Activation of Stimulus-Secretion Coupling in Pancreatic β-Cells by Specific Products of Glucose Metabolism

EVIDENCE FOR PRIVILEGED SIGNALING BY GLYCOLYSIS*

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The energy requirements of most cells supplied with glucose are fulfilled by glycolytic and oxidative metabolism, yielding ATP. In pancreatic β-cells, a rise in cytosolic ATP is also a critical signaling event, coupling closure of ATP-sensitive K⁺ channels (K<sub>ATP</sub>) to insulin secretion via depolarization-driven increases in intracellular Ca<sup>2+</sup> ([Ca<sup>2+</sup>]<sub>i</sub>). We report that glycolytic but not Krebs cycle metabolism of glucose is critically involved in this signaling process. While inhibitors of glycolysis suppressed glucose-stimulated insulin secretion, blockers of pyruvate transport or Krebs cycle enzymes were without effect. While pyruvate was metabolized in islets to the same extent as glucose, it produced no stimulation of insulin secretion and did not block K<sub>ATP</sub>. A membrane-permeant analog, methyl pyruvate, however, produced a block of K<sub>ATP</sub>, a sustained rise in [Ca<sup>2+</sup>]<sub>i</sub>, and an increase in insulin secretion 6-fold the magnitude of that induced by glucose. These results indicate that ATP derived from mitochondrial pyruvate metabolism does not substantially contribute to the regulation of K<sub>ATP</sub> responses to a glucose challenge, supporting the notion of subcompartmentation of ATP within the β-cell. Supranormal stimulation of the Krebs cycle by methyl pyruvate can, however, overwhelm intracellular partitioning of ATP and thereby drive insulin secretion.

In pancreatic islets of Langerhans, glucose uptake by the β-cell initiates a cascade of events culminating in insulin release. One of the key components in the insulin release process is the generation of ATP from glucose metabolism (1, 2). Cytosolic ATP blocks ATP-sensitive K⁺ (K<sub>ATP</sub>) channels leading to depolarization of the cell membrane, opening of voltage-gated Ca<sup>2+</sup> channels, and Ca<sup>2+</sup> influx (3, 4). Depolarization also induces release of Ca<sup>2+</sup> from endoplasmic reticulum (ER) stores, further contributing to the elevation in intracellular calcium ([Ca<sup>2+</sup>]<sub>i</sub>) (5, 6). Furthermore, the consequent Ca<sup>2+</sup> depletion of the ER activates a plasma membrane non-selective cationic channel that results in further depolarization and enhancement of Ca<sup>2+</sup> influx (7–9). The interplay between the ER Ca<sup>2+</sup> stores and plasma membrane ion channels underlies the glucose-stimulated coupling of membrane potential and [Ca<sup>2+</sup>]<sub>i</sub>, oscillations (8–12). Insulin secretion results from these changes in [Ca<sup>2+</sup>]<sub>i</sub>, in a process that remains unclear.

A general principle of physiology holds that metabolism and energy requirements are tightly coupled. Additionally, the pancreatic β-cell utilizes the precise measurement of its own metabolism, via the degree of activation of K<sub>ATP</sub>, to regulate insulin secretion. The dual role of metabolism in this cell type necessitates discrete regulation of these specific functions. By virtue of the fact that oxidative metabolism takes place in the mitochondria, whereas the glycolytic enzymes are cytosolic or plasma membrane-associated, compartmentation of metabolism can theoretically occur, leading to functional intracellular microdomains of ATP (13).

