How do reducing equivalents increase insulin secretion?

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

Cytosolic NADPH has also been tested as a pathway for driving pyruvate-mediated insulin secretion. 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 knock-out of cytosolic malic enzyme does not diminish GSIS (9). Additionally, inhibition of the malate-aspartate shuttle does not inhibit GSIS (10).

Glucose stimulation of insulin secretion in pancreatic β cells involves cell depolarization and subsequent opening of voltage-dependent Ca2+ 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.

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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), \( \beta \)-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).

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 in \( \beta \) cells. Ferdaoussi, Dai, and colleagues found that infusion of 10 \( \mu \)M GSH into \( \beta \) 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 intermolecular 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), \( \beta \)-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), syntaxotagmins, and synapto-somal-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 syntaxotagmin isoforms in cells have putative SUMOylation motifs (16). In the case of syntaxotagmin 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 syntaxotagmin.

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 NAPD+ 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 research into the possible role of the GSH/SUMO1 pathway in glutamate-evoked insulin secretion and perhaps for secretion in response to other insulin secretagogues.

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