Correlated Oscillations in Glucose Consumption, Oxygen Consumption, and Intracellular Free Ca\(^{2+}\) in Single Islets of Langerhans

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Micron-sized sensors were used to monitor glucose and oxygen levels in the extracellular space of single islets of Langerhans in real-time. At 10 mM glucose, oscillations in intraislet glucose concentration were readily detected. Changes in glucose level correspond to changes in glucose consumption by glycolysis balanced by mass transport into the islet. Oscillations had a period of 3.1 ± 0.2 min and amplitude of 0.8 ± 0.1 mM glucose (n = 21). Superimposed on these oscillations were faster fluctuations in glucose level during the periods of low glucose consumption. Oxygen level oscillations that were out of phase with the glucose oscillations were also detected. Oscillations in both oxygen and glucose consumption were strongly dependent upon extracellular Ca\(^{2+}\) and sensitive to nifedipine. Simultaneous measurements of glucose with intracellular Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_{i}\)) revealed that decreases in [Ca\(^{2+}\)]\(_{i}\) preceded increases in glucose consumption by 7.4 ± 2.1 s during an oscillation (n = 9). Conversely, increases in [Ca\(^{2+}\)]\(_{i}\) preceded increases in oxygen consumption by 1.5 ± 0.2 s (n = 4). These results suggest that during oscillations, bursts of glycolysis begin after Ca\(^{2+}\) has stopped entering the cell. Glycolysis stimulates further Ca\(^{2+}\) entry, which in turn stimulates increases in respiration. The data during oscillation are in contrast to the time course of events during initial exposure to glucose. Under these conditions, a burst of oxygen consumption precedes the initial rise in [Ca\(^{2+}\)]\(_{i}\). A model to explain these results is described.

Glucose-stimulated insulin secretion is of paramount importance in maintenance of glucose homeostasis. Defects in this process are a critical component of type 2 diabetes and further understanding of stimulus-secretion coupling is required to enable better management of this prevalent disease. Unlike other secretory pathways that rely on receptor binding to initiate secretion, glucose-stimulated insulin secretion requires metabolism of the sugar to generate appropriate intracellular signaling events. The intertwining of metabolism with secretion results in a complex control process, which has yet to be completely elucidated. The present model stipulates that glucose is rapidly transported into the β-cells through glucose transporter-2. Glycolysis ultimately produces ATP, which activates K\(_{\text{ATP}}\) channels causing the cell to depolarize. Cellular depolarization opens L-type Ca\(^{2+}\) channels, which allow entry of Ca\(^{2+}\) and subsequent triggering of exocytosis (see Ref. 1 for review). Recent reports point to NADH produced during glycolysis and its activation of the NADH shuttle in mitochondria as a key step in initiating secretion (2, 3). According to this model, metabolism should be activated prior to Ca\(^{2+}\) entry into the cell and several lines of evidence support this view. Glucose-stimulated pancreatic β-cells exhibit rises in ATP/ADP ratio and/or oxygen consumption, which precede increases in the Ca\(^{2+}\) levels (4, 5). In addition, pyridine and flavin nucleotide fluorescence increases precede rises in intracellular Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_{i}\)) (6, 7).

At the same time, however, for many cells Ca\(^{2+}\) has been shown to be a primary activator of mitochondrial respiration by activation of Ca\(^{2+}\)-dependent dehydrogenases (8). Indeed, evidence supports the possibility of activation of key dehydrogenases by Ca\(^{2+}\) in the β-cell (9–11). In addition, simultaneous measurements of [Ca\(^{2+}\)]\(_{i}\) and NAD(P)H fluorescence have illustrated that increasing [Ca\(^{2+}\)]\(_{i}\), by K\(^{+}\)-induced depolarization will pace metabolism (12). Recent measurements of intracellular free ATP by a luciferase assay in single β-cells have revealed that ATP levels increase without Ca\(^{2+}\), but increase further with Ca\(^{2+}\) indicative of both mechanisms occurring in β-cells (13).

