MEMRI of Glucose Activated Human Pancreatic Islets

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Introduction

Recently we have shown that Manganese (Mn) enhanced MRI (MEMRI) can be successfully employed to assess glucose activation in isolated rodent islets (1). However, for this technique to be clinically relevant, it has to be implemented on human pancreatic islets. Although both rat and human islets exhibit pulsatile insulin secretion, they are quite different in terms of cell composition and architecture (2). Therefore findings in rodent models may not necessarily translate to human islets.

Here, the validity of MEMRI in assessing glucose induced human pancreatic islet activation was investigated. Glucose stimulated influx of calcium into ß-cells is necessary for insulin release. When present during glucose stimulation, extracellular Mn can enter ß-cells through voltage-gated calcium channels and its accumulation alters T_1 and T_2 relaxation times and results in increased MR image contrast (3). As with rodent islets, high-resolution MR micro imaging of glucose activated isolated human islets showed a significant increase in MR contrast. However, while maximum contrast in rat islets was achieved at 2.5 µM and 25 µM Mn, in human islets it was achieved at 50 µM Mn and higher. Although concerns may exist about Mn toxicity, the doses employed did not exhibit any acute negative effects as glucose stimulation indices were normal.

Materials and Methods

Isolated human islets were obtained and two sets of about 30 islets each were first incubated in Krebs Ringer Buffer (KRB) solution at 1.67 mM glucose for 30 minutes. Samples were then switched to KRB with 50 µM Mn, however one set was incubated in 2 mM glucose while other in 14 mM glucose .Islets were then rinsed three times with KRB and loaded into 2 cm long micro capillary tube of 570µm ID. The sample tubes were mounted on home built double loop Archimedes spiral coil with OD of 750 µm and inserted into the Bruker Micro 5 Imaging Probe (triple axes gradients of maximum strength 2000 gauss/cm) (1). All experiments were conducted in a 56-mm vertical bore 11.7 T magnet using a Bruker DRX Avance Spectrometer (Bruker, Billerica, MA). The typical imaging parameters were: TE = 8 ms, TR = 500 ms, Mx = 256, NEX = 15, Slice thickness = 0.3 mm, FOV = 0.3 mm, In-plane resolution = 10 µM. 3D images acquisition parameters were: TE = 8 ms, TR = 800 ms, Mx = 128, NEX = 2, Slice thickness = 0.3 mm, FOV = 0.4 mm. The MR images acquired were viewed and processed using ImageJ (National Institutes of Health, USA). Islet secretory response in the presence of Mn was scored by stimulation index (ratio of insulin release in high glucose vs. low glucose). Groups of 5 sets of 5 islets each were incubated at 37^0C in modified Krebs-Ringer buffer (KRB) at 1.67 mM glucose and 0 µM, 2.5 µM, 25 µM or 100 µM Mn for 1 hour and 30 minutes. Supernatants were collected and islets resuspended in KRB as specified above at 16.7 mM glucose for 90 minutes. Supernatants were collected and aliquots of both fractions were tested for insulin content via ELISA (Mercodia, Upsala, Sweden).

Results and Discussion

Paramagnetic contrast agents such as Mn reduce the T1 relaxation time of the tissues and increase the contrast in comparison from the signal from non-target tissues for T1-weighted MR images. The 3D image of Mn signal enhancement of stimulated human islets is shown in Figure 1. Contrast was achieved for Mn concentrations ranging between 50 µM and 100 µM. As opposed to rodent islets for which highest contrast was achieved between 2.5 µM to 25 µM, no contrast was seen at lower Mn concentrations. This could be due to several factors including higher sensitivity to the isolation process as well as structural differences. T1 measurements were performed using the spin echo saturation recovery method. Exponential equations were used to fit the curve between the recovery time and intensity and figure 2 illustrates representative tracings. A T1 relaxation map was then produced from these data using Matlab (The Mathworks Inc, Natick, MA). The T1 values of stimulated islet was observed to be in the order of 500 to 600 msec, while the values for control islets were between 800 to 900 msec *(data not shown)*. Despite the higher Mn concentrations employed compared to rodent islets, no statistically significant toxic effects on insulin secretion were observed as demonstrated by insulin stimulation indices (Figure 3).

Conclusions

Our data confirm that MEMRI used in combination with high resolution MRI is a viable technique to non-invasively assess the functionality of human islets, thus enabling possible future clinical applications. Differences were found between rodent and human islets and need to be further investigated. Because of the high heterogeneity of human islets, it is believed that ongoing inter-islet as well as intra-islet characterization currently being performed by our group will help explain such dissimilarities.

References:

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3. Lin YJ, Koretsky AP. Manganese ion enhances T1-weighted MRI during brain activation: an approach to direct imaging of brain function. Magn Reson Med 1997.

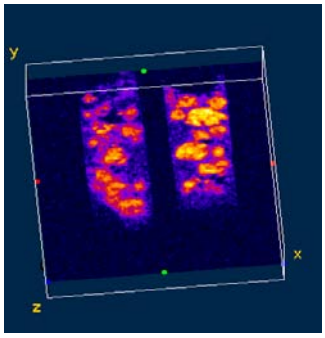

Figure 1. High resolution MR image of unstimulated vs. stimulated isolated human pancreatic islets. Islets were exposed to 50 µM[0] Mn and 2mM (left) and 14 mM (right) glucose respectively.

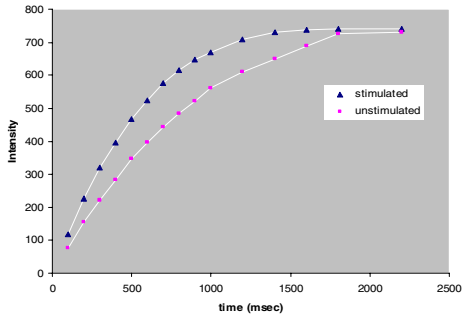

Figure 2. T1 plot of unstimulated vs. stimulated isolated human pancreatic islets. Islets were exposed to 50 µM[0] Mn and 2mM and 14 mM glucose respectively.

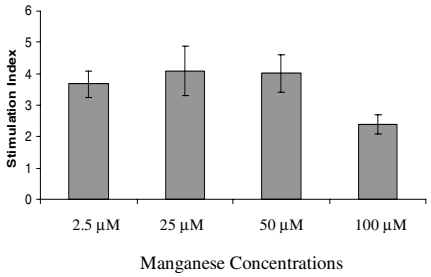

Figure 3. Insulin Stimulation Indices of human pancreatic islets exposed to 2.5 µM, 25 µM, 50 µM, and 100 µM Mn. Islets were preconditioned in 1.67 mM glucose KRB and then stimulated in 16.7 mM glucose. Stimulation indicies were calculated as the ratio of insulin secreted in high glucose media vs. low (Mean +/- SEM; n=5).