Glucose-induced pulsatile insulin release from single islets at stable and oscillatory cytoplasmic Ca\textsuperscript{2+}

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Bergsten, Peter. Glucose-induced pulsatile insulin release from single islets at stable and oscillatory cytoplasmic Ca\textsuperscript{2+}. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E796–E800, 1998.—The cytoplasmic Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]i) and insulin release were measured simultaneously in mouse pancreatic islets cultured overnight. [Ca\textsuperscript{2+}]i was 105 nM and insulin release 3 pmol·g\textsuperscript{-1}·s\textsuperscript{-1} at 3 mM glucose. An increase to 7 mM glucose reduced [Ca\textsuperscript{2+}]i transiently, whereas insulin release doubled and was pulsatile with a frequency of 0.47 min\textsuperscript{-1}. [Ca\textsuperscript{2+}]i oscillations with similar frequency appeared at 11 mM glucose associated with increased amplitude of the insulin oscillations, raising the secretory rate 10-fold. In the presence of 16 and 20 mM glucose, [Ca\textsuperscript{2+}]i was 300 nM and showed no oscillations apart from two islets, which demonstrated [Ca\textsuperscript{2+}]i oscillations with small amplitude at 16 mM glucose. Insulin release with maintained frequency increased by 46 and 31%, respectively. When the glucose concentration was increased from 3 to 11 mM, [Ca\textsuperscript{2+}]i decreased with a nadir that appeared significantly earlier than when the glucose concentration was raised from 3 to 7 mM. Glucose-induced insulin release from the isolated islet is pulsatile both at stable and oscillatory [Ca\textsuperscript{2+}]i, with changes in secretory rate caused by the sugar also when [Ca\textsuperscript{2+}]i is unchanged.

Results

The [Ca\textsuperscript{2+}]i and insulin release were measured simultaneously in individual mouse pancreatic islets cultured overnight in 5.5 mM glucose. In the presence of 3 mM glucose [Ca\textsuperscript{2+}]i was 105 nM and insulin release 3 pmol·g\textsuperscript{-1}·s\textsuperscript{-1} (Fig. 1 and Table 1). Increase of the glucose concentration to 7 mM reduced [Ca\textsuperscript{2+}]i transiently with a nadir after 2.1 min followed by return to levels observed in the presence of 3 mM glucose. In parallel measurements of insulin release the secretory rate was doubled and showed pulses with a frequency of 0.47 min\textsuperscript{-1}. When the glucose concentration was changed to 11 mM, [Ca\textsuperscript{2+}]i oscillations appeared after 0.3 min with a frequency of 0.49 min\textsuperscript{-1} and with a doubling of the mean [Ca\textsuperscript{2+}]i. The measurements of

MATERIAL AND METHODS

Materials. Reagents of analytic grade and deionized water were used. Collagenase, HEPES, and BSA (fraction V) were obtained from Boehringer Mannheim (Mannheim, Germany). Tetramethylbenzidine, insulin-peroxidase, and poly-L-lysine (P-5899) were supplied by Sigma Chemical (St. Louis, MO). FCS was provided by Gibco (Paisley, UK). The acetoxy-methyl ester of fura 2 was from Molecular Probes (Eugene, OR), and IgG-certified microtiter plates were from Nunc (Roskilde, Denmark).

Preparation and culture of islets. Islets were collagenase isolated from ob/ob-mice and cultured overnight in the presence of 5.5 mM glucose in RPMI 1640 medium supplemented with 10% FCS. Subsequent experimental handling was performed in a medium containing (in mM) 125 NaCl, 5.9 KCl, 1.2 MgCl\textsubscript{2}, 2.56 CaCl\textsubscript{2}, and 25 HEPES, titrated to pH 7.4 with NaOH. The medium was supplemented with 3, 7, 11, 16, or 20 mM glucose. BSA (0.5 mg/ml) was present in the medium except when the islets were loaded with the Ca\textsuperscript{2+} indicator fura 2.

