Thinking acidic Ca^{2+} stores

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One-sentence summary: Foster *et al*. show that mobilization of acidic Ca^{2+} stores by the intracellular messenger NAADP links metabotropic glutamate receptor signaling to synaptic plasticity in the hippocampus.

Abstract:

Glutamate signaling regulates neuronal activity and synaptic plasticity, which underlies learning and memory. In this issue of *Science Signaling*, Foster *et al*. found that metabotropic glutamate receptors mediate long-term potentiation in hippocampal neurons by mobilizing acidic Ca^{2+} stores through the intracellular messenger NAADP and the endo-lysosomal two-pore channels.

Metabotropic glutamate receptors (mGluRs) are G-protein coupled receptors for glutamate, the major excitatory neurotransmitter in the nervous system. They regulate numerous cellular processes, including synaptic plasticity that underlies learning and memory. And their malfunction is implicated in many psychiatric and neurological disorders (*1*). Group I mGluRs comprising mGluR1 and mGluR5 subtypes are traditionally thought to signal by releasing Ca^{2+} from endoplasmic reticulum (ER) Ca^{2+} stores. Foster and colleagues (*2*), investigated the functional role of the so-called "acidic Ca^{2+} stores" during mGluR signalling in the hippocampus.

Acidic Ca^{2+} stores is an umbrella term that describes a cross-kingdom collection of organelles rich in H^+ and Ca^{2+} that are critical components of the Ca^{2+} signalling network (*3*). They include lysosomes which are highly acidic (pH~4.5) and maintain a luminal Ca^{2+} concentration similar to the ER (~0.5 mM). Central to signalling through acidic stores of Ca^{2+} is the Ca^{2+} mobilizing messenger NAADP and the two-pore channel (TPC) family of endo-lysosomal ion channels (*4, 5*). Earlier studies showed that NAADP-evoked Ca^{2+} signals regulate neuronal processes such as neurotransmission, neuronal differentiation and neurite growth, which is consistent with the presence of NAADP-binding sites throughout the brain *(7).* Our current understanding of TPCs in neuronal function, however, is relatively limited.

Using pyramidal neurons from hippocampal slice cultures, Foster *et al*. showed that cell-permeable NAADP caused depolarization that depended on both acidic organelles and ryanodine receptors (RyRs) on the ER. So-called "chatter" between acidic and ER Ca^{2+} stores has been repeatedly documented *(3, 4)*. Depolarization appeared unique to NAADP as intracellular delivery of other Ca^{2+} mobilizing messengers had no effect on membrane potential. NAADP-mediated depolarization exhibited a bell-shaped concentration dependence where by high concentrations of NAADP were functionally ineffective. This is a hallmark feature of NAADP-evoked Ca^{2+} signals in mammalian cells (*4*), but few studies have correlated this with Ca^{2+}-dependent output. Electrical stimulation following pharmacological isolation of metabotropic glutamate receptors or bath application of glutamate also evoked depolarization

which again was dependent on acidic organelles. In these experiments, the lysosomotropic agent GPN was used to disrupt lysosomes. Further pharmacological analyses pointed to mGluR1 as the glutamate receptor subtype responsible for the depolarizing effects of glutamate. Importantly, similar to NAADP, depolarization upon direct glutamate application required ryanodine but not IP_3 receptors although the actions of glutamate were curiously slow given the relative rapid increases in NAADP and Ca^{2+} upon glutamate stimulation (*9*). Glutamate responses were blocked by the fast Ca^{2+} chelator BAPTA but not the slower chelator EGTA, suggesting that local but not global Ca^{2+} changes are functionally relevant. Together, these data corroborate previous findings linking NAADP-evoked Ca^{2+} signals to both depolarization (*8*) and metabotropic glutamate receptor stimulation (*9*) and point to a signaling micro-domain in hippocampal neurons comprising mGluR1, acidic Ca^{2+} stores, and RyRs.

Functionally, Foster *et al*. examined the contribution of acidic Ca^{2+} stores to mGluR1-dependent long-term potentiation (LTP). LTP was induced by a spike timing dependent plasticity protocol. Consistent with a role for acidic Ca^{2+} stores, LTP was blocked by desensitizing NAADP receptors with high concentrations of NAADP (other pharmacological perturbations were not tested). Strong evidence linking acidic Ca^{2+} stores to LTP was obtained using TPC knockout mice. Mice and humans possess two TPCs (TPC1 and TPC2), unlike most other animals which possess three (*10*). Knockout of either TPC1 or TPC2 prevented NAADP-mediated depolarization and mGluR-mediated LTP. The former result is particularly notable in light of claims that TPCs are NAADP-insensitive. Intriguingly, in the TPC-knockout animals, the stimulation protocol induced long-term depression (LTD). This is a remarkable finding suggesting that acidic Ca^{2+} stores likely regulate both forms of plasticity in a context-dependent manner.