While glycolysis in the β-cell produces only one-sixth of the total amount of ATP derived from the complete oxidation of glucose (14), the contribution of the much larger mitochondrial Krebs cycle-derived ATP to β-cell glucose signaling remains controversial (15–18). Recently, it has been reported that NADH derived from glycolysis and processed into ATP by the operation of the transmembrane NADH shuttles supplies the essential rise in cytosolic ATP necessary to initiate block of K<sub>ATP</sub> and induce membrane depolarization and [Ca<sup>2+</sup>]<sub>i</sub>, oscillations (19). Using measurements of K<sub>ATP</sub> from single β-cells and [Ca<sup>2+</sup>]<sub>i</sub>, and insulin release from whole islets, we have further investigated the critical steps in ATP production necessary for activation of stimulus-secretion coupling. Our results suggest that glycolysis is critically involved in glucose-stimulated insulin secretion, whereas the Krebs cycle appears to play an insignificant role. The additional findings that a membrane-permeant ester of pyruvate nonetheless is able to induce insulin secretion indicate that ATP produced during the suprastimulated operation of the Krebs cycle is able to initiate the β-cell stimulus-secretion coupling signaling cascade but that in the normal course of glucose metabolism this does not take place.

MATERIALS AND METHODS

Islets of Langerhans were isolated from 3-7-month-old C57BL/KsJ mice by collagenase digestion and cultured for 24 h for batch islet insulin studies or for 2–10 days on glass coverslips for [Ca<sup>2+</sup>]<sub>i</sub>, studies, as described elsewhere (5, 7, 8). Single β-cells were dispersed as described previously (7, 8).

ATP-dependent K⁺ Channel Recordings—Single β-cells were voltage clamped using perforated patch techniques (19). For whole-cell recordings, patch electrodes contained (in mM) 80 potassium aspartate, 50 KCl, 5 NaCl, 5 MgCl<sub>2</sub>, 10 HEPES, pH 7.2, with KÖH and 120 μM glutamin. The perfusion medium was a modified Krebs-Ringer solution. All experiments were performed at 33–35 °C. Currents were acquired at 10 KHz. The currents were confirmed to be K<sub>ATP</sub> by their sensitivity to glucose or their suppression by 500 μM gliburide. For single K<sub>ATP</sub> channel recordings, patch electrodes contained (in mM) 80 KCl, 10 HEPES, 1 MgCl<sub>2</sub>, 2 CaCl<sub>2</sub>, pH 7.4, while the perfusion medium contained 140 KCl, 10 HEPES, 0.3 MgCl<sub>2</sub>, 0.3 ADP, 0.3 MgATP, pH 7.4. Following patch excision, single K<sub>ATP</sub> channel unitary currents were observed. Under these conditions, single channel activity was consistently maintained for >30 min and was characterized by single channel

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1 The abbreviations used are: K<sub>ATP</sub>, ATP-sensitive K⁺ channel; ER, endoplasmic reticulum; IAA, iodoacetate; CHC, a-cyano-4-hydroxycinnamate; DCA, dichloroacetate.
conduction, K<sup>-</sup>-selectivity and inhibition by increasing ATP, glyburide (500 mM), as well as activation by diazoxide (100 μM).

Islets (Ca<sup>2+</sup>).—Islets were loaded with fura-2 by a 25-min incubation at 37°C in Krebs-Ringer buffer with 2 mM glucose, previously bubbled with 95% O<sub>2</sub>, 5% CO<sub>2</sub>, containing 5 μM acetoxyethyl ester of fura-2 (Molecular Probes, Inc.) (5, 7–9). The specimen holder (voltage 1 mV) was mounted on a temperature-controlled stage (Medical Systems Inc.) and was continuously perfused with Krebs-Ringer buffer at 37°C at pH 7.4 at a rate of 2.5 ml/min. Fura-2 dual excitation (340 and 380 nm) and fluorescence detection (510 nm) were accomplished using a Photoscan-2 ratio fluorescence photometry system (Nikon Inc.). Calcium is expressed in all records as the 340/380 nm ratio (5). Stock solutions of methyl pyruvate were made up just prior to use, due to its slight instability at 37°C.

