

# Metabolic Oscillations in $\beta$ -Cells

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Whereas the mechanisms underlying oscillatory insulin secretion remain unknown, several models have been advanced to explain if they involve generation of metabolic oscillations in  $\beta$ -cells. Evidence, including measurements of oxygen consumption, glucose consumption, NADH, and ATP/ADP ratio, has accumulated to support the hypothesis that energy metabolism in  $\beta$ -cells can oscillate. Where simultaneous measurements have been made, these oscillations are well correlated with oscillations in intracellular  $[Ca^{2+}]$  and insulin secretion. Considerable evidence has been accumulated to suggest that entry of  $Ca^{2+}$  into cells can modulate metabolism both positively and negatively. The main positive effect of  $Ca^{2+}$  is an increase in oxygen consumption, believed to involve activation of mitochondrial dehydrogenases. Negative feedback by  $Ca^{2+}$  includes decreases in glucose consumption and decreases in the mitochondrial membrane potential.  $Ca^{2+}$  also provides negative feedback by increasing consumption of ATP. The negative feedback provided by  $Ca^{2+}$  provides a mechanism for generating oscillations based on a model in which glucose stimulates a rise in ATP/ADP ratio that closes ATP-sensitive  $K^+$  ( $K_{ATP}$ ) channels, thus depolarizing the cell membrane and allowing  $Ca^{2+}$  entry through voltage-sensitive channels.  $Ca^{2+}$  entry reduces the ATP/ADP ratio and allows reopening of the  $K_{ATP}$  channel. *Diabetes* 51 (Suppl. 1):S152–S161, 2002

**I**nulin release in vivo is normally pulsatile showing a period of 4–13 min (1–3). Pulsatile insulin release is believed to provide a more robust signal to target tissues because application of exogenous insulin in pulses is more effective at reducing glucose levels than the same amount of insulin given at a constant dose (4,5). The effectiveness of pulsatile insulin may result from the insulin receptor dose-response curve and prevention of receptor desensitization. Interest in the cause of pulsatile insulin secretion has been heightened by the observation that loss of regular oscillatory insulin release is an early symptom of type 2 diabetes (6,7). Although some reports dispute this link (8), it is apparent that oscillatory insulin release is a normal part of  $\beta$ -cell function and is ultimately important in normal glucose homeostasis.

The current model of glucose-stimulated insulin secre-

tion has been described in several excellent reviews (9–14) and will only briefly be discussed here. Glucose enters  $\beta$ -cells through the GLUT2 glucose transporter. Glucose is consumed almost exclusively by glycolysis (15) with glucokinase believed to be the glucose sensor by virtue of its 10 mmol/l  $K_m$  matching the half-maximal concentration for secretion (16,17). Glycolysis provides NADH via mitochondrial NADH shuttles and the substrates pyruvate and  $\alpha$ -glycerol phosphate to the mitochondria (18–20). The resulting increase in mitochondrial respiration has been shown to be critical for insulin release (18). The increase in intracellular [ATP] to [ADP] ratio (ATP/ADP) produced by respiration and/or glycolysis closes the ATP-sensitive  $K^+$  ( $K_{ATP}$ ) channel resulting in membrane depolarization (21–23) and opening of voltage-gated  $Ca^{2+}$  channels (24). The resulting rise in intracellular  $Ca^{2+}$  concentration ( $[Ca^{2+}]_i$ ) triggers exocytosis. These steps, sometimes called the  $K_{ATP}$  channel-dependent pathway, constitute the consensus model for glucose-stimulated insulin secretion.

Other pathways have recently been demonstrated to be important in glucose-stimulated insulin secretion. For example, it has been demonstrated that glucose can augment secretion aside from its effect on membrane potential and  $[Ca^{2+}]_i$ . Thus, during experiments in which the  $K_{ATP}$  channel is blocked open and the membrane is depolarized with high  $K^+$ , glucose can amplify secretion, suggesting production of other messengers besides  $Ca^{2+}$  to sustain and amplify exocytosis (25,26). Glutamate has been identified as a potential glucose-metabolism-derived messenger that can increase secretion and may be involved in the  $K_{ATP}$  channel-independent pathway at a step proximal to exocytosis (27), although its role has been disputed (28). Changes in metabolism are also important in glucose-stimulated insulin release. As glucose levels rise, a switch from free fatty acid oxidation to glucose oxidation occurs; this is facilitated by malonyl-CoA, which inhibits the transport of long-chain acyl-CoA (LC-CoA) into the mitochondria for oxidation (29–31). LC-CoA itself may be a regulator of insulin secretion by direct effects on the exocytosis machinery and other regulatory enzymes (32). The significance of the LC-CoA pathway has been questioned after the observation that blockade of the glucose-induced rise in the malonyl-CoA level has no effect on insulin secretion in INS-1-derived  $\beta$ -cells (33). Thus, glutamate, malonyl-CoA, and LC-CoA are among the second messengers implicated in glucose-stimulated insulin secretion in addition to the more established roles of cytosolic  $Ca^{2+}$  and ATP/ADP.

This model can explain many aspects of glucose-stimulated insulin secretion; however, it does not explain the oscillatory nature of insulin release. Dynamic studies on groups of islets (34–36) and single islets (37–40) have

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$[Ca^{2+}]_i$ , intracellular  $Ca^{2+}$  concentration;  $\Delta\Psi$ , mitochondrial membrane potential;  $K_{ATP}$ , ATP-sensitive  $K^+$ ; LC-CoA, long-chain acyl-CoA; PFK, phosphofructokinase.

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demonstrated inherent pulsatility of islet insulin secretion. Thus, islets are capable of generating oscillations in secretion without *in vivo* inputs such as neuronal or hormonal signals. Insulin secretion oscillations observed at single islets, when measured with high temporal resolution, have a period of 0.5–3 min. These inherent oscillations observed at single islets are believed to underlie *in vivo* insulin oscillations and, in this review, we focus on them; however, oscillations observed from groups of islets usually have longer periods (5–20 min), which more closely approximate the oscillations observed *in vivo* (34–36). The relationship between the slower oscillations *in vivo* and at groups of islets to the faster ones in single islets has not been explored and could involve different regulatory mechanisms; however, the observation of 4-min oscillations *in vivo* suggests that discrepancies may be related to methods used for measurement rather than inherent differences in the period among the different systems (3).

In addition to insulin secretion, it is well known that  $[Ca^{2+}]_i$  oscillates in islets in response to increases in glucose (41–43). Simultaneous measurements of  $[Ca^{2+}]_i$  and secretion from single islets has revealed that increases in  $[Ca^{2+}]_i$  correspond well with increases in secretion during oscillations, suggesting that the  $Ca^{2+}$  pulses drive secretory pulses as expected from the  $K_{ATP}$  channel-dependent pathway (39,44). Interestingly,  $[Ca^{2+}]_i$  oscillations have complex patterns including fast oscillations with a period of 10–20 s, slow oscillations with a period of 0.5–3 min, and mixed oscillations in which the fast pulses are superimposed on the slower waves (45). The fast pulses are believed to arise from bursts of action potentials, whereas the mechanism of the slower wave is uncertain. The fast-only oscillations arise when the slower oscillations begin to overlap. The difficulty of measuring insulin release with similar temporal resolution has prevented correlation of the faster waves of  $[Ca^{2+}]_i$  with pulses of insulin secretion, although fast pulses of insulin release have been detected (46). In addition, single cells show bursting membrane potential behavior (47), which correlates with  $[Ca^{2+}]_i$  increases (48). This bursting activity has been linked to the  $K_{ATP}$  channel conductance changes (48). Given the above observations, it is reasonable to believe that membrane potential oscillates by activity of the  $K_{ATP}$  channel, resulting in  $[Ca^{2+}]_i$  and secretory oscillations. What is not clear from these observations, however, is how feedback occurs that is necessary for oscillatory behavior.

