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Stress-induced dissociations between intracellular calcium signaling and insulin secretion in pancreatic islets

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ABSTRACT

In healthy pancreatic islets, glucose-stimulated changes in intracellular calcium ($[Ca^{2+}]_i$) provide a reasonable reflection of the patterns and relative amounts of insulin secretion. We report that $[Ca^{2+}]_i$ in islets under stress, however, dissociates with insulin release in different ways for different stressors. Islets were exposed for 48 h to a variety of stressors: cytokines (low-grade inflammation), 28 mM glucose (28G, glucotoxicity), free fatty acids (FFAs, lipotoxicity), thapsigargin (ER stress), or rotenone (mitochondrial stress). We then measured $[Ca^{2+}]_i$ and insulin release in parallel studies. Islets exposed to all stressors except rotenone displayed significantly elevated $[Ca^{2+}]_i$ in low glucose, however, increased insulin secretion was only observed for 28G due to increased nifedipine-sensitive calcium-channel flux. Following 3–11 mM glucose stimulation, all stressors substantially reduced the peak glucose-stimulated $[Ca^{2+}]_i$ response (first phase). Thapsigargin and cytokines also substantially impacted aspects of calcium influx and ER calcium handling. Stressors did not significantly impact insulin secretion in 11 mM glucose for any stressor, although FFAs showed a borderline reduction, which contributed to a significant decrease in the stimulation index (11:3 mM glucose) observed for FFAs and also for 28G. We also clamped $[Ca^{2+}]_i$ using 30 mM KCl + 250 μM diazoxide to test the amplifying pathway. Only rotenone-treated islets showed a robust increase in 3–11 mM glucose-stimulated insulin secretion under clamped conditions, suggesting that low-level mitochondrial stress might activate the metabolic amplifying pathway. We conclude that different stressors dissociate $[Ca^{2+}]_i$ from insulin secretion differently: ER stressors (thapsigargin, cytokines) primarily affect $[Ca^{2+}]_i$ but not conventional insulin secretion and 'metabolic' stressors (FFAs, 28G, rotenone) impacted insulin secretion.

1. Introduction

The primary function of beta cells is to synthesize and secrete insulin, a critical regulator of blood glucose. At the level of the individual beta-cell, the 'Consensus Model' provides a detailed description of the cellular response to glucose stimulation as summarized in Refs. [1,2]. In this model, the beta cell is electrically silent at low glucose concentrations (<~5 mM, representing fasting conditions), secreting insulin at a low, basal rate. In response to sharp increases in blood glucose, beta cells take up glucose through glucose transporters. During this time, the endoplasmic reticulum (ER) and mitochondria take up $[Ca^{2+}]_i$ in response to increased glucose metabolism, which causes an overall dip in $[Ca^{2+}]_i$. The resulting increase in the ATP to ADP ratio closes ATP-sensitive potassium channels (K_{ATP}-channels). As the dominant resting conductance of the beta cell, K_{ATP}-channels normally hyperpolarize the beta-cell membrane under basal glucose conditions. The

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closure of K_{ATP}-channels in response to increased glucose metabolism depolarizes the cell membrane. This initiates the repetitive firing of calcium-dependent action potentials and the influx of calcium into the beta cell, resulting in increased calcium in the mitochondria, ER, and nucleus. A large spike in calcium influx leads to the exocytosis of a readily releasable pool of docked insulin granules, which is termed first phase insulin release. Following first phase insulin release, $[Ca^{2+}]_i$ and insulin secretion remain elevated throughout the second phase response for as long as glucose remains elevated.

Because $[Ca^{2+}]_i$ is a strong trigger of exocytosis, both glucose-stimulated $[Ca^{2+}]_i$ (GSCa) and glucose-stimulated insulin secretion (GSIS) show similar trajectories under these conditions. GSCa can thus be used to assess the physiological response of islets to glucose stimulation. $[Ca^{2+}]_i$ imaging is advantageous because it provides high temporal precision of real-time changes in response to stimuli at the level of the individual islet [3]. Changes in the latency, trajectory, and amplitude of the triphasic GSCa response may indicate specific defects in stimulus-secretion coupling or other aspects of islet dysfunction. However, there are also amplifying processes that operate in parallel with the pathways of the Consensus Model to couple glucose uptake and metabolism with insulin exocytosis [4–6]. The amplifying pathway allows additional insulin release to occur independently of changes in $[Ca^{2+}]_i$ and thus provide a means by which the processes of insulin release and $[Ca^{2+}]_i$ signaling can dissociate from one another.