Batch Iset Inulin Secretion.—Islets were pre-incubated for 1 h in 2 mM glucose RPMI medium with 10% fetal bovine serum, at 37°C, and then washed in 40 ml of a modified Krebs-Ringer buffer containing (in mM) 119 NaCl, 4.7 KCl, 2.5 CaCl<sub>2</sub>, 1.2 MgSO<sub>4</sub>, 1.2 KH<sub>2</sub>PO<sub>4</sub>, 25 NaHCO<sub>3</sub>, with 0.5% bovine serum albumin as described previously (20). Groups of 10 islets were picked into wells of 24-well plates containing 0.5 ml of test solutions in Krebs-Ringer buffer with 0.5% bovine serum albumin and were incubated at 37°C for 1 h. Supernatant samples (250 μl) were collected and assayed for insulin by Specific Proximity Assay kit (Amersham Corp.) and calibrated using rat insulin (Novo) as a standard. Statistical analysis was performed using Student’s t-test.

Microphysiometry Readings—Using a Cytosensor (21) (Molecular Devices Inc.), small fluctuations in metabolic rate were determined via alterations in extracellular pH. Standard solutions were utilized with the exception that extracellular pH buffers were omitted. Experiments were performed at 37°C.

Pyruvate Oxidation Measurements.—Pyruvate oxidation measurements were determined by measuring the generation of CO<sub>2</sub> from [U-14C]pyruvate by the method of Bernstein and Wood (22), described in full elsewhere (23). Approximately 50 islets from Wistar rats were pipetted into wells containing either 20 mM pyruvate or 20 mM pyruvate and 1 mM α-CHC in Krebs-Ringer bicarbonate buffer. They were incubated at 37°C for 5 min, followed by the addition of [U-14C]pyruvate (Amersham Corp.) to a specific activity of 1 cpmp. Parallel experiments were carried out using the methyl ester of [U-14C]pyruvate, but we observed breakdown of several preparations of the isotope in the absence of protein at 37°C over the time course of the experiment. The blank rates we obtained were nearly equivalent to the rates observed in the presence of islets. More importantly, these blank rates were severalfold higher than the total rates we obtained for pyruvate oxidation, which caused us to question their validity. Because we were unable to obtain a preparation of the [U-14C]methyl ester, which had blank rates low enough to give us confidence in the oxidation rates obtained in the presence of islets, the data obtained for methyl pyruvate oxidation are not reported here.

RESULTS AND DISCUSSION

Through the subcellular localization of key enzymes, glycolysis takes place in the cytosol, whereas the oxidation of the glycolytic end product, pyruvate, takes place in the mitochondria. While the plasma membrane is freely permeable to pyruvate, entry of the carboxylic acid into the mitochondrion is regulated by a specific transporter (24). Fig. 1 summarizes the relative effects of glucose and pyruvate on whole-cell K<sub>ATP</sub> recorded from a single β-cell using perforated patch techniques. While glucose caused a large suppression of K<sub>ATP</sub>, pyruvate, at concentrations as high as 20 mM, had no effect. These data are in agreement with the lack of stimulatory effect of pyruvate on insulin secretion in rat islets (15) and on [Ca<sup>2+</sup>]<sup>2</sup> in mouse islets (7). Furthermore, the observations are consistent with the lack of effect of the mitochondrial pyruvate transport inhibitor α-CHC on glucose-induced closure of K<sub>ATP</sub> in mouse β-cells (18) or on glucose-stimulated elevations in [Ca<sup>2+</sup>]<sup>2</sup> in single HIT cells (25).

These observations suggested that, in the normal course of events, either mitochondrial metabolism of glucose-derived pyruvate to form ATP does not occur in β-cells or, alternatively, operation of the Krebs cycle does not contribute directly to the initial steps in the glucose-dependent stimulus-secretion coupling process. Consistent with either notion was the lack of

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attenuated [Ca$^{2+}$] steps in the earlier findings of the suppressive effects of IAA on proximal insulin secretion, compared to control (Fig. 5), consistent with exposure to 3 mM sodium azide, an effective inhibitory concentration in $\beta$-cells (26), methyl pyruvate produced a greatly attenuated [Ca$^{2+}$] rise, an effect that was reversible following sodium azide removal (Fig. 4). In addition, the sustained elevation of [Ca$^{2+}$], induced by 20 mM methyl pyruvate was reversed by addition of sodium azide (Fig. 4).

