Extracellular pH shifts and their consequences for secretory epithelial cells

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Introduction. Most, if not all, secretory granules maintain an acidic pH. which in many types of granules this is used to drive secondary-active uptake of contents into the granule (*e.g.* neurotransmitter uptake). In peptidergic granules the acid lumen is thought to act as a charge screen on between the proteins enabling a tighter packing of granule contents. Upon granule fusion, during exocytosis, protons are lost through the open fusion pore prior to the loss of other, heavier granule content. In this way, the loss of the acid gradient is one of the first events of exocytosis. Given the high mobility of the protons, release from a small granule into a large extracellular volume would be expected not to change the extracellular pH significantly, if at all. Where the extracellular environment is restricted, this might not be the case. There are however many extracellular environments where this might not be the case, particularly where the extracellular volume is restricted. Within hollow organs, it is conceivable that exocytotic release of protons from granules might contribute to the intra-organ pH environment. In organs with a restricted extracellular volume, regulated pH changes have been shown to occur as a result of the transport of acid into the extracellular environment (Chu & Montrose, 1995). Indeed in organs lined with mucous, pH changes within the restricted mucous volume have been shown and may well arise as part of the exocytic activity. We here test the hypothesis that whether exocytosis can lead to pH changes in the lumen of the exocrine pancreas.

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 & Parker, 2005 for details). The tissue fragments were bathed in extracellular fluorescent dyes and imaged live with 2-photon microscopy. Cell exocytic responses were stimulated with cholecystokinin (15, 20 or 100 pM). Upon exocytosis the extracellular fluorescent dye enters and therefore labels the granules. We used two different extracellular dyes; sulforhodamine B (SRB, an inert dye), 8-Hydroxypyrene-1,3,6-trisulfonic acid (HPTS, a pH sensitive dye, see Schwiening & Willoughby, 2002) and 8-Methoxypyrene-1,3,6-trisulfonic acid (MPTS used as an inert control for HPTS). We also used an intracellular calcium sensor, Fluo-4 AM. We calibrated the pH sensitivity of HPTS in the 2-photon microscope with 950 nm excitation light. Our estimated Kd, derived from the calibration was 6.79.

Results. Initial experiments were performed in the presence of extracellular 7 mM HEPES. Here we observed single exocytic events in response to 15 pM CCK. These events were seen as a sudden increase in SRB and HPTS fluorescence in the granule. In regions of interest in the lumens, immediately adjacent to the exocytic events, we observed little change in the SRB signal (in some cases a small increase due to dye binding to released proteinaceous content, Thorn & Parker, 2005). In contrast, we consistently observed small, transient decreases in HPTS fluorescence indicative of possible acidification. Applying our HPTS calibration to this data gave us an estimated mean decrease from 7.4 to 7.24 ± 0.03 (n = 68). To assess the unbuffered pH changes we removed HEPES from the extracellular solution. Again the luminal SRB changes were small or showed a slight increase. Now HPTS recorded greater pH changes (from 7.4 to a mean of 7.02 ± 0.03 n = 52). These luminal changes preceded the influx of SRB into the granule suggesting release of protons from the granule through an initial fusion pore too small to allow SRB entry. Control experiments with MPTS showed no changes. Experiments stimulating the cells with high CCK (100 pM) showed very large luminal acidifications. To determine if these extracellular pH changes affected cell responses we measured cytosolic calcium responses to CCK (with Fluo-4 AM) +/- extracellular HEPES. The responses were very different. For example the frequency of calcium oscillations in HEPES was 0.42 ± 0.03 Hz (n = 43) compared to 0.65 ± 0.06 Hz (n = 38) in the absence of HEPES (p < 0.001), supporting the idea that extracellular pH changes do have functional consequences for the cell.

Conclusions. What we show here is that proton release from secretory granules significantly acidifies the primary secretory output with pH drops of up to 0.4 pH units. This 10 fold increase in protons is an unprecedented change for an extracellular ion and we show this pH change is capable of modulating intracellular calcium levels. We conclude that the acid content of secretory granules has the potential for significant effects when released. In the pancreas we reveal a novel negative feedback mechanism in the integrative control of organ function.

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