NADH Shuttle and Insulin Secretion

In their report (1), Eto et al. hypothesize that nicotinamide adenine dinucleotide (NADH) is the signal for the coupling of glycolytic and mitochondrial glucose metabolism that triggers insulin secretion. Their conclusions, based on their innovative and meticulous experiments, do not consider the possibility that the glycolytic end-product in the cytosol is not pyruvate, but lactate. Thus, when lactate enters the mitochondrial tricarboxylic acid cycle, it must first be converted to pyruvate by the lactate dehydrogenase (LDH) reaction that also produces NADH. The LDH reaction is an important source of NADH that allows cellular utilization of lactate as the sole oxidant substrate (2). Whether or not the LDH-produced NADH plays a role in triggering insulin secretion could be determined by use of the well-designed system described in the report (1). Incubation of mGPDH−−/− islets with 22.2 mM glucose + aminooxyacetate (AOA) + (20 to 40 mM) lactate should provide ample amounts of LDH-produced NADH. Alternatively, incubation of wild-type (WT) islets with 22.2 mM glucose + iodoacetic acid (IAA) + AOA + (20 to 40 mM) lactate should work as well. Instead of IAA, the use of a pharmacological dose of the glucose analog 2-deoxy-D-glucose might work. Performing these experiments, one should be able to determine if the LDH reaction produces an NADH signal and, if so, whether or not such a signal is sufficient to trigger insulin secretion.

Avital Schurr
University of Louisville
School of Medicine
Louisville, KY 40292, USA
E-mail: atischur01@ulkyvm.louisville.edu

Ralphiel S. Payne
University of Louisville
School of Medicine
Louisville, KY 40292, USA
E-mail: rpayn01@ulkyvm.louisville.edu

References

18 March 1999; accepted 7 September 1999

Response: Schurr and Payne note that lactate can be an alternative substrate that provides the cytosol with NADH and pyruvate through a reaction catalyzed by LDH. However, lactate at 40 mM could not restore the 22.2 mM glucose-induced insulin secretion in mGPDH−−/− islets treated with 5 mM AOA, where activities of both the NADH shuttles were blocked. Two possibilities might explain this result. One is that LDH activity in the islets was too low to generate ample NADH and pyruvate from lactate in the cytosol. Indeed, LDH activity reportedly must be quite low to ensure aerobic glucose metabolism in β cells (1). The other is that although LDH activity was sufficiently high to utilize lactate, the products, NADH and pyruvate—which in combination may stimulate insulin secretion—were unable to do so because these conditions block the NADH shuttle system.

Glucose-induced insulin secretion is known to be abolished in the presence of IAA, which inhibits glycolysis at a step catalyzed by glyceraldehyde-3-phosphate dehydrogenase, before generation of glucose-derived NADH (2). In wild-type islets treated with 1 mM IAA, lactate at 40 mM was also unable to restore the secretion. In this case, the inability to restore secretion can again be explained by low LDH activity in β cells, but not by the blocking of the NADH shuttle system when LDH activity is sufficiently

Finally, some new information suggests that the conventional thinking about the glycolytic phosphate shuttle as only using NADH may be oversimplified. At the normal pH of 7.1, the islet cytosolic glycerol phosphate dehydrogenase (cGPD) prefers NADPH as a cofactor over NADH, and there is evidence that enzyme–enzyme interaction influences the preference of cGPD for NADPH over NADH (6). Furthermore, islets possess at least one shuttle capable of exporting NADPH equivalents from the mitochondria (7). Thus, in the β cell, a major role of cGPD might be to supply cytosolic NADP.
technical comments

Department of Metabolic Diseases
Graduate School of Medicine
University of Tokyo
7-3-1 Hongo, Bunkyo-ku
Tokyo, 113-8655, Japan
E-mail: kadowaki-3im@h.u-tokyo.ac.jp