#### POSSIBLE SOURCES OF FEEDBACK

Theories describing the feedback and oscillatory mechanisms can be divided into two categories. In one model, the oscillation is driven by inherent oscillations in metabolism (49). According to this theory, glycolysis, which has been demonstrated to have oscillatory properties in several systems, serves as a pacemaker and inherently oscillates to give rise to oscillations in ATP/ADP, membrane potential,  $[Ca^{2+}]_i$ , and insulin secretion. The second model that has been advanced is that  $Ca^{2+}$  entry into cells provides negative feedback on further  $Ca^{2+}$  entry by an action involving the  $K_{ATP}$  channel (50). This model was originally proposed based on the observation that  $Ca^{2+}$  regulation of the period of oscillations was mediated by

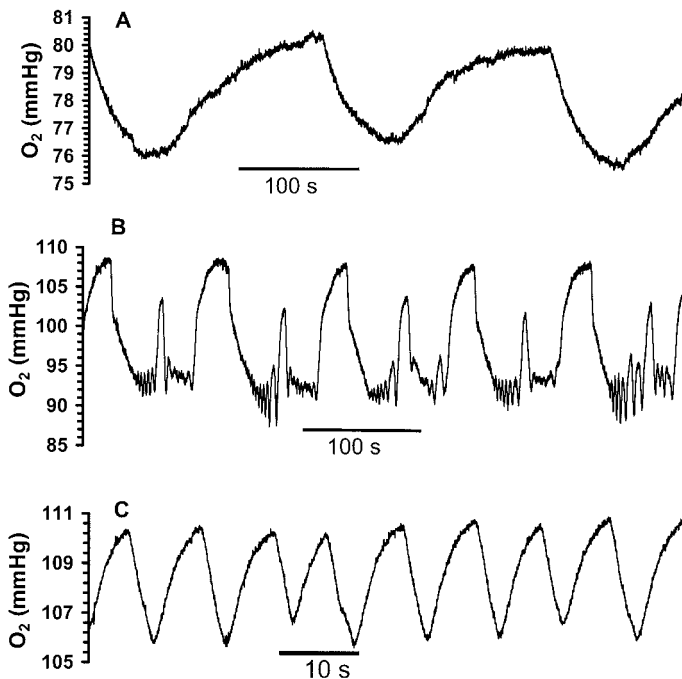
$K_{ATP}$  channel conductance (50). Several mechanisms of feedback can be envisioned including  $[Ca^{2+}]_i$ -induced decreases in ATP production (51,52) or increases in ATP consumption (53), which would decrease ATP/ADP, reopen  $K_{ATP}$  channels, and begin a new cycle. In this article, we consider these theories in light of recent data generated by dynamic measurements of metabolic parameters. Our focus is first on summarizing data supporting the occurrence of metabolic oscillations, a requirement for many of the models. We then consider the hypothesis that metabolic oscillations occur by an interaction with  $Ca^{2+}$ . We emphasize this model because of recent data and the mathematical modeling that supports it and because reviews of the inherent glycolytic oscillation model have already appeared (49).

#### EVIDENCE FOR METABOLIC OSCILLATIONS

Evidence that metabolism can oscillate in islets was first obtained by measurements of oxygen level from groups of perfused islets (54). These data demonstrated that oxygen levels oscillate with a period of ~10 min, which is similar to that observed for  $Ca^{2+}$  and insulin secretion in the same system. Greater detail on the oxygen oscillations has been obtained by using sensors that can be implanted directly into single islets or positioned near single cells (55–59). For single islet measurements, the intimate contact of sensor and tissue allows better sensitivity and temporal resolution than measurements of bath levels. Sensors implanted into single islets have revealed that oxygen levels oscillate with patterns remarkably similar to the known  $[Ca^{2+}]_i$  fluctuations. This is illustrated in Fig. 1, which shows that oxygen levels have both fast and slow oscillations and mixed oscillations in which the slow oscillations appear to be composed of burst of faster pulses. Measurements at single clonal  $\beta$ -cells have revealed both slow oscillations (2–4 min) (HIT cells) (58) and fast oscillations (INS-1 cells) (57) in oxygen consumption. Oscillations in oxygen consumption have been correlated with oscillations in insulin secretion, demonstrating the relevance of the metabolic oscillation to secretion (59).

Other metabolic parameters have also been observed to oscillate. NADH levels, measured by native fluorescence in single  $\beta$ -cells, have been shown to oscillate with a period of ~50 s in a subpopulation of single rat  $\beta$ -cells in response to 8.3 mmol/l glucose (60), although this result has not always been reproduced. In addition, oscillations in whole-islet NADH levels have not been reported despite numerous measurements (61,62). Mitochondrial membrane potential ( $\Delta\Psi$ ), measured by the fluorescence of rhodamine 123, has been shown to oscillate with a period of ~50 s in groups of  $\beta$ -cells upon step changes from 0.5 to 15 mmol/l glucose (63). Finally, glucose consumption, taken as a measure of glycolytic rate because glucose is primarily used by glycolysis in  $\beta$ -cells, oscillates with both short and long periods in single mouse islets, as detected using a glucose microsensor (Fig. 2) (56).

