Contribution of the endoplasmic reticulum to the glucose-induced \([\text{Ca}^{2+}]_c\) response in mouse pancreatic islets

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ARREDOUANI, Abdelilah, Jean-Claude Henquin, and Patrick Gilon. Contribution of the endoplasmic reticulum to the glucose-induced \([\text{Ca}^{2+}]_c\) response in mouse pancreatic islets. Am J Physiol Endocrinol Metab 282: E982–E991, 2002.—Thapsigargin (TG), a blocker of \(\text{Ca}^{2+}\) uptake by the endoplasmic reticulum (ER), was used to evaluate the contribution of the organelle to the oscillations of cytosolic \(\text{Ca}^{2+}\) concentration \((\text{[Ca}^{2+}]_c)\) induced by repetitive \(\text{Ca}^{2+}\) influx in mouse pancreatic \(\beta\)-cells. Because TG depolarized the plasma membrane in the presence of glucose alone, extracellular \(\text{K}^+\) was alternated between 10 and 30 mM in the presence of diazoxide to impose membrane potential (MP) oscillations. In control islets, pulses of \(\text{K}^+\), mimicking regular MP oscillations elicited by 10 mM glucose, induced \([\text{Ca}^{2+}]_c\) oscillations whose nadir remained higher than basal \([\text{Ca}^{2+}]_c\). Increasing the depolarization phase of the pulses while keeping their frequency constant (to mimic the effects of a further rise of the glucose concentration on MP) caused an upward shift of the nadir of \([\text{Ca}^{2+}]_c\) oscillations that was reproduced by raising extracellular \(\text{Ca}^{2+}\) (to increase \(\text{Ca}^{2+}\) influx) without changing the pulse protocol. In TG-pretreated islets, the imposed \([\text{Ca}^{2+}]_c\) oscillations were of much larger amplitude than in control islets and occurred on basal levels. During intermittent trains of depolarizations, control islets displayed mixed \([\text{Ca}^{2+}]_c\) oscillations characterized by a summation of fast oscillations on top of slow ones, whereas no progressive summation of the fast oscillations was observed in TG-pretreated islets. In conclusion, the buffering capacity of the ER in pancreatic \(\beta\)-cells limits the amplitude of \([\text{Ca}^{2+}]_c\) oscillations and may explain how the nadir between oscillations remains above baseline during regular oscillations or gradually increases during mixed \([\text{Ca}^{2+}]_c\) oscillations, two types of response observed during glucose stimulation.

GLUCOSE STIMULATES INSULIN SECRETION by producing, triggering, and amplifying signals in pancreatic \(\beta\)-cells (22). The triggering signal, an increase in cytosolic free \(\text{Ca}^{2+}\) concentration \((\text{[Ca}^{2+}]_c)\), is the outcome of the following sequence of events: the acceleration of metabolism augments the ATP/ADP ratio, which closes ATP-sensitive \(\text{K}^+\) channels (\(\text{K}^+\)-ATP channels) in the plasma membrane, leading to membrane depolarization, opening of voltage-dependent calcium channels, and \(\text{Ca}^{2+}\) influx (5, 22, 39).

Although single-cell studies have been extremely informative on the regulation of ionic currents and \(\text{Ca}^{2+}\) handling in \(\beta\)-cells (4, 10, 20), intact islets, in particular mouse islets, are a more physiological preparation, permitting comparison of the changes in membrane potential and \([\text{Ca}^{2+}]_c\) (13, 38, 43). The validity of the model is attested by the similarity of the changes in electrical activity and \([\text{Ca}^{2+}]_c\) in vitro and in vivo (15). Stimulation by a sudden increase in the glucose concentration typically induces an initially small lowering of \([\text{Ca}^{2+}]_c\), followed by a biphasic rise consisting of a pronounced first peak, lasting 1–3 min, and a second phase consisting of a sustained elevation with superimposed \([\text{Ca}^{2+}]_c\) oscillations (3, 13, 45). These oscillations are regular and rapid (frequency of \(\sim 2.5/\text{min}\)), regular and slow (frequency of \(\sim 0.2/\text{min}\)), or mixed, with rapid oscillations superimposed on slow ones (23). They are associated with periodic depolarizations of the \(\beta\)-cell membrane (10, 13, 43) and abolished by omission of extracellular \(\text{Ca}^{2+}\) or blockade of voltage-dependent \(\text{Ca}^{2+}\) channels (10, 13).

