Metabolomics Applied to Islet Nutrient Sensing Mechanisms

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Abstract

After multiple decades of investigation, the precise mechanisms involved in fuel-stimulated insulin secretion are still being revealed. One avenue for gaining deeper knowledge is to apply emergent tools of "metabolomics", involving mass spectrometry and nuclear magnetic resonance-based profiling of islet cells in their fuel-stimulated compared to basal states. The current article summarizes recent insights gained from application of metabolomics tools to the specific process of glucose-stimulated insulin secretion, revealing two new mechanisms that may provide targets for improving insulin secretion in diabetes.

Keywords

Introduction

The pancreatic islets of Langerhans play a critical role in metabolic physiology by translating key markers of nutritional status into hormonal changes that orchestrate systemic energy and substrate balance. The secretion of the primary hormonal mediators, insulin and glucagon, are regulated in a reciprocal fashion such that the anabolic hormone insulin is induced and secreted in the fed condition, whereas the catabolic hormone glucagon is preferentially released in the fasted state. A key signal of the fasted to fed transition is a rise in circulating glucose levels that triggers insulin secretion from islet β-cells. The mechanism of glucose-stimulated insulin secretion (GSIS) has been investigated for decades, and a canonical view of the process has emerged to broad acceptance. The central model holds that glucose induces insulin secretion via its own glycolytic and oxidative metabolism, leading to an increase in ATP:ADP ratio, inhibition of ATP-sensitive K^+ (K_{ATP}) channels, activation of voltage-gated Ca^{2+} channels, and influx of extracellular Ca^{2+} to stimulate insulin granule exocytosis.[1] In more recent years, a modified version of the central model has emerged, in

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which the K_{ATP} channel-dependent pathway is viewed as the primary mediator of the "triggering" or first phase of insulin secretion, whereas other signals are likely to be key drivers of the more prolonged "amplifying" or second phase of secretion[2]. Consistent with these ideas, several studies have demonstrated a significant residual insulin secretion response to glucose in islets with pharmacologic or molecular suppression of K_{ATP} channels, [2,3] strongly implicating K_{ATP}-channel independent signaling mechanisms in mediating the full response. Our laboratory and others have been focused on identifying the signaling pathways and mediators involved in the sustained second phase of insulin secretion.[4–6]

In type 1 diabetes (T1D), β-cells are destroyed by the immune system, creating complete insulin deficiency. The long-practiced clinical intervention of insulin injection is life-saving, but can lead to hyperglycemic and hypoglycemic episodes due to the lack of regulation of insulin delivery by normal physiologic signals. Cell-based approaches to insulin replacement involving human islets or stem-cell derived β-cells have been actively investigated. Strategies for inducing differentiation of human stem cells to insulin-producing cells have advanced steadily, including recent reports of cells that exhibit some level of GSIS.[7,8] However, it is unlikely that these cells fully mimic the glucose response mechanisms of normal human islets, and further engineering is likely to be required. In type 2 diabetes (T2D), the transition from the obese, insulin resistant, pre-diabetic state to full blown diabetes is triggered by β-cell failure, a general term that encompasses loss of normal nutrient signaling, an inability to maintain a high rate of insulin secretion to compensate for impaired insulin action, and eventual loss of β-cell mass.[9] A clearer understanding of the complete mechanism of GSIS is essential for ultimate success in creation of surrogate cells for insulin replacement therapy in T1D, as well as development of more efficacious drugs for treatment of T2D.

Metabolomics Technology

The approach taken by our group to gain a deeper understanding of pancreatic islet cell nutrient signaling is to apply evolving tools of comprehensive metabolic analysis, or "metabolomics". With the advent of more sensitive mass spectrometers and accompanying chromatographic strategies, researchers are able to profile an increasingly broad set of metabolic intermediates in response to controlled systems perturbations. These technologies, when coupled to nuclear magnetic resonance (NMR)- or mass spectrometry (MS)-based metabolic flux analysis, provide a comprehensive view of the metabolic fates of glucose and other nutrient secretagogues in islet cells. These technologies can be applied to relatively simple and well-controlled in vitro experiments in which cultured pancreatic islets or insulinoma cell lines are exposed to basal or stimulatory levels of glucose and other fuels for varying time periods, followed by comprehensive profiling of metabolites that differ in the basal compared to stimulated states. Inclusion of stable isotope-labeled secretagogues (e.g. ^{13}C-labeled glucose) allows analysis of enrichment of downstream metabolites and calculation of metabolic flux rates.