Measurements of cytoplasmic Ca\textsuperscript{2+}. Individual islets were loaded with fura 2 during 40 min of incubation at 37°C with 2 µM of fura 2-acetoxyethyl ester in the presence of 3 mM glucose. After rinsing, each islet was placed in a 15-µl perfusion chamber as previously described (3). The chamber was placed on the stage of an inverted microscope (Nikon Diaphot) within a climate box maintained at 37°C and perfused at a rate of 150–200 µl/min. The microscope was equipped for [Ca\textsuperscript{2+}]i measurements by dual wavelength fluorometry with excitation at 340 and 380 nm and emission measured at 510 nm from which [Ca\textsuperscript{2+}]i was calculated (17).

Measurements of insulin release. Insulin secretion from individual islets was determined from measurements of the hormone in 5-s fractions of the perfusate (3). After perfusion the islets were freeze-dried overnight and weighed on a quartz fiber balance, and secretion was expressed as picomole insulin per gram dry islet weight per second. The dry weights of the islets were 8–15 µg. Insulin was assayed by competitive ELISA as previously described (7).

Statistical analysis. Differences between paired and unpaired observations were evaluated with Student's t-test.

RESULTS

The mean [Ca\textsuperscript{2+}]i and insulin release were measured simultaneously in individual mouse pancreatic islets cultured overnight in 5.5 mM glucose. In the presence of 3 mM glucose [Ca\textsuperscript{2+}]i was 105 nM and insulin release 3 pmol·g\textsuperscript{-1}·s\textsuperscript{-1} (Fig. 1 and Table 1). Increase of the glucose concentration to 7 mM reduced [Ca\textsuperscript{2+}]i transiently with a nadir after 2.1 min followed by return to levels observed in the presence of 3 mM glucose. In parallel measurements of insulin release the secretory rate was doubled and showed pulses with a frequency of 0.47 min\textsuperscript{-1}. When the glucose concentration was changed to 11 mM, [Ca\textsuperscript{2+}]i oscillations appeared after 0.3 min with a frequency of 0.49 min\textsuperscript{-1} and with a doubling of the mean [Ca\textsuperscript{2+}]i. The measurements of
insulin release showed oscillations with a similar frequency and a 10-fold increase of the secretory rate. In the presence of 16 mM glucose the mean [Ca\(^{2+}\)]\(_i\) increased by ~50%. In two of five islets the [Ca\(^{2+}\)] oscillations persisted with the same frequency but appeared from a higher [Ca\(^{2+}\)] level and with decreased amplitude. In the remaining three islets [Ca\(^{2+}\)] was stable and elevated. Insulin release was pulsatile in all five islets with maintained frequency and a 46% increase of the secretory rate. When the glucose concentration was raised to 20 mM, all islets displayed a stable and elevated [Ca\(^{2+}\)] level with no further increase of the mean [Ca\(^{2+}\)] (Fig. 1 and Table 1). The corresponding insulin release measurements rose by 31% with maintained frequency of the insulin oscillations.

The [Ca\(^{2+}\)] was monitored alone in individual islets cultured overnight in 5.5 mM glucose. After initial exposure to 3 mM glucose the sugar concentration was increased to 11 mM, which decreased [Ca\(^{2+}\)] transiently with the nadir appearing after 1.6 min, significantly earlier (P < 0.01) than in the presence of 7 mM glucose (Figs. 1 and 2; Tables 1 and 2). The magnitude of the lowering in [Ca\(^{2+}\)] was similar for both sugar concentrations, however. The first oscillation appeared after 2.8 min, significantly later (P < 0.001) than when the sugar concentration was raised from 7 to 11 mM. However, both the peak [Ca\(^{2+}\)] of the first oscillation as well as the frequency of the following oscillations and the mean [Ca\(^{2+}\)] were not affected by 7 mM glucose preceding 11 mM of the sugar. In the presence of 16 or 20 mM glucose, neither the oscillations nor the means in [Ca\(^{2+}\)] were affected by the omission of 7 mM glucose (Figs. 1 and 2; Tables 1 and 2).

