Pancreatic Islet β-Cells Transiently Metabolize Pyruvate*

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Pancreatic β-cell metabolism was followed during glucose and pyruvate stimulation of pancreatic islets using quantitative two-photon NAD(P)H imaging. The observed redox changes, spatially separated between the cytoplasm and mitochondria, were compared with whole islet insulin secretion. As expected, both NAD(P)H and insulin secretion showed sustained increases in response to glucose stimulation. In contrast, pyruvate caused a much lower NAD(P)H response and did not generate insulin secretion. Low pyruvate concentrations decreased cytoplasmic NAD(P)H without affecting mitochondrial NAD(P)H, whereas higher concentrations increased cytoplasmic and mitochondrial levels. However, the pyruvate-stimulated mitochondrial increase was transient and equilibrated to near-base-line levels. Inhibitors of the mitochondrial pyruvate-transporter and malate-aspartate shuttle were utilized to resolve the glucose- and pyruvate-stimulated NAD(P)H response mechanisms. These data showed that glucose-stimulated mitochondrial NAD(P)H and insulin secretion are independent of pyruvate transport but dependent on NAD(P)H shuttling. In contrast, the pyruvate-stimulated cytoplasmic NAD(P)H response was enhanced by both inhibitors. Surprisingly the malate-aspartate shuttle inhibitor enabled pyruvate-stimulated insulin secretion. These data support a model in which glycolysis plays a dominant role in glucose-stimulated insulin secretion. Based on these data, we propose a mechanism for glucose-stimulated insulin secretion that includes allosteric inhibition of tricarboxylic acid cycle enzymes and pH dependence of mitochondrial pyruvate transport.

β-Cell metabolic flux increases in response to elevated extracellular glucose levels, which leads to increased cellular NAD(P)H (1–3) and [ATP]/[ADP] ratio (4). The current model of glucose-stimulated insulin secretion (GSIS)1 by pancreatic islets predicts that a change in β-cell redox state increases the [ATP]/[ADP] ratio, which leads to closure of ATP-sensitive K+ channels and depolarization of the plasma membrane. Membrane depolarization results in Ca2+ influx through voltage-gated channels and subsequent insulin release (5). Thus, any metabolite causing an increase in [ATP]/[ADP] should result in insulin secretion. However, this model fails to describe why the glycolytic intermediates glyceraldehyde 3-phosphate and dihydroxyacetone phosphate (6) and the mitochondrial substrates leucine (7) and methyl pyruvate (8), fail to mimic the insulin secretion effects of glucose. Furthermore, this model does not explain why other mitochondrial metabolites (e.g. pyruvate and succinate) fail to stimulate insulin secretion. Despite its inability to induce insulin secretion, pyruvate can potentiate GSIS (5), a phenomenon known as the “pyruvate dilemma.” The potentiation of GSIS indicates that pyruvate is metabolized in pancreatic islets, but the extent and mechanism of this metabolism remains unclear.

Because pyruvate is a mitochondrial fuel, the pyruvate dilemma has led to the suggestion that glycolysis and mitochondrial NADH shuttles play the primary role in GSIS (9). Previous studies have shown that glucose-induced Ca2+ influx is independent of mitochondrial pyruvate transport but depends on the electron transport chain (9). However, pancreatic islets from mice containing a deletion of the mitochondrial glyceraldehyde 3-phosphate dehydrogenase gene, which is critical for the glycero phosphate shuttle, demonstrate normal GSIS until this response is abolished by treatment with a malate-aspartate shuttle inhibitor (10). Therefore, it appears that GSIS is more dependent on the malate-aspartate shuttle for ATP production.

