How do reducing equivalents increase insulin secretion?

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Glucose stimulation of insulin secretion in pancreatic β cells involves cell depolarization and subsequent opening of voltage-dependent Ca^{2+} channels to elicit insulin granule exocytosis. This pathway alone does not account for the entire magnitude of the secretory response in β cells. In this issue, Ferdaoussi, Dai, and colleagues reveal that insulin secretion is amplified by cytosolic isocitrate dehydrogenase–dependent transfer of reducing equivalents, which generates NADPH and reduced glutathione, which in turn activates sentrin/SUMO-specific protease-1 (SENP1). β Cell–specific deletion of *Senp1* in murine models reduced the amplification of insulin exocytosis, resulting in impaired glucose tolerance. Further, their studies demonstrate that restoring intracellular NADPH or activating SENP1 improves insulin exocytosis in human β cells from donors with type 2 diabetes, suggesting a potential therapeutic target to augment insulin production.

Glucose metabolism and insulin secretion

Insulin secretion is stimulated by the metabolism of glucose within pancreatic β cells (1). The majority of glucose metabolism occurs through the glycolytic pathway, which produces pyruvate. As insulin-secreting β cells express low levels of lactate dehydrogenase (2), pyruvate metabolism in the mitochondria is critical for glucose-stimulated insulin secretion (GSIS). Indeed, overexpression of lactate dehydrogenase in β cells attenuates insulin secretion in response to added glucose (3). Several pathways downstream of pyruvate have been invoked as the mediators of insulin secretion. β Cells maintain high rates of pyruvate carboxylation to sustain anaplerotic metabolism. In the presence of high glucose concentrations, pyruvate oxidation is decreased via pyruvate dehydrogenase; however, pyruvate carboxylation is increased, resulting in production of oxaloacetate (4). Oxaloacetate can be metabolized to form citrate, malate, aspartate, or phosphoenolpyruvate (PEP); therefore, pathways downstream of these metab-

olites have been assessed for their influence on GSIS in β cells (Figure 1, see inset).

Citrate export and subsequent cleavage to cytosolic acetyl-CoA for de novo lipogenesis was proposed to amplify GSIS via fatty acyl-CoA, malonyl-CoA, and/or glycerolipids (5). However, inhibition of citrate lyase or fatty acid synthase does not suppress GSIS (6). Monoacylglycerol has been shown to amplify insulin secretion through its interaction with the exocytosis effector Munc13-1 (7), but the fatty acyl-CoA substrate for monoacylglycerol appears to be derived from lipolysis rather than de novo lipogenesis (7).

While β cells express very low levels of cytosolic PEP carboxykinase (PEPCK) (8), they produce high levels of the mitochondrial isoform (PEPCK$_m$). A large proportion of PEP is derived from mitochondrial oxaloacetate via PEPCK$_m$, and PEP substantially increases in the presence of elevated glucose (8). Moreover, silencing of PEPCK$_m$ effectively inhibits GSIS, suggesting that formation of mitochondrial PEP by this route is critical for GSIS (8).

Pyruvate cycling, via formation of malate, and its oxidative decarboxylation, via malic enzyme, back to pyruvate has also been tested as a pathway for driving pyruvate-mediated insulin secretion. Such a mechanism would involve export of mitochondrial NADH-derived reducing equivalents by malate. These reducing equivalents would then be converted to cytosolic NADPH via cytosolic malic enzyme. However, suppression or knockout of cytosolic malic enzyme does not diminish GSIS (9). Additionally, inhibition of the malate-aspartate shuttle does not inhibit GSIS (10).

Linking reducing equivalents to amplification of insulin secretion

Cytosolic NADPH is also produced from a reaction catalyzed by cytosolic isocitrate dehydrogenase (ICDc). Reduction of isocitrate transport from the mitochondria (6) or knockdown of ICDc (11) decreases GSIS. These observations point to the products of ICDc, NADPH and α-ketoglutarate, as the mediators of GSIS amplification.