**DISCUSSION**

Glucose-induced increase of the amplitude of insulin pulses has been explained by recruitment of β-cells into the secretory phase by transformation of [Ca\(^{2+}\)] from the low and stable to the oscillatory mode (18, 20, 23). Whereas some cells show such a response already at 8 mM glucose (14), most cells and islets require 10–11 mM glucose (3, 4, 12–14, 18, 20, 34, 35) and some need

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**Table 1.** Cytoplasmic Ca\(^{2+}\) and insulin release from isolated islets in response to 4 different glucose concentrations

<table>
<thead>
<tr>
<th>Glucose, mM</th>
<th>Mean, nM</th>
<th>Frequency, osc/min</th>
<th>Nadir, nM</th>
<th>Peak, nM</th>
<th>Mean, pmol·g(^{-1})·s(^{-1})</th>
<th>Frequency, osc/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>105 ± 14</td>
<td></td>
<td></td>
<td></td>
<td>3 ± 1</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>110 ± 18</td>
<td></td>
<td></td>
<td></td>
<td>7 ± 2*</td>
<td>0.47 ± 0.04</td>
</tr>
<tr>
<td>11</td>
<td>215 ± 12†</td>
<td>0.49 ± 0.05</td>
<td>76 ± 12†</td>
<td>2.1 ± 0.1</td>
<td>258 ± 18†</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>16</td>
<td>315 ± 30†</td>
<td>0.51 ± 0.07§</td>
<td></td>
<td></td>
<td>343 ± 31†</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>20</td>
<td>342 ± 21</td>
<td></td>
<td></td>
<td></td>
<td>130 ± 12*</td>
<td>0.48 ± 0.04</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 islets from 5 different animals. Islets were cultured overnight in 5.5 mM glucose. After loading with fura 2 at 3 mM glucose, islets were perfused in presence of 3, 7, 11, 16, and 20 mM glucose. Cytoplasmic Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) was analyzed with dual wavelength fluorometry and insulin release from measurements of insulin in the perfusate by ELISA. osc, Oscillations. *P < 0.05, †P < 0.01, and ‡P < 0.001 vs. preceding glucose concentration. §Based on 2 of 5 islets.
Glucose metabolism is oscillatory and modulated by alterations in the sugar already at glucose concentrations below 3–4 mM (10, 22), which could explain the pulsatile insulin release in the presence of such low glucose concentrations, when there is no entry of Ca$^{2+}$ (35). The secretory activity probably resides with a metabolically responsive subpopulation of the β-cells. When β-cells were sorted according to their metabolic activity (32), glucose-induced stimulation of insulin release in the presence of 4.2 mM was seen only in this subpopulation (33). The metabolically recruited β-cells also show [Ca$^{2+}$] oscillations if the glucose concentration is further elevated (3, 4, 12–14, 18, 31, 34), which increases the metabolism leading to the critical rise in the ATP/ADP ratio (1, 8, 9, 30). Although oscillations in [Ca$^{2+}$], could be pacing the secretory pulses via the Ca$^{2+}$-sensitive mitochondrial dehydrogenases (28), it seems more likely that [Ca$^{2+}$] oscillations are coupled to and act synergistically with metabolic oscillations via fluctuations in the ATP/ADP ratio to amplify the insulin oscillations. Metabolic and secretory oscillations with similar frequency in the absence of [Ca$^{2+}$], oscillations (10, 35) argue in favor of an oscillatory metabolism generating the pulses in insulin release. Furthermore, when the glucose concentration is raised to promote [Ca$^{2+}$], oscillations, changes in metabolic parameters precede the rise in [Ca$^{2+}$], the metabolic parameters are not further altered by the rise in [Ca$^{2+}$], (27, 30), and no change in the frequency of the insulin oscillations is observed with the onset of [Ca$^{2+}$], oscillations (34).