Static metabolic profiling ("snap shots" at a single time point) with MS-based methods can be performed in a targeted or non-targeted fashion. Non-targeted metabolomics is used to compare two biological conditions with coverage of as many metabolites as possible

regardless of the chemical class of the metabolites. It is best suited as a discovery tool for identifying metabolites that change in response to manipulation of a biological system rather than providing the specific concentration of a known metabolite. In contrast, targeted metabolomics provides more quantitative analysis by focusing on measurement of known metabolites in clusters with similar chemical structures (e.g. amino acids, organic acids, etc). The method often involves addition of stable isotope-labeled standards (usually ^{2}H or ^{13}C-labeled) to samples, and therefore provides precise quantification of a targeted analyte relative to known concentration of the added standard.[10–13] Identification and measurement of metabolites by MS is typically coupled to a separation technique such as gas chromatography (GC-MS), liquid chromatography (LC-MS), ultra-performance liquid chromatography (UPLC-MS), or capillary electrophoresis (CE-MS).[14,15] MS ionizes chemical species and sorts the ions based on their mass to charge ratio. Chromatography techniques help to separate metabolites based on their chemical properties. In contrast, NMR uses the magnetic properties of certain atomic nuclei, and detects spectral features originating from molecules that contain carbon or hydrogen.[16] In general, MS methods have advantages of high sensitivity and small sample volumes, whereas NMR has little chemical bias and can be used directly on the sample with no need for extraction and derivatization. Metabolomics methods developed specifically for use in β-cell lines include an extraction protocol determined by statistical design prior to GC-MS analysis,[17] and an extraction procedure developed for adherent cells compatible with LC-MS.[18] In terms of analytical techniques, a 2D capillary LC-MS strategy has been devised to improve metabolite coverage in small tissue samples,[19] and direct matrix-assisted laser desorption/ionization (MALDI)-TOF analysis of single islets has also been reported.[20] More complete discussion of metabolomics methods and their use in disease research can be found in several recent reviews. [6,12,14–16,21]

Our laboratory has used a combination of NMR- and MS-based metabolic flux analysis coupled with targeted MS-based static profiling of key intermediates of glucose, lipid, and amino acid metabolic pathways to gain insights into mechanisms of GSIS. Here we focus on our experiences in use of metabolomics for advancing our understanding of islet biology, and the implications of this new information for translational applications.

Metabolic Flux Analysis Implicates Anaplerotic Metabolism of Glucose in Stimulus-Secretion Coupling

Our early work focused on subclones of the rat insulinoma cell line INS-1 with varying capacity for GSIS.[22] We reasoned that understanding of metabolic differences between robustly versus poorly glucose responsive cell lines might unveil novel metabolic pathways or factors that regulate glucose sensing. We first applied NMR-based mass isotopomer analysis to measure relative fluxes through the oxidative enzyme pyruvate dehydrogenase (PDH) and the anaplerotic enzyme pyruvate carboxylase (PC), using U-^{13}C glucose as substrate. The studies showed that variable GSIS across a panel of INS-1-derived cell lines was tightly correlated to pyruvate anaplerosis rather than pyruvate oxidation.[23] Flux through PC-catalyzed anaplerosis actually exceeds flux through PDH in isolated islets,[24] and the strong correlation of anaplerotic metabolism of pyruvate with GSIS that we found with