We recently reported that the endoplasmic reticulum (ER) takes up \(\text{Ca}^{2+}\) during the upstroke of the \([\text{Ca}^{2+}]_c\) rise induced by an imposed depolarization and releases it slowly upon repolarization (12). Because this slow release prolongs the period of \([\text{Ca}^{2+}]_c\) elevation, we hypothesized that it might prevent \([\text{Ca}^{2+}]_c\) from returning to basal levels when oscillations occur at a high frequency and thus underlie the sustained elevation of \([\text{Ca}^{2+}]_c\), on top of which the oscillations appear. The aim of the present study was thus to evaluate the possible contribution of the ER to the \(\beta\)-cell \([\text{Ca}^{2+}]_c\) response to glucose. Thapsigargin (TG) was used to inhibit the sarco-endoplasmic reticulum \(\text{Ca}^{2+}\)-ATPase (SERCA) pump (41) and prevent the uptake of \(\text{Ca}^{2+}\) by the ER. However, \(\text{Ca}^{2+}\) emptying of the ER by TG causes sustained membrane depolarization and stable elevation of \([\text{Ca}^{2+}]_c\) in glucose-stimulated \(\beta\)-cells (6, 12, 33, 45). The contribution of the ER to \([\text{Ca}^{2+}]_c\) oscillations cannot be studied under these conditions. Therefore,
[Ca^{2+}]_c oscillations similar to those produced by glucose were imposed by repetitive depolarizations of the plasma membrane with elevated concentrations of K\(^+-\) in the presence of diazoxide, an opener of K\(^+-\)ATP channels. The results demonstrate that the ER markedly dampens the amplitude of [Ca^{2+}]_c oscillations and contributes to maintain a steady-state elevation of [Ca^{2+}]_c when [Ca^{2+}]_c oscillations occur at a frequency (2–3/min) similar to that of the fast oscillations produced by glucose.

MATERIALS AND METHODS

Preparation of islets and islet cell clusters. The research project was approved by, and the experiments were conducted in accordance with, the guidelines of the Commission d'Ethique d'Expérimentation Animale of the University of Louvain School of Medicine. Islets were aseptically isolated after collagenase digestion of the pancreas of fed female NMRI mice (25–30 g) killed by decapitation. When needed, they were dispersed into clusters of islet cells as previously described (25). Islets or clusters were cultured for 1–3 days in RPMI 1640 culture medium (GIBCO, Paisley, UK) containing 10% heat-inactivated fetal calf serum, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 10 mM glucose. Except for the experiments of Figs. 1A, 6A, and some control tests performed with overnight cultured islets, most experiments were carried out with islets or cells cultured for 2–3 days. This culture period was necessary for the tissue to firmly attach to glass coverslips and sustain frequent solution changes at a high flow rate used subsequently during [Ca^{2+}]_c measurements. Suction with glass micropipettes proved unsatisfactory to hold the islets motionless. However, the few successful experiments performed with this technique showed that the results were not different between islets cultured overnight or for 2–3 days.

Solutions and drugs. The medium used for the isolation of islets and for all experiments was a bicarbonate-buffered solution containing (in mM) NaCl 120, KCl 4.8, K\(_5\)C\(_6\) 2.5, CaCl\(_2\) 1.2, MgCl\(_2\) 24, and NaHCO\(_3\) 12. It was gassed with O\(_2\)-CO\(_2\) (94:6) to maintain pH 7.4 at 37°C. Except for electrophysiological experiments, it was supplemented with 1 mg/ml BSA (fraction V, Boehringer Mannheim, Mannheim, Germany). When the concentration of KCl was increased, that of NaCl was decreased accordingly to keep the osmolarity of the medium unchanged. TG was obtained from Sigma (St. Louis, MO) and diazoxide from Schering-Plough Avondale (Rathdrum, Ireland).

[Ca^{2+}]_c measurements. Cultured islets or clusters of islet cells were loaded with, respectively, 2 μM fura PE3-AM (Teflabs, Austin, TX) for 90–120 min or 1 μM fura 2-AM (Molecular Probes, Eugene, OR) for 40 min at 37°C in a bicarbonate-buffered solution containing 10 mM glucose. When needed, 1 μM TG was added to the loading solution. The coverslip was then used as the bottom of a 200-μl temperature-controlled perfusion chamber (Intracell, Royston, Herts, UK) mounted on the stage of an inverted microscope. The flow rate was 2 ml/min, and the temperature within the chamber was 37°C. The different solutions were introduced into the chamber through separate quartz capillaries placed just in front of the studied islet or cluster and were rapidly changed by computer-controlled Iso-Latch valves (Parker Hannifin, Fairlly, NY). Control experiments with two solutions containing different concentrations of K\(^+\) showed that as little as 0.2 s was enough to start changing [Ca^{2+}]_c in β-cells with this system. [Ca^{2+}]_c was measured at 10 Hz by dual-wavelength (340 and 380 nm) excitation spectrofluorimetry, using a photometric-based system (Photon Technologies International, Princeton, NJ) to capture the emitted fluorescence at 510 nm. [Ca^{2+}]_c, was calculated by comparing the ratio of the 510-nm signals successively acquired at 340 and 380 nm with a calibration curve based on the equation of Grynkiewicz et al. (18) and was established by filling the chamber with an intracellular type of solution containing 10 μM fura PE3 or fura 2 free acid, and ~10 mM or <1 nM free Ca\(^{2+}\). The dissociation constants, or K\(_d\), for the fura PE3-Ca\(^{2+}\) and fura 2-Ca\(^{2+}\) complexes of, respectively, 290 (44) and 224 nM (18) were used. Previous experiments have shown that the Ca\(^{2+}\) probe is not significantly compartmentalized in islet cells and that the changes in fluorescence report changes in [Ca^{2+}]_c within the cytosol, not the ER (12).