In pancreatic islets, ~90% of glucose-derived pyruvate enters the mitochondria for oxidation (11), although it has been proposed that the tricarboxylic acid cycle of β-cells does not actively metabolize pyruvate until Ca2+-dependent dehydrogenases are “primed” by Ca2+ influx (10). In this model, glycolytic NADH induces a proton gradient across the mitochondrial membrane and consequently stimulates mitochondrial Ca2+ influx. However, this model is inconsistent with previous NAD(P)H measurements that showed an increase in mitochondrial metabolism well before Ca2+ influx (1). It has also been proposed that pyruvate is not sufficiently transported across the β-cell plasma membrane due to low expression of monocarboxylate transporter (MCT) and that this effect inhibits β-cell pyruvate metabolism enough to abrogate secretion (12, 13). To address these models, we examined the fate of exogenous pyruvate as well as glucose-derived pyruvate in the β-cells of pancreatic islets.

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1 The abbreviations used are: GSIS, glucose-stimulated insulin secretion; oCHC, α-hydroxysucciniminate, AOA, aminoxyacetic acid, BSA, bovine serum albumin; MCT1, monocarboxylate transporter 1; ADH, alcohol dehydrogenase.
In the present study, we have used two-photon microscopy of NAD(P)H autofluorescence to monitor metabolism of living β-cells within intact pancreatic islets. The metabolic measurements were performed in parallel with insulin secretion measurements by an islet perfusion system. Two-photon NAD(P)H microscopy has proven successful for monitoring β-cell redox state in pancreatic islets (1–3). This technique provides adequate spatial and temporal resolution to follow both cytoplasmic and mitochondrial NAD(P)H responses from individual β-cells within intact islets during stimulated insulin secretion (1). Glucose-induced increases in mitochondrial NAD(P)H levels can be assessed by malate-aspartate shuttling of glycolytically produced NAD(P)H and tricarboxylic acid cycle metabolism of the glycolytically produced pyruvate. Because the glycerophosphate shuttle utilizes flavoproteins to transfer cytoplasmic NADH electrons to the electron transport chain, it is not expected to directly increase mitochondrial NAD(P)H levels. To delineate mitochondrial NAD(P)H changes due to either glycolysis or tricarboxylic acid cycling, we utilized two inhibitors: α-hydroxy-4-cinnamic acid (αCHC), an inhibitor of mitochondrial pyruvate transport (9, 14), and aminoxyacetate (AOA), an inhibitor of the malate-aspartate shuttle (10, 15). Importantly, glycolysis is unaffected by αCHC (16) or AOA (10), and glucose oxidation is unaffected by AOA (10). Therefore, with αCHC treatment, observed changes in mitochondrial NAD(P)H would be mainly due to malate-aspartate shuttling, whereas observed changes in the presence of AOA would be related to pyruvate metabolism in the tricarboxylic acid cycle. The NAD(P)H response could then be compared quantitatively with insulin secretion by islets in the presence and absence of these inhibitors.

**EXPERIMENTAL PROCEDURES**

**Islet Isolation and Culture**—Islets were extracted from 6- to 12-week-old C57BL/6 male mice by collagenase (Roche Molecular Biochemicals) digestion (17, 19). Prior to perfusion studies, islets were cultured in untreated culture dishes for 24 h in RPMI 1640 media (Invitrogen) with 11 mM glucose at 37 °C and 5% CO2. For NAD(P)H imaging, isolated pancreatic islets were placed on Cell-Tak (BD PharMingen) coated 35-mm glass bottom dishes (MatTek Corp.) as previously described for microscopy analysis (3).

**Islet Culture and Flattening on Human Extracellular Matrix**—Islets were also routinely cultured on cell matrix (BD PharMingen). After ~14 days the cell matrix promotes spreading, and the islets flatten in appearance. These islets have been shown to function normally, and in particular, they exhibit normal NAD(P)H and insulin secretion responses to glucose (1, 20). Prior to experiments, these islets were equilibrated in BMHH buffer (125 mM NaCl, 5.7 mM KCl, 2.5 mM CaCl2, 1.2 mM MgCl2, 10 mM HEPES, and 0.1% bovine serum albumin, pH 7.4), and metabolites were added as indicated.