NMR-based flux analysis in INS-1-derived cell lines was subsequently confirmed by MS-based methods applied to primary pancreatic islets.[16] During active glucose stimulation of β-cells, anaplerotic metabolism of pyruvate through PC fills the TCA cycle, allowing excess intermediates to exit the mitochondria to engage with cytosolic enzymes for use in various biosynthetic pathways. This includes possible diversion of TCA cycle intermediates into one of the three "pyruvate cycling" pathways that involve extramitchondrial citrate, isocitrate or malate.[4,25] Systematic study of each of these pathways with recombinant adenoviruses containing shRNAs specific to key enzymes in each of the pathways implicated the cytosolic NADP-dependent isocitrate dehydrogenase (ICDc or IDH1) as a key extra-mitochondrial step (Figure 1).[26] Supporting this idea, shRNA-mediated suppression of the citrate/isocitrate carrier (responsible for export of citrate and isocitrate from the mitochondria) or IDH1 results in significant impairment of GSIS.[4,26,27] IDH1 knockdown also impairs secretion in response to glutamine in the presence of the glutamate dehydrogenase activator BCH.[28]

Our more recent work suggests that the IDH1 reaction plays two key roles in mediating insulin release. First, it produces NADPH needed for the glutathione reductase reaction, which converts oxidized glutathione (GSSG) to its reduced form (GSH).[29,30] Reduced GSH in turn reduces glutaredoxin, which interacts with various cellular proteins to influence their activity via reduction of disulfide (cysteine-cysteine) bridges. Second, it generates α-ketoglutarate that can be transaminated to glutamate, a building block of glutathione. The latter finding emerged through targeted MS/MS profiling of amino acids during glucose stimulation of β-cells, demonstrating significant changes in levels of only 3 of 15 amino acids profiled. Among these, we observed a clear decrease in aspartate and a rise in glutamate in response to stimulatory glucose.[29] Treatment of INS-1-derived with the general aminotransferase inhibitor AOA blocked the glucose-induced changes in both amino acids and partially blocked GSIS.[29] These findings suggest that α-ketoglutarate generated by IDH1 is transaminated to glutamate by aspartate aminotransferase, providing a key substrate for de novo glutathione synthesis. Consistent with this idea, glucose stimulation of 832/13 cells increases both the total (GSH + GSSG) and reduced (GSH) pools of glutathione, an effect blocked by shRNA-mediated suppression of IDH1. The increase in reduced GSH was also demonstrated by cytosolic expression of a GSH biosensor (Grx1-roGFP) in intact cells.[29]

A key remaining element is to define how and why maintenance of robust pools of reduced glutathione (GSH) and glutaredoxin stimulate insulin secretion. These biochemical changes appear to be linked to insulin granule exocytosis via reduction of the desumoylation enzyme SENP1. MacDonald and co-workers have demonstrated that SENP1 triggers insulin granule exocytosis via deSUMOylation of secretory granule proteins, including tomosyn1.[29,31,32] Glucose-dependent deSUMOylation of tomysyn1 influences its interactions with the granule trafficking proteins syntaxin1A and secretagogin. Glutaredoxin acts to reduce cysteine residues in SENP1, leading to activation of the enzyme. A direct connection between intermediates in the emergent glucose/isocitrate signaling pathway and SENP1 was demonstrated in studies of patch-clamped rodent and human β-cells perfused with isocitrate, NADPH or GSH. These intermediates, but not NADH or α-ketoglutarate, were as effective as glucose for stimulation of exocytosis,[29,30] an effect lost in mouse islets with transgenic

deletion of SENP1 or human islets with shRNA-mediated suppression of SENP1 expression.
[29] (See Figure 1 for overall schematic summary).

Do these findings have translational implications for rescue of GSIS in T2D? Importantly, the ability of glucose to drive insulin granule exocytosis in patch-clamped normal human β-cells is lost in β-cells from human donors with T2D. However, infusion of isocitrate or NAPDH rescues the exocytotic response in these cells.[29] These findings suggest that islets from humans with T2D may be defective at a step (or steps) of anaplerotic metabolism of glucose upstream of the IDH1 reaction. Further studies will be required to define the specific molecular lesions in anaplerotic metabolism of glucose in islets from T2D subjects.