An important feature of glucose stimulation that is not readily explained is the oscillatory nature of insulin secretion. Since oscillatory secretion is lost in type II diabetes (14, 15), considerable attention has been focused on the cause and regulation of the oscillation. Such oscillations can be observed in vivo (14, 15), in groups of islets in vitro (16–19), and at single islets (18, 20, 21). In addition to oscillations in insulin secretion, oscillations in oxygen consumption (19, 22, 23), [Ca\(^{2+}\)]\(_{i}\) (19–21, 24, 25), membrane potential (26), and K\(_{\text{ATP}}\) channel activity (27) have all been observed. One attractive hypothesis explaining these data is that oscillatory insulin secretion is driven by oscillatory glycolysis (28). This hypothesis has been supported by several lines of evidence including the observation that oxygen and [Ca\(^{2+}\)]\(_{i}\), oscillate with similar frequency in similar preparation (19); however, to date no simultaneous measurements of metabolic oscillations and [Ca\(^{2+}\)]\(_{i}\) oscillations have been made with appropriate temporal resolution to verify the expected temporal relationships. A stipulation of this model, as currently formulated, is that Ca\(^{2+}\) entry into the cell, while possibly amplifying secretory oscillations, does not affect metabolic rates (28). Alternative models have also been proposed (29).

In this work, we have correlated the temporal changes of glucose level, oxygen level, and [Ca\(^{2+}\)]\(_{i}\), in single islets. While techniques for oxygen (22, 23) and [Ca\(^{2+}\)]\(_{i}\), measurements (19–

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1 The abbreviations used are: [Ca\(^{2+}\)]\(_{i}\), intracellular free Ca\(^{2+}\) concentration; K\(_{\text{ATP}}\), ATP-sensitive K\(^{+}\) channel; PFK, phosphofructokinase; PFK-M, muscle isoform of PFK; GOx, glucose oxidase; FIA, flow injection analysis.
21, 24, 25) in single islets have been described, this is the first report of measuring glucose in single islets. The glucose sensor used here is based on immobilizing glucose oxidase (GOx) on the surface of a Pt microelectrode. GOx-based sensors have been described before (30, 31), but we have miniaturized the sensor to a total tip diameter of <5 μm, which allows implantation into a single islet with minimal damage. The sensor derives its selectivity and signal generation from GOx, a flavoprotein that is specific for oxidation of glucose (32, 33). The following reactions occur in the presence of glucose and enzyme,

\[ \beta\text{-d-glucose + GOx/FAD} \rightarrow \text{glucono-δ-lactone + GOx/FADH}_2 \]  
\[ \text{GOx/FADH}_2 + O_2 \rightarrow \text{GOx/FAD} + H_2O_2 \]  
\[ H_2O_2 \rightarrow O_2 + 2H^+ + 2e^- \]

resulting in an anodic current that is directly proportional to glucose concentration. Sensor design for use in real systems must account for nonlinear response, potential effects of low oxygen level (low oxygen levels can slow the formation of H₂O₂ and lead to artificially low signals), and possible fouling of the electrode surface by the tissue. In this report, these issues are addressed by recessing the electrode tip in a glass case, coating the electrode with a polymer, and ensuring a sufficient oxygen level in the system.

**EXPERIMENTAL PROCEDURES**

**Chemicals and Regents**—Pt wire (diameter 25 μm) was from Johnson Matthey (Ward Hill, MA). Silver epoxy was from Epoxy Technology, Inc. (Billerica, MA). Epoxy was from Miller-Stephenson Chemical Co. (Danbury, CT). Glucose oxidase (type X-S from Aspergillus niger) was from Sigma. Deionized water as obtained from a Milli-Q Plus system water purifier (Millipore Co., Bedford, MA) was used to make all solutions. All cell culture media and solutions were from Life Technologies, Inc. (Grand Island, NY). All other chemicals were obtained from Aldrich or Sigma.

**Islet Isolation and Culture**—Islets of Langerhans were isolated from mice weighing 20–30 g using controlled collagenase perfusion via the duct as described previously (34). The isolated islets were put into RPMI 1640 solution allowed to recover overnight in an incubator at 37 °C in a humidified 95% air and 5% CO₂.

**Fabrication of Glucose and Oxygen Microsensors**—Oxygen microsensors were prepared as described previously (23). The final product consists of a Pt wire recessed inside a glass pipette tip, sealed with epoxy, and coated with cellulose acetate as illustrated in Fig. 1B. Oxygen is detected at these electrodes by reduction at platinum. The characteristics and performance of the oxygen microsensors were reported previously (23). Glucose sensors were prepared as recessed Pt electrodes using the same initial steps as for preparation of oxygen sensors; however, before coating with cellulose acetate, the Pt surface was electrochemically platinized at –0.2 V versus Ag/AgCl in an aqueous solution of 10 mM hexachloroplatinate in lead acetate (1.6 mM) for 1 min to form Pt particles inside the recess (see Fig. 1A). GOx was immobilized onto the Pt particles by immersing the electrodes in 5% (w/v) aqueous GOx solution for 10 min and 2% glutaraldehyde for 1 min. Once the enzyme was immobilized, the electrodes were dip-coated with 10% (w/v) cellulose acetate (30 kDa) in dimethylformamide for ~5 s and then allowed to dry for 10 min. Dipping could be repeated to achieve a thicker coat of polymer. The total tip diameter of the resulting sensors was 3–5 μm, which is much smaller than the islets as illustrated in Fig. 1C.

