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Sphingolipids modulate docking, Ca^{2+} sensitivity and membrane fusion of native cortical vesicles.

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Running Title: Sphingolipids modulate regulated exocytosis.

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Vesicles

Abstract

Docking, priming, and membrane fusion of secretory vesicles (i.e. regulated exocytosis) requires lipids and proteins. Sphingolipids, in particular, sphingosine and sphingosine-1-phosphate, have been implicated in the modulation of exocytosis. However, the specific exocytotic steps that sphingolipids modulate and the enzymes that regulate sphingolipid concentrations on native secretory vesicle membranes remain unknown. Here we use tightly coupled functional and molecular analyses of fusion-ready cell surface complexes and cortical vesicles isolated from oocytes to assess the role of sphingolipids in the late, Ca^{2+}-triggered steps of exocytosis. The molecular changes resulting from treatments with sphingolipid modifying compounds coupled with immunoblotting analysis revealed the presence of sphingosine kinase on native vesicles; the presence of a sphingosine-1-phosphate phosphatase is also indicated. Changes in sphingolipid concentrations on vesicles altered their docking/priming, Ca^{2+}-sensitivity, and ability to fuse, indicating that sphingolipid concentrations are tightly regulated and maintained at optimal levels and ratios to ensure efficient exocytosis.

Abbreviations: α-NP, α-naphthylphosphate; CB, cerebrosides; DMS, dimethylsphingosine; FFM, fundamental fusion mechanism; Fin, fingolimod; PFM, physiological fusion machine; PMA, phorbol 12-myristate 13-acetate; Sph, sphingosine; S1P, sphingosine-1-phosphate; SphK, sphingosine kinase

Keywords: Exocytosis, Membrane Fusion, Docking, Sphingolipids, Lipid Phosphorylation, Sphingosine kinase (SphK), Sphingosine-1-phosphate (S1P), Calcium Sensitivity, Secretory Vesicles

1 Introduction

Regulated exocytosis enables the release of select intracellular compounds into the extracellular space, a process that serves several diverse and essential functions. Secretory vesicle fusion (i.e., the merging of apposed vesicle and plasma membranes leading to release of vesicle content, or mixing of vesicle content that occurs in homotypic or compound vesicle fusion) – the final step of exocytosis – is known