Metabolomics Identifies Novel Nucleotide Secretagogues

The classical K_{ATP}-channel dependent pathway of GSIS invokes an increase in ATP:ADP ratio as a key signal for suppression of K_{ATP} channel activity, leading to membrane depolarization, activation of voltage-gated Ca^{2+} channels, and triggering of insulin granule release by Ca^{2+} signaling. However, recent non-targeted metabolomics studies suggested that glucose also alters the levels of a variety of other purine and nucleotide pathway intermediates in islet cells,[33–36] although the functional significance of these changes was not defined. To investigate this further, our group developed a targeted, quantitative LC-MS/MS method for profiling of 37 different nucleotides and purine pathway intermediates.[37] The method was applied to the robustly glucose responsive INS-1-derived cell line 832/13 [22] exposed to basal or stimulatory glucose concentrations. Glucose stimulation caused a clear rise in NADPH and NADH levels, consistent with operation of the glucose/isocitrate pathway of GSIS discussed earlier. Stimulatory glucose also caused a clear decrease in inosine monophosphate (IMP), and an equally striking increase in adenylosuccinate (S-AMP).[37] IMP and S-AMP are the substrate and product of a single enzymatic reaction in which IMP is converted to S-AMP by adenylosuccinate synthase (ADSS), suggesting that this enzyme could play a regulatory role in β-cell glucose sensing (Figure 1). Indeed, pharmacologic suppression of ADSS with alanosine, or molecular suppression of enzyme function by delivery of an ADSS-specific shRNA, caused impairment of GSIS with an attendant decrease in S-AMP, effects that could be overcome by addition of adenine. Moreover, shRNA-mediated suppression of adenylosuccinate lyase (ADSL), a more proximal enzyme in the S-AMP biosynthetic pathway, impaired GSIS and lowered S-AMP levels. The effect of ADSL suppression to lower S-AMP was independent of decreases in other adenine nucleotides or GTP, implying a specific role for S-AMP in regulation of insulin secretion. Indeed, infusion of S-AMP into patch-clamped β-cells from normal (non-diabetic) human donors at low glucose (1 mM) revealed that it is equipotent to stimulatory glucose (12 mM) for activation of exocytosis. Moreover, similar to intermediates in the glucose/isocitrate pathway, S-AMP rescues exocytosis in glucose-unresponsive β-cells from humans with T2D. Also, analogous to the glucose/isocitrate pathway, the stimulatory effect of S-AMP on exocytosis in patch-clamped human β-cells is impaired by shRNA-mediated suppression of SENP1.[37]

The new glucose/isocitrate and S-AMP pathways of GSIS summarized above are viewed as complementary to the classical K_{ATP}-channel-dependent initiating pathway. It remains to be

determined if the newly described pathways are additive or synergistic with each other and/or the K_{ATP} channel-dependent mechanism in control of GSIS. Our studies on the glucose/isocitrate pathway also imply a particular impact of NADPH generated by the IDH1 reaction, relative to other NADPH producing enzymes such as malic enzyme or the pentose monophosphate shunt enzymes glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH). With regard to the pentose pathway enzymes, inhibition of G6PDH with dehydroepiandrosterone (DHEA),[35] or 6PGDH by siRNA or the chemical inhibitor 6-AN,[38] were both reported to result in impaired GSIS. The effects of 6PGDH inhibition were ascribed to accumulation of early PPP intermediates and activation of p-ERK,[38] whereas DHEA treatment caused a decrease in GSH levels, although effects on NADPH were not reported.[35] These findings appear inconsistent with our findings that shRNA-mediated suppression of IDH1 is sufficient to ablate the glucose-induced increment in GSH and GSH:GSSG ratio in islet cells.[29] In addition, the finding that S-AMP has a direct effect to activate exocytosis in normal islets, and is able to rescue dysfunctional exocytosis in human T2D islets seems more aligned with the concept that S-AMP is the key regulator of GSIS produced by the pentose shunt.[37] However, these issues will require further investigation.