**Testing of Sensors**—Calibration and measurement of the response times and flow sensitivities of the sensors were measured in an electrolyte consisting of 50 mM HEPES buffer with 0.15 m NaCl at pH 7.4 using flow injection analysis (FIA). The FIA system consisted of a reservoir for electrolyte connected to a pneumatically actuated two-position, six-port valve (Valco AC6/UHC) equipped with a 1-ml sample loop. The outlet of the valve was connected to a glass cell via tubing of 0.25-mm inner diameter. The sensors were positioned in the outlet of the tubing using a micromanipulator. The electrolyte solution was fed by gravity at 1 ml/min. The entire FIA system was housed in a Faraday cage.

Amperometric data were collected using an EI-400 bipotentiostat (Ensman Instrumentation, Bloomington, IN) which allowed measurements to be made with two sensors simultaneously. In experiments involving single sensors, a Keithley 428 Current Amplifier was used because it generated lower noise. Amperometric data were collected using a National Instruments (Austin, TX) multifunction board and an IBM compatible personal computer at 300 Hz and low-pass filtered with a cutoff frequency of 20 Hz using filters on the potentiostat or current amplifier. For all measurements, glucose sensors and oxygen sensors were poised at +0.60 V and –0.60 V versus Ag/AgCl reference electrode, respectively.

**Glucose and Oxygen Measurements in Single Islets**—Experiments were performed 1 to 3 days after islet isolation, when the islets had adhered to the surface of culture dishes. To perform measurements, islets in Petri dishes were rinsed three times with a modified Krebs-Ringer buffer (KRB) consisting of 118 mM NaCl, 5.4 mM KCl, 2.4 mM CaCl₂, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, and 20 mM HEPES at pH 7.4. The dish was filled with 4 ml of KRB and placed in a microincubator (Medical Systems, Co., Greenvale, NY) on the stage of a Zeiss Axiosvert 35 microscope at 37 °C. For measurements, the glucose sensor inside an islet, a stream of oxygen was continuously flowed above the solution containing the islet to raise oxygen levels in the KR. As discussed under “Results,” this was necessary to prevent low oxygen levels from interfering with the glucose measurement. For most measurements, the islets were maintained in a static solution on the microscope stage to minimize noise in the electrochemical measurements. Drugs or other additions were made by injecting drug dissolved in KRB into the incubation chamber to generate the desired final concentration. For some experiments, the islets were constantly perfused at 32 μl/s and changes were made by switching solutions that flowed over the islet. In this case, the total volume of the incubation chamber was 400 μl. The response time of the perfusion system, defined as the time to completely change the buffer content of the incubation chamber on the microscope stage, was 38.5 ± 1.2 s (mean ± S.D., n = 5). For all measurements, sensors were positioned normal to the islet surface and advanced to the islet with the use of a micromanipulator (Burleigh, PC-1000). Data collection and electrode potentials were the same as for sensor testing described above.

**Intracellular Calcium Measurements in Single Islets**—Calcium indicator was loaded by incubating islets in RPMI 1640 containing 2 μM Fura-2/AM (Molecular Probes, Eugene, OR) at 37 °C in a humidified
95% air and 5% CO₂ for 30 min. After loading, an islet was attached to the central part of circular 25-mm coverslip coated with poly-L-lysine and incubated in dye-free KRB at 37 °C. The complex of [Ca²⁺-(Fura-2)] was alternately excited at 340 and 380 nm and the resulting fluorescence from individual islets was collected at 1 Hz through a Pulsar 40× oil immersion objective (Zeiss), band pass filter (510 ± 10 nm), and 20-µm pinhole aperture onto a photomultiplier tube using a SPEX CMX cation measurement system and DM3000M data acquisition software (Instruments SA) (35). The ratio of the emission intensities from 340 and 380 nm (F₃₄₀/F₃₈₀) was used as a measure of [Ca²⁺], in the islet. In some experiments, the signal was calibrated to allow measurement of actual [Ca²⁺⁺] (36). All values are reported as mean ± S.E. unless stated otherwise. Two-tailed Student’s t tests are used to evaluate significant differences observed.

