^{2+} is not a wonder drug for brain injury. A recent large phase 2 clinical trial for head injury found that moderate intravenous Mg^{2+} treatment significantly worsened outcome (Temkin et al., 2007).

MgT, nevertheless, represents a very promising lead for drug development, and shows that the BBB can be pharmacologically surmounted to therapeutically increase the uptake of metal ions by the brain. This is also the principal of recent Alzheimer's disease (AD) candidate disease-modifying ionophore compounds clioquinol and PBT2, which facilitate the uptake of Zn^{2+} and Cu^{2+} captured from amyloid pathology into cortical parenchymal cells (Adlard et al., 2008). PBT2 showed beneficial effects in a recent phase 2 clinical trial in AD (Lannfelt et al., 2008). Therefore, the medical chemistry of brain interstitial metal ion concentrations seems to be rapidly developing as a promising avenue of potential intervention for cognitive disorders.

It should be interesting to test MgT in models of neurological injury wherein Mg^{2+} has been implicated, such as stroke, head-injury, epilepsy, AD, and Parkinson's disease. One issue facing the planning of potential clinical trials is the large dose that was effective in the current studies: 604 mg/kg/d (50 mg eq Mg). Even when allowing for a body surface area correction when translating (Reagan-Shaw et al., 2008), the estimated human dose for early phase trials would be 98 mg/kg/d or about 7 g of MgT per day, which could be an impractical dose for a trial. However, this work should spur the exploration of further novel Mg^{2+} ionophores or organic chaperones. In any case, work like this enhances the appreciation of the inorganic milieu of the brain beyond the preoccupation with calcium. Hence, "Kalzium ist nicht alles."

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