Future studies should include attempts to estimate the relative contributions of different pathways for NADPH formation, using recently described methods.[39] In this approach, pentose pathway flux is measured by the classical method of the difference between $^{14}CO_2$ production from [1-^{14}C] and [6-^{14}C] glucose. The contribution of the pentose shunt to NADPH synthesis is then measured by fractional enrichment (FE) of the NADPH and G6P pools in cells incubated with [3-D] glucose.[39] Using these methods, a surprisingly large contribution of folate metabolism to total NAPDH production was recently reported in HEK293T cells.[39] Application of similar methods, possibly including use of different substrates with strategic deuterium labeling, to pancreatic islet cells should allow the fractional contributions of various NADPH producing pathways to be quantified. Performance of such flux experiments in the presence and absence of strategic suppression of contributory enzymes such as G6PDH and IDH1 will add to our knowledge of key operative pathways for control of GSIS.

Acknowledgments

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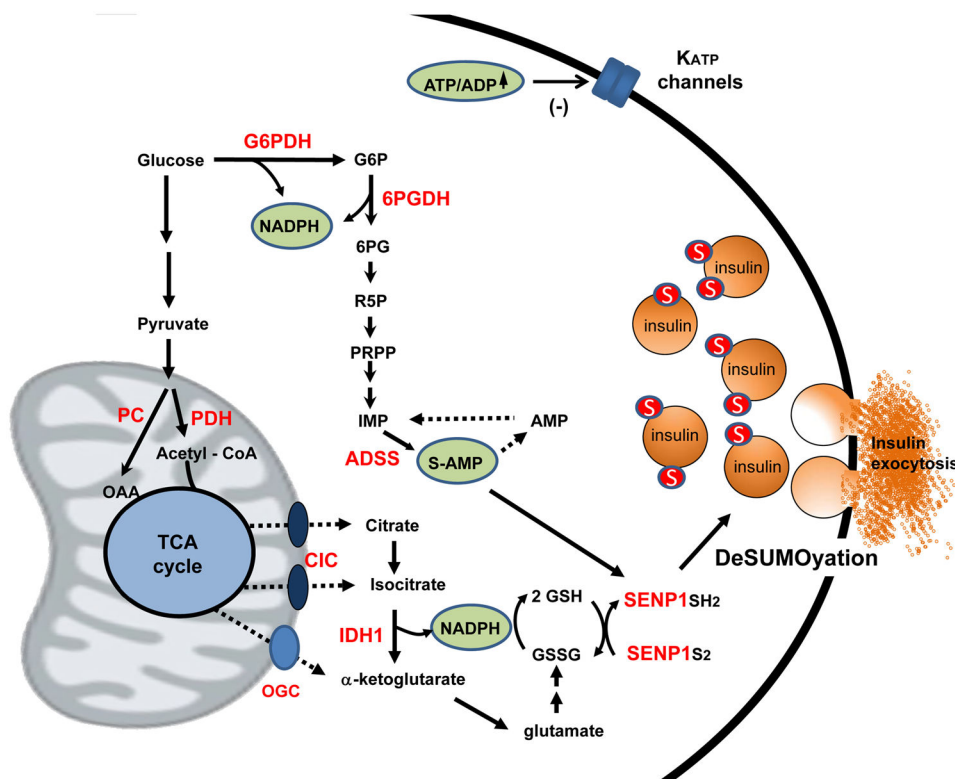
Figure 1. New mechanisms of glucose-stimulated insulin secretion revealed by metabolomics
In addition to the classical pathway involving glycolytic and oxidative metabolism of glucose to increase ATP:ADP ratio and inhibit K_{ATP} channels, our recent metabolomics studies identify two pathways that play complementary roles. First, anaplerotic metabolism of glucose via pyruvate carboxylase (PC) rather than pyruvate dehydrogenase (PDH) creates excess TCA cycle intermediates that leave mitochondria in the form of citrate and isocitrate via the citrate/isocitrate carrier (CIC). Isocitrate engages with the cytosolic, NADP-dependent isoform of isocitrate dehydrogenase (IDH1) to form NADPH and reduce glutathione, leading to reduction and activation of the deSUMOylase enzyme SENP1. In addition, metabolism of glucose via the pentose shunt enzymes glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH) contributes to nucleotide synthesis, particularly the conversion of IMP to S-AMP via adenylosuccinate synthase (ADSS). S-AMP also stimulates insulin secretion in a SENP1-dependent manner. Figure modified from reference 37 and used with permission.