The exposure of islets to stress can further dissociate calcium signaling and insulin release. In this study, we systematically examined the effects of five putative triggers of islet stress in type 2 diabetes, each of which affected a different target: (1) rotenone to partially inhibit mitochondrial complex I [7,8], (2) thapsigargin to disrupt ER function by blocking sarco(endo)plasmic calcium ATPase (SERCA) pump activity [9,10], (3) cytokines to target the inflammatory response [11,12], (4) high glucose to mimic a state of hyperglycemia and induce hypersecretion of insulin [13,14], or (5) a combination of palmitate, oleate, and linoleate to target free fatty acid metabolism [15]. At much higher concentrations, each of these treatments is associated with toxicity and cell death, but we chose concentrations that approximated physiological levels (or subpharmacological levels) and did not induce cell death. Following 48-h treatment with these various stressors, we measured $[Ca^{2+}]_i$ and insulin secretion in low (3 mM) and stimulatory (11 mM) glucose to examine calcium entry through voltage-gated calcium channels, $[Ca^{2+}]_i$ handling by the ER, and the consensus pathway and amplifying pathways of insulin secretion. We show that all stressors reduced the first phase calcium response to glucose stimulation, an established marker of early beta-cell decline for insulin in type 1 diabetes [16] and type 2 diabetes [17,18], to a similar degree, yet glucose-stimulated insulin secretion was not affected in all cases. Our data suggest that stressors related to nutrient metabolism (FFAs, glucose, and rotenone) have greater impact on insulin secretion, whereas stressors associated with the ER (cytokines and thapsigargin) produced substantial changes in $[Ca^{2+}]_i$ handling without affecting insulin secretion.

2. Materials and methods

2.1. Mice

Studies were conducted using outbred CD-1 mice at ages of 8–12 weeks (Charles River Laboratories, MA). All animal procedures were approved by the University of Virginia (UVA) Institutional Animal Care and Use Committee.

2.2. Islet isolation and treatment

Pancreatic islets were isolated by collagenase-P digestion (Roche Diagnostics, Indianapolis, IN) followed by centrifugation with Histopaque 1100 (Sigma–Aldrich, St. Louis, MO) as previously described [19]. Islets were incubated overnight in RPMI 1640 medium (Invitrogen) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin to allow recovery from collagenase digestion before further treatment. Various islet stressors were prepared as follows: rotenone and thapsigargin were purchased from Sigma–Aldrich and prepared in stocks of DMSO to final concentrations less than 0.1%. Glucose-free RPMI medium (10% FBS and 1% penicillin/streptomycin) was supplemented with 1 M glucose stock to produce the 28G condition. Stocks of the murine forms of IL-1B and IL-6 (purchased from R&D Systems, Inc., Minneapolis, MN) were prepared in PBS with 0.1% BSA. Palmitate, oleate, and linoleate were purchased from Sigma–Aldrich and were prepared in 100 mM stock concentrations in methanol and stored in −80 °C. To treat the islets, the required amount of each fatty acid was taken in a glass tube and dried under a stream of nitrogen to remove methanol. The dried mixture of fatty acids was then resuspended in the RPMI medium with 0.1% BSA followed by vortexing and sonication. Islets were exposed to final concentrations of each stressor as follows: 28G/glucotoxicity (28 mM glucose), free fatty acids (50 μM palmitate + 100 μM oleate + 50 μM linoleate), ER stress (100 nM thapsigargin), low-grade inflammation (10 pg/ml IL-1β + 20 pg/ml IL-6), or mitochondrial stress (20 nM rotenone). Following 48-h exposure, islets were removed from the treatment conditions and tested for function by measuring glucose-stimulated changes in $[Ca^{2+}]_i$ and insulin release. These doses had effects on islet function but did not produce cell death as measured by propidium iodide fluorescence ($P > 0.38$ for each condition compared to basal cell death in untreated control islets; $n = 18–37$ islets/condition).

2.3. Glucose-stimulated calcium measurements

$[Ca^{2+}]_i$ was measured using the ratiometric $[Ca^{2+}]_i$ indicator fura-2 AM as previously described [20,21]. Briefly, Cell Tracker Red CMTPX (CTR, Invitrogen), a membrane penetrating fluorescent probe, was used in order to distinguish stressor-treated islets from control islets by selectively labeling only one of the two groups, thus enabling a simultaneous comparison of the two treatment groups (see [21] for additional details). All islets were loaded with 1 μM fura-2 AM in KRB solutions containing 3 mM glucose for 30 min (with one of two groups for each recording also loaded with 200 nM CTR and the other not), washed, and then transferred to a recording chamber. Islets were washed of excess surface fura-2AM by perifusing islets with KRB solution containing 3 mM glucose for ~15-min at ~400 μl/min using a peristaltic pump (Minipuls 2, Gilson, Middleton, WI). Islets were recorded with a Hamamatsu ORCA-ER camera (Hamamatsu Photonics, Japan) attached to an Olympus BX51WI fluorescence microscope (Olympus, Tokyo, Japan) using 340 and 380 nm excitation light and 510 nm emission as previously described [22]. Data were recorded and analyzed with IP Lab software Version 4.0 (Scanalytics, Rockville, MD).

2.4. Glucose-stimulated insulin secretion

Islets were tested for glucose-stimulated insulin secretion as described previously [20]. Briefly, islets were pre-incubated at 37 °C and 5% CO_2 for one hour in a standard KRB solution containing no glucose in order to reduce initial variance in insulin release rates among islets. Islets were then washed and incubated in KRB supplemented with 3 mM glucose for one hour followed by