RESULTS

Characterization of Glucose Microsensor—The response time (time for signal to change from 10% of the maximum to 90% of the maximum) for glucose sensors was 0.8 ± 0.1 s (n = 8) as determined by FIA. A FIA trace for injection of 20 mM glucose is shown in Fig. 2A for a sensor that had a response time of 0.7 s. The sensors typically yielded a linear response up to 10 mM glucose while some deviation was observed with the sensors of high sensitivity (see Fig. 2B). As illustrated by Fig. 2B, the sensitivity was variable for different sensors yielding an average sensitivity of 4.8 ± 0.7 pA/mM (n = 38). The relatively wide linear dynamic range of the sensors was achieved by slowing transport of glucose to the electrode through the use of a recessed electrode and cellulose acetate coating (37).

An important consideration in using oxidase-based enzyme sensors in biological systems is their oxygen dependence. At a low glucose to oxygen ratio, the signal at the sensor is only dependent on glucose because sufficient oxygen is available to rapidly form H₂O₂; however, at a higher glucose to oxygen ratio, the oxygen level may not be high enough to rapidly form H₂O₂ (reaction 2) resulting in a lower signal for the glucose level (37, 38). The oxygen dependence of the microsensors used here is shown in Fig. 2C. For a glucose concentration of 10 mM, the sensor signal is independent of the oxygen level as long as the oxygen level is above 150 mm Hg; however, below this level the glucose signal is decreased. We have previously shown that under typical incubation conditions, the oxygen level in the islet interior can be as low as 67 mm Hg (23). Therefore, to avoid this artifact in islet measurements, the oxygen level in islet media was increased by flowing oxygen over the islet chamber. This precaution maintained oxygen in the medium at 405 ± 12 mm Hg (n = 7) and within islets at 214 ± 9 mm Hg (n = 7) even at 20 mM glucose. These levels are sufficiently high to prevent any effect of oxygen on the glucose measurement. Oxygen was added only for experiments involving glucose measurements.

Glucose Measurements in Single Islets—Fig. 3A shows a typical time-dependent glucose recording inside a single islet upon changing the perfusion medium from 3 to 10 mM glucose. As expected, the signal increases up to nearly 10 mM glucose. The increase is not instantaneous, requiring 7.4 ± 0.6 min (n = 9) to achieve a steady state level, as the glucose must diffuse into the islet and is constantly being taken up by islet cells and consumed. This delay to a steady level has been predicted based on the effects of mass transport and metabolism of glucose inside islets (39). To demonstrate that the signals observed are due exclusively to glucose and not an intervent, an electrode identical to the sensor but without the GOX enzyme was also implanted in the islet. As shown in Fig. 3A, the current at this “dummy” electrode was unaffected by the glucose addition indicating that the signal is due exclusively to glucose.

An interesting feature of the glucose signal is that upon reaching its maximum level, it appears to oscillate with a period of 2.4 min. Such oscillations were not seen at the control electrode indicating that the oscillation is not an artifact from the system but rather a real fluctuation in the glucose level (see Fig. 3A). To determine if this oscillation was metabolic in origin, the effect of 10 mM mannoheptulose, an inhibitor of glucokinase (40, 41), was tested as illustrated in Fig. 3B. In Fig. 3B, as in all trials (n = 5), mannoheptulose caused an abrupt halt to oscillations and small increase in glucose level. Mannoheptulose was verified to not interfere with the sensor operation in separate experiments.

Regular fluctuations in glucose level were observed in 82 of 104 islets exposed to 10 mM glucose. A variety of patterns were observed as shown in Fig. 4. In 74 of the islets, we observed a “slow” oscillation with a period of 3.1 ± 0.2 min and an amplitude of 0.8 ± 0.1 mM (n = 21). Frequently (68 of 74 islets), the slow oscillation was accompanied by faster fluctuations during the times of relatively high glucose level (i.e. low glucose consumption). In some cases, the fast oscillations frequently started small with a short period and then increased in ampli-
Glucose fluctuations, the fast oscillations of oxygen occur at low oxygen levels. As shown, much like glucose, both slow and fast oscillations can occur. Slow oscillations had a period of 12.1 ± 0.6 min and an amplitude of 9.7 ± 6.5 mm Hg. Fast oscillations had a period of 6.5 ± 2.1 mm Hg (n = 6). Fast oscillations had a period of 6.5 ± 2.1 mm Hg (n = 6). The periods and amplitudes of the slow oscillations are significantly longer (p < 0.001) and larger (p < 0.01) than those of the fast oscillations. In contrast to the glucose fluctuations, the fast oscillations of oxygen occur at low oxygen levels, i.e., during high oxygen consumption. When two oxygen electrodes were placed at different positions in the same islet, we observed that the oxygen levels fluctuated synchronously (Fig. 5B). This synchronous relationship was observed in all trials (n = 9) and was independent of the relative electrode position. Such synchrony has previously been observed for [Ca^2+], oscillations in islets by Ca^{2+} imaging techniques (24). These results indicate that small differences in the placement of the electrodes should not affect the observed phase relationship between different measurements.