Electrophysiology. The membrane potential of a single β-cell within an islet was continuously recorded with a high-resistance (~200-MΩ) intracellular microelectrode (32). β-Cells were identified by the typical electrical activity that they display in the presence of 10 mM glucose.

Presentation of the results. The experiments are illustrated by traces (means ± SE) or by recordings that are representative of results obtained with the indicated number of islets from at least three different preparations. The statistical significance between means was assessed by paired or unpaired Student’s t-test as appropriate. Differences were considered significant at P < 0.05.

RESULTS

Design of the experimental conditions to test the effect of TG. Because TG depolarizes β-cells and usually abolishes the oscillations of membrane potential and [Ca^{2+}]_c induced by glucose (6, 12, 33, 45), the drug cannot be used to evaluate directly the contribution of the ER to glucose-induced [Ca^{2+}]_c oscillations. Therefore, all experiments were performed in the presence of 250 μM diazoxide, which, by opening K\(^+-\)ATP channels (42), clamps the plasma membrane at the resting potential and prevents the depolarizing effect of TG (12, 33). Because diazoxide also prevents the depolarizing effect of glucose, islet cells were cyclically depolarized and repolarized to potentials close to those of oscillations of the membrane potential induced by glucose in the absence of diazoxide. The K\(^+\) concentrations that best mimicked these potential changes were determined by recording the β-cell membrane potential with intracellular microelectrodes. After initial recordings in the presence of 10 mM glucose alone, diazoxide was added, and the K\(^+\) concentration of the medium was changed stepwise (4.8, 8, 10, 12, 30, and 45 mM). Each step caused a depolarization of the β-cell membrane (Fig. 1A). At concentrations of K\(^+\) ≥ 10 mM, the membrane potential varied linearly with the logarithm of the K\(^+\) concentration. The slope of the best linear fit for this relationship was 59 mV for a 10-fold change of the K\(^+\) concentration, which is close to the theoretical value of 61.5 mV (at 37°C) given by the Nernst equation (Fig. 1B). This value is higher than that of 47 mV measured in experiments performed in 0–2.8 mM glucose (32), probably because diazoxide opens K\(^+-\)ATP channels more efficiently than the simple omission of glucose. Below 10 mM K\(^+\), the slope decreased, indi-
cating that the membrane potential of β-cells is not exclusively determined by the K⁺ permeability.

The resting potential in the presence of 4.8 mM K⁺, 10 mM glucose, and diazoxide averaged −71 ± 4 mV (n = 3). The concentration of 10 mM K⁺ was selected to bring the membrane potential to a similar value (−61 ± 3 mV, n = 6) to that measured during the repolarization intervals between glucose-induced membrane potential oscillations without diazoxide (−62 ± 3 mV, n = 8) (Fig. 1A). The concentration of 30 mM K⁺ (in the presence of diazoxide) was selected to depolarize the membrane to a potential (−32 ± 2 mV, n = 13) between the peak and the foot of the spikes occurring on the plateau of membrane potential oscillations (Fig. 1A). In the presence of diazoxide, TG did not affect the absolute values or the kinetics of the changes in membrane potential induced by switches between low- and high-K⁺ solutions (12).

Role of the ER on [Ca²⁺]ᵢ in conditions mimicking a change of the glucose concentration from 3 to 10 mM. Figure 2A shows [Ca²⁺]ᵢ changes occurring in a mouse islet stimulated by a rise in the glucose concentration from 3 to 10 and then 15 mM. A small initial drop was followed by a large increase, and eventually by rapid oscillations occurring above a sustained elevation. These [Ca²⁺]ᵢ changes are known to result from concomitant changes in membrane potential (13, 38, 45).

To mimic the changes in β-cell membrane potential induced by 10 mM glucose, the islets were submitted, in the presence of 250 μM diazoxide, to cycles of depolarization-repolarization, following a protocol that is illustrated in Fig. 2B (top). When glucose was increased from 3 to 10 mM, the islets were depolarized for 1 min with 30 mM K⁺ to reproduce the initial phase. Thereafter, the concentration of K⁺ was repetitively changed between 30 mM (for 8 s) and 10 mM (for 16 s) to impose oscillations of the membrane potential of similar duration and frequency (2.5/min) to those induced by 10 mM glucose itself under control conditions (16, 21). At the end of the experiment, the islets were continuously perifused with 10 mM K⁺.

