This Week in The Journal

Polyphosphate Protects Neurons from Excitotoxicity

Marta Maiolino, Nathanael O'Neill, Vincenzo Lariccia, Salvatore Amoroso, Sergiy Sylyantsev, et al.

(see pages 6038–6048)

Inorganic polyphosphate, a linear polymer of 5–1000s of phosphate units, is abundant throughout the body, and it participates in a variety of cellular processes, including mitochondrial metabolism, calcium handling, and protein folding. Polyphosphate levels are relatively high in the nervous system, particularly in astrocytes. Bath application of exogenous polyphosphate activates P2Y1 receptors in astrocytes, leading to increases in intracellular calcium levels and release of endogenous polyphosphate from intracellular vesicles. The effects of polyphosphate on neurons is less clear: when bath applied, it had little effect on calcium levels in most neurons, but when puffed on, it induced neuronal firing, likely because, as a polyanion, it alters the membrane surface charge and thus affects the function of voltage-sensitive sodium, calcium, and potassium channels (Stotz et al., 2014 *Mol Brain* 7:42).

Maiolino et al. now report that polyphosphate also inhibits glutamate-induced calcium elevation in neurons. In cortical neurons cocultured with astrocytes, bath application of polyphosphate—particularly chains shorter than 15 units—reduced neuronal calcium elevation in response to bath application of physiological levels of glutamate. Polyphosphate also blocked calcium elevation induced by AMPA, but not by NMDA. Short-chain polyphosphates also reduced the toxic effects of high concentrations of glutamate, including delayed calcium deregulation, mitochondrial depolarization, and neuronal death. The effects of polyphosphate on neurons were blocked by an antagonist of P2Y1 receptors and were mimicked by a P2Y agonist.

These results suggest that polyphosphate protects neurons from excitotoxicity by reducing glutamate-induced calcium elevation. The fact that short polyphosphate chains were more effective than longer chains is important, because previous work has shown that long chains induce mitochondrial depolarization and apoptosis

(Angelova et al., 2016 *Biochem Soc Trans* 44:40–45). Whether the effects stem from activation of P2Y1 receptors in neurons or astrocytes is unclear. It is possible, for example, that activation of astrocytic P2Y1 receptors triggers the release of gliotransmitters that regulate neuronal glutamate responses. Future work should clarify these mechanisms.

Axon terminals are more highly branched in synaptobrevin-deficient motor neurons (right) than in controls (left). See Liu et al. for details.

Synaptobrevin Limits Spontaneous Vesicle Release

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(see pages 6049–6066)

Synaptic vesicle release is driven by a zipper-like interaction between SNARE proteins in the vesicular and active-zone membranes. In the resting state, vesicles are docked at the plasma membrane by a protein complex that includes SNARE proteins, a calcium-sensor, and other proteins. When calcium binds to the sensor, the SNARE zipper closes, forcing the vesicular and plasma membranes together. Many vesicles fuse simultaneously when an action potential invades the terminal and causes calcium channels to open. Single vesicles can fuse with the plasma membrane between action potentials, but these spontaneous fusion events are thought to

involve different vesicles, vesicular SNAREs, and calcium sensors than evoked release (Kavalali, 2017 *J Neurosci Res* 96:331).

Synaptobrevins are the main vesicular SNAREs involved in evoked release. Consistent with this, Liu et al. found that knocking out Syb1 and Syb2, the primary synaptobrevins in the mouse nervous system, greatly reduced evoked release at neuromuscular junctions (NMJs). At the same time, however, synaptobrevin knock-out increased the frequency and amplitude of spontaneous release events at NMJs. This occurred even though the vesicular SNAREs thought to drive spontaneous release were barely detectable in embryonic motor neurons and their expression was unaffected by synaptobrevin knock-out. Moreover, unlike in wild-type neurons, spontaneous release was independent of intracellular and extracellular calcium levels in synaptobrevin-deficient neurons.

Spontaneous vesicle release is thought to regulate neurite growth and synapse formation during embryonic development. Indeed, synaptobrevin knock-out greatly increased the extent of motor-axon branching and the size and number of motor endplates in muscle fibers. In contrast, the number of vesicles and the synaptic contact length in presynaptic terminals were lower in mutant mice than in controls.

These results confirm that synaptobrevins are essential for evoked synaptic vesicle release at NMJs. The reduction in evoked release from synaptobrevin-deficient motor axons might contribute to their exuberant growth through homeostatic mechanisms; the resulting increase in endplate size and number likely contributes to increases in the number of spontaneous release events measured in muscle fibers. But the results also reveal a role for synaptobrevins in conferring calcium dependency to spontaneous release. The loss of this dependency likely contributes to the increase in spontaneous events and exuberant growth as well. Which of these mechanisms dominates and how synaptobrevin contributes to the calcium dependence of spontaneous release remain to be discovered.

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