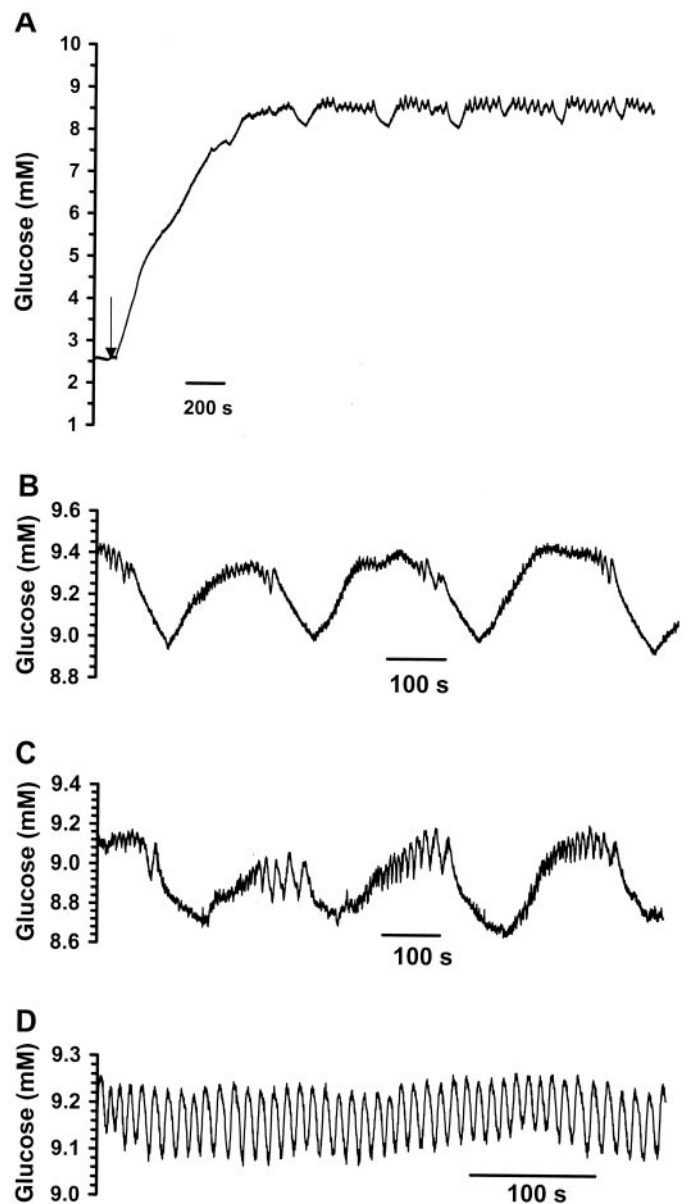
An important consideration is the ATP level or the ATP/ADP ratio, since this is taken as the parameter that translates metabolic changes into electrical activity and secretion. Measurements of ATP by a bioluminescent method have not revealed consistent oscillatory levels (64,65) with glucose concentrations >16 mmol/l. On the



**FIG. 1.** Glucose-stimulated oxygen oscillations. Oxygen measurements were made with a 1- to 3- $\mu\text{m}$  diameter sensor implanted 50–75  $\mu\text{m}$  deep into individual mouse islets. Islets were perfused with 10 mmol/l glucose in Krebs-Ringer buffer containing 2.4 mmol/l  $\text{Ca}^{2+}$  at 37°C. Recordings are shown after regular oscillations began. Oxygen levels represent the balance of consumption and resupply by diffusion through the islet. The decreases in oxygen level correspond to increases in consumption relative to transport into the islet. Once consumption decreases, the oxygen level increases as diffusion into the islet restores the original balance of transport and consumption. The different patterns of oxygen oscillations observed in single islets are slow oscillations with a period of ~3 min (A), slow oscillations with fast fluctuation occurring during the decreases (B), and fast oscillations with a period of 8–30 s (C).

other hand, the conductance of  $\text{K}_{\text{ATP}}$  channels has been shown to oscillate (48), which would suggest oscillations in ATP or ATP/ADP. Finally, ATP/ADP, measured by a direct biochemical assay of cells sampled from a suspension of dissociated *ob/ob* islets, was found to oscillate with a period of ~5 min after stimulation with 20 mmol/l glucose and synchronization with clonidine and high (5 mmol/l)  $\text{Ca}^{2+}$ ; however, these oscillations were apparently not sustained over a long period (66), and the infrequent sampling time makes it difficult to correlate these oscillations with the  $[\text{Ca}^{2+}]_i$  oscillations observed in intact islets. The discrepancy among different ATP and ATP/ADP measurements could be due to several factors. For example, the bioluminescent method has only been used with higher glucose concentrations, which tend to promote continuous elevation of  $[\text{Ca}^{2+}]_i$  and membrane depolarization and not oscillations. It is also possible that ADP level is more important in changing activity of  $\text{K}_{\text{ATP}}$  channels, which would mean that a measure of ATP level may not reveal oscillations. Finally, factors other than ATP or ADP may regulate the  $\text{K}_{\text{ATP}}$  channel and, if they oscillate, could give rise to oscillations in  $\text{K}_{\text{ATP}}$  channel conductance.

To summarize, unequivocal evidence has been amassed to support the notion that metabolism oscillates in  $\beta$ -cells. The most robust measurements appear to be oxygen consumption; however, the limited  $\Delta\Psi$  and glucose consumption measurements are also strong. Direct measure-



**FIG. 2.** Oscillations in glucose level. Glucose measurements were made in a similar way to the oxygen measurements, but a glucose enzyme electrode was used for measurements (53). A: The islet was perfused with 3 mmol/l glucose; then at the arrow, the perfusion fluid was switched to 10 mmol/l glucose. The resulting pulses were only observed at the elevated glucose level. The resulting pulses were only observed at the elevated glucose level. Different patterns of glucose oscillation are observed, including slow (B), slow with fast superimposed (C), and fast (D). The periods are similar to those observed for oxygen. B and C are adapted with permission from Jung et al. (56).

ments of ATP or ATP/ADP have not strongly demonstrated oscillations, although indirect measurements based on the  $\text{K}_{\text{ATP}}$  channel conductance do. Importantly, the recent measurements have demonstrated exquisite moment-to-moment modulation of metabolism to meet the needs and function of the cell.

**DOES  $\text{Ca}^{2+}$  PROVIDE THE FEEDBACK NECESSARY FOR METABOLIC OSCILLATIONS?**

The above discussion demonstrates that metabolism oscillates within islets and the oscillations are correlated with secretion. Although this evidence is important, it does not distinguish among the different theories that purport to

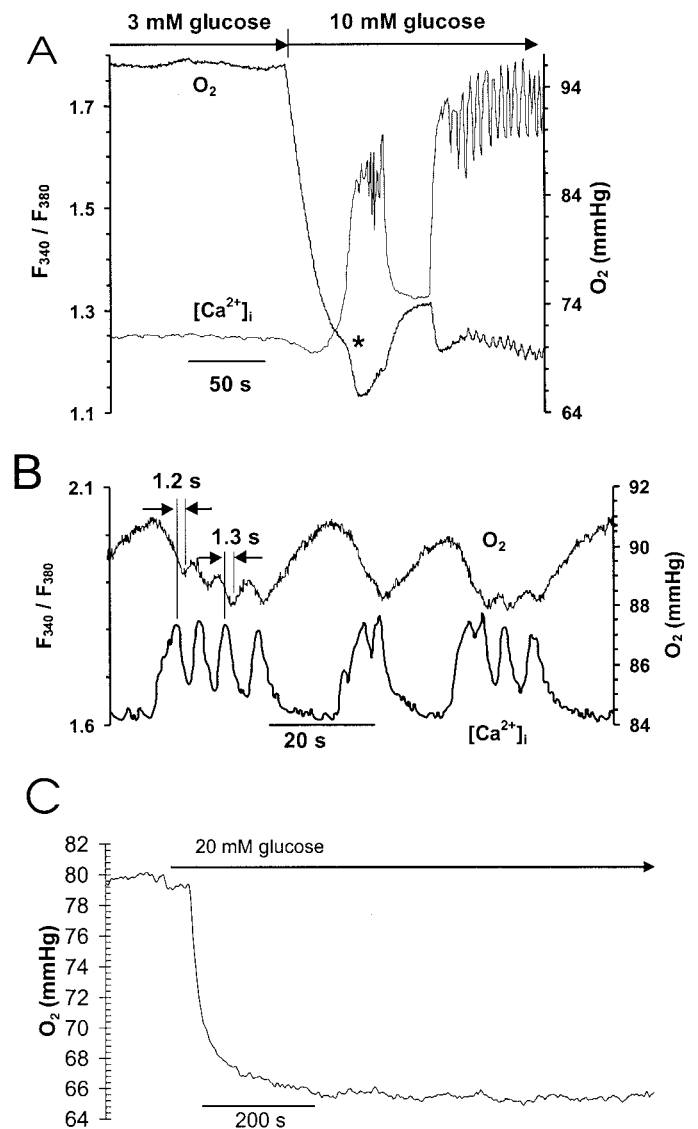
describe oscillatory insulin secretion. Testing of these hypotheses has been aided by modeling and dynamic simultaneous measurements of multiple parameters in single islets and single cells. In this discussion, we consider the evidence that  $\text{Ca}^{2+}$  is a significant modulator of metabolic oscillations.

