Distinct characteristics of exocytosis in mouse pancreatic acinar cells

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Exocytosis, the fusion of a vesicle with the plasma membrane is the principal way a cell can release lipophobic substances to the outside environment. It is probable that the basic machinery of exocytosis is similar across different cell types. But recent studies have shown the process of exocytosis may be differently regulated in different cells. Here we describe novel characteristics of the prolonged (many minutes) exocytotic events in exocrine cells of the mouse pancreas.

Mice were humanely killed (in accord with local guidelines) and the pancreas gland removed. The gland was then incubated in collagenase (Worthington CLSPA) for 5-10 minutes at 37°C. The tissue was then resuspended in extracellular solution (containing [mM] NaCl 145, KCl 5, $MgCl_2$ 1, $CaCl_2$ 1, HEPES 10 – pH 7.4 NaOH) and gently triturated to produce a preparation of large clusters of acinar cells. The clusters were then placed on Poly-l-lysine coated coverslips. The cell clusters were imaged using a custom-built 2-photon microscope. Images were then processed using Metamorph software (Universal Imaging).

We imaged lobules and smaller fragments of mouse pancreatic tissue that retained the typical morphology of the intact exocrine glands. Inclusion of a fluorescent probe (Sulphorhodamine B or Oregon Green, Molecular Probes) in the extracellular bathing medium labelled acinar ducts and the extracellular space between cells, but dyes were excluded from the cell interior. Addition of ACh or the uncaging of caged CCh, with a flash of UV light, rapidly evoked fluorescence spots in the cell. These fluorescent spots were exclusively observed in the apical regions of cells, had the same diameter as secretory granules and had similar kinetics to the release of digestive enzymes. Our observations are therefore consistent with fluorescence labelling of zymogen granules. Using fluorescence recovery after photobleaching (FRAP) techniques we show that the fusion pore remains open for protracted periods of time (minutes) to allow free exchange between the aqueous granule lumen and the outside. Although, at later times, we show that granules do not take up extracellular dye indicating that the fusion pore can close. Finally, using lipophillic dyes, we show no evidence for interchange of lipid between the plasma membrane and the vesicle membrane during the lifetime of the vesicle.

We propose that these distinct characteristic of exocytosis in exocrine glands may represent adaptations to the characteristic physiological responses of these cells.