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PERSPECTIVES

VGLUT soothes the sour synapse

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When it comes to cations that regulate neurotransmitter release, Ca^{2+}, Na^+ and K^+ get all the attention. In a sense this is not surprising because the movement of these three cations across presynaptic plasma membranes (PMs) is vital for several aspects of neurotransmission. Whereas flux of Na^+ and K^+ establishes the resting membrane potential and ensures faithful propagation of action potentials, Ca^{2+} is arguably most critical for neurotransmitter release. Since an adequate elevation in presynaptic $[Ca^{2+}]$ is *sufficient* to trigger synaptic vesicle (SV) exocytosis, $[Ca^{2+}]$ is very tightly regulated in that compartment. This regulation involves the combined action of multiple proteins including voltage gated Ca^{2+} channels (VGCCs), which elevate presynaptic $[Ca^{2+}]$ and trigger SV exocytosis, and plasma membrane Ca^{2+}-ATPases (PMCAs), which extrude and restore presynaptic $[Ca^{2+}]$ to resting levels. An oft overlooked aspect of Ca^{2+} extrusion is that PMCAs are actually ATP-driven Ca^{2+}/H^+ exchangers (Trapp *et al.* 1996). Thus, elevation of presynaptic $[H^+]$ is an integral byproduct of SV release. Since cytosolic acidification severely impacts cellular function, it stands to reason that mechanisms must exist to restore the pH balance in the presynaptic compartment during neurotransmission.

Multiple proteins have been documented to mediate the extrusion of presynaptic H^+ including PM-resident Na^+/H^+ exchangers (NHEs) and vesicular H^+-ATPases (vATPase) (Zhang *et al.* 2010). In this issue of *The Journal of Physiology*, Rossano *et al.* describe a novel role for the vesicular glutamate transporter (VGLUT) in extrusion of presynaptic H^+ at the *Drosophila* neuromuscular junction (NMJ) (Rossano *et al.* 2017). In a study that is nothing short of a technical *tour de force*, the authors describe the development and *in situ* validation of a genetically encoded, cytosolic pH indicator called pHerry – a chimeric protein similar to ClopHensor (Arosio *et al.* 2010) that comprises the pH-sensitive super-eclipitic-pHluorin fused to mCherry via a short linker (Fig. 1*A*). As cytosolic pH in cells expressing pHerry is lowered, the fluorescence intensity of super-eclipitic-pHluorin (green channel) decreases, but that of mCherry (red channel) remains unchanged (Fig. 1*B*). Thus, the ratio of green to red fluorescence is an elegant ratiometric approach to detect dynamic changes in cytosolic pH *in situ* in a manner similar to that of previously published work (Koivusalo *et al.* 2010) employing pHluorin–mCherry fusions *in vitro* (Fig. 1*B*). When expressed in *Drosophila* motor neurons, this marker localized to the presynaptic compartment of the NMJ and displayed predicted responses to alterations in cytosolic pH. The authors used pHerry to confirm that neuronal activity causes presynaptic acidification proportional to the frequency of action potentials (APs), and noted that following rapid acidification, cytosolic pH gradually returned to baseline.

Interestingly, the authors found that evoked release of SVs was required for re-alkalinization, but not the initial acidification, of the cytosol. Whereas blocking SV exocytosis delayed the recovery of cytosolic pH, prolonged fusion of SVs led to precocious re-alkalinization. These data prompted the authors to focus on the SV as the carrier of the protein(s) that mediate the extrusion of presynaptic H^+. Using a pharmacogenetic approach, the authors show that VGLUT mediates the extrusion of presynaptic H^+ in a manner that appears independent of glutamate transport. The shift from glutamate to Na^+ transport exhibited by VGLUT in SVs and PM, is likely to be a function of the differences in concentrations of glutamate and Na^+ in the presynaptic compartment *versus* the synaptic cleft. Importantly, the authors expressed *Drosophila* VGLUT in

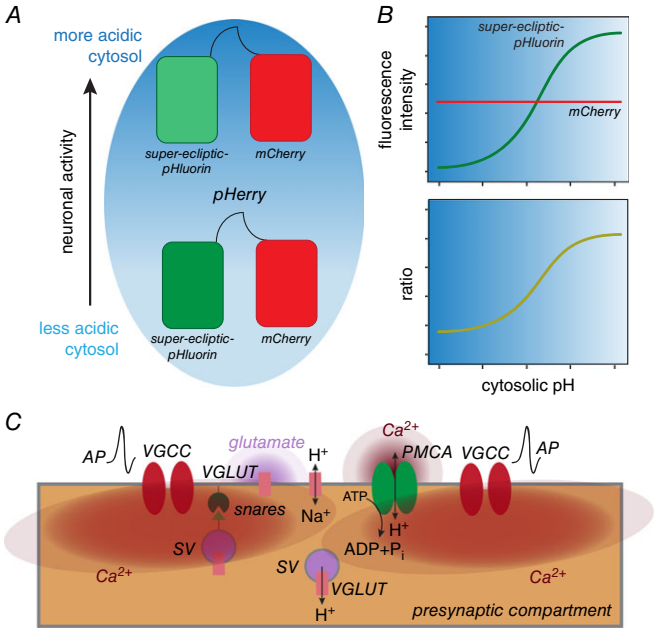

Figure 1. VGLUT mediates H^+ efflux at presynaptic terminals
A and *B*, pHerry is a genetically encoded cytosolic pH indicator. *C*, action potential (AP)-induced VGCC opening results in cytosolic Ca^{2+} (red) elevation, which triggers snare-dependent SV exocytosis and glutamate (light purple) release. PMCA actively pumps out cytosolic Ca^{2+} while increasing cytosolic $[H^+]$. The resulting cytosolic acidification is reversed by VGLUT inserted at the PM during SV exocytosis, which functions as a Na^+/H^+ exchanger to mediate H^+ efflux.

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Xenopus oocytes and directly demonstrated that the protein possesses intrinsic Na^+/H^+ exchange activity, which explains its role in presynaptic H^+ extrusion. Together, these studies establish that, at least in *Drosophila*, VGLUT is also a Na^+/H^+ exchanger involved in presynaptic re-alkalinization after being inserted into the PM (Fig. 1*C*). Since these findings are consistent with the notion that SVs bear the proteins involved in presynaptic re-alkalinization, their elegant model explains how the presynaptic compartment scales its capacity for cytosolic re-alkalinization with the strength of the initiating stimulus.

This study also raises some important questions for future investigation. For instance, it remains to be addressed whether mammalian VGLUTs are also Na^+/H^+ exchangers at the PM. The authors also found that pharmacological inhibition of both NHEs and vATPases altered the dynamics of cytosolic re-alkalinization independent of VGLUT. However, these pharmacological findings might not be sufficient to completely distinguish involvement of NHEs or vATPases in intrinsic and activity-enhanced presynaptic acid extrusion. Indeed, VGLUT, NHEs and vATPases could all mediate H^+ extrusion in a context-dependent manner that is constrained by the unique features of the synapse under observation. Further investigation will be needed to tease apart the functions of these proteins in presynaptic H^+ extrusion. Finally, given that genetic studies in both *Drosophila* and mammals have described the role of the VGLUT proteins in synaptic plasticity as well as excitotoxicity (Daniels *et al.* 2011; Granseth *et al.* 2015), it would be worthwhile to examine whether VGLUT-dependent regulation of presynaptic pH impacts synaptic plasticity and/or excitotoxicity-induced neurodegeneration.

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Additional information

Competing interests

None declared.

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