In order to determine the temporal relationship between glucose and oxygen fluctuations, both glucose and oxygen electrodes were simultaneously implanted into single islets during exposure to 10 mM glucose. Typical traces are illustrated in Fig. 6. During simultaneous measurements, oxygen oscillations were observed; however, they were not as distinct because of the elevated oxygen level required to obtain reliable glucose measurements (see above). The elevated oxygen level tends to damp out the oxygen oscillation because of both the higher level and increased diffusive flux. Nevertheless, it was consistently observed that both glucose and oxygen oscillated with the same period but out of phase. That is, high glucose consumption correlated with low oxygen consumption and vice versa. This result is consistent with the observation made with independent measurements of oxygen and glucose, which showed no significant difference in the period of glucose and oxygen oscillations. (The out of phase correlation of glucose and oxygen...
confirms that the glucose fluctuation is not an artifact of the oxygen level change. This is because if the artifact did occur, then decreases in oxygen level would cause a decrease in the glucose signal resulting in an in phase correlation.)

**Relationship of Glucose and Oxygen Consumption to \([\text{Ca}^{2+}]\).**—To study the relationship between \([\text{Ca}^{2+}]\), and glucose consumption, glucose measurements were performed in media without \([\text{Ca}^{2+}]\) (Fig. 7A). Upon addition of 2.4 mM \([\text{Ca}^{2+}]\) to the extracellular media, the glucose level abruptly decreased indicating a strong dependence of glucose consumption on extracellular \([\text{Ca}^{2+}]\) level. Furthermore, oscillations in glucose level appeared 6.2 ± 1.4 min \((n = 8)\) after addition of \([\text{Ca}^{2+}]\). These effects could essentially be replicated by blocking L-type \([\text{Ca}^{2+}]\) channels with 40 \(\mu\text{M}\) nifedipine, a selective L-type \([\text{Ca}^{2+}]\) channel blocker (42), as illustrated in Fig. 7B. Nifedipine induced an increase in glucose level, indicative of a decrease in glucose consumption, and eliminated the oscillations (Fig. 7B). Measurements of oxygen consumption in single islets under these conditions have revealed a similar pattern (22). That is, large increases in oxygen consumption were accompanied by addition of \([\text{Ca}^{2+}]\) to the media with eventual oscillations and addition of nifedipine abruptly damped oscillations and decreased overall consumption at 10 mM glucose (22).

**Fig. 5.** A, recording of oxygen level at an oxygen sensor in a single islet exposed to 10 mM glucose. This islet was typical in generating slow oscillations with faster oscillations during the low oxygen level. Other patterns have also been observed as reported elsewhere (22, 23). B, recording made synchronously at two oxygen sensors in a single islet. Sensors were implanted 70 \(\mu\text{m}\) into the islet with a 80- \(\mu\text{m}\) gap between them as illustrated in the drawing below the trace. The straight lines highlight the synchrony observed in peaks of oxygen consumption. Trace is shown as current rather than oxygen level to allow an offset of the current traces. For both A and B, islets were incubated in 10 mM glucose in KRB at 37 °C for 5–10 min before the sensor was implanted. Traces shown after stable oscillations were recorded. The potential applied to the oxygen sensors was −0.6 V versus Ag/AgCl. Islets were incubated as described for Fig. 3B except the oxygen level in the incubation medium was air-saturated and was not enhanced.

**Fig. 6.** Simultaneous measurements of oxygen and glucose at islets incubated in 10 mM glucose. A and B are recordings made at two different islets to illustrate both slow (A) and fast (B) oscillations. Sensor positioning and incubation conditions were the same as for Fig. 3B. Potential at glucose and oxygen sensors was +0.6 V and −0.6 V versus Ag/AgCl, respectively. Oxygen level in the medium was maintained at 400 mm Hg as described under “Experimental Procedures.” The high oxygen level, necessary for reliable glucose sensing, suppressed the oxygen oscillations making them less prominent than those recorded in Fig. 5. Note the higher oxygen level in these traces compared with those in Fig. 5.

