Focal secretion of insulin within intact islets of langerhans

J. Low, J. Mitchell, J. Bax and P. Thorn , School of Biomedical Sciences, The University of Queensland, St Lucia, QLD 4072, Australia.

The biochemical pathways that link glucose sensing in pancreatic β-cells to insulin secretion are well understood. However, in a more physiological context it is not clear how glucose controls/coordinates insulin secretion from the many hundreds of β-cells within the islets of Langerhans. Here we have set out to determine the basis of glucose sensing from β-cells within intact mouse pancreatic islets. With two-photon imaging of whole islets we identify each individual exocytic event within individual cells, induced by a range of different glucose concentrations.

We used 2-photon microscopy to measure insulin granule fusion from intact living islets isolated from CD-1 mice. The mice were humanely killed according to local ethics guidelines. The islets were isolated by a collagenase digestion procedure and cultured for 2-3 days prior to use. Insulin granule fusion was recorded in response to a range of glucose concentrations. We observed a glucose dose-dependence in the numbers of fusion events that is very similar to our measured dose-dependence of insulin secretion. A Chi-squared test proves (P<0.01) that the exocytic responses are not evenly distributed; a few cells show many fusion events and ~54% of cells (for example at 15 mM glucose) apparently have none. To explore this result we captured responses at 1 plane, then moved focus 5.5 μm and captured responses at a 2nd plane. We reasoned that if cells were non-responsive, then doubling the sampling volume should not disclose a response. Our data however show that now 44% of cells have no responses suggesting that cells are responding and that exocytosis must be unevenly distributed across single β cells.

To test this we calculated the ratio of numbers of fusion events in one plane compared to the other. An even distribution of exocytosis around a cell would give a ratio of 1:1. In contrast, we get a ratio of 1:6.6 which provides evidence for strong polarization of exocytosis in the β cells. This conclusion means our measurements of maximal exocytic activity must be in regions of hot spots of secretion within that cell. Here the measured fusion density is 1 granule per 2.29 μm^2. We then measured insulin secretion and based on estimates of granule insulin content and numbers of β cells within islets we calculate that each β cell secretes 132 granules (in 20 minutes of 15 mM glucose). If all this exocytosis occurs within a focal hot-spot then 132 granules would fuse over an area of 302 μm^2. The total surface area of a β cell is 784 μm^2.

We conclude that insulin granule exocytosis is focused within a small area, 38%, of β cells within intact islets.