## Differential regulation of two modes of exocytosis by protein phosphatases

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Phosphorylation and dephosphorylation of nerve terminal proteins are involved in the regulation of neurotransmitter release. However, it is now clear that there are multiple modes of exocytosis in neurons and the specific roles for different kinases and phosphatases remain unknown. The most well characterised mode of exocytosis involves docking and fusion of a synaptic vesicle with the plasma membrane followed by full incorporation of the vesicle into the plasma membrane, so called full fusion exocytosis. Vesicles are subsequently recovered by Clathrin-mediated endocytosis. However, an alternative mode, termed kiss-and-run, has now been demonstrated, where neurotransmitter is released without complete fusion of the vesicle with the plasma membrane, and the vesicle is rapidly retrieved and refilled with neurotransmitter. Kiss-and-run therefore accelerates the turnover of the limited pool of synaptic vesicles in neurons and may have both beneficial and pathological outcomes. In studies to identify the molecular events underlying these different modes, we have shown that serine/threonine and potentially tyrosine phosphatases have specific regulatory roles.

We used selective pharmacological inhibitors of different protein phosphatases to investigate their roles in the different modes of exocytosis in neurons and mast cells. Two chemical depolarising agents were used (KCl and 4-aminopyridine) that can selectively induce full fusion and kiss-and-run exocytosis, and exocytosis was measured by two complementary assays that can distinguish between these 2 modes of exocytosis. Measurement of endogenous, soluble, glutamate release detects both full fusion and kiss-and-run modes of exocytosis. In contrast, measuring the release of the lipophilic styryl dye FM 2-10 , reflects the time dependent dissociation of the dye from vesicle membranes, and is therefore much less capable of detecting the rapid, transient exocytosis that occurs during kiss-and-run. Our results suggest that protein phosphatase 2A positively regulates the full fusion mode of exocytosis, whilst protein phosphatase 2B, in addition to its recognised role in regulating endocytosis, negatively regulates the kiss-and-run mechanism of exocytosis.

We have also studied the role of the Src family of tyrosine kinases in regulating these two modes of exocytosis. Inhibition of the Src family kinases, using the specific inhibitor, PP1 (10μM), significantly increased kiss-and-run release, but had no effect on full fusion release. The inactive analog, PP3, had no effect on either mode. Measurement of depolarisation induced changes in synaptosomal protein tyrosine phosphorylation did not show any association between Src kinase activity and kiss-and-run exocytosis. This indicates that the effect of Src kinase inhibition is either to remove a constitutive phosphorylation dependent restraint on exocytosis, perhaps mimicking an endogenous tyrosine dephosphorylation event that promotes the kiss-and-run mode, or to inhibit a depolarisation dependent activation of a member of the Src kinase family that is not Src.

Since the fundamental molecular machinery involved in full fusion exocytosis is highly conserved, we have also used a mast cell model to further investigate the molecular events underlying the control of full fusion exocytosis by PP2A. These studies indicate that translocation of PP2A from cytosolic to membrane-associated locations within the cell and the formation of transient complexes with myosin are critical for the regulation of exocytosis.