In control islets, the depolarization protocol induced [Ca²⁺]ᵢ changes similar to those elicited by glucose in the absence of diazoxide, with a first [Ca²⁺]ᵢ peak followed by [Ca²⁺]ᵢ oscillations occurring above a sustained elevation (Fig. 2B). During the pulses of K⁺30/ K⁺10 (8/16 s), [Ca²⁺]ᵢ oscillations had an average amplitude of 186 ± 23 nM, with a nadir at 173 ± 21 nM, i.e., 118 ± 19 nM above basal [Ca²⁺]ᵢ, in the presence of 4.8 mM K⁺ (Table 1, line 1). In TG-pretreated islets, basal [Ca²⁺]ᵢ at 4.8 mM K⁺ was not modified (Table 1, lines 1 and 2), whereas both initial [Ca²⁺]ᵢ peak and [Ca²⁺]ᵢ oscillations induced by 30 mM K⁺ were about two times larger than in control islets (Fig. 2, B and C; Table 1, lines 1 and 2). Moreover, the nadir of [Ca²⁺]ᵢ oscillations during the pulses of K⁺30/K⁺10 (8/16 s) was at 95 ± 10 nM, similar to the average [Ca²⁺]ᵢ, (107 ± 14 nM) during final repolarization with 10 mM K⁺, and much closer to basal [Ca²⁺]ᵢ in 4.8 mM K⁺ (Δ) than in control islets (Table 1, compare line 2 with line 1).

Mouse islets contain ~20% non-β-cells (25) that are preferentially located at the periphery of the organ and might thus contribute to the recorded [Ca²⁺]ᵢ signal. To ascertain that the results obtained in islets with the imposed depolarizations correctly reflect changes in β-cells, the same protocol was repeated with clusters of ~10 islet cells, a preparation that contains a low proportion of non-β-cells (9% in all clusters, with 58% of clusters entirely composed of β-cells) (25). The depolarizing protocol induced similar qualitative results as in islets (Fig. 3 and Table 1, lines 5 and 6). Again, TG was without effect on steady-state [Ca²⁺]ᵢ during continuous perifusion with 4.8 and 10 mM K⁺, but it affected [Ca²⁺]ᵢ oscillations during K⁺ pulses. The amplitude of [Ca²⁺]ᵢ oscillations was increased from 371 ± 34 to 921 ± 13 nM, and the nadir was lowered from 276 ± 23
Ca\textsuperscript{2+} is released from the ER at the end of each oscillation (12).

Role of the ER on \([Ca^{2+}]_c\) in conditions mimicking a change of the glucose concentration from 10 to 15 mM. Membrane potential recordings of \(\beta\)-cells within an islet have shown that raising the glucose concentration of the medium from 10 to 15 mM under control conditions increases the duration of the depolarization phase without affecting the frequency of membrane potential oscillations (21). Figure 2A illustrates \([Ca^{2+}]_c\) changes in an islet in response to a similar rise of the glucose concentration. The peak of \([Ca^{2+}]_c\) oscillations was increased, and the nadir between the oscillations was slightly shifted upward. To mimic these changes in islets treated with diazoxide, the frequency of K\textsuperscript{+} pulses was not changed, but the duration of the depolarizing phase (K\textsuperscript{+}30) was increased while that of the repolarizing phase (K\textsuperscript{+}10) was decreased, both to 12 s, in accordance with the measurements of the membrane potential of islets perfused with 15 mM glucose (21). The glucose concentration was increased from 10 to 15 mM simultaneously with the change in pulse protocol. Neither in control nor in TG-pretreated islets did this protocol affect the amplitude of \([Ca^{2+}]_c\) oscillations (Table 1, lines 1 and 2). However, the nadir between the oscillations became more elevated, the difference being larger \((P < 0.05)\) in the absence of TG \((33 \pm 7 \text{nM}, n = 4)\) than in its presence \((12 \pm 4 \text{nM}, n = 5)\). Similar qualitative results were obtained in clusters of islet cells. Thus the rise of the nadir brought about by the change of the protocol was larger in control than in TG-pretreated cells \((88 \pm 14 \text{nM}, n = 4 \text{ vs. } 14 \pm 7 \text{nM}, \text{ respectively}, n = 4, P < 0.01; \text{Table 1, lines 5 and 6})\). This highlights again the role of the ER in the sustained elevation of \([Ca^{2+}]_c\) above which the oscillations of \([Ca^{2+}]_c\), occur in control \(\beta\)-cells.

