Pre-synaptic pH changes and vesicle fusion at the *Drosophila* neuromuscular junction

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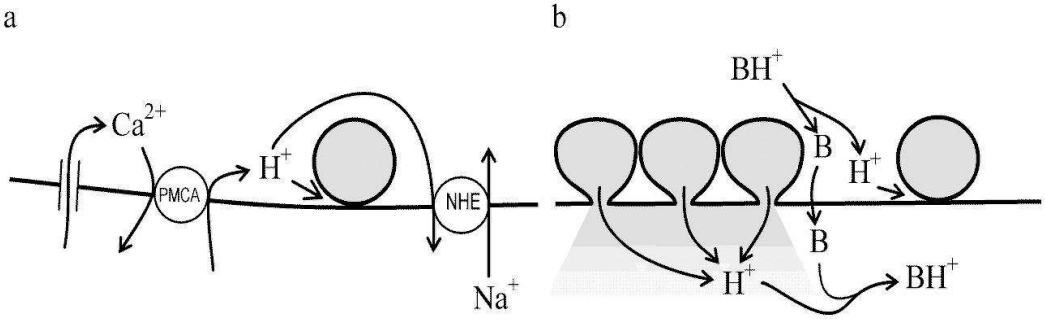
It is generally accepted that exocytosis is triggered by an influx of calcium and the activation of protein complexes with synaptotagmin acting as one of the calcium sensors. However, exocytosis can also be induced in the absence of calcium using low pH (Cohen & Van Der Kloot, 1976; Drapeau & Nachshen, 1988) or hypertonic solutions (Suzuki *et al.*, 2002; Vyleta & Smith, 2011) with the suggestion that the vesicles released are from the same pool as those triggered by calcium (Rosenmund & Stevens, 1996). Whilst the calcium-independent mechanisms are poorly understood and not ascribed a physiological role, we have sought to test whether signals exist that might activate them when calcium is available such that they contribute to vesicle release.

Calcium extrusion by the plasma membrane calcium pump (PMCA) is known to result in intracellular acidification in post-synaptic regions (Schwiening *et al.*, 1993). Using the pH-sensitive dye HPTS we have recorded pre-synaptic, depolarization-evoked, pH shifts (~0.1 pH units) at the *Drosophila* neuromuscular junction which were both reduced in size and slowed by eosin (an inhibitor of the PMCA). In the absence of added calcium weak acid addition (20 mM propionate), or weak base removal (ammonium or TMA) both induced increases in spontaneous vesicle fusion rate (miniatures recorded using sharp microelectrodes). Furthermore, EIPA (50μM), a Na^+:H^+ exchanger inhibitor, induced a rise in fusion rate and enhanced the rise in rate caused by the weak acid addition or base removal. This is consistent with changes in intracellular pH, rather than tonicity, inducing vesicle fusion. Finally, in some cells, we found that in the presence of ammonium there were periodic rises in vesicle fusion rate.



We propose that pH might have a role in stimulating vesicle fusion (Figure 1) in two ways. First, calcium extrusion on the PMCA may cause local acidifications that stimulate vesicle fusion. Second, acid released from synaptic vesicles into the cleft may, by altering the concentration of uncharged acid or base, cross the pre-synaptic membrane causing intracellular pH changes and alter release rate. This would act as a positive feedback mechanism acid-induced acid release driving vesicle release to high rates. Such a positive feedback mechanism might help explain why the nervous system function is so sensitive to changes in pH or pH buffering.

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