**Two-photon Microscopy and Analysis**—The two-photon microscope instrument, described previously (3), uses 150 fs pulses at 710 nm light from a Coherent Mira laser and fills the back aperture of a 40× Plan Neofluor 1.3 NA objective lens (Zeiss). The laser illumination used provides ~3 milliwatts at sample, which does not cause observable damage to islets (21) even after 60 min of continuous imaging (3). The NAD(P)H fluorescence was collected with a custom 380- to 550-nm filter. Images were analyzed using Scion Image release version beta 3B (Scion Corp.). Mitochondrial and cytoplasmic intensity extraction images were collected at the edge of flattened islets using a 0.1-μm/pixel resolution. With flattened pancreatic islets, each image contained ~10–50 cells. Image stacks were filtered using a 7 × 7 mean filter, and intensity thresholds were set that highlighted the bright areas known to be mitochondria (1). With this threshold, the “Analyze Particles” function was used to obtain the maximum intensity of each of these bright areas. The brightest 15–50 areas were used to calculate mitochondrial intensity. These areas represent the best in focus mitochondria. For cytoplasmic intensity, a threshold was set on these filtered images to levels that highlighted moderately bright areas. The threshold values were assigned by first determining the bottom (nuclear) and top (mitochondrial) levels in each image. Once this range was set the average intensity of the thresholded image was measured. This process was carried out three times to obtain the most precise measure of the cytoplasmic intensity. This threshold method for intensity extraction is faster and more robust than the manual sampling method used previously (1), but both methods produce similar values when used on identical images. Mitochondrial and cytoplasmic NAD(P)H concentrations were determined from an NADH-yeast alcohol dehydrogenase (ADH) (Sigma) standard curve. A solution composed of 2 mg/ml ADH, 6 mM semicarbazide, 10 mM ethanol, 100 mM isobutyramide, and 10 mM HEPES pH 8.0 was titrated with a freshly made 10 mM NADH solution. Images were collected at 0, 150, 200, 250, 300, 350, and 400 μM NADH.

**Pancreatic Islet Insulin Secretion**—Insulin secretion was assayed by altering the extracellular glucose and pyruvate in a cell perfusion system. In these studies, 50–60 mouse islets were placed in each column. Islets were equilibrated in 2.8 mM glucose, 125 mM NaCl, 5.7 mM KCl, 2.5 mM CaCl2, 1.2 mM MgCl2, and 10 mM HEPES (pH 7.4) with 0.2% bovine serum albumin (BSA) (Sigma). Raising the extracellular glucose concentration from 2.8 to 20 mM and monitoring insulin secretion was used to assess islet function and integrity. A flow rate of 1 ml/min was used with each fraction containing 3 ml of effluent. Radioimmunoassays were done in duplicate on each fraction using insulin antibody-coated tubes (INC Pharmaceuticals, Inc., Costa Mesa, CA).

**Lactate Assay**—Lactate production from pancreatic islets was measured as described (18) except NADH production was measured fluorometrically, using a L-format fluorescence spectrometer (Photon Technology International). Islet incubations were done using ~40 islets in a total volume of 350 μl at 37 °C for 4 h.

**RESULTS**

**Pancreatic Islet NAD(P)H Response**—The NAD(P)H response of pancreatic islets was followed during glucose and pyruvate metabolism (Fig. 1). In these studies, a single pancreatic islet was equilibrated on the microscope stage at 37 °C. The images shown were collected at 0, 180, and 590 s after the addition of 20 mM pyruvate (Fig. 1, A, B, and C). This same islet was treated with 20 mM glucose at 600 s, and the final image collected was at 880 s (Fig. 1D). Addition of pyruvate caused an increase in NAD(P)H across the pancreatic islet (Fig. 1, A versus B), although this increase was transient (Fig. 1, A versus C). Addition of glucose to this same islet increased the NAD(P)H intensity (Fig. 1, C versus D). Fig. 1E summarizes the NAD(P)H responses of five separate islets treated similarly to Fig. 1, A–D. These data were collected from the islet center (~75% of the pancreatic islet) because the perimeter of the islet also contains α- and δ-cells (21). The addition of 20 mM pyruvate caused an initial decrease in intensity, which then increased to a maximum of 1.5-fold base line at ~180 s. This increase was transient because the intensity decreased to 1.2-fold base line at ~600 s. Addition of glucose at 600 s increased NAD(P)H intensity to a maximum 1.8-fold within 150 s. In contrast to pyruvate, glucose caused a sustained NAD(P)H response.