Two series of experiments were then performed to test which of the two parameters, the change of the glucose concentration or the change in the pulse protocol, affected more the nadir of \([Ca^{2+}]_c\) oscillations. When the glucose concentration was increased from 10 to 15 mM while the pulse protocol remained unchanged \((8 \text{s of K}^{+}30 \text{ and } 16 \text{s of K}^{+}10)\), the nadir and the amplitude of imposed \([Ca^{2+}]_c\) oscillations did not change either in control or TG-pretreated islets (3 experiments, data not shown). In the other series of experiments, the glucose concentration of the medium was kept constant at 10 mM, but the pulse protocol \((K^{+}30/K^{+}10)\) was changed from 8/16 s to 12/12 s (Table 1, lines 3 and 4). This resulted in a significant upward shift of the average nadir, which again was larger in control than in TG-pretreated islets \((39 \pm 7 \text{nM}, n = 3 \text{ vs. } 5 \pm 2 \text{nM}, n = 3, \text{ respectively}, P < 0.05; \text{Table 1, compare lines 3 and 4})\). Similar results were obtained in clusters of islet cells, as shown by the mean traces presented in Fig. 3. Thus TG pretreatment virtually suppressed the upward shift of the nadir provoked by the increase of the depolarizing phase \((86 \pm 11 \text{nM} \text{ in control cells}, n = 3 \text{ vs. } 8 \pm 4 \text{nM in TG-pretreated cells}, n = 3, P < 0.01; \text{Table 1, lines 7 and 8})\).

**Fig. 2.** Contribution of the endoplasmic reticulum to cytosolic Ca\textsuperscript{2+} concentration \(([Ca^{2+}]_c)\) changes occurring in pancreatic islets under conditions mimicking glucose-induced \([Ca^{2+}]_c\) oscillations. A: spontaneous \([Ca^{2+}]_c\) changes induced in a control mouse islet by stepwise increases of the glucose (G) concentration in the perfusion medium. This recording is representative of results obtained in 4 different islets. B and C: mouse islets were perfused with a medium containing 250 

\(\mu\text{M} \text{Dz}\) throughout. Glucose and K\textsuperscript{+} concentrations \(([Ca^{2+}]_c)\) in the medium were changed as indicated. Protocol of depolarization was imposed \([Ca^{2+}]_c\) oscillations are shown \((B, \text{ top})\). After 1-min perfusion with 4.8 mM K\textsuperscript{+}, islets were depolarized with 30 mM K\textsuperscript{+} for 1 min. They were then submitted to 30 or 20 cycles of 8/16 s or 12/12 s of depolarization/repolarization with 30 and 10 mM K\textsuperscript{+}, respectively. The frequency of the imposed oscillations was thus kept constant \((2.5/\text{min})\). After 30 min, islets were perfused with 10 mM K\textsuperscript{+} until the end. Changes in pulse protocol at 1, 14, and 22 min coincided with the indicated change in glucose concentration. B: \([Ca^{2+}]_c\); changes in control islets; C: \([Ca^{2+}]_c\); changes in islets pretreated with 1 \(\mu\text{M} \text{thapsigargin (TG)}\) during the period of loading with fura PE-3. Traces are means of results obtained in 4 \((B)\) and 5 \((C)\) islets.

to 142 \pm 11 \text{nM}, i.e., to a similar average \([Ca^{2+}]_c\) \((115 \pm 9 \text{nM})\) to that during prolonged repolarization with 10 mM K\textsuperscript{+} (Table 1, line 6).

All of these observations indicate that the sustained \([Ca^{2+}]_c\) elevation \((\Delta)\) above which \([Ca^{2+}]_c\) oscillations occur in untreated \(\beta\)-cells is dependent on a functional ER. They are fully compatible with our suggestion that
Table 1. Effects of thapsigargin on \( \text{[Ca}^{2+}\text{]}_c \) changes induced by various protocols of depolarization

<table>
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<tr>
<th>Line no.</th>
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<th>Oscillation amplitude ( \Delta ) (Nadir - K4.8)</th>
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<td>G10 K30/K10 (8/16)</td>
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<td>186 ± 23 118 ± 19</td>
<td>179 ± 23 151 ± 24</td>
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<td>5 50 ± 6 107 ± 14</td>
<td>362 ± 52* 44 ± 7†</td>
<td>391 ± 28† 56 ± 10†</td>
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<td>3</td>
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<td>3 64 ± 11 142 ± 8</td>
<td>151 ± 19 146 ± 26</td>
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<td>5 53 ± 3 130 ± 1</td>
<td>287 ± 43* 63 ± 7</td>
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<tr>
<td>5</td>
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<td>4 112 ± 18 174 ± 29</td>
<td>371 ± 34 164 ± 8</td>
<td>337 ± 7 252 ± 11</td>
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<td>6</td>
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<td>921 ± 13* 47 ± 4†</td>
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<tr>
<td>8</td>
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<td>3 87 ± 9 126 ± 15</td>
<td>885 ± 94* 66 ± 19†</td>
<td>808 ± 12† 74 ± 15†</td>
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Values are means ± SE from n experiments. Control cells or cells preincubated with 1 μM thapsigargin (TG) during period of loading with fura PE-3 were perfused with a medium containing 250 μM dazoxodic, throughout, or 10 mM glucose, and the indicated concentration of K+. Absolute steady-state cytosolic \( \text{[Ca}^{2+}\text{]}_c \) concentration (\( \text{[Ca}^{2+}\text{]}_c \)) values correspond to initial and final periods of experiment in the presence of the indicated stable Gbc (G) and K+ concentrations. \( \text{[Ca}^{2+}\text{]}_c \), during imposed oscillations (K10/K30, at a frequency of 2.5/min) is shown as \( \Delta \) between peak and nadir of oscillation (amplitude) or between nadir and initial basal level in 4.8 mM K+. \( \text{[Ca}^{2+}\text{]}_c \) values for protocols 1 (islets) and 2 (clusters of islet cells) have been taken from experiments illustrated in Figs. 1 and 2, respectively. * P = 0.05 or † P = 0.01, respectively, vs. corresponding control (preceding line), by unpaired t-test.
slowly develops during mixed \([\text{Ca}^{2+}]_c\) oscillations in control islets also involves the ER.