It would seem, therefore, that glycolytic but not Krebs cycle processing of glucose-derived substrates was necessary to initiate changes in K$_{ATP}$ channel activity and [Ca$^{2+}$], prerequisites for downstream initiation of insulin secretion. Nevertheless, a membrane-permeant analog of pyruvate was able to largely reproduce the effects of glucose on K$_{ATP}$ and [Ca$^{2+}$] by stimulating mitochondrial ATP synthesis.

At physiological concentrations, both glucose and the early glycolytic intermediate, D-glyceraldehyde, stimulate insulin synthesis and secretion in whole islets (24, 27). Iodoacetate (IAA) inhibits glyceraldehyde phosphate dehydrogenase, blocking glycolysis and halting further glucose oxidation prior to the production of NADH and ATP (28). When islets were exposed to 1 mM IAA, there was a 78% inhibition of glucose-stimulated insulin secretion, compared to control (Fig. 5), consistent with earlier findings of the suppressive effects of IAA on proximal steps in the $\beta$-cell glucose stimulus-secretion coupling cascade (19). Basal insulin secretion in the presence of 2 mM glucose was, however, elevated by exposure to IAA (Fig. 5). Since this effect was only seen in low glucose, it is unlikely to be a consequence of the net depletion of ATP produced by the unaffected proximal phosphorylation steps in glycolysis. An alternative explanation is that in a manner similar to the related glyceraldehyde phosphate dehydrogenase inhibitor iodoacet-
amide, low concentrations (0.5 mM) might activate glyceraldehyde phosphate dehydrogenase, especially at lower glucose concentrations (29).

Glycolysis produces ATP directly through dephosphorylation reactions catalyzed by the actions of phosphoglycerate kinase on 1,3-diphosphoglycerate and pyruvate kinase on phosphoenolpyruvate (14). To investigate the role played by these ATPs on insulin secretion, we utilized arsenate, which uncouples ATP production by phosphoglycerate kinase without halting glycolysis (30). In the presence of 1 mM arsenate, there was a 70% reduction in glucose-stimulated insulin secretion (Fig. 5). We previously reported that arsenate pretreatment had no effect on glucose-induced closure of $K_{ATP}$ (19). However, the insulin secretion process, unlike the early steps in the glucose signaling pathway, is an energy-consuming process and may therefore be expected to be more sensitive to small changes in ATP production. On the other hand, prolonged exposure to arsenate is known to inhibit mitochondrial oxidative phosphorylation (31), so the different results obtained in the insulin secretion experiment (90 min exposure) and $K_{ATP}$ measurements (10–20 min exposure) may reflect this fact. However, in contrast to the effects of IAA, there was no increase in basal insulin secretion (Fig. 5). Finally, exposure to 1 mM sodium fluoride, an inhibitor of enolase, which catalyzes the formation of the pyruvate precursor, phosphoenolpyruvate (14), reduced glucose-stimulated insulin secretion from 367 ± 80 to 265 ± 85 pM/islet/h (mean ± S.E., n = 3) with no effect on basal (2 mM glucose) insulin secretion (18 ± 3 versus 23 ± 6). These results emphasize the importance of glycolytic ATP in the insulin release process and suggest that a small decrease in ATP production has a marked effect on insulin secretion.

![FIG. 5. Inhibition of glycolysis reduces, whereas inhibition of pyruvate utilization has no effect on glucose-stimulated insulin secretion. Shown are the insulin secretion responses to low (2 mM glucose, filled bars) and high (12 mM glucose, cross-hatched bars) glucose exposure in the absence and presence of a series of inhibitors of glycolysis and pyruvate transport. These include IAA, an inhibitor of glyceraldehyde-3-dehydrogenase, arsenate, an uncoupler of glycolytic ATP production, and CHC, an inhibitor of pyruvate transport. Note that arsenate and IAA both inhibited glucose-stimulated insulin secretion; since IAA halts glycolysis but arsenate only uncouples glycolytic ATP production and does not therefore prevent downstream metabolism of pyruvate, this result suggests that glycolysis alone plays a critical role in glucose-stimulated insulin secretion. On the other hand, CHC had a mild potentiating effect on glucose-stimulated insulin secretion, an effect also observable following the combined addition of arsenate and CHC. These observations with CHC may reflect the preferential conversion of pyruvate to lactate under conditions of impaired mitochondrial pyruvate transport, with enhanced reformation of NAD to prime the residual production of glycolytic ATP still taking place in the presence of the inhibitors.](http://www.jbc.org/)