To explore the initial decrease in pyruvate-stimulated NAD(P)H responses, we acquired pyruvate dose-response data (Fig. 1F). At 2, 4, and 8 mM pyruvate, the decrease was transient with the intensity recovering to an equilibrium just below 1.0-fold NAD(P)H intensity by 250 s. At higher pyruvate concentrations (16 and 20 mM), the 0.6- and 0.7-fold intensity decreases at 40–50 s were followed by large transient increases in NAD(P)H intensity. The NAD(P)H intensity rose to a maximum of 1.3- and 1.4-fold intensity for 16 and 20 mM pyruvate, respectively, at 180 s before falling to intensities of 1.2- and 1.3-fold at 270 s. Therefore, pyruvate caused observable NAD(P)H changes at all concentrations studied, and all of the observed responses were transient.

**Mitochondrial and Cytoplasmic Dose Responses to Glucose and Pyruvate**—Dose responses of NAD(P)H levels were determined in flattened islets to measure mitochondrial and cytoplasmic responses (Fig. 2). Islets were treated with glucose (Fig. 2, A and C) or pyruvate (Fig. 2, B and D), and images were collected at 0, 80, and 270 s. Glucose above 4 mM induced a significant cytoplasmic NAD(P)H response (Fig. 2A). The cyto-
plasmic glucose-induced NAD(P)H response reached near-maximal intensities within 80 s and was sustained throughout the experiment. The mitochondrial glucose-induced NAD(P)H response also increased dramatically above 4 mM glucose (Fig. 2C). At all concentrations studied above 4 mM glucose (8–20 mM), the mitochondrial response was saturated inducing final NAD(P)H levels in a range of ~400 μM.

Pyruvate caused a decrease in cytoplasmic NAD(P)H at concentrations from 2 to 8 mM (Fig. 2B). Above 8 mM, pyruvate caused dramatic increases in cytoplasmic NAD(P)H within 80 s, and these increases remained stable at 270 s. Mitochondrial NAD(P)H levels were not changed significantly by 2–8 mM pyruvate although a slight increase was observed with 8 mM pyruvate at 270 s. Above 8 mM pyruvate, we observed significant increases in mitochondrial NAD(P)H levels within 80 s, but the responses were transient and thus greatly diminished at 270 s. Therefore, pyruvate, above a threshold concentration of approximately ~8 mM, induced large cytoplasmic and mitochondrial NAD(P)H increases with a transient response in the mitochondria.

To further clarify the fate of pyruvate, we measured lactic acid production from pancreatic islets. When exposed to 2 mM pyruvate islets produced 0.46 ± 0.09 nmol/islet/h of lactic acid, which was slightly larger than when exposed to 2 mM glucose (0.25 ± 0.13 nmol/islet/h). In contrast, 20 mM pyruvate caused a significant increase in the amount of lactic acid produced (2.44 ± 0.19 nmol/islet/h).

**Temporal NAD(P)H Response of Pancreatic Islets**—In these studies, mitochondrial and cytoplasmic NAD(P)H responses to 20 mM glucose or 20 mM pyruvate were followed over time (Fig. 3). Islets were divided into 4 groups, no pretreatment or pretreated with CHC (a mitochondrial pyruvate transport inhibitor), AOA (a malate-aspartate shuttle inhibitor), or both of these inhibitors. Previous studies in pancreatic islets have shown that CHC inhibits the oxidation of glucose and pyruvate in pancreatic islets without affecting glycolysis (16) and that AOA does not inhibit glycolysis or glucose oxidation (10).