References

Applied 5 mM AOA to mGPDH−/− mice islets, glucose-induced insulin secretion was almost completely abolished. The inhibition by AOA was dose-dependent, and the half-maximal inhibitory concentration was ~1 mM at 22.2 mM glucose (6). The apparent difference in the ~15% inhibitory effect of 5 mM AOA on insulin secretion in wild-type islets observed by us (3) and the ~60% inhibition observed by other workers (5, 7, 8) may have its roots in the relative activity of the glycerol phosphate shuttle compared with that of the AOA-sensitive malate-aspartate shuttle. The activity of the glycerol phosphate shuttle may have been decreased during their incubation of rat islets (detailed procedures of islet culture after isolation were not described). We used the mouse islets within a few hours after isolation for insulin secretion experiments. It is notable that 15-mM glucose-stimulated insulin secretion is inhibited by more than 85% in clonal INS-1 β cells treated with as low as 0.25 mM AOA (9).

Oxidation of [6-14C]glucose is thought to reflect the TCA (trichloroacetic acid) cycle activity more precisely than that of [U-14C]glucose, mainly because the isotope-labeled carbons of [3-14C]glucose and [4-14C]glucose are lost at a step catalyzed by pyruvate dehydrogenase before entering the TCA cycle, although some portion of them enters the cycle as [1-14C]oxaloacetate for oxidation (10). This is not the case with glucose labeled in position 1, 2, 5, or 6. In addition, oxidation of [1-14C]glucose produces 14CO2 during the oxidative part of the pentose phosphate pathway.

In contrast to the prevailing hypothesis, our report clearly demonstrates that glycolysis can proceed when the NADH shuttle system is halted, a proposition that might be explored through measurement of NAD+/NADH ratio in the cytosol. If the ratio is comparable to wild-type islets, reoxidation of NADH to NAD+ may be compensated for by other dehydrogenases present in the cytosol, one candidate being the reaction catalyzed by LDH. However, if that reaction predominates, [U-14C]glucose oxidation in mitochondria should be decreased, which we did not observe (3). Thus, dehydrogenases other than LDH are more probable candidates. If the ratio is low, it may follow that glyceroldehyde-3-phosphate dehydrogenase functions well enough to maintain the normal glycolytic flux under the reduced cytosolic conditions.

Kazuhiro Eto
Yoshiharu Tsunamoto
Takashi Kadowaki

Department of Metabolic Diseases
Graduate School of Medicine
University of Tokyo
7-3-1 Hongo, Bunkyo-ku
Tokyo, 113-8655, Japan
E-mail: kadowaki-3im@h.u-tokyo.ac.jp

If the ample supply of NADH and pyruvate is not sufficient to cause insulin secretion, then a third, hitherto unknown glycolysis-derived signal, possibly originating from between 1,3-bisphosphoglycerate and phosphoeneolpyruvate, may be required for reconstitution of glucose-induced insulin secretion, a possibility that might be investigated by overexpressing LDH in β cells.

Turning to the comments of MacDonald and Fahien, we note that our report (3) demonstrated that the NADH shuttle system is essential for glucose-induced insulin secretion by coupling glycolysis with activation of mitochondrial metabolism, thereby promoting efficient adenosine triphosphate generation in mitochondria. MacDonald (4, 5) suggested the potential role of the glycerol phosphate shuttle or the malate-aspartate shuttle in glucose-induced insulin secretion. The absence of an inhibitor of the malate-aspartate shuttle, partially inhibits glucose-induced insulin secretion. The absence of an inhibitor of the glycerol phosphate shuttle, however, has made it difficult to determine the role of the NADH shuttle system, composed of the two distinct shuttles. We have directly demonstrated the role of the system in glucose-induced insulin secretion by coupling glycolysis with activation of mitochondrial metabolism, thereby promoting efficient adenosine triphosphate generation in mitochondria. MacDonald (4, 5) suggested the potential role of the system in glucose-induced insulin secretion, noting that mGPDH demonstrates the role of the system in glucose-induced insulin secretion, noting that mGPDH demonstrates the role of the system in glucose-induced insulin secretion.
NADH Shuttle and Insulin Secretion
Avital Schurr and Ralphiel S. Payne

Science 287 (5455), 931.
DOI: 10.1126/science.287.5455.931a