The second source of glycolytic ATP is generated indirectly through the mitochondrial processing of NADH derived from the oxidation of glyceraldehyde-3-phosphate. The NADH reducing equivalents must cross the mitochondrial membrane to generate ATP through oxidative phosphorylation. This is accomplished by means of two electron shuttle systems: the malate-aspartate shuttle and the glycerol phosphate shuttle, which deposit their electrons at sites 1 and 2 of the electron transport chain, respectively (19, 32). Rotenone and antimycin A block these respective sites (19, 33, 34) and were previously found to inhibit a glucose-dependent block of $K_{ATP}$ and elevations in $[Ca^{2+}]=_{i}$ in mouse pancreatic $\beta$-cells and islets, respectively (19). Rotenone (50 nM) caused a 63% inhibition of insulin release, and antimycin A (200 nM) caused a 59% inhibition of insulin release in 12 mM glucose-stimulated islets compared to control (Fig. 6).

Our findings with rotenone support the suggested importance of the malate-aspartate shuttle in islets (32, 35). Aminoxyacetate inhibits the malate-aspartate shuttle by suppressing aspartate aminotransferase (36). Consistent with this action, 5 mM aminoxyacetate produced a 53% inhibition of insulin secretion in the presence of 12 mM glucose, from 371 ± 103 to 176 ± 34 pM/islet/h (mean ± S.E., n = 3; p < 0.05), with no effect on basal insulin release (13 ± 2 versus 12 ± 2 pM/islet/h). As far as the alternative NADH shuttle is concerned, mitochondrial glycerol phosphate dehydrogenase activity is 60-fold greater in the islet than in the liver and 10-fold greater in $\beta$-cells than in non-$\beta$-islet cells (37). To further investigate the significance of the operation of this shuttle to glucose-stimulated insulin secretion, we used 2-aminobicyclo[2,2,2]heptane-2-carboxylate, which reportedly increases the flux through the glycerol phosphate shuttle most likely by a direct activation of the FAD-linked mitochondrial glycerophosphate dehydrogenase (38). In the presence of 12 mM glucose, 5 mM 2-aminobicyclo[2,2,2]heptane-2-carboxylate produced a 76% stimulation of insulin secretion over control (412 ± 69 versus 230 ± 16 pM/islet/h, n = 3, p < 0.02) and no significant change in basal release (34 ± 12 versus 12 ± 9 pM/islet/h, n = 3). These
data suggest that a significant contribution is played by glycolytic NADH-derived ATP in the regulation of glucose-dependent insulin secretion even though it only contributes one-fifth the number of reduced equivalents produced during mitochondrial oxidation of glucose-derived substrates.

To investigate the contribution of pyruvate-derived ATP to the insulin secretion response in glucose-stimulated islets, we blocked pyruvate utilization with α-CHC. In accordance with our other findings, exposure to 1 mM α-CHC had no stimulatory effect on basal or glucose-stimulated insulin secretion (Fig. 7); in fact, a slight stimulatory effect in the presence of 12 mM glucose was often seen. Monofluoroacetate blocks the action of the Krebs cycle enzyme aconitase, halting the Krebs cycle prior to the production of reduced nucleotides (19, 39), halting the Krebs cycle substrate, succinate, also stimulating insulin secretion even though it only contributes one-fifth the number of reduced equivalents produced during mitochondrial oxidation of glucose-derived substrates.