The concept that metabolic oscillations could be driven by an interaction with cytoplasmic  $\text{Ca}^{2+}$  oscillations has previously been dismissed based on the observation that such a scenario would contradict the basic model of stimulus-secretion coupling, which holds that increases in metabolism lead to  $\text{Ca}^{2+}$  influx and secretion (49). Indeed, when glucose is increased to stimulatory levels, ATP/ADP (53,67), NADH levels (68), and  $\Delta\Psi$  (69) all rise before increases in  $[\text{Ca}^{2+}]_i$ . A limitation of using these observations in evaluating the role of  $\text{Ca}^{2+}$  in an oscillation is that they have not been performed during oscillatory conditions.

Simultaneous measurements of oxygen, glucose, or  $\Delta\Psi$  with  $[\text{Ca}^{2+}]_i$  during glucose-stimulated oscillations have revealed that changes in metabolism do not always precede the rise in  $[\text{Ca}^{2+}]_i$ . This is illustrated in Fig. 3, which shows that during a glucose challenge, oxygen consumption increases dramatically before the  $[\text{Ca}^{2+}]_i$  increase in agreement with the accepted model of stimulus secretion coupling. However, after the  $\text{Ca}^{2+}$  increases, a new dynamic emerges. As shown in Fig. 3, once  $[\text{Ca}^{2+}]_i$  rises, an acceleration of oxygen consumption occurs. Furthermore, once the oscillations begin, it appears that the  $[\text{Ca}^{2+}]_i$  rises are in phase with oxygen oscillations with the  $[\text{Ca}^{2+}]_i$  rise, possibly slightly preceding the increase in oxygen consumption (Fig. 3B). In contrast, with low  $\text{Ca}^{2+}$  present in the extracellular fluid, glucose gives rise to an increase in oxygen consumption but no apparent oscillatory behavior (Fig. 3C). Oscillations can be restored by addition of  $\text{Ca}^{2+}$  to the medium (56,57). Similarly, oxygen oscillations are greatly dampened or nonexistent in the presence of L-type  $\text{Ca}^{2+}$ -channel blockers (56,57). Glucose oscillations also trailed  $[\text{Ca}^{2+}]_i$  oscillations by a few seconds and had a similar  $\text{Ca}^{2+}$  dependency; however,  $\text{Ca}^{2+}$  appeared to evoke a decrease in glucose consumption (56).

Similar observations have been made in small groups of cells when simultaneously measuring mitochondrial membrane potential and  $[\text{Ca}^{2+}]_i$  (63) (Fig. 4). In this case, the membrane was observed to hyperpolarize, indicative of an increase in the driving force for ATP production, before the rise in  $[\text{Ca}^{2+}]_i$ ; however, during subsequent oscillations, increases in  $[\text{Ca}^{2+}]_i$  preceded decreases in membrane potential by about 4 s.  $\text{Ca}^{2+}$  entry into the cell was found to evoke similar decreases in the membrane potential. It was also demonstrated that  $\text{Ca}^{2+}$  entry alone could decrease  $\Delta\Psi$ . (In this study, the  $[\text{Ca}^{2+}]_i$  rise evoked a decrease in  $\Delta\Psi$ , whereas in other studies, oxygen consumption was seen to increase with increasing  $[\text{Ca}^{2+}]_i$ . This apparent paradox will be discussed below.)

In an effort to further explore the connection between  $[\text{Ca}^{2+}]_i$  and oscillations in metabolism, we recently performed experiments in which  $[\text{Ca}^{2+}]_i$  was clamped at high and low levels. These experiments were performed because it was apparent that prior work demonstrated strong  $\text{Ca}^{2+}$  dependency for oxygen consumption oscillations but did not prove that  $[\text{Ca}^{2+}]_i$  oscillations are driving the



**FIG. 3.** *A:* Simultaneous measurement of oxygen and  $[\text{Ca}^{2+}]_i$  in a single islet as glucose is changed from 3 to 10 mmol/l. Conditions for measurement were similar to that in Fig. 1 except the islet was loaded with fura-2/AM, and  $[\text{Ca}^{2+}]_i$  was measured as the ratio of fluorescence at 340 nm and 380 nm ( $F_{340}/F_{380}$ ) (53). The initial decrease in oxygen precedes the initial increase in  $\text{Ca}^{2+}$  by 20 s. The asterisk indicates an increase in oxygen consumption that occurs after  $\text{Ca}^{2+}$  rises. *B:* Enlarged view of simultaneous oxygen and  $[\text{Ca}^{2+}]_i$  during oscillation period. The fast pulses of  $\text{Ca}^{2+}$  correspond well with the fast decreases in oxygen. In general, the  $\text{Ca}^{2+}$  pulse appears ahead of the oxygen pulse by  $\sim 1$  s. This close proximity is in contrast to the initial rise seen in *A*. *C:* Effect of a change from 3 to 20 mmol/l glucose on oxygen in the presence of 0.1 mmol/l  $\text{Ca}^{2+}$ . The islet was initially perfused with 3 mmol/l glucose, and at the time indicated, glucose was changed to 20 mmol/l. The oxygen consumption increases; however, no acceleration or development of significant oscillations occurs. *A* and *B* are adapted with permission from Jung et al. (56).

oscillations, since such oscillations may require the presence of  $\text{Ca}^{2+}$ . In these experiments, we first demonstrated that addition of diazoxide eliminated any oscillation in glucose or oxygen consumption (Fig. 5), which illustrates that the metabolic oscillations were mediated by changes in the activity of the  $K_{\text{ATP}}$  channel and not the other way around.