**To gain a better understanding of the temporal relationships between \([\text{Ca}^{2+}]\), level, glucose consumption, and oxygen consumption, simultaneous measurements of \([\text{Ca}^{2+}]\), and glucose and \([\text{Ca}^{2+}]\), and oxygen were made as illustrated in Fig. 8. In many cases, it was possible to observe both fast and slow waves in \([\text{Ca}^{2+}]\), as previously reported (25). Slow \([\text{Ca}^{2+}]\), oscillations had a period of 3.0 ± 0.7 min and an amplitude of 184 ± 35 nM \((n = 9)\). Fast oscillations occurred during the slow oscillations and had a period of 11.8 ± 1.5 s and an amplitude of 62 ± 17 nM \((n = 9)\). The periods and amplitudes of the slow oscillations are significantly longer \((p < 0.001)\) and larger \((p < 0.01)\) than those of the fast oscillations. The data in Fig. 8 show that oscillations in glucose consumption and \([\text{Ca}^{2+}]\), level were strongly correlated. Specifically, it appears that as the \([\text{Ca}^{2+}]\), begins to decrease, glucose consumption begins to increase and as the \([\text{Ca}^{2+}]\), begins to rise, glucose consumption decreases. On average, the \([\text{Ca}^{2+}]\), change preceded the change in glucose consumption by 7.4 ± 2.1 s \((n = 9)\) during a slow-wave portion of an oscillation. During the faster fluctuations, the phase relationship did not always remain constant. It is suspected that this may be due to slower recovery for glucose level due to the requirement of diffusion through the islet to the sensor or to noise affecting the glucose measurement. Glucose and \([\text{Ca}^{2+}]\), oscillations were simultaneously eliminated by addition of nifedipine (Fig. 8B).

Oxygen consumption and \([\text{Ca}^{2+}]\), were also correlated such
that increases in oxygen consumption closely followed increases in [Ca\textsuperscript{2+}]. Close inspection of the data reveal that even the fast spikes of [Ca\textsuperscript{2+}], observed are correlated with brief increases in oxygen consumption (Fig. 8C). Although the changes are close in time, it is apparent that the increase in [Ca\textsuperscript{2+}], precedes the increase in oxygen consumption in all cases. On average, the peak in [Ca\textsuperscript{2+}], occurred 1.5 ± 0.2 s (n = 24) before the peak in oxygen consumption. This time difference is significantly different from that between [Ca\textsuperscript{2+}], and glucose consumption (p < 0.005).

**Correlation during Initial Respiration Burst**—The data in Fig. 8 illustrate that during oscillations, the [Ca\textsuperscript{2+}], increases precede respiration increases. Furthermore, the strong Ca\textsuperscript{2+} dependence and nifedipine sensitivity of oscillations indicate that Ca\textsuperscript{2+} entry through voltage-gated Ca\textsuperscript{2+} channels is important in enhancing respiration and allowing for development of regular oscillations (22). These observations contrast with observations that the ATP/ADP ratio rises prior to [Ca\textsuperscript{2+}], increases and that such rises can occur without extracellular Ca\textsuperscript{2+} (4, 5). In addition, such results would apparently contradict the model that increases in metabolism lead to increases in ATP/ADP ratio, cellular depolarization, Ca\textsuperscript{2+} entry, and insulin secretion. Therefore, we investigated oxygen consumption and [Ca\textsuperscript{2+}], that occurred during the initial change of 3 to 10 mM glucose by simultaneous measurements. During the initial use of oxygen, indicating a respiratory burst, the increase in oxygen consumption is ahead of the [Ca\textsuperscript{2+}], increase by 20 ± 7 s (n = 6) (see Fig. 9, for example). Interestingly, during the initial [Ca\textsuperscript{2+}], increase, once the [Ca\textsuperscript{2+}], increases to a certain level, an apparent enhancement of oxygen consumption begins as well (see * in Fig. 9).

**DISCUSSION**

**Glucose Measurements in Single Islets**—Intraislet oxygen and [Ca\textsuperscript{2+}], have been measured before and their interpretation as oxygen consumption (22) and [Ca\textsuperscript{2+}], have been discussed (25); however, these data presented here are the first measurements of intraislet glucose. The results show that the novel glucose sensor measures exclusively glucose in the islets and artifacts associated with changes in oxygen, temperature, and other conditions do not contribute to the signals observed. Once a steady state of glucose is achieved in an islet, changes in glucose level represent changes in the balance of the rate of glucose consumption and the rate of diffusive mass transport into the islet such that decreases in glucose signal correspond to increases in glucose consumption. Since the sensor measures extracellular glucose, glucose consumption could be governed either by transport to the intracellular space or by glycolysis. Glucose transport is rapid relative to metabolism so that the intracellular and extracellular levels equilibrate quickly (43) implying that increased consumption should not be due to transport across the membrane but to glycolysis. This conclusion is supported by the fact that oscillations were eliminated with the glycolysis inhibitor mannose, which decreases glucose consumption due to glycolysis. As long as the glycolytic rate exceeds diffusion, glucose levels will decrease. Once the glycolysis rate slows, the glucose level increases as diffusion into the islet restores the original balance of transport and consumption.

