Spatial relationships influence stimulus-secretion coupling in secretory epithelial cells

J. Low and P. Thorn, School of Biomedical Science, University of Queensland, QLD 4072, Australia.

Introduction. Stimulus secretion coupling in excitable cells primarily utilizes the influx of calcium through cell-surface calcium channels as the trigger for exocytosis. Work has characterized the spatial relationships between the calcium channels and the sites of exocytic release. Many secretory cells, however, do not use influx of calcium as the trigger for exocytosis and instead use calcium release from intracellular calcium stores. In these cells, and a good example is the pancreatic acinar cells, there is little information as to how the calcium release sites are spatially related to the sites of exocytosis. We know in these cells that exocytosis occurs exclusively along the lumen and that relatively high calcium concentrations are required to trigger exocytosis (Ito *et al.,* 1997). We also know that calcium release is focused at the lumen and this has led us to speculate that the calcium release apparatus may be closely apposed to the sites of exocytosis (Thorn *et al.,* 1993). Here we test this hypothesis.

Methods. Male CD-1 mice were killed according to the approved ethical procedures of The University of Queensland. The pancreas was excised and collagenase-digested to produce fragments of pancreatic tissue (see Thorn *et al.,* 2004 for details). The tissue fragments were bathed in fluorescent dye, sulforhodamine B (SRB) and imaged live with 2-photon microscopy. Cell exocytic responses were typically stimulated with cholesystokinin (CCK, 15 pM). Upon exocytosis the extracellular fluorescent dye enters and labels the granules. Cells were loaded with Fura-2 (AM) and the fluorescence recorded to determine the calcium response. Finally in some experiments we loaded the cells with NP-EGTA (caged calcium) and used a UV light source, with flash duration controlled electronically, to uncaged calcium within the cells.

Results. The first question we asked was, whether under conditions of uniform calcium elevation we could observe clustering in the sites of exocytic release. To characterize the exocytotic response we uncaged calcium from NP-EGTA. Varying the UV photolyisis flash duration (from 5 to 200 ms) released calcium in a graded manner (as recorded with Fura-2). At the shorter flash durations we observed limited to no exocytic activity. With the longest durations of 100 ms large calcium responses triggered massive exocytic responses (measured as events per lumen length per minute 1.61 events/nm/minute ± 0.35) with short latencies (9.459s ± 0.525). In these experiments the calcium signal rises uniformly across the cell. Spatial analysis of the exocytic response revealed a modal value for granule-to-granule distance apart of 3 μm with evidence to suggest that this distance is not due to any spatial localization of release sites but rather due to the limited lengths of the lumens in each cell. Further analysis showed that the latency of response was similar along the lumen length, reinforcing the idea of uniformity of exocytic release sites along the lumen.

We then asked the question as to whether, in response to CCK, where there is a clear structure to the calcium response, that this signal structure affected the spatial organization of exocytic responses. CCK-induced oscillations start at a specific locus within the apical region of the cell and then travel as a wave to all other regions of the cell. Past work suggests that these focal hot-spots of calcium release are due to regions of particularly active inositol trisphosphate receptors. Here we determined the focal hot-spot by a first derivative analysis of the calcium responses and recorded the exocytic responses, studying the latency to respond and the location of exocytosis. Measuring the distance between the focal hot-spot of release and the triggered exocytic responses revealed ~50% of granules are released within 4 μm of the calcium release site. Surprisingly, very few exocytic responses were seen < 1 μm from the calcium release hot-spot possibly suggesting steric exclusion of granular exocytosis.

Conclusions. We here describe for the first time the spatial relationship between the calcium signal and the exocytic response in cells primarily reliant on release of calcium from intracellular stores. Our data show that the exocytic release sites have no preferential clustering along the lumen. But we show that the structure of the agonist-evoked calcium response does influence the spatial extent of exocytic release.

Ito K, Miyashita Y & Kasai H. (1997) *EMBO Journal* **16:** 242-51.
Thorn P, Lawrie AM, Smith PM, Gallacher DV & Petersen OH. (1993) *Cell Calcium* **14:** 746-57.
Thorn P, Fogarty KE & Parker I. (2004) *Proceedings of the National Academy of Sciences USA* **101:** 6774-9