When a similar experiment is performed, in which 30 mmol/l  $\text{K}^+$  is pulsed to depolarize the membrane after diazoxide has been added to cease the oscillations, then a

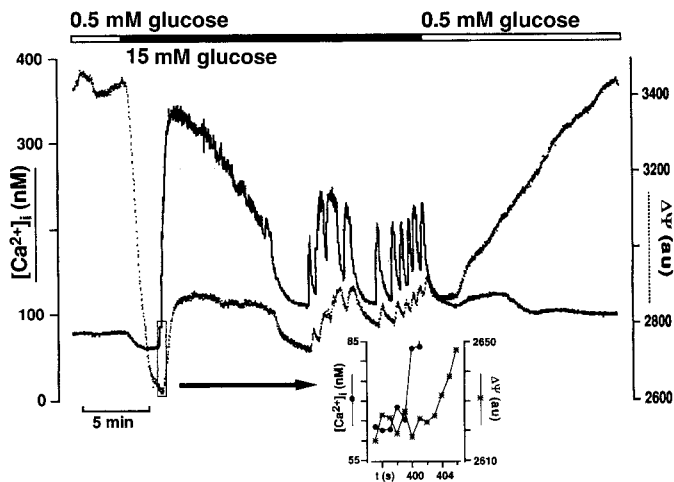


FIG. 4. Effect of 0.5 and 15 mmol/l glucose on cytosolic calcium activity (solid line) and  $\Delta\Psi$  (dotted line). Solutions with different glucose concentrations were present for the time indicated by horizontal bars. Cells were loaded with fura-2/AM and rhodamine 123 (Rh123). Changes in  $[Ca^{2+}]_c$  and  $\Delta\Psi$  were recorded simultaneously. An increase in Rh123 fluorescence corresponds to a depolarization of  $\Delta\Psi$ . The inset shows scaled signals for Rh123 fluorescence and  $[Ca^{2+}]_c$  at a higher time resolution for the period indicated by the rectangle. au, arbitrary units. Reprinted with permission from Krippeit-Drews et al. (63).

pulse of metabolic change is observed (Fig. 6). For oxygen, we observe that depolarization results in a strong increase in oxygen consumption that eventually lessens, even though the  $Ca^{2+}$  remains elevated. Upon returning to 5 mmol/l  $K^+$ , the oxygen returns to the prepolarization level. For glucose, we observed that glucose level increases briefly during the depolarization pulse, but this is followed by a return to baseline or even a decrease to below baseline, as shown in the Fig. 6. When  $K^+$  is returned to basal level, a small sustained increase in glucose consumption is observed. Oscillations are insignificant for both glucose and oxygen if the 30 mmol/l  $K^+$  is kept on the islet for longer periods (data not shown). Thus, in the presence of 10 mmol/l glucose, spontaneous oscillations in  $[Ca^{2+}]_i$ , oxygen consumption, and glucose consumption are observed. However, if  $[Ca^{2+}]_i$  is kept low by treatment with diazoxide or if  $[Ca^{2+}]_i$  is clamped high, with  $K^+$ /diazoxide treatment, no oxygen or glucose oscillations are observed. In contrast, if  $Ca^{2+}$  is pulsed in by a  $K^+$  depolarization in the presence of 10 mmol/l glucose/diazoxide, then a pulse of metabolic changes will consist initially of a decrease in glucose uptake, which is tentatively attributed to an inhibition of glycolysis, and an increase in oxygen consumption.

These results demonstrate that the  $K_{ATP}$  channel-independent pathway does not contribute to metabolic oscillations. More important to the current discussion, however, these results demonstrate that metabolism, measured as oxygen or glucose consumption, exhibits little or no oscillation in single islets without  $[Ca^{2+}]_i$  oscillations whether  $[Ca^{2+}]_i$  is held low or high; however, metabolic changes will accompany pulses of  $[Ca^{2+}]_i$ . These data lend evidence to the argument that  $[Ca^{2+}]_i$  changes substantially modulate metabolic changes seen during oscillatory behavior.

The conclusion from these observations is that initially metabolism accelerates independently of  $Ca^{2+}$  influx, but once  $Ca^{2+}$  enters the cell, it begins to modulate metabo-

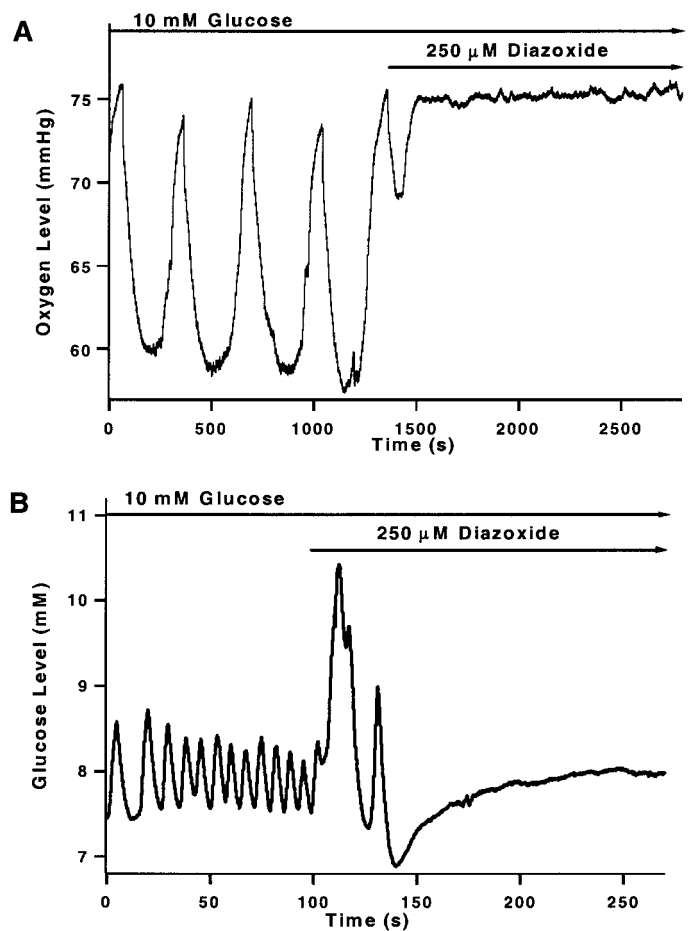


FIG. 5. Effect of diazoxide on oxygen oscillations (A) and glucose oscillations (B). Recordings were made similarly to those in Figs. 1 and 2 but at different islets. At the time indicated, the perfusion buffer was changed to a buffer containing 250  $\mu$ mol/l diazoxide. Data are representative of experiments at five different islets for each type of measurement.

lism by increasing oxygen consumption, decreasing  $\Delta\Psi$ , and altering glucose consumption. Several other observations support the idea that  $Ca^{2+}$  can affect energy metabolism in the  $\beta$ -cell. Increases in NADH in response to  $Ca^{2+}$  entry induced by  $K^+$  or glucose (60,61,68) have also been observed. Measurements of intracellular ATP by bioluminescence in MIN6  $\beta$ -cells during glucose stimulation showed that ATP levels increased before  $[Ca^{2+}]_i$  but then increased more after  $Ca^{2+}$  entry, suggesting that ATP production was augmented by increased  $[Ca^{2+}]_i$  (64). Similar measurements in INS-1 cells showed that treatment with depolarizing concentrations of  $K^+$  had a net effect of decreasing cellular ATP by 10% (70).

