Author’s response to reviews

Title: Nutrient-stimulated insulin secretion in mouse islets is critically dependent on intracellular pH: a randomized controlled study

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PDF covering letter
Responses to Reviewer’s Comments

We appreciate your comments, and have revised the manuscript accordingly. Additions and modifications in the manuscript text are denoted in blue font.

1. It is well known that there are marked differences between cytosolic and intragranular pH in beta cells and it is not clear whether the methodology measures an average that includes both (or whether these can be distinguished). An explanation should be provided to indicate how intragranular pH contributes to the measurements recorded and the possible impact of this on the conclusions drawn.

The following explanation is incorporated into paragraph 1 (lines 8-14) and paragraph 4 (lines 11-17) of the Discussion.

Carboxy SNARF-AM esters, loaded under the conditions in this study, remain localized in the cytosol (as opposed to the more complex carboxy SNARF-calcein-AM form, which accumulates in cellular organelles). Carboxy SNARF-AM esters diffuse through the cell membrane and are hydrolyzed by cytosolic esterases, consequently getting trapped in the cytosol with minimal leakage or compartmentalization (ref.52-56). Thus, we expect the current pH-measurements to principally reflect the cytosolic pH and not that of granules or other organelles.

It would, however, be very interesting to see how intragranular pH and intramitochondrial pH affects different secretory functions. Manipulating the loading conditions or using different SNARF-AM derivatives can promote compartmentalization of the dye into organelles, but more sophisticated techniques are necessary to distinguish different organelles from each other.

2. A difficulty with the technique employed is that it requires that the islet architecture is disrupted prior to imaging. This presumably alters the cell-cell communication mechanisms that are believed to be critical to correct secretory function. Thus, the authors should discuss the extent to which this may influence the interpretation of studies designed to evaluate the effects of manipulation of intracellular pH. Indeed, is it possible that the loss of secretory response seen upon islet dissociation may reflect impaired intracellular pH regulation?

Explanation is added to the Methods section: Subheading “Culture of islets”: lines 8-14.

The culture technique used in this study does not significantly disrupt islet architecture. Although the islet cells spread out over the matrix, the cells do not separate from each other, and the gap junctions between islets continue to function. As described in previous studies (ref. 42,39,41), islets cultured under these conditions exhibit normal responses of Ca^{2+}, NAD(P)H and insulin release to glucose stimulation. If cultured for prolonged
periods (6-10 weeks) the islet cells do disperse eventually. However, in the 2-week culture period used in this study the islets remain intact. Thus, this technique only changes the shape of the islet making it easier to image, but does not significantly alter islet cell-cell interactions or other cellular functions.

They suggest that the dye (SNARF5) was efficiently loaded into the "beta cell rich" region of the plated islets. How was this determined (how enriched was this region)?

Explanation is added to paragraph 1 of Discussion (lines 16-21)

Mouse islets have alpha and delta cells on the outside, while the interior is made up almost entirely of beta cells. With the relatively short loading time used in this study, SNARF5 loads into the periphery of the islet but the center is usually left unloaded. The pH-calculations were done using regions-of-interest chosen from selected well-loaded areas closer to the center of each islet.

3. Insulin secretion experiments appear to have been conducted with freshly isolated (intact?) islets whereas pH measurements were made in cultured (dissociated?) islets. Are the authors confident that it is valid to extrapolate between these two conditions?

Explanation is added to paragraphs 1 (lines 8-14) and 4 (lines 11-17) of Discussion.

The culture conditions used here do not cause dissociation of islet cells or disruption of cell-cell interactions. As described in literature (ref 41,42) islets cultured under these conditions exhibit normal insulin release, Ca\(^{2+}\), and NAD(P)H responses to glucose stimulation. Thus, this culture technique provides an excellent model for imaging studies whose results can be extrapolated to fresh islets.

4. The data shown in Fig 3 appear to be repeated in Fig 4a. Thus, Fig 3 is redundant. In addition, the 2.8mM glucose plus DMA control should be included in the figure.

Both figures are necessary because they demonstrate two different effects of alteration of intracellular pH. Figure 3 shows the effect of pH\(_i\) on glucose-induced TDP (memory effect), while figure 4A shows the effect of pH\(_i\) on direct insulin secretion by glucose. We believe Figure 3 is of particular importance because it demonstrates a major finding in this study, that a lowered pH\(_i\) can unmask TDP, a function believed to be absent in mouse islets.

Both figures (3 and 4A) have been modified to include the controls of 2.8 mM glucose with DMA.
Discretionary Revisions (which the author can choose to ignore)

1. It would be interesting to learn the time-course of the TDP response observed when mouse islets are exposed to glucose and DMA. How long does TDP persist after removal of 16.7mM glucose plus DMA?

   Our preliminary studies show that TDP effect lasts for at least 70 minutes after removal of glucose and DMA. Experiments with longer rest periods have not been done yet.

2. The postulate that manipulation of pHi might be used to correct the secretory defect seen in type 2 diabetes is interesting and potentially important. Is there any evidence that beta cell pHi is altered in type 2 diabetes?

   Currently we are studying islets from several mouse models of type 2 diabetes. Preliminary work on KK/Upj/Ay/j mice from Jackson Laboratory (hyperglycemic and hyperinsulinemic) show slightly elevated basal intracellular pH, slower recovery from acid/base load and/or overcompensation to acid/base load. These changes were seen in 70% of the islets tested, while 30% of the islets behaved similar to wild type islets. Studies on two or more hypo-insulinemic models of NIDDM will be conducted over the next few months.

   We hope the above responses were to your satisfaction. Thank you.

   Sincerely yours,

   Subhadra Gunawardana.