**DISCUSSION**

The present study demonstrates that the ER limits the amplitude of \([\text{Ca}^{2+}]_c\) oscillations and keeps \([\text{Ca}^{2+}]_c\) elevated between oscillations when these are induced by repetitive \([\text{Ca}^{2+}]_c\) influx through voltage-dependent \([\text{Ca}^{2+}]_c\) channels in pancreatic \(\beta\)-cells. A summation of \([\text{Ca}^{2+}]_c\) transients has been reported in patch-clamped single \(\beta\)-cells submitted to short depolarization steps applied at a high frequency (\(\geq 30/\text{min}\)) to mimic rapid action potentials (2, 37). Here, the concentration of extracellular \(K^+\) was repetitively changed at a frequency of \(2.5/\text{min}\) to mimic the oscillations of the membrane potential occurring in \(\beta\)-cells within islets during stimulation with glucose. This resulted in \([\text{Ca}^{2+}]_c\) oscillations whose nadir remained well above basal \([\text{Ca}^{2+}]_c\). In other words, the imposed \([\text{Ca}^{2+}]_c\) oscillations appeared on top of a sustained elevation of \([\text{Ca}^{2+}]_c\), as do glucose-induced \([\text{Ca}^{2+}]_c\) osc-

![Clusters of islet cells](image1)

**Fig. 3.** Contribution of the endoplasmic reticulum to \([\text{Ca}^{2+}]_c\) changes occurring in clusters of islet cells during imposed depolarizations of different durations. Clusters of 10 mouse islet cells were perfused with a medium containing 250 \(\mu\text{M}\) Dz throughout. Protocol was similar to that in Fig. 2, except that the glucose (\(G\)) concentration of the medium was kept at 10 mM during application of the depolarizing pulses. A: \([\text{Ca}^{2+}]_c\) changes in control clusters. B: \([\text{Ca}^{2+}]_c\) changes in clusters pretreated with 1 \(\mu\text{M}\) TG during the period of loading with fura 2. Each trace is the mean of results obtained in 3 clusters.

Suppression of this elevation by TG identifies the ER as a major contributor to its development.

We have previously suggested that periodic influx of \(Ca^{2+}\) into \(\beta\)-cells induces parallel oscillations of the \(Ca^{2+}\) concentration in both cytosol and ER (12). Oscillations of the \(Ca^{2+}\) concentration in the ER are attributed to \(Ca^{2+}\) uptake during the upstroke of \([\text{Ca}^{2+}]_c\) oscillations, followed by \(Ca^{2+}\) release when \(Ca^{2+}\) influx stops. The sustained \([\text{Ca}^{2+}]_c\) elevation above which \([\text{Ca}^{2+}]_c\) oscillations occur would result from differences in the kinetics of uptake and release of \(Ca^{2+}\) by the ER, the uptake being much faster than the release (12). The fast kinetics of the uptake by the ER can be inferred from the observation that inactivation of the SERCA by TG nearly doubled the amplitude of the \([\text{Ca}^{2+}]_c\) rise produced by short depolarizations (3 s). The mechanism of the slow release process has not been identified, but \(Ca^{2+}\) - or inositol phosphate-induced \(Ca^{2+}\) release, two fast processes, are not involved (12). We suggest that it reflects slow leakage of...
Ca\(^{2+}\) from the ER. Although such a mechanism may seem surprising, it is fully compatible with numerous observations showing that blockade of the SERCA pump by TG in a Ca\(^{2+}\)-free medium induces a transient rise in [Ca\(^{2+}\)]\(_{c}\) by emptying of the ER (28, 33–35). Because of its slow kinetics, this passive Ca\(^{2+}\) release from the ER prolongs [Ca\(^{2+}\)]\(_{c}\) oscillations when influx of Ca\(^{2+}\) has stopped and prevents [Ca\(^{2+}\)]\(_{c}\) from returning to basal levels during fast [Ca\(^{2+}\)]\(_{c}\) oscillations like those induced by glucose.