**HOW DOES  $Ca^{2+}$  AFFECT MITOCHONDRIAL FUNCTION?**

$Ca^{2+}$  could potentially have several competing effects on metabolism at the level of the mitochondria. Metabolism could be accelerated by activation of mitochondrial dehydrogenases. Activation of mammalian mitochondrial dehydrogenase by increases in cytoplasmic  $Ca^{2+}$  is a well-known phenomenon (71) that has been demonstrated in  $\beta$ -cells (72-74). This idea has been further supported by the observation (75-78) that increases in cytosolic  $Ca^{2+}$  are followed by increases in mitochondrial  $Ca^{2+}$  to the

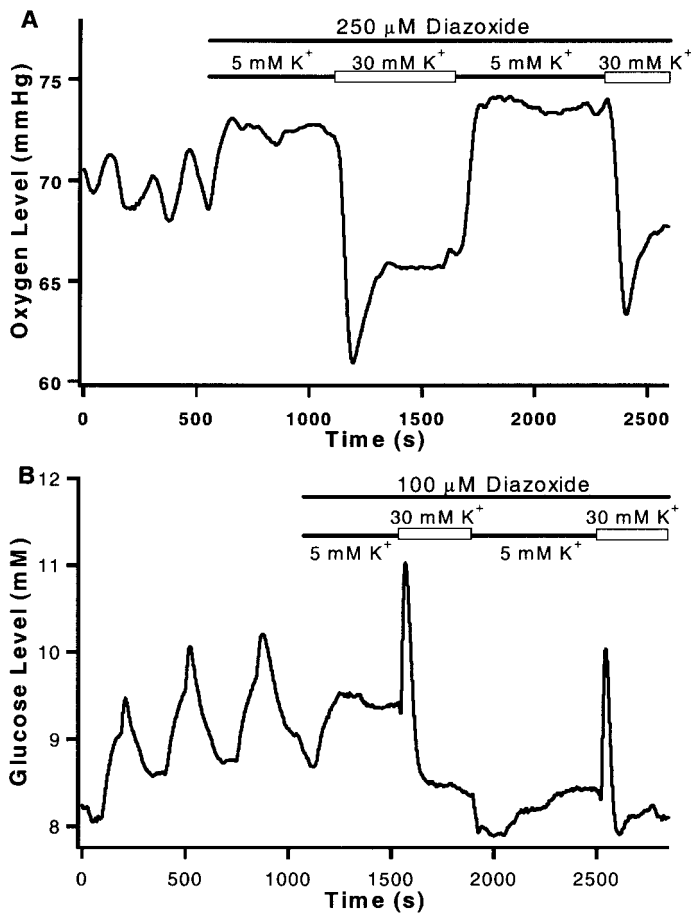


FIG. 6. Effect of  $K^+$ -induced depolarization on oxygen consumption (A) and glucose consumption (B). Experimental conditions were similar to those in Figs. 1 and 2. After establishing oscillations at 10 mmol/l glucose, the islet was perfused with buffer containing 100 or 250  $\mu$ mol/l diazoxide to block oscillations. As shown, the buffer was periodically switched between 5 and 30 mmol/l  $K^+$  to alternately depolarize and repolarize the plasma membrane. Switches to 30 mmol/l  $K^+$  evoked a short-lived decrease in glucose consumption followed in some cases, such as the one shown here, by a small increase in consumption. Oxygen consumption showed a strong increase that lessened with time. Opposite effects occurred with a switch to 5 mmol/l  $K^+$ . Oxygen and glucose measurements were made in separate islets.

micromolar range, a concentration that is sufficient to act on dehydrogenases within the mitochondria. This activation would explain the  $Ca^{2+}$ -induced oxygen consumption, ATP production, and NADH production discussed above.  $Ca^{2+}$  could also accelerate metabolism by activating the glycerol phosphate NADH shuttle to increase the flux of reducing equivalents from the cytosol to the mitochondria (79). However, a role for this process in oscillations seems unlikely given that knockout of glycerol-3-phosphate dehydrogenase, the key  $Ca^{2+}$ -sensitive enzyme in the shuttle, had little effect on oscillatory  $[Ca^{2+}]_i$  (80). It has also been proposed (51,52) and demonstrated (63) that  $Ca^{2+}$  entering the mitochondria could exert negative effects on the metabolic rate by collapsing the  $\Delta\Psi$ , which would decrease the driving force for ATP production.

These competing effects have been taken into account in a quantitative model, which includes, among several mitochondrial variables, the possibility that as  $Ca^{2+}$  enters the mitochondria, it initially increases dehydrogenase activity; this increases ATP production and oxygen consumption, but as it continues to rise in the mitochondria, it begins to

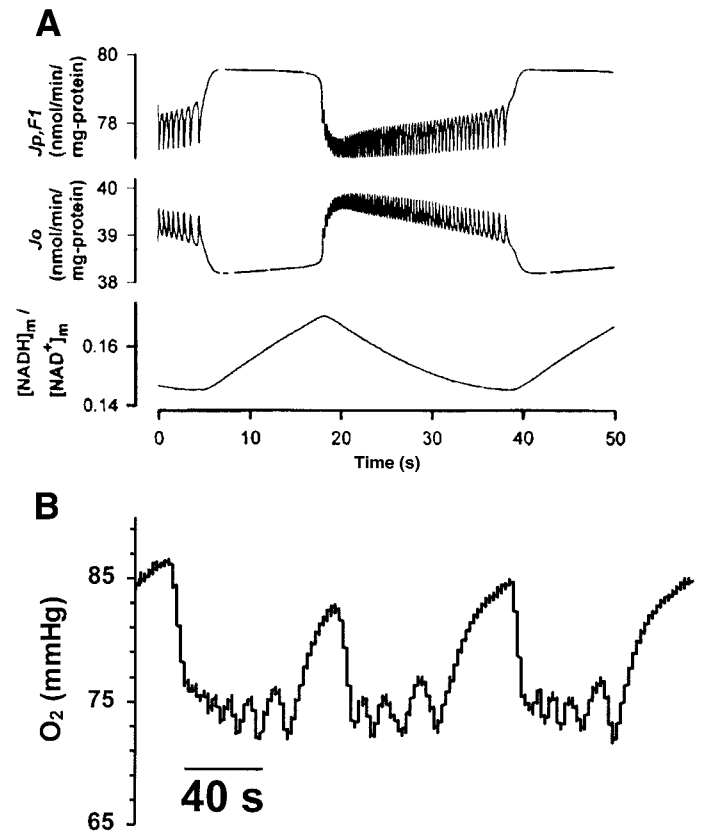


FIG. 7. A: Theoretical predictions of ATP production by oxidative phosphorylation ( $J_{p,F1}$ ), oxygen flux ( $J_o$ ), and mitochondrial NADH/NAD<sup>+</sup> ratio ( $[NADH]_m/[NAD^+]_m$ ) from the model described by Magnus and Keizer (52) at 13.9 mmol/l glucose. Oxygen flux is expected to be roughly the inverse of oxygen level. B: View of oscillations of oxygen level taken during conditions described in Fig. 1. Similarities of predicted  $J_o$  and oxygen oscillations observed include the appearance of fast pulses with an increasing period superimposed on the slow oscillations. A was adapted with permission from Magnus and Keizer (52).

have a suppressive effect by decreasing  $\Delta\Psi$  (52). A variety of experimental observations support the possibility of a biphasic effect. For example, the model produced predictions of oxygen consumption that are similar to those observed, as shown in Fig. 7. Although the model does not match the data exactly, several key observations are predicted, such as fast pulses of oxygen consumption with an increasing period during the slow wave. Some discrepancies, such as the time between slow waves and frequency of fast waves, may result from the slow recovery of oxygen into the islet by diffusion. Also, it is intriguing that whereas oxygen consumption appears to follow very closely the increases in  $[Ca^{2+}]_i$  during an oscillation (Fig. 3B), the mitochondrial membrane depolarization lags by several seconds (Fig. 4). It is reasonable to speculate that this reflects the biphasic nature of the effect of  $Ca^{2+}$  on the mitochondria. In the  $K^+$  pulse experiments illustrated in Fig. 6, oxygen consumption increased but eventually returned to a lower level even though  $[Ca^{2+}]_i$  remained elevated. The initial respiratory burst may reflect  $Ca^{2+}$  activation of dehydrogenases, whereas the subsequent inhibition may reflect the effects of  $Ca^{2+}$  on  $\Delta\Psi$ . Finally, in mitochondria from  $\beta$ -cells of *ob/ob* mice, ATP production is activated at low  $Ca^{2+}$  levels, but inhibited at higher  $Ca^{2+}$  levels (81,82). The evidence supports the hypothesis that  $Ca^{2+}$  entry into the cell is rapidly reflected in the mito-

chondria, resulting initially in an activation of respiration followed closely by inhibition of ATP production by decreases in  $\Delta\Psi$ .