Since the sensor effectively detects changes in glycolytic rate, the oscillations observed demonstrate that glycolysis oscillations occur within the islet. The electrode was always placed in the interior of the islet so the oscillations in glycolysis are likely due to β-cells, which make up the core of the islet (44). What is not clear from these results, and warrants further study, is which glycolytic enzyme(s) governs the signal. Glucokinase is one candidate as this enzyme is the “glucose sensor” of the β-cell and it exerts the most control over the rate of glycolysis in this cell (43). The other likely candidate enzyme is phosphofructokinase (PFK) which has also been shown to exert some control over the glycolytic rate in β-cells (43). PFK also has the interesting property of demonstrable oscillatory activity (45), which could underlie the oscillations observed here. PFK can be made to oscillate by the AMP-dependent, autocalytic activation of the enzyme by its product, fructose-1,6-P\textsubscript{2}. A burst of PFK activity is generated once the ATP/ADP ratio decreases to a certain level. The burst of glycolytic activity raises ATP/ADP. Once the ATP/ADP is decreased to low levels, the cycle is repeated (45).

**Temporal Relationship of [Ca\textsuperscript{2+}], Glucose, and Oxygen during Initial Respiratory Burst**—Our data clearly show that during the initial phases of exposure to high glucose, respiration is enhanced (oxygen levels decrease) before Ca\textsuperscript{2+} enters the cell (see Fig. 9). This sequence of events is in accord with the general model of stimulus secretion modeling in the β-cell and several reports on the temporal relationship between increases in ATP/ADP ratio and [Ca\textsuperscript{2+}], (4, 5). Presumably this increase in [Ca\textsuperscript{2+}], is a result of glycolysis plus respiration with the respiration being stimulated by provision of substrates to the mitochondria. It has been demonstrated elsewhere that the initial respiratory burst is associated with a large increase in the ATP/ADP ratio, which depolarizes the cell by closing $K_{\text{ATP}}$.
channels and allows Ca\(^{2+}\) entry via L-type channels (4). It seems likely that glycolysis also increased prior to the Ca\(^{2+}\) entry on the change from 3 to 10 mM glucose; however, our measurement technique did not allow observing this effect. This is because during the change from 3 to 10 mM glucose, the increase in glucose level was convoluted with any decreases due to consumption.

Temporal Relationship of Ca\(^{2+}\), Glucose, and Oxygen during Oscillation—The high temporal resolution and use of simultaneous measurements reported here have allowed us to observe that the temporal relationship of [Ca\(^{2+}\)], and respiration during an oscillation follows a different pattern than what occurs
during the initial respiratory burst. Most importantly, the [Ca\(^{2+}\)]\(_i\) increases precede the pulses in oxygen consumption during the oscillation. This result could be explained by Ca\(^{2+}\) activation of mitochondrial dehydrogenases (8, 9, 46). That Ca\(^{2+}\) is driving the enhanced respiration is apparent not only from the temporal relationship but also from the strong dependence of the oxygen oscillations on extracellular Ca\(^{2+}\) and sensitivity to nifedipine. It should be emphasized that these oscillations occur on a background of Ca\(^{2+}\) that was increased by metabolism as illustrated in Fig. 9; thus, the Ca\(^{2+}\)-induced enhancement in respiration is dependent upon the initial respiratory burst and does not contradict the prevailing hypothesis of stimulus-secretion coupling. These results are in accord with the view that substrate activation of mitochondrial ATP production is enhanced by subsequent Ca\(^{2+}\) activation of ATP production (13).

Glycolysis on the other hand, is out of phase with oxygen consumption so that increased [Ca\(^{2+}\)]\(_i\) is associated with decreases in glucose consumption and vice versa. This relationship is especially apparent on the slower oscillation. While the [Ca\(^{2+}\)]\(_i\) changes and glycolysis changes are close in time, it appears that the [Ca\(^{2+}\)]\(_i\) change precedes the glucose change so that [Ca\(^{2+}\)]\(_i\) begins to decrease before glucose consumption increases. The importance of Ca\(^{2+}\) to the oscillation is again made apparent by the strong dependence of the glycolysis oscillation on extracellular Ca\(^{2+}\) and nifedipine sensitivity. It is possible that oscillations in glycolysis and respiration occur without Ca\(^{2+}\) entry in the cells; however, we were not able to detect oscillations with the regular period observed in the presence of Ca\(^{2+}\).