An important contribution of this study was to show that the unexplained mixed [Ca\(^{2+}\)]\(_{c}\) oscillations observed in islets (23) and clusters of \(\beta\)-cells (25, 36) stimulated by glucose can be reproduced by trains of depolarizations interrupted by longer pauses. It is therefore tempting to ascribe them to the mixed oscil-

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**Fig. 5.** Gradual contribution of the endoplasmic reticulum to [Ca\(^{2+}\)]\(_{c}\) changes occurring in islets during imposed depolarizations of different durations. Mouse islets were perfused with a medium containing 250 \(\mu\)M Dz and 10 mM glucose (G10) throughout. Protocol of depolarization used to impose [Ca\(^{2+}\)]\(_{c}\) oscillations is shown (A, top). After 2 min of perfusion with 10 mM K\(^{+}\), islets were submitted to cycles of depolarization and repolarization with 30 and 10 mM K\(^{+}\), respectively. After 58 min, they were perfused with 30 mM K\(^{+}\) until the end. The duration of the depolarization and repolarization phases was changed by steps of 3 s after each series of 20 cycles, as shown by the expanded pulses. The frequency of the imposed oscillations was thus kept constant (2.5/min). A: [Ca\(^{2+}\)]\(_{c}\) changes in control islets; B: [Ca\(^{2+}\)]\(_{c}\) changes in islets pretreated with 1 \(\mu\)M TG during the period of loading with fura PE-3. Each trace is representative of results obtained in 4 (A) and 5 (B) islets. C: average [Ca\(^{2+}\)]\(_{c}\) at the nadir between the oscillations; D: average amplitude of [Ca\(^{2+}\)]\(_{c}\) oscillations measured during the last 5 min of each pulse protocol and plotted as a function of the percentage of the time of depolarization with 30 mM K\(^{+}\) (means \pm SE). *P < 0.05 and **P < 0.01 for the difference between control islets and islets pretreated with TG (Thapsi). Data in C and D were calculated for all experiments like those shown in A and B.

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**Fig. 6.** The endoplasmic reticulum contributes to the pattern of mixed [Ca\(^{2+}\)]\(_{c}\) oscillations in islets. A: spontaneous mixed [Ca\(^{2+}\)]\(_{c}\) oscillations induced by 10 mM glucose (G10) in a mouse islet. This recording is representative of results obtained in 15 different islets. B and C: mouse islets were perfused with a medium containing 250 \(\mu\)M Dz and 10 mM glucose throughout. The protocol of depolarization used to impose [Ca\(^{2+}\)]\(_{c}\) oscillations is shown (B, top). After 2 min of perfusion with 10 mM K\(^{+}\), islets were sequentially submitted to 6 series of 5 cycles of depolarization and repolarization with 30 and 10 mM K\(^{+}\), respectively. Between each series, islets were perfused with 10 mM K\(^{+}\). Duration of depolarization and repolarization phases (in s) is indicated above each representative pulse. The frequency of the imposed oscillations was either 2.5/min (first 3 series of pulses) or 5/min (last 3 series of pulses). B: [Ca\(^{2+}\)]\(_{c}\) changes in control islets; C: [Ca\(^{2+}\)]\(_{c}\) changes in islets pretreated with 1 \(\mu\)M TG during the period of loading with fura PE-3. Each trace is the mean of results obtained in 3 (B) and 4 (C) islets.
lations of the membrane potential that occur in some β-cells during glucose stimulation (8, 24, 36), and to the activity of the ER that permits summation of these [Ca²⁺]ᵣ signals. This does not imply that summation of fast oscillations is the only mechanism to generate slow oscillations. Indeed, slow oscillations can occur in the absence of fast oscillations, probably as a result of long bursts of action potentials (13). The mechanisms that we propose to explain the mixed pattern of [Ca²⁺]ᵣ oscillations are thus different from those suggested by Liu et al. (29). From studies using ob/ob mouse islets, these authors also emphasize the role of depolarization-induced Ca²⁺ influx, but they attribute the mixed pattern of slow and superimposed fast oscillations to separate cell populations, each showing one type of response. They also invoke spiking Ca²⁺ release from the ER in some cells to coordinate the fast [Ca²⁺]ᵣ oscillations. We do not believe that this proposal is applicable to islets from normal mice for three reasons. First, [Ca²⁺]ᵣ transients resulting from Ca²⁺ mobilization in response to glucose and cAMP-producing agents are much more common in β-cells from these leptin-deficient ob/ob mice than in normal mice (1). Second, experiments using two intracellular microelectrodes in the same islet have shown that the electrical activity is similar and synchronized in all β-cells from normal mice (11, 31). Third, mixed [Ca²⁺]ᵣ oscillations have been observed in clusters of 2–20 cells (25, 36) and even in single β-cells (26) from normal mice.