#### INTERACTIONS OF $\text{Ca}^{2+}$ WITH GLYCOLYSIS

Most measurements of metabolic parameters in  $\beta$ -cells have focused on events that primarily reflect mitochondrial metabolism. This is reasonable because the vast majority of ATP production, oxygen consumption, and NADH production occurs in the mitochondria. However, because respiration may be substrate-limited under some conditions, it is important to consider glycolytic flux as well. Besides the observation of oscillations in glucose consumption mentioned above, little evidence exists for glycolytic oscillations in intact islet cells except for the observation that glucose-6-phosphate levels pulse under certain conditions (clonidine synchronization and 5 mmol/l  $\text{Ca}^{2+}$ ) in dispersed *ob/ob* islet cells (66).

Glucose consumption oscillations have a strong  $\text{Ca}^{2+}$  dependency and occur in parallel with  $\text{Ca}^{2+}$ , such that increases in  $[\text{Ca}^{2+}]_i$  correspond to decreases in glucose consumption during the oscillation (56). In addition, pulses of  $\text{Ca}^{2+}$  induced by  $\text{K}^+$  depolarization (Fig. 6) briefly suppress glucose consumption. Suppression of glycolysis by  $\text{Ca}^{2+}$  could provide negative feedback to further  $\text{Ca}^{2+}$  entry via a decrease in substrate supply to the mitochondria, which would decrease ATP/ADP; however, it is not clear if the decrease in glycolysis is sufficient to decrease the substrate supply. For example, whereas glycolysis is briefly inhibited, oxygen consumption increases, suggesting no limitation of substrate (Fig. 6). Thus, it remains to be determined if  $\text{Ca}^{2+}$  effects on glucose consumption are significant with regard to generating oscillations in secretion or if they are simply a response to  $\text{Ca}^{2+}$  oscillations.

While  $\text{Ca}^{2+}$  entry under some conditions appears to evoke a decrease in glucose consumption (see Fig. 6 and discussion above), the interactions of  $\text{Ca}^{2+}$  and glucose consumption are complex. Most obviously, the glucose consumption suppression is only temporary with depolarization. In addition, when nifedipine is added to oscillating islets, oscillations in glucose consumption and  $[\text{Ca}^{2+}]_i$  cease, but the glucose level rises, indicating less glucose consumption as  $[\text{Ca}^{2+}]_i$  falls (56). In addition, switches from  $\text{Ca}^{2+}$ -free conditions to 2.4 mmol/l  $\text{Ca}^{2+}$  at 10 mmol/l glucose causes an increase in glucose consumption followed by initiation of oscillations (56). These discrepancies have not been investigated, but they suggest the possibility for a biphasic effect of  $\text{Ca}^{2+}$  on glucose consumption, where low intracellular  $\text{Ca}^{2+}$  is required for glucose consumption but higher concentrations are inhibitory. The effects of  $\text{Ca}^{2+}$  on glucose consumption could be direct by effects of  $\text{Ca}^{2+}$  on glycolytic enzymes or could be mediated by ATP. In the latter case,  $\text{Ca}^{2+}$  induces changes in ATP, which then feed back to affect the glycolytic rate (83).

#### $\text{Ca}^{2+}$ -INDUCED ATP CONSUMPTION

Thus far, we have shown how  $\text{Ca}^{2+}$  could modulate ATP production at the level of glycolysis and mitochondrial metabolism. It is also possible that  $\text{Ca}^{2+}$  could exert negative feedback via the ATP/ADP by activating numer-

ous ATP-consuming processes such as ion pumping and secretion (53). Thus, it has been demonstrated that  $\text{Ca}^{2+}$  influx induced by  $\text{K}^+$  or tolbutamide evokes decreases in ATP level (53,70). This hypothesis is not mutually exclusive with the modulation of energy production discussed above.

#### INHERENT GLYCOLYTIC OSCILLATIONS

The data discussed so far provide evidence that cytosolic  $\text{Ca}^{2+}$  can and does exert strong effects on both ATP production and consumption in  $\beta$ -cells and islets. As mentioned above, it has also been suggested that inherent oscillations in glycolysis drive the metabolic oscillations by a mechanism involving phosphofructokinase (PFK). In a glycolytic oscillation, a burst of PFK activity is generated once the ATP/ADP ratio decreases to a certain level. The burst is terminated once the supply of high-energy phosphate donors is exhausted, allowing the ATP/ADP ratio to decay (83). Supporting the idea that glycolysis may oscillate is the presence of PFK-M, the PFK isoform with known oscillatory behavior, in islets (84). Interestingly, mutations in this enzyme are associated with impairment of oscillatory insulin release in humans (85). A key feature of this model is that high ATP/ADP ratios inhibit glycolysis.

According to this model,  $\text{Ca}^{2+}$  may modulate oscillations; however, oscillatory glycolysis will proceed without a necessary interaction with  $\text{Ca}^{2+}$ . Evidence for an inherent oscillation, without a requirement for  $\text{Ca}^{2+}$  interaction, has been reported. Oscillations in  $\text{K}_{\text{ATP}}$  channel activity (86) and oxygen tension (59) have been detected at nonstimulatory glucose levels. Slow oxygen oscillations were detected in single HIT cells in 15 mmol/l glucose without added  $\text{Ca}^{2+}$ , although these oscillations were amplified by extracellular  $\text{Ca}^{2+}$  (58). In our own laboratory, we observed irregular pulsatile oxygen (but not glucose) levels under  $\text{Ca}^{2+}$ -free or low-glucose conditions; however, these fluctuations did not rise above the signal-to-noise ratio. Because of these contradictory observations, it would be premature to conclude that all oscillations, especially slower metabolic oscillations, are entirely  $\text{Ca}^{2+}$ -dependent. However, we emphasize that metabolic oscillations are greatly amplified under conditions where  $[\text{Ca}^{2+}]_i$  can oscillate (see Fig. 5 and comparison of Fig. 3A and 3C). Furthermore, it is apparent that rises in  $[\text{Ca}^{2+}]_i$  can create rapid changes in metabolism like those observed during oscillations (Fig. 6). It is also possible, as mentioned above, that the inhibition in glycolysis observed with  $\text{Ca}^{2+}$  entry is mediated by an increase in ATP/ADP brought about by the increased oxygen consumption (and presumably ATP/ADP) associated with increases in  $[\text{Ca}^{2+}]_i$ . In this case, the glycolytic oscillation mechanism would come into play but would require an interaction with  $\text{Ca}^{2+}$ .