The time-resolved cytoplasmic (Fig. 3, A and B) and mitochondrial responses (Fig. 3, C and D) are shown for both glucose (Fig. 3, A and C) and pyruvate (Fig. 3, B and D) stimulation.

**Glucose-induced Cytoplasmic Response**—The glucose-induced cytoplasmic NAD(P)H response in CHC- and AOA-treated islets (Fig. 3A, 35–95 μM within 30 s) was similar to the response in non-treated islets but was slightly decreased when these inhibitors were used in combination.

**Glucose-induced Mitochondrial Response**—The glucose-induced mitochondrial response (Fig. 3C) showed a rapid increase in NAD(P)H (~<50 s) from 260 to 430 μM. This concentration increased further to 530 μM by 250 s. Treated the islets with CHC slowed the overall rate of mitochondrial increase but only slightly lowered the final concentration (520 μM). The mitochondrial NAD(P)H response of AOA-treated islets was also slowed in comparison to non-treated islets, but AOA treatment also lowered the maximal mitochondrial NAD(P)H levels (400 μM at 250 s). Therefore, CHC and AOA both slowed the rate of mitochondrial NAD(P)H increase, which indicates that
early in the metabolism of glucose both the tricarboxylic acid cycle and glycolysis contribute to this rise. In contrast, the equilibrium concentration decreased with AOA only, indicating that glycolysis determines the later mitochondrial NAD(P)H levels.

**Pyruvate-induced Cytoplasmic Response**—The pyruvate-induced cytoplasmic response reached a maximum of 80 μM within 40 s, and this response was stable beyond 250 s (Fig. 3B). αCHC treatment increased the cytoplasmic response dramatically (105 μM within 100 s), but this increase was transient and equilibrated to 85 μM. AOA treatment also increased the pyruvate-induced response dramatically (120 μM within 100 s), but this increase was stable beyond 250 s. Islets treated with both αCHC and AOA showed cytoplasmic responses similar to those from non-treated islets.

**Pyruvate-induced Mitochondrial Response**—The pyruvate-induced mitochondrial response showed a rapid increase in NAD(P)H (~20 s) to 475 μM (Fig. 3D). This concentration dropped after 150 s. Treating the islets with αCHC slowed the NAD(P)H response (>100 s) without changing the equilibrium levels, effectively removing the transient nature of pyruvate metabolism. AOA treatment also slowed the mitochondrial NAD(P)H response from 20 to 70s, but unlike non-treated islets, this large increase was stable beyond 250 s. The mitochondrial response was completely abolished during combined inhibitor treatment. Therefore, both mitochondrial pyruvate transport and the malate-aspartate shuttle contribute to the initial rate of NAD(P)H increase. The equilibrium NAD(P)H concentration in αCHC-treated islets was similar to non-treated islets, whereas NAD(P)H was decreased in AOA-treated islets. In all cases, pyruvate stimulation caused lower maximal mitochondrial concentrations than glucose (475 versus 530 μM), and this increase was transient in all but the AOA-treated islets.

**Pancreatic Islet Glucose- and Pyruvate-stimulated Insulin Secretion**—Glucose- and pyruvate-stimulated insulin secretions were examined in the presence or absence of inhibitors (Fig. 4). Initial glucose stimulation was given to ensure the health of the islets in each study as assessed by insulin secretion levels (data not shown). The islets were equilibrated at low glucose with each inhibitor for 15 min prior to stimulation, and no increase in base-line insulin secretion was observed in any of the experiments. GSIS was significantly larger with αCHC-treated pancreatic islets than with non-treated islets (20 mM glucose + αCHC versus 20 mM glucose, p < 0.05, Student’s t test). In contrast, GSIS was decreased with AOA-treated pancreatic islets (20 mM glucose + AOA versus 20 mM glucose, p < 0.05) and abolished with both inhibitors (20 mM glucose + both inhibitors). As expected, pyruvate did not stimulate insulin secretion from these islets (20 mM glucose versus 20 mM pyruvate). Pyruvate stimulation of αCHC-treated islets also caused no significant insulin secretion response. Interestingly, pyruvate stimulated a significant insulin secretion from AOA-treated islets (20 mM pyruvate + AOA versus 2.8 mM glucose, p < 0.01). This response was smaller than observed with GSIS (20 mM glucose versus 20 mM pyruvate + AOA) but comparable with levels observed with glucose-stimulated AOA-treated islets. Pyruvate stimulation with the combined inhibitor-treated islets sometimes caused insulin secretion (2 of 3 experiments). When this response was observed however, it did not decrease upon removal of pyruvate. In addition, the insulin release was not peaked as seen for the other traces but was more of a slow rise in secretion. This increased baseline is suggestive of inhibitor toxicity rather than pyruvate-stimulated insulin secretion.