In the physiological range of glucose concentrations, when the sugar is raised from 4 to 11 mM glucose, the number of metabolically activated β-cells increases sigmoidally two- to threefold to ~70% (25). Although a similar sigmoidal increase is observed in insulin release the rise in seeration is at least 10-fold (2, 7, 34, 36), manifested as an increase in the amplitude of the insulin oscillations (7, 34). Within the same glucose range, [Ca$^{2+}$] is transformed from the low and stable to the oscillatory mode in as many as 85% of the β-cells (20). This demonstrates the important role of [Ca$^{2+}$] in the amplitude regulation by glucose of the insulin oscillations in the physiological range of the sugar. However, the almost doubling in secretory rate when the glucose concentration is further raised from 11 to 20 mM glucose observed in this and other studies (3, 4, 7)

### Table 2. Cytoplasmic Ca$^{2+}$ in isolated islets in response to 3 different glucose concentrations

<table>
<thead>
<tr>
<th>Glucose, mM</th>
<th>Mean, nM</th>
<th>Frequency, osc/min</th>
<th>Nadir, nM</th>
<th>Peak, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>111 ± 8</td>
<td>0.41 ± 0.04</td>
<td>87 ± 8*</td>
<td>288 ± 26†</td>
</tr>
<tr>
<td>11</td>
<td>217 ± 18†</td>
<td>0.52 ± 0.08‡</td>
<td>1.6 ± 0.2</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td>16</td>
<td>350 ± 27*</td>
<td></td>
<td>371 ± 30†</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>20</td>
<td>381 ± 32</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 islets from 7 different animals. Islets were cultured overnight in 5.5 mM glucose. After loading with fura 2 at 3 mM glucose the islets were perfused in presence of 3, 11, 16, and 20 mM glucose. [Ca$^{2+}$] was analyzed with dual wavelength fluorometry. *P < 0.01 and †P < 0.001 vs. preceding glucose concentration. ‡Based on 2 of 8 islets.
excludes a mere recruitment of β-cells accounting for the glucose-induced amplification of insulin release.

The glucose-induced increase in insulin release at high glucose concentrations could reflect elevation of the ATP content because the exocytosis of insulin-containing granules is an ATP-dependent process (11). In tolbutamide-stimulated islets a high glucose concentration maintains the amplitude of the insulin oscillations, whereas a decline in the amplitude is seen in the presence of a low glucose concentration despite a sustained and elevated free Ca2+ (34). Changes in the ATP/ADP ratio are probably not only important for the induction of [Ca2+]i oscillations (1, 8, 23, 30) and synchronized with insulin oscillations (23) in the normal glucose range but also play an important role in regulating insulin release at high glucose concentrations. In fact, such changes in the ATP/ADP ratio caused by high glucose concentrations have been observed (9). The cellular content of cAMP shows a similar glucose-dependent increase in isolated islets (15) and may well participate in the potentiation of the amplitudes of insulin pulses (7) at a stage distal to the elevation of [Ca2+], (16). The glucose-induced increase in cAMP could also be important for the potentiation of the amplitudes of insulin pulses by affecting the recruitment phenomenon per se because addition of glucagon decreases the glucose concentration required for induction of [Ca2+]i oscillations in isolated β-cells (14). In this context it can be argued that isolated islets could have a higher cellular content of cAMP through paracrine secretion of glucagon than isolated β-cells. Although no detailed comparisons have been made there is no evidence to support that isolated islets require lower glucose concentrations for the induction of [Ca2+]i oscillations (2, 4, 12–14, 18, 34, 35).

In conclusion, transformation from low and stable to oscillatory [Ca2+]i in the individual β-cell, which occurs at different glucose concentrations for different β-cells, is an important factor for explaining the glucose-modulated amplification of pulsatile insulin release. However, apart from inducing [Ca2+]i oscillations, increase in the glucose concentration may also be capable of amplifying the secretory response of the individual β-cell for glucose concentration below or above this threshold concentration (21). To determine whether the individual β-cell can modulate its secretory response at low and high glucose concentrations, when no changes in [Ca2+]i are observed, an assay capable of monitoring dynamically the secretory activity of the pancreatic β-cell over sufficient time and with enough sensitivity would be required.

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