Despite these negative data relating to the importance of Krebs cycle-derived ATP to glucose-dependent insulin secretion, 20 mM methyl pyruvate produced a 6-fold greater stimulation of insulin release over controls in 12 mM glucose (Fig. 8). An equivalent stimulation of insulin secretion by methyl pyruvate was observed in the presence of 2 or 12 mM glucose, suggesting that the secretory process was maximally stimulated by the pyruvate ester. Furthermore, addition of glucose in the presence of methyl pyruvate did not further elevate [Ca\textsuperscript{2+}]; (Fig. 9). The potent secretagogue and [Ca\textsuperscript{2+}], elevating effects of methyl pyruvate suggested that the pyruvate ester had provided the β-cell access to a large source of ATP unavailable through normal glucose oxidation. Mono and dimethyl esters of the Krebs cycle substrate, succinate, also stimulate insulin release (40, 41), while succinate itself is ineffective (40).

It seemed unlikely that the esterified form of pyruvate would be a more favorable metabolic substrate than pyruvate itself. Since β-cells are freely permeable to pyruvate (42, 43), it is also unlikely that methyl pyruvate stimulation results from an increase in cytosolic, de-esterified pyruvate. It seemed more probable that methyl pyruvate traversed the mitochondrial membrane, circumventing the pyruvate transporter, and was then de-esterified, liberating pyruvate at its site of oxidation. To test this hypothesis, we measured the effects of methyl pyruvate on islets in the presence of 1 mM α-CHC. The mito-
Methyl pyruvate, but not glucose-stimulated insulin secretion, is potentiated by activation of the Krebs cycle. Shown are the insulin secretion responses to low (2 mM glucose, filled bars) and high (12 mM glucose, cross-hatched bars) glucose exposure in the absence and presence of 1 mM DCA, an activator of the pyruvate dehydrogenase complex. Also shown are the effects of DCA on the secretagogue activity of 10 mM methyl pyruvate (MP). Note that while the methyl pyruvate response was potentiated by dichloroacetic acid, there was no effect on glucose-stimulated insulin secretion.

Chondrial transport inhibitor had no effect on [Ca\(^{2+}\)] response to 20 mM methyl pyruvate (Fig. 9). Furthermore, α-CHC exposure did not alter the insulin secretory responses to methyl pyruvate (Fig. 8), indicating that mitochondrial, and not cytosolic de-esterification, enables methyl pyruvate to produce its effects on [Ca\(^{2+}\)] and insulin secretion.

To confirm that the potent insulin secretagogue effects of the pyruvate ester were due to the delivery of pyruvate to the site of action of Krebs cycle enzymes within the mitochondria, we tested the effects of dichloroacetate (DCA), a well characterized activator of the pyruvate dehydrogenase enzyme complex (44, 45). In the presence of a submaximal concentration of methyl pyruvate, insulin secretion was augmented by 33% by DCA (Fig. 10). On the other hand, the presence of 1 mM DCA produced no appreciable increase in glucose-stimulated insulin secretion (Fig. 10). This lack of potentiating effect of DCA on glucose-dependent insulin secretion from mouse islets is in agreement with a previous report using rat islets (15) and is consistent with the idea that DCA bypasses the mitochondrial transport inhibitor utilized in the previous studies (15). We considered whether DCA bypassed the mitochondrial transport inhibitor utilized in the previous studies (15).

The observations with methyl pyruvate are not consistent with previous reports on the lack of an insulinotropic effect of pyruvate. Exposure of islets to 10 mM pyruvate produced intracellular levels of pyruvate 5-fold higher than those found following exposure to 16.7 mM glucose (15). Despite this, pyruvate did not initiate insulin release (15, 17) and failed to show a significant increase in NADH or ATP levels, even though pyruvate oxidation was taking place (15). We considered whether the discrepancy between the effects of glucose and pyruvate were due to differing rates of metabolism. Consistent with previous reports (15), we found on the basis of changes in pH detected using a microphysiometer (Fig. 12) or direct measurements of \(^{13}C\)O\(_2\) production from \(^{13}C\)pyruvate that pyruvate was oxidized as effectively as glucose. Thus, 20 mM of \(^{13}C\)pyruvate yielded 51 pmol of \(^{13}C\)O\(_2\)/h/islet, comparable to rates of oxidation measured with glucose (22). Incubation with 1 mM α-CHC reduced the pyruvate oxidation rate to 8 pmol/h/islet, indicating that the concentration of the pyruvate transport inhibitor utilized in the K\(_{ATP}\), [Ca\(^{2+}\)], and insulin secretion assays was sufficient to block pyruvate transport and was in agreement with other findings demonstrating inhibition by α-CHC of \(^{14}C\)O\(_2\) release from labeled pyruvate in HIT-T15 insulinoma cells (25).