**DISCUSSION**

The failure of pyruvate to stimulate insulin secretion, despite its ability to potentiate GSIS, has not been adequately explained (5, 6). To address this issue, we measured cellular metabolic fluxes and both glucose- and pyruvate-stimulated insulin secretion. Two-photon excitation microscopy of NAD(P)H allowed us to examine glucose- and pyruvate-stimulated metabolic fluxes in both the cytoplasm and mitochondria of β-cells within intact mouse islets. These data allow for new insights about β-cell metabolism and the inability of pyruvate to induce insulin secretion.

Our data combined with that from previous studies (5, 9, 24) support a dominant role for glycolysis in GSIS (Fig. 5). Glycolysis produces ATP, NAD(P)H, and pyruvate. However, β-cells lacking mitochondrial DNA do not exhibit GSIS (24), and electron transport chain inhibition reduces glucose-stimulated Ca2+ influx (9). Thus, glycolytically derived ATP is insufficient for K⁺ channel closure and insulin secretion. The necessary ATP for K⁺ channel closure must therefore be derived through either glycolytic NAD(P)H or metabolism of pyruvate in the tricarboxylic acid cycle. Cytoplasmic NAD(P)H can be shuttled into the mitochondria through the glycerophosphate or the malate-aspartate shuttles (Fig. 5A). Because pancreatic islets from mice lacking the glycerophosphate shuttle demonstrate normal GSIS until treated with AOA (10), we do not expect the glycerophosphate shuttle to play a major role in these events. Glucose-derived pyruvate can also be transported into mitochondria for tricarboxylic acid cycle metabolism. Our data (Fig. 3A) show that the initial glucose-stimulated mitochondrial
NAD(P)H rise depends on both the malate-aspartate shuttle and mitochondrial pyruvate transport. This suggests that both shuttling of NAD(P)H into the mitochondria and tricarboxylic acid cycle metabolism of glycolytically produced pyruvate begin immediately (Fig. 5A). The transient mitochondrial pyruvate metabolism (Fig. 2D and 3C) and the malate-aspartate shuttle dependence of glucose-stimulated mitochondrial NAD(P)H levels (Fig. 3A) indicate that pyruvate metabolism by the tricarboxylic acid cycle slows with time. In contrast, cytoplasmic NAD(P)H remains elevated during glucose stimulation suggesting that glycolysis continues to metabolize glucose. GSIS depends on the malate-aspartate shuttle but is stimulated by blocking mitochondrial pyruvate transport (Fig. 4). Therefore, it is likely that glycolytic NAD(P)H continues to supply electrons to the electron transport chain through the malate-aspartate shuttle and the glycerophosphate shuttle during glucose stimulation. This continuing supply of electrons sustains the elevated [ATP]/[ADP] ratio, which in turn continues stimulating Ca²⁺ influx and insulin secretion.

Contrary to previously proposed models of pyruvate not entering β-cells (12, 13) or not being metabolized in β-cells (10), our data clearly demonstrate that pyruvate enters β-cells and is rapidly metabolized in the mitochondria. However, pyruvate-stimulated changes in metabolic flux are transient, which indicates that pyruvate alone is insufficient to sustain tricarboxylic acid cycle metabolism.