The MacDonald lab has pursued the trail of reducing equivalents, in particular NADPH, in β cells. They used microdialysis to introduce membrane-impermanent metabolites into β cells, and membrane capacitance was measured to monitor exocytosis in response to these metabolites. In previous studies, direct introduction of NADPH into β cells was shown to enhance insulin exocytosis (12). In this issue, Ferdaoussi, Dai, and colleagues have made a novel connection between metabolites upstream and downstream of NADPH (13). Importantly, microdialysis of 100 μM isocitrate amplified insulin exocytosis in β cells as effectively as did 10 mM glucose. Moreover, knockdown of ICDc completely blocked GSIS, placing this enzyme downstream of glucose. ICDc converts isocitrate into α-ketoglutarate and NADPH; however, only microdi-
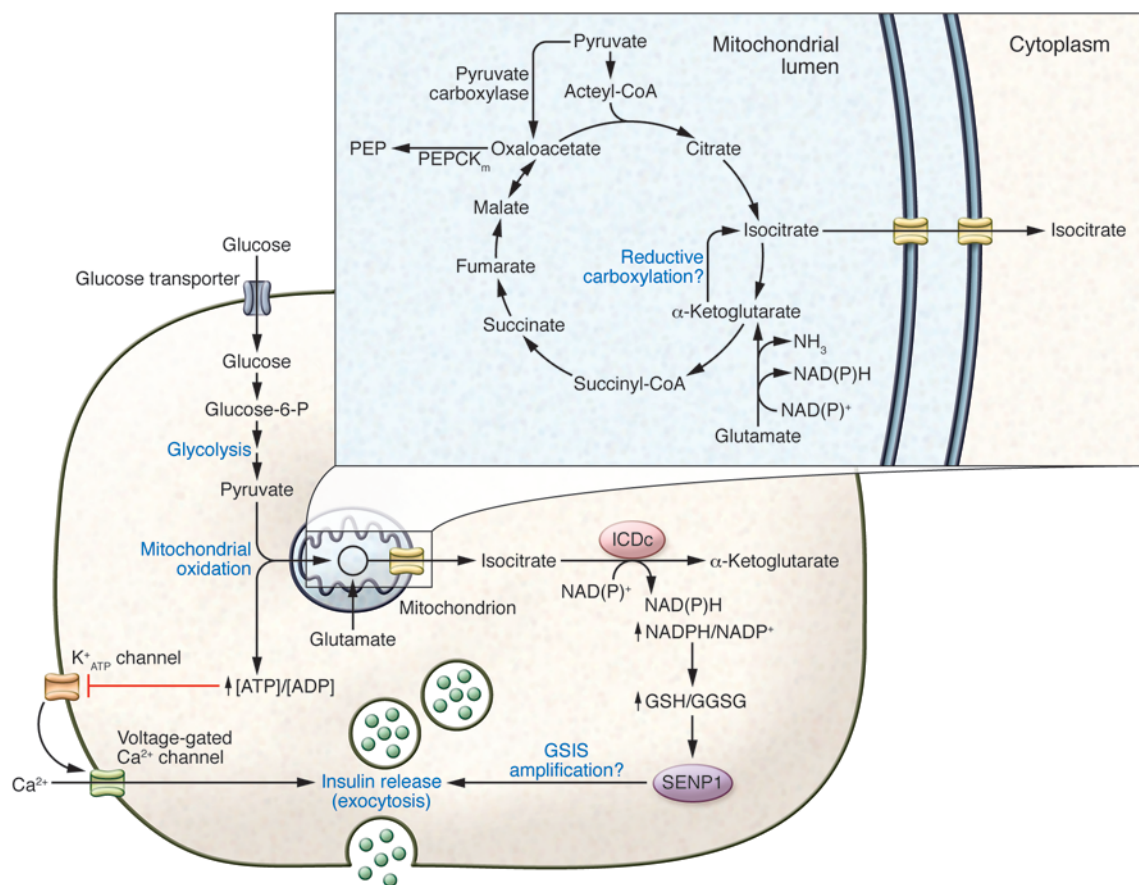
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Figure 1. Triggering and amplification of glucose-stimulated insulin secretion. In β cells, glucose uptake and metabolism result in depolarization and subsequent opening of voltage-gated Ca^{2+} channels, which in turn triggers insulin granule secretion. Amplification of insulin secretion requires pyruvate metabolism in the mitochondria, which in β cells is driven by high rates of pyruvate carboxylation and generation of oxaloacetate (inset). Several metabolites downstream of oxaloacetate have been evaluated for their ability to amplify GSIS, including citrate, malate, aspartate, and PEP. Of these, generation of PEP in the mitochondria is critical for GSIS. In this issue, Ferdaoussi, Dai, and colleagues demonstrate that the transfer of reducing equivalents mediated by the conversion of isocitrate to α-ketoglutarate by ICDc in the cytosol generates NADPH and maintains glutathione in its reduced form, GSH. GSH then activates SENP1, which deSUMOylates a target protein (not yet identified), thus promoting (or derepressing) insulin release. Glutamate metabolism is also involved in insulin secretion, and mutations in the inhibitory GTP-binding site of GDH, which converts glutamate to α-ketoglutarate in the mitochondria, increase insulin secretion. Reductive carboxylation of α-ketoglutarate in the mitochondria generates isocitrate and is thus a potential pathway through which glutamate metabolism may feed into the ICDc/SENP1-mediated amplification pathway.

alysis of NADPH, but not α-ketoglutarate or NADH, mimicked the amplifying effect of 10 mM glucose. Together, these results suggested the presence of a redox switch, one likely affecting cysteine thiols, that is responsive to the cytosolic NADPH/ $NADP^+$ ratio in β cells.