**Model for Oscillations**—An important parameter in understanding the oscillation is the temporal changes in ATP/ADP ratio. While such measurements have not been made with the temporal resolution used here, several related studies shed light on how the ATP/ADP ratio may change. First, activity of the K\(_{ATP}\) channel has been shown to oscillate in response to enhanced glucose level suggesting oscillations in ATP/ADP ratio (27). In addition, a recent study has shown that while Ca\(^{2+}\) may activate respiration (in agreement with our results and others), its entry into the islet has a net effect of decreasing the ATP/ADP ratio (29). This net decrease is presumed to result from the activation of numerous ATP consuming processes such as secretion and ion-pumping. These observations, combined with the detection of PFK-M (47), an isofrom of PFK first found in muscle that responds to changes in ATP/ADP, in β-cells, suggests the model depicted in Fig. 10 for oscillatory metabolism/secretion in islets.

According to this picture, the initial respiratory burst enhances respiration causing a quick rise in ATP/ADP. This rise in ATP/ADP suppresses glycolysis causing the decrease in glucose consumption seen during the oscillation. The rise in ATP/ADP also blocks the K\(_{ATP}\) channel and depolarizes the cell allowing Ca\(^{2+}\) entry through the L-type channels. Ca\(^{2+}\) entry enhances respiration causing increased oxygen consumption to follow Ca\(^{2+}\) increases. As Ca\(^{2+}\) stays in the cell, however, it begins to decrease the ATP/ADP ratio eventually repolarizing the cells and preventing further Ca\(^{2+}\) entry. The reduction of [Ca\(^{2+}\)]\(_i\) leads to less oxygen consumption seen during an oscillation. The lowered ATP/ADP ratio also disinhibits glycolysis and initiates a new cycle. This leads to the enhanced glucose consumption following decreases in [Ca\(^{2+}\)]\(_i\) (Fig. 8A).

Thus, this model supposes that glycolytic oscillations underlie the oscillations seen; however, detectable glycolytic oscillations can only occur if Ca\(^{2+}\) is allowed to enter the cells and stimulate use and synthesis of ATP which provides feedback to

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**Fig. 9.** Simultaneous recording of [Ca\(^{2+}\)]\(_i\) (F\(_{340}/F_{380}\)) and oxygen level in a single islet during a transition from 3 to 10 mM glucose. The islet was perfused at 32 μl/s at 37 °C with Krebs-Ringer buffer. At the time indicated, the glucose level was switched from 3 to 10 mM. All other recording conditions are the same as in Fig. 8C. Note although the oxygen level decreases before the initial increase in [Ca\(^{2+}\)]\(_i\), the oxygen level decrease is enhanced by increases in [Ca\(^{2+}\)]\(_i\) at the time indicated by the asterisk (*).

**Fig. 10.** Model of interactions between glycolysis, respiration, and Ca\(^{2+}\) during oscillation. Arrowheads indicate positive feedback or an enhancing effect and blocked lines indicate negative feedback or an inhibitory effect. Thus, glycolysis provides substrates for the mitochondria (mito.) which enhance the ATP/ADP ratio providing negative feedback to glycolysis. Enhanced ATP/ADP blocks the K\(_{ATP}\) channel allowing Ca\(^{2+}\) entry, which further activates the mitochondria, exocytosis of secretory granules (SG), and eventually leads to a decrease in the ATP/ADP ratio. The decrease in the ATP/ADP ratio allows cellular repolarization and disinhibition of glycolysis.
the glycolytic reactions. Without Ca^{2+} acting in this way, oscillations, if they occur, are greatly damped and irregular. This model essentially is a synthesis of two recently proposed models (28, 29). In one case, inherent oscillations in glycolysis have been supposed to occur which are independent of [Ca^{2+}] (28). In another proposal, Ca^{2+} feedback on ATP/ADP use and synthesis with no interaction with glycolysis has been proposed (29). Our data show that Ca^{2+}/glycolysis interactions occur and must be accounted for by the model. Besides the interactions outlined here, other interactions may also occur. For example, in some cells Ca^{2+} has been shown to have direct effects on glycolytic enzymes (48, 49), thus such effects, if they occurred in ß-cells, could possibly be compatible with our data and help explain the oscillation.

The rather complicated pattern shows that any number of defects could alter or eliminate the oscillations. Defects in Ca^{2+} handling, respiration, or glycolysis could all upset the balance that leads to oscillations. Analysis of simultaneous, temporally resolved measurements in islets from diabetic models will be required to determine which steps are important in loss of oscillations in diabetes.

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