The discrepancy between the rates of pyruvate oxidation and lack of ATP signaling and an allied insulinotropic effect is puzzling but has several explanations. First, some cytosolic...
pyruvate may be shunted off into lactate, thereby consuming reduced nucleotides destined for ATP production in the process. Second, pyruvate preferentially inhibits the oxidation of endogenous nutrients, reducing the apparent yield of ATP compared to glucose (15). Indeed, when correcting for such phenomena, the net generation of reducing equivalents by glucose and pyruvate became strictly proportional to their secretagogue activities (15). Yet another explanation is that the results could reflect the oxidation of pyruvate by non-β-cells in the whole islets used, which would suggest that measurement of pyruvate oxidation as it applies to islet β-cells is overstated. This controversy as to whether pyruvate is actually metabolized in β-cells has, however, been recently resolved by the demonstration in cell-sorted rat β-cells of efficient mitochondrial pyruvate oxidation as well as oxidation of acetyl-CoA in the Krebs cycle (46).

The contrastingly potent secretagogue effects of pyruvate in HIT insulinoma cells (25) indicates that they differ markedly from normal β-cells in their pyruvate metabolism pathways. Indeed, the hypersensitivity of HIT cells to glucose (4) may reflect the more efficient utilization of glucose-derived pyruvate for ATP generation and stimulation of insulin secretion or altered expression of key enzymes involved in glucose metabolism. For instance, islets lack phosphoenolpyruvate carboxykinase (47, 48), an enzyme which permits pyruvate to re-enter glycolysis. When Escherichia coli-derived phosphoenolpyruvate carboxykinase is expressed in β-cells, pyruvate becomes capable of promoting insulin synthesis (49). This further supports the singular importance of glycolysis in regulating glucose-stimulated signal transduction in β-cells.

Our findings with methyl pyruvate suggest that when pyruvate is delivered directly to the mitochondria, a large stimulation of ATP production and insulin secretion takes place. Assuming that de-esterification of the methyl pyruvate primarily takes place in the mitochondrion, then certain metabolic costs associated with the processing of glucose-derived pyruvate will be saved. Thus, loss of substrate by conversion to lactate will not occur, since this process only takes place in the cytosol. Furthermore, the energy expended in operating the mitochondrial pyruvate transporter will also be saved. The question remains, however, whether this is an adequate enough explanation to reconcile the contrasting effects of pyruvate and methyl pyruvate.

It is important to distinguish at this point the dual effects of ATP in β-cells. Clearly, ATP is an important regulator of insulin secretion by virtue of its critical role in initiating the stimulus-secretion cascade by blocking K_ATP channels. Additionally, the processing of ATP is necessary to fuel the energy-consuming aspects of the insulin secretion process. These disparate roles of ATP in the β-cell can explain the divergent effects on metabolism and secretion of some of the agents that we have employed. Thus, while pyruvate is ineffective in initiating insulin secretion by failing to inhibit K_ATP channels (Fig. 1), it is able to potentiate glucose-stimulated insulin secretion (15), presumably by supplementing ATP destined for energy-consuming purposes. By the same token, the inhibitory effects of arsenate on insulin secretion are explicable on the basis of depleting ATP destined for energy requirements without the impairing glucose-induced block of K_ATP (Ref. 19 and Fig. 5).