Glutathione (GSH) functions as a critical redox buffer in a variety of cells, and the ratio of reduced GSH to the oxidized disulfide-bridged dimer (GSSG) is directly related to the NADPH/$NADP^+$ ratio. Ferdaoussi, Dai, and colleagues found that infusion of 10 μM GSH into β cells amplifies insulin secretion as effectively as 10 mM glucose. Based on these results, ICDc appears to be essential for

glucose amplification of insulin secretion through its production of NADPH and GSH. But what is downstream of GSH?

The MacDonald laboratory previously showed that SUMOylation negatively regulates GSIS (14) and that this brake on insulin secretion is reversed by expression of sentrin/SUMO-specific protease-1 (SENP1). Additionally, knockdown of SENP1 in human and murine pancreatic islets almost completely abolished glucose-stimulated amplification of exocytosis, placing the SUMOylation pathway downstream of glucose metabolism. The deSUMOylation activity of SENP1 is dependent on an active-site cysteine thiol. This residue can be oxidized to form an intermolecu-

lar disulfide with SUMO1 (15). Thus, the activity of SENP1 is sensitive to its ambient redox environment. Ferdaoussi, Dai, and colleagues show that glucose increases the thiol exposure and enzymatic activity of SENP1 (13). These glucose-dependent changes required NADPH and GSH.

As Ferdaoussi et al. predicted (13), β cell–specific *Senp1* deletion decreased GSIS in mouse models, and SENP1 deficiency also decreased insulin exocytosis in response to GSH. Together, the results of Ferdaoussi, Dai, and colleagues establish a causal connection between glucose metabolism, isocitrate availability for the ICDc reaction, NADPH generation, GSH maintenance, and SENP1 activation (Figure 1).

Conclusions and future directions

One of the key questions remaining is what is the downstream substrate of SENP1 that enhances (or derepresses) a late step in exocytosis? Exocytosis in β cells involves formation of a complex composed of vesicle-associated membrane protein 2 (VAMP2), synaptotagmins, and synaptosomal-associated protein 25 (SNAP-25). A variety of proteins have been shown to bind to these complex proteins and modulate their function. As SUMOylation modulates protein-protein interactions, this modification has the potential to regulate the function of insulin secretory granules. Syntaxin 1A, SNAP-25, and at least two synaptotagmin isoforms in cells have putative SUMOylation motifs (16). In the case of synaptotagmin 7, mutation of one of its SUMOylation sites rescues β cells from the inhibitory effect of SUMO1 on insulin secretion (14). Another potential candidate is tomosyn (tomosyn-1 or tomosyn-2), which is a known inhibitor of regulated secretion. Recent studies show that tomosyns inhibit GSIS (17, 18) and are substrates for SUMOylation (19). Therefore, SUMOylation would be predicted to promote the binding of tomosyn to its targets, syntaxin and/or synaptotagmin.

It is an intriguing observation that NADPH specifically derived from ICDc, and not from cytosolic malic enzyme, seems to be critical for GSIS. This apparent metabolic compartmentalization could indicate that ICDc itself is in a complex that channels its NADPH product to the GSH reductase reaction.

Glutamate metabolism also plays an important role in insulin secretion. Mutations in the inhibitory GTP-binding site of glutamate dehydrogenase (GDH) increase insulin secretion and lead to hyperammonemia and hypoglycemia. There has been some controversy about the direction of the GDH reaction in β cells (20, 21); however, the fact that the gain-of-function GDH mutations lead to hyperammonemia together with hyperinsulinemia suggests that the reaction occurs in the oxidative deamination direction. GDH resides in the mitochondria, produces α-ketoglutarate, and can use either NAD^+ or $NADP^+$ as the electron acceptor. Perhaps the α-ketoglutarate from GDH is exported to the cytosol and converted back to glutamate via transamination and serves as a major source of glutamate for GSH production. Another recently discovered pathway, the reductive carboxylation of α-ketoglutarate to form isocitrate (22), raises the possibility that there is an isocitrate cycling pathway that involves GDH.

Together, the findings of Ferdaoussi, Dai, and colleagues are game changing for improving our understanding of the factors that mediate and amplify GSIS. The results of this study will likely motivate investigations into the possible role of the GSH/SUMO1 pathway in glutamate-evoked insulin secretion and perhaps for secretion in response to other insulin secretagogues.

Acknowledgments

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