A rise in the glucose concentration lengthens the depolarized, active phase and shortens the repolarized, silent phase of membrane potential oscillations without affecting their frequency (21). It also causes an upward shift of the nadir between [Ca²⁺]ᵣ oscillations (i.e., prevents [Ca²⁺]ᵣ from intermittently returning to basal levels) (14, 38). A similar shift occurs when islets are subjected to a depolarizing protocol that mimics the changes in membrane potential normally produced by raising glucose from 10 to 15 mM. This elevation of the nadir cannot be explained by too short a repolarization for extrusion of all Ca²⁺ from the cytosol after Ca²⁺ influx has stopped, or by insufficient repolarization for abolition of Ca²⁺ influx. Thus, in TG-pretreated islets, short periods of repolarizations (9 s) are usually sufficient for restoration of low [Ca²⁺]ᵣ during the nadir, although the kinetics of the changes in membrane potential induced by high and low K⁺ solutions are not different from those in control islets (12).

When the external Ca²⁺ concentration was raised stepwise without changing the pulse protocol, the nadir between [Ca²⁺]ᵣ oscillations was progressively shifted to higher values in control but not in TG-pretreated islets. Our interpretation is that the ER takes up more Ca²⁺ when Ca²⁺ influx is larger at higher extracellular Ca²⁺ and therefore releases more Ca²⁺ during the repolarization intervals. This is consistent with the observations that high K⁺ raises the Ca²⁺ concentration in the ER of insulin-secreting rat insulinoma cells (30) and ob/ob β-cells (40), and that the filling state of the ER is directly proportional to the steady-state [Ca²⁺]ᵣ level (7, 9, 12, 34). We suggest that the same mechanism explains the upward shift of the nadir observed when the fraction of depolarization time increases, for instance when the glucose concentration of the medium is raised from 10 to 15 mM. Under these conditions, Ca²⁺ pumping into the ER probably results from a direct activation of SERCA by Ca²⁺. An increase in cell metabolism is unlikely to be involved, because the change in glucose concentration (10 to 15 mM) did not affect the nadir of [Ca²⁺]ᵣ oscillations when the pulse protocol was kept constant. This is not surprising, because glucose-induced filling of the ER is nearly maximal at 10 mM (40).

The strong buffering capacity of the ER is illustrated by the much smaller amplitude of [Ca²⁺]ᵣ oscillations in control than in TG-pretreated islets. Because voltage-dependent Ca²⁺ currents are not affected by TG (12), this difference cannot be ascribed to greater Ca²⁺ influx. Whereas the buffering properties of the ER are evident during intermittent Ca²⁺ influx and [Ca²⁺]ᵣ oscillations, they are not during sustained Ca²⁺ influx, probably because the Ca²⁺ concentration within the ER is then in equilibrium with a steady-state [Ca²⁺]ᵣ ensured by extrusion mechanisms in the plasma membrane.

In conclusion, our study demonstrates that the ER has a major role in the control of [Ca²⁺]ᵣ oscillations induced by repetitive Ca²⁺ influx through voltage-dependent Ca²⁺ channels. The ER limits the amplitude of [Ca²⁺]ᵣ oscillations by a fast uptake and a slow release of the ion. This slow release keeps the nadir of regular [Ca²⁺]ᵣ oscillations elevated (higher than basal [Ca²⁺]ᵣ) or gradually sums the Ca²⁺ signals to result in mixed [Ca²⁺]ᵣ oscillations. This buffering effect of the ER with cycles of Ca²⁺ uptake and release could serve several functions. Prolongation of [Ca²⁺]ᵣ elevation between oscillations might recruit insulin secretory granules into a pool from which they can be released once [Ca²⁺]ᵣ is high enough to promote exocytosis, e.g., during the next [Ca²⁺]ᵣ oscillation (17). Pumping of Ca²⁺ into the ER could prevent [Ca²⁺]ᵣ from reaching too high levels that might be cytotoxic (27) and refill the organelle in which Ca²⁺ affects processes such as protein synthesis or maturation. Thus the proteolytic processing of proinsulin within the ER involves specific Ca²⁺-dependent maturation steps, and depletion of intracellular Ca²⁺ pools impairs proinsulin conversion and intracellular transport (19). The Ca²⁺ content of the ER might also modulate cell survival, because depletion of ER Ca²⁺ stores triggers apoptosis (46). Finally, because the Ca²⁺ content of the ER (45) influences the membrane potential, the periodic emptying and refilling of the ER during [Ca²⁺]ᵣ oscillations may participate in feedback mechanisms controlling membrane potential oscillations (12).

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