Endocytosis in secretory epithelial cells

C. Soekmadji and P. Thorn, School of Biomedical Science, University of Queensland, St Lucia, QLD 4072, Australia.

The process of regulated secretion involves fusion of granules with the cell membrane followed by endocytic recovery of membrane. Our recent work in epithelial cells shows that granule fusion can be complex and that there are multiple routes endocytosis. Mouse pancreas was excised and collagenase-digested to produce fragments of pancreatic tissue. The tissue fragments were bathed in extracellular fluorescent dyes and either imaged live with 2-photon microscopy or after paraformaldehyde fixation with confocal microscopy. Cell exocytic responses were stimulated with acetylcholine (0.1-1 μM) its action terminated by the application of Atropine (10 μM). Upon exocytosis the extracellular fluorescent dye enters and labels the granules. Using different dyes and different times of dye addition we have developed methods to enable positive identification of whether the fusion pores are open or closed. With a range of acetylcholine concentrations we now show that secretion is not only regulated by the control of the numbers of fusion events but also by the dynamics of granule fusion itself. In further experiments we have used the dynamin inhibitor, dynasore (80 μM) to investigate if dynamin is a regulator of endocytosis. In control experiments we prove this drug is effective in inhibiting transferrin uptake in cultured cells. Using dynasore as a tool in pancreatic acinar cells we show that the fusion pore is a site of regulation and is closed by dynamin (most likely dynamin 2). In live-cell experiments we are currently investigating whether dynamin inhibition affects endocytosis. Our data provide evidence for whole-granule recapture that is independent of dynamin. We thus conclude that there are dynamin dependent and dynamin independent mechanisms of endocytosis in secretory epithelial cells.