Dynamics of the fusion pore during exocytosis in mouse pancreatic acinar cells

O. Larina[1] and P. Thorn [2], [1]Department of Physiology, Development & Neuroscience, University of Cambridge, UK and [2]School of Biomedical Sciences, University of Queensland, Australia.

After fusion with plasma membrane the zymogen granules of pancreatic acinar cells remain close to the plasma membrane for many minutes with the fusion pore open. In the present study we have set out to determine if this fusion pore can subsequently close. In control experiments Lysine-fixable Texas Red (TR) and Fluorescein dextran (FD) were added to the extracellular solution surrounding fragments of mouse pancreas. These extracellular dyes enter and label granules that undergo ACh-evoked exocytosis. The tissue fragments, fixed in 4% paraformaldehyde, were visualized with a confocal microscope. In experiments designed to study fusion pore dynamics, TR and FD application were separated in time. TR was present throughout but, to probe for fusion pore closure, FD was added 2, 6 or 11 minutes after cell stimulation. With simultaneous application of FD and TR the FD/TR fluorescence ratio within a granule had a mean value of 0.84 ± 0.28 (mean ± SEM, n=175 granules) indicating that the dyes filled the granules approximately equally. When FD was added at later times the FD/TR ratio changed with a predominance of low FD/TR ratios. For example, application of FD 2 minutes after stimulation gave a mean FD/TR ratio of 0.78 ± 0.43 (mean ± SEM, n=113) and now 12.4% granules had a ratio of less than 0.2. A Shapiro-Wilk test showed the control FD/TR ratio distribution was not significantly different from Gaussian ($p=0.2$) but when FD was added after 2 minutes the distribution was significantly skewed ($p<0.05$). The low FD/TR ratios indicate granules predominantly filled with TR dye and demonstrate that fusion pore must have closed preventing entry of FD. In conclusion, we show the fusion pore in acinar cells is dynamic. We hypothesize that this behavior may be important in the regulation of enzyme release.