As ever, these exciting new findings summarised in Fig. 1, pose many questions. In particular, what are the sequelae of events distal to mobilization of acidic Ca^{2+} stores to regulate LTP?

The authors provided evidence that inhibition of SK channels is a downstream consequence of store mobilization. These channels are perhaps best known for mediating Ca^{2+}-dependent after-hyperpolarisation. However, they have also been implicated in mGluR-mediated depolarization and LTP (*11*). In accord, glutamate-mediated depolarization was blocked by the SK channel blocker, apamin. But apamin did not affect LTP. However, the authors argued that SK channels would already be inhibited by mGluR activation according to their model. Consistent with this, apamin reversed the inhibitory effect of mGluR1 antagonists on LTP. In other words, inhibition of SK channels was sufficient to induce LTP under these conditions, although alone it did not affect membrane potential.

But how are SK channels physiologically inhibited upon mobilization of acidic Ca^{2+} stores? They cannot be direct targets for Ca^{2+} in this context as Ca^{2+} would activate them causing hyperpolarisation. Instead, the authors proposed that the Ca^{2+}-sensitive phosphatase PP2A was the target for mobilized Ca^{2+}, because the PP2A inhibitor okadaic acid also prevented LTP, and there is evidence that PP2A is a negative regulator of SK channels. But a scheme where mobilization of acidic Ca^{2+} stores locally activates PP2A to inhibit SK channels causing depolarization remains speculative. There is not yet any direct evidence showing that mobilization of acidic Ca^{2+} stores is sufficient to activate PP2A or inhibit SK channels, or that PP2A affects the phosphorylation state of SK channels and if it does, whether this is solely responsible for depolarization (PP2A has many targets). Such uncertainties highlight more generally the relative lack of knowledge at present concerning the Ca^{2+} sensors coupled to acidic Ca^{2+} stores.

In sum, there is now ample evidence documenting the importance of acidic Ca^{2+} stores and their resident ion channels such as TPCs in regulating Ca^{2+}-dependent output (*5*). Synaptic

plasticity can now be added to this list. Lysosome dysfunction is increasingly implicated in neuronal dysfunction and a number of studies have delineated lysosomal Ca^{2+} defects in diseases such as Parkinson's disease (*12*). Of relevance here is exaggerated NAADP and mGluR signalling upon pharmacological lysosomal insult (*13*). In Alzheimer's disease, defects in synaptic plasticity are an early feature of the disease, manifesting prior to overt neurodegeneration, and this observation is fuelling therapeutic efforts to target mGluRs. The work of Foster *et al*. identifying acidic Ca^{2+} stores as key players in mGluR1 signalling and neuronal plasticity thus identifies new targets of potential clinical benefit in patients with associated disorders.

