PI-3 kinase type II C2α is essential for ATP-dependent priming of neurosecretory granules prior to exocytosis

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Phosphatidylinositol 3-kinases (PI3K) are implicated in a variety of synaptic functions including axonal guidance and long-term depression and potentiation (reviewed in Osborne *et al*., 2001). However, a direct involvement of this class of enzymes and their lipid products in neuroexocytosis has been questioned (Chasserot-Golaz *et al.*, 1998), based on the low sensitivity of exocytosis to PI3K inhibitors wortmannin and LY294002 (Martin *et al.*, 1997; Wiedemann *et al.*, 1996).

Neurotransmitter release from synaptosomes and hormonal secretion from chromaffin cells are only sensitive to high concentrations of the PI3K inhibitors wortmannin and LY294002, pointing to a possible role for the less sensitive PI3K-C2α. In support of this, PI3K-C2α was detected on a subpopulation of mature secretory granules abutting the plasma membrane in neurosecretory cells. Furthermore, both PI3K inhibitors and sequestration of PI3K-C2α with specific antibodies selectively prevented ATP-dependent priming in permeabilised chromaffin cells.

Transient over-expression of PI3K-C2α in PC12 cells potentiated evoked secretion, whereas its dominant negative mutant abolished exocytosis, suggesting PtdIns3*P*, the main catalytic product of this enzyme plays a role in neuroexocytosis. Consistent with this, treatment of PC12 cells transiently expressing PtdIns3*P*-sequestering FYVE domain with low concentrations of wortmannin selectively abolished early endosomal staining and revealed a full co-localisation of the FYVE domain with PI3K-C2α on PC12 granules. Finally sequestration of PtdIns3*P* by the FYVE domain also abolished secretion from PC12 cells demonstrating that PtdIns3*P* production is needed in the process of acquisition of fusion competence secretory vesicles undergo, during or following docking to the plasma membrane.

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