Low pyruvate concentrations caused a decrease in cytoplasmic NAD(P)H levels (Figs. 1 and 2C). There are many possible explanations of this effect, which we cannot rule out at this time. Despite the very low lactate dehydrogenase activity (11–13) in β-cells, the presence of pyruvate-induced lactic acid shows that pyruvate may be converted to lactate, thus lowering NAD(P)H levels. Another possible explanation is that the stability of NAD(P)H is decreased at lower pH. Addition of pyruvate, a weak acid that lowers cellular pH, could decrease NAD(P)H levels by destabilizing the reduced form of the molecule. Regardless of the mechanism of the NAD(P)H decrease, the observed redox change shows that pyruvate enters the cells even at low concentrations.

Cytoplasmic NAD(P)H was increased in response to pyruvate only at concentrations that caused a mitochondrial increase (Fig. 2, B and D), and the temporal cytoplasmic response...
reaches maximal levels only after maximal levels are obtained in the mitochondria (Fig. 3, B versus D). Our results also suggest that cytoplasmic response to pyruvate is dependent on mitochondrial metabolism. This mitochondrial metabolism is likely to include pyruvate/malate cycling (22, 25) and reversal of the malate-aspartate shuttle (Fig. 5 B), although neither of these occurs to a large extent because the pyruvate-induced cytoplasmic NAD(P)H level was significantly smaller than glucose-induced levels (Fig. 3, A versus B, 80 versus 95 μM). Therefore, with the transient pyruvate-induced NAD(P)H response and malate-aspartate shuttle reversal, few electrons are available to the electron transport chain at later time points for ATP production.

Because pyruvate enters β-cells, the transient nature of the mitochondrial response to pyruvate must result from the inhibition of either mitochondrial pyruvate metabolism or transport. Increased NADH and ATP can allosterically inhibit various tricarboxylic acid cycle enzymes, including NADH inactivation of pyruvate dehydrogenase and α-ketoglutarate dehydrogenase and ATP inactivation of pyruvate dehydrogenase, citrate synthase, and isocitrate dehydrogenase (26). This negative feedback mechanism describes the glucose-stimulated NAD(P)H and insulin secretion data but does not clearly explain why expression of hamster MCT1 in rat pancreatic islets (13) and treatment of islets with AOA would cause pyruvate to become a secretagogue. Alternatively, pyruvate is symported into the mitochondria with a proton, and this transport depends on the pH gradient across the mitochondrial membrane (27). Glucose metabolism increases cytoplasmic pH due to tricarboxylic acid metabolism (28, 29), which could inhibit mitochondrial pyruvate transport. During pyruvate metabolism, non-facilitated diffusion into the cell temporarily decreases cytoplasmic pH until equilibrium is achieved. The combination of pyruvate equilibration across the membrane and its metabolism in the tricarboxylic acid cycle would increase cytoplasmic pH and could therefore inhibit mitochondrial pyruvate transport. This mechanism can also explain the pyruvate-stimulated insulin secretion in MCT1-expressing islets (13) and AOA-treated pancreatic islets. MCT1 also co-transport a proton resulting in a prolonged pyruvate-induced pH decrease in mitochondria (Fig. 3, B versus D).
MCT1-expressing pancreatic islets (13). Furthermore, in MCT1-expressing pancreatic islets decreases in cytoplasmic pH are likely to stimulate further membrane pyruvate transport and proton influx. Inhibition of the malate-aspartate shuttle by AOA (Fig. 5C) could lead to a buildup of cytoplasmic protons, providing the necessary precursors for mitochondrial pyruvate transport. Our evidence supports this model because AOA treatment allowed pyruvate stimulation to cause both a transient rise in cytoplasmic NAD(P)H and NAD(P)H increase (Fig. 3D) and insulin secretion (Fig. 4). It should be noted that both the mitochondrial and insulin responses were lower than the normal glucose-stimulated responses. Therefore, it is reasonable to consider that both the negative feedback and pH mechanisms are involved in the slowed tricarboxylic acid cycle metabolism because they are not mutually exclusive.

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