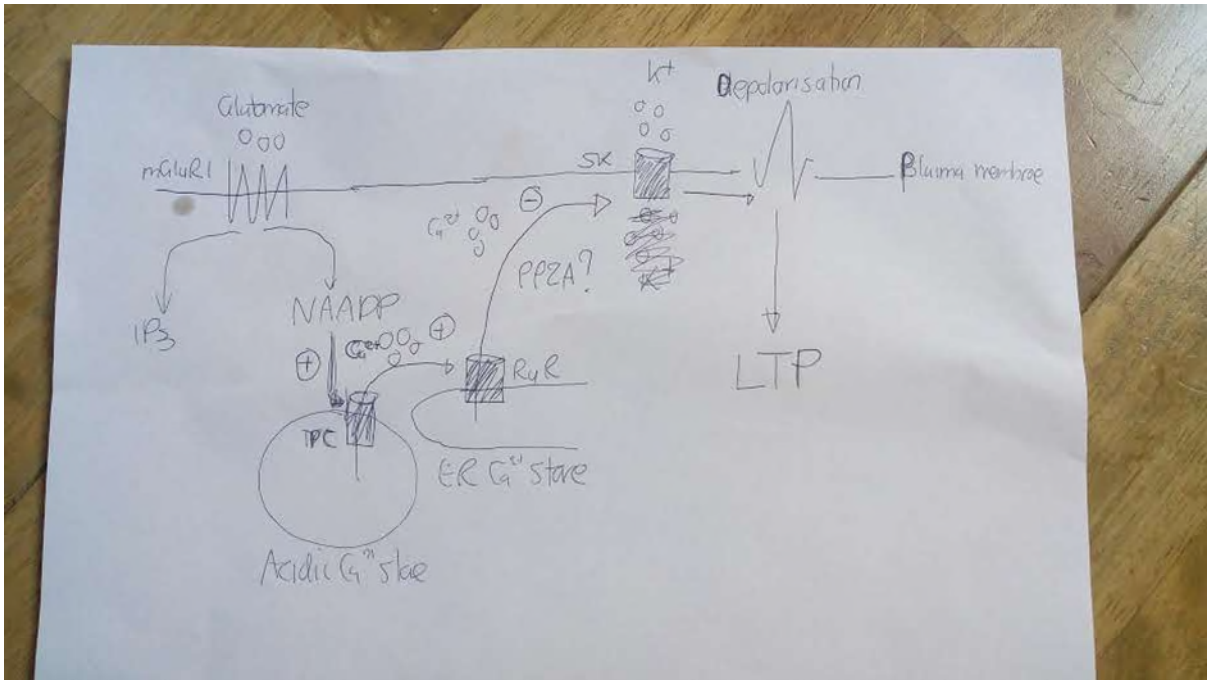

Figure 1: Glutamate to LTP through acidic Ca^{2+} stores. Extracellular glutamate activates plasma membrane mGluR1 resulting in the synthesis of Ca^{2+}-mobilizing messengers NAADP and IP_3. NAADP activates two-pore channels (TPCs) on acidic Ca^{2+} stores, resulting in the release of Ca^{2+}. This activates ryanodine receptors (RyRs) on ER Ca^{2+} stores, resulting in further Ca^{2+} release. This in turn inhibits SK channels on the plasma membrane possibly through the phosphatase activity of PP2A. The ensuing inhibition of K^+ flux depolarizes the cell, thereby initiating LTP.

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References and Notes

1. C. M. Niswender, P. J. Conn, Metabotropic glutamate receptors: physiology, pharmacology, and disease. *Annual review of pharmacology and toxicology* **50**, 295-322 (2010).
2. Foster et al
3. S. Patel, X. Cai, Evolution of acid Ca^{2+} stores and their resident Ca^{2+}-permeable channels. *Cell Calcium* **57**, 222-230 (2015).
4. A. Galione, A primer of NAADP-mediated Ca signalling: From sea urchin eggs to mammalian cells. *Cell Calcium* **58**, 27-47 (2015).
5. S. Patel, Function and dysfunction of two-pore channels. *Sci. Signal* **8**, re7 (2015).

7. S. Patel, G. C. Churchill, T. Sharp, A. Galione, Widespread distribution of binding sites for the novel Ca^{2+}-mobilizing messenger, nicotinic acid adenine dinucleotide phosphate, in the brain. *J. Biol. Chem* **275**, 36495-36497 (2000).
8. G. C. Brailoiu *et al.*, NAADP-mediated channel "chatter" in neurons of the rat medulla oblongata. *Biochem. J* **419**, 91-97 (2009).
9. V. Pandey *et al.*, Recruitment of NAADP-sensitive acidic Ca^{2+} stores by glutamate. *Biochem. J* **422**, 503-512 (2009).
10. X. Cai, S. Patel, Degeneration of an intracellular ion channel in the primate lineage by relaxation of selective constraints. *Mol. Biol. Evol* **27**, 2352-2359 (2010).
11. C. M. Tigaret, V. Olivo, J. H. Sadowski, M. C. Ashby, J. R. Mellor, Coordinated activation of distinct Ca(2+) sources and metabotropic glutamate receptors encodes Hebbian synaptic plasticity. *Nature communications* **7**, 10289 (2016).
12. L. N. Hockey *et al.*, Dysregulation of lysosomal morphology by pathogenic LRRK2 is corrected by TPC2 inhibition. *J. Cell Sci* **128**, 232-238 (2015).
13. G. D. Dickinson, G. C. Churchill, E. Brailoiu, S. Patel, Deviant NAADP-mediated Ca^{2+}-signalling upon lysosome proliferation. *J. Biol. Chem* **285**, 13321-13325 (2010).

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