Thus, an alternative solution for the differing importance of glycolytic and Krebs cycle processing of glucose-derived metabolites in β-cell signal transduction depends on the concept of functional compartmentation of ATP production. There are numerous reports of cells taking advantage of the compartmentalization of ATP production for signaling purposes (13, 50). For instance, Weiss and colleagues (51, 52) have amply demonstrated a similar dependence on glycolysis for regulation of K_ATP in cardiac myocytes and a close physical association between functional glycolytic enzymes and these channels (51). Another report indicates that glycolytic and oxidative ATP account for different phases of K_ATP channel activation (53) and that different types of muscle fibers rely specifically on glycolytic or oxidative metabolism (54). Yet another manifestation of intracellular compartmentation of ATP production could be achieved if there was heterogeneity of mitochondria. Mitochondrial heteroplasmy has been suggested to underlie a number of heritable diseases, and the evidence for functionally different mitochondria being present within a single cell type is accumulating (55–59). For instance, one recent report supports the existence of altered pyruvate carboxylase expression within mitochondria of the same cell resulting in completely different Krebs cycle operations (60). Thus, it is conceivable that mitochondria within the β-cell might have different capacities for utilizing pyruvate and that with a restricted subcellular distribution could result in the preferential role of non-Krebs cycle-dependent glucose metabolism in the regulation of insulin secretion.

The potent effects of methyl pyruvate may result from its concentration in the mitochondria. It is known that free acids formed from esterified precursors, for instance the acetoxy ester of fura-2, can accumulate in intracellular compartments at concentrations several orders of magnitude higher than the bathing ester concentration (61). This occurs by virtue of the large concentration gradient for the esterified form created by the continual de-esterification by esterases present in the intracellular compartment and the consequent trapping of the free acid. Thus, pyruvate would be expected to accumulate to extremely high levels in mitochondria and cause such an over-stimulation of ATP production that the cell would be flooded with ATP, overcoming any functional microdomains of ATP that might exist and directly interacting with plasma membrane-localized K_ATP. By contrast, the transport of glycolytically produced pyruvate would be rate-limited by the operation of the mitochondrial transporters as well as being subject to shunting off to form lactate. The large stimulation of insulin secretion produced by methyl pyruvate supports this hypothesis; on the basis of molar equivalents, methyl pyruvate pro-
duced a 6-fold greater increase in insulin release than glucose. Direct evidence to support enhanced Krebs cycle ATP production is, however, lacking. Attempts to measure \([U-^{14}C]\)methyl pyruvate oxidation were unsuccessful as the ester was too unstable to allow reliable measurements to be made. In the future, experiments utilizing oxygen-burst measurements in islets with methyl pyruvate may be able to confirm this proposition.

In conclusion, increases in glycolytic ATP alone are primarily responsible for glucose-dependent insulin secretion, although this pool accounts for only one-sixth of the total ATP that can be produced by the complete oxidation of glucose. Our results with \(\alpha\)-CHC and DCA on glucose-and methyl pyruvate-induced stimulation of insulin secretion further suggest that the \(\beta\)-cell does not fully utilize the ATP-generating capabilities that would follow from the complete metabolism of glucose. The \(\beta\)-cell may be taking advantage of the compartmentalization of ATP production, utilizing glycolytic ATP to regulate the following downstream events. The \(\beta\)-cell’s primary role is to sense the amount of glucose present and to respond by releasing an appropriate amount of insulin. Where sensing of glucose is important, glycolysis is no doubt capable of producing a measured rise in ATP. If this sensing system needed to be fine tuned enough to detect small changes in glucose concentration coupled to a suitable insulin response, large amounts of ATP from the complete oxidation of glucose might well overwhelm that sensing system. The disadvantage, however, of such a singular dependence of the \(\beta\)-cell on glycolysis-derived ATP for glucose signaling is the inherent loss of signal plasticity. This heightens the likelihood that minor defects in glycolytic processing may translate into a \(\beta\)-cell-specific breakdown in glucose sensing, a hallmark of non-insulin-dependent diabetes mellitus.