The discrepancies in metabolic oscillation detection mentioned above highlight the experimental difficulties that can hamper studies of oscillations. Artifactual oscillations can be created by temperature fluctuations or pulsatile flow created by pumps and plumbing used for perfusion. On the other hand, a system that is not sensitive enough may not detect weaker oscillations. Finally, oscillations require a delicate balance of electrical activity and

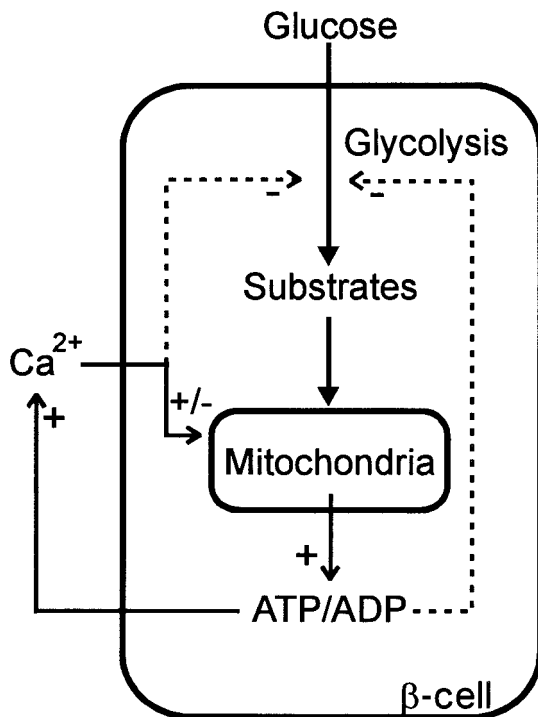


FIG. 8. Summary of model proposed. Glucose initiates an increase in ATP/ADP, which promotes  $\text{Ca}^{2+}$  entry via depolarization of the plasma membrane and  $\text{Ca}^{2+}$  entry through voltage-sensitive  $\text{Ca}^{2+}$  channels.  $\text{Ca}^{2+}$  initially exerts positive feedback on the ATP/ADP ratio by activating dehydrogenases in the mitochondria; however, this is quickly followed by negative feedback via a decrease in  $\Delta\Psi$  as the  $\text{Ca}^{2+}$  continues to increase.  $\text{Ca}^{2+}$  can also affect glycolysis by an unknown mechanism (dashed line). The increase in ATP/ADP ratio associated with initial  $\text{Ca}^{2+}$  increase or sustained glycolysis can also lead to negative feedback on glycolysis by direct inhibition on glycolytic enzymes such as PFK. Also shown is the decrease in ATP/ADP ratio evoked by  $\text{Ca}^{2+}$  activation of ATP-consuming processes.

biochemical messengers. Therefore, the type of  $\beta$ -cell tissue and its metabolic state could play an important role in the observation of oscillations under different conditions.

#### SYNCHRONY

For a whole islet to exhibit an oscillation in  $[\text{Ca}^{2+}]_i$ , metabolism, and secretion implies that the cells of the islet are synchronized. Imaging  $[\text{Ca}^{2+}]_i$  within islets (41,61) and use of multiple oxygen sensors in one islet (56) have both indicated that oscillations occur in synchrony throughout the islet. The mechanism for synchronization is not known; however, electrical coupling through gap junctions and chemical coupling through diffusible factors have been suggested (87). Evidence for chemical coupling has mainly been that changes in flow rate appear to change the period of oscillations (36). Many compounds have been investigated as possible chemical couplers, including pyruvate, lactate, insulin, and ATP, without clear effects on oscillations, thus the mechanism of synchronization remains unknown.

#### $\text{Ca}^{2+}$ -INDEPENDENT OSCILLATORY INSULIN SECRETION

Another observation that remains to be explained is oscillatory insulin secretion without oscillatory  $[\text{Ca}^{2+}]_i$ . Thus, oscillations in insulin secretion have been observed in the absence of glucose (88), with  $[\text{Ca}^{2+}]_i$  clamped at

elevated levels by depolarizing  $\text{K}^+$  and diazoxide in the presence of glucose (36), and in the absence of oscillatory  $[\text{Ca}^{2+}]_i$  (89). Such pulses apparently involve oscillations in pathways other than the  $\text{K}_{\text{ATP}}$ -dependent pathway emphasized here. The significance of these oscillations remains uncertain because they tend to be much weaker than those observed with oscillatory  $[\text{Ca}^{2+}]_i$ . Furthermore, imposed metabolic oscillations give rise to small insulin secretion oscillations when compared with imposed  $[\text{Ca}^{2+}]_i$  oscillations (90).

#### CONCLUSION

We have presented a review of the existence of metabolic oscillations in  $\beta$ -cells and their interaction with  $[\text{Ca}^{2+}]_i$  changes. The picture that has emerged is summarized in Fig. 8: during a glucose challenge, metabolism accelerates independent of extracellular  $\text{Ca}^{2+}$ , producing a rise in the ATP/ADP ratio, closure of the  $\text{K}_{\text{ATP}}$  channel, membrane depolarization, and entry of  $\text{Ca}^{2+}$  through L-type  $\text{Ca}^{2+}$  channels. Once entering the cell, cytosolic  $\text{Ca}^{2+}$  increases are mirrored in the mitochondria where further acceleration of metabolism (increased oxygen consumption) occurs followed by reduction of ATP production (reduced  $\Delta\Psi$ ). The rise in cytosolic  $\text{Ca}^{2+}$  might also result in a temporary inhibition of glycolysis. While the mechanism for such an inhibition is unclear, it could involve a direct effect of  $\text{Ca}^{2+}$  on glycolytic enzymes or inhibition by the increased ATP evoked by  $\text{Ca}^{2+}$  entry. The rise in  $\text{Ca}^{2+}$  may also activate ATP-consuming processes to further decrease ATP/ADP. The net result of  $\text{Ca}^{2+}$  entry is negative feedback via ATP/ADP by a reduction of metabolism (less glucose consumption and lower  $\Delta\Psi$ ) and an increase in ATP consumption. The relative importance of these effects remains to be determined. These combined effects decrease the ATP/ADP ratio, causing a reopening of the  $\text{K}_{\text{ATP}}$  channel, repolarization of the cell, and a reduction in  $[\text{Ca}^{2+}]_i$ . With reduction in  $[\text{Ca}^{2+}]_i$ , the process can begin again. This model is consistent with much of the obtained data on dynamic processes occurring in  $\beta$ -cells and is supported by a mathematical model that incorporates most of these features. The resulting changes in  $\text{Ca}^{2+}$  and ATP/ADP would seem to explain the glucose-stimulated insulin secretion oscillations seen in single islets under most conditions. This model diverges from the proposal that inherent glycolytic oscillations drive all processes in the  $\beta$ -cell; however, the possibility that changes in ATP/ADP underlie  $\text{Ca}^{2+}$ -dependent changes of glycolytic rate provides an intriguing link between the models.

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