

Uptake and Release of Ca^{2+} by the Endoplasmic Reticulum Contribute to the Oscillations of the Cytosolic Ca^{2+} Concentration Triggered by Ca^{2+} Influx in the Electrically Excitable Pancreatic B-cell*

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The role of intracellular Ca^{2+} pools in oscillations of the cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_c$) triggered by Ca^{2+} influx was investigated in mouse pancreatic B-cells. $[\text{Ca}^{2+}]_c$ oscillations occurring spontaneously during glucose stimulation or repetitively induced by pulses of high K^+ (in the presence of diazoxide) were characterized by a descending phase in two components. A rapid decrease in $[\text{Ca}^{2+}]_c$ coincided with closure of voltage-dependent Ca^{2+} channels and was followed by a slower phase independent of Ca^{2+} influx. Blocking the SERCA pump with thapsigargin or cyclopiazonic acid accelerated the rising phase of $[\text{Ca}^{2+}]_c$ oscillations and increased their amplitude, which suggests that the endoplasmic reticulum (ER) rapidly takes up Ca^{2+} . It also suppressed the slow $[\text{Ca}^{2+}]_c$ recovery phase, which indicates that this phase corresponds to the slow release of Ca^{2+} that was taken up by the ER during the upstroke of the $[\text{Ca}^{2+}]_c$ transient. Glucose promoted the buffering capacity of the ER and amplified the slow $[\text{Ca}^{2+}]_c$ recovery phase. The slow phase induced by high K^+ pulses was not affected by modulators of Ca^{2+} - or inositol 1,4,5-trisphosphate-induced Ca^{2+} release, did not involve a depolarization-induced Ca^{2+} release, and was also observed at the end of a rapid rise in $[\text{Ca}^{2+}]_c$ triggered from caged Ca^{2+} . It is attributed to passive leakage of Ca^{2+} from the ER. We suggest that the ER displays oscillations of the Ca^{2+} concentration ($[\text{Ca}^{2+}]_{\text{ER}}$) concomitant and parallel to $[\text{Ca}^{2+}]_c$. The observation that thapsigargin depolarizes the membrane of B-cells supports the proposal that the degree of Ca^{2+} filling of the ER modulates the membrane potential. Therefore, $[\text{Ca}^{2+}]_{\text{ER}}$ oscillations occurring during glucose stimulation are likely to influence the bursting behavior of B-cells and eventually $[\text{Ca}^{2+}]_c$ oscillations.

The physiological response to a stimulus is often transduced by oscillations of the cytosolic free Ca^{2+} concentration ($[\text{Ca}^{2+}]_c$). In electrically nonexcitable cells, $[\text{Ca}^{2+}]_c$ oscillations are mainly driven by antiparallel changes of the Ca^{2+} concentration within intracellular Ca^{2+} stores. In electrically excitable cells, $[\text{Ca}^{2+}]_c$ oscillations are generally produced by intermittent influx of Ca^{2+} through voltage-dependent Ca^{2+} channels in the plasma membrane. In some of these cells, such as muscle cells and neurons, release of Ca^{2+} from intracellular stores can also contribute to the changes in $[\text{Ca}^{2+}]_c$ (1, 2).

The insulin-secreting pancreatic B-cell is electrically excitable. Its main physiological stimulus, glucose, triggers insulin secretion by increasing $[\text{Ca}^{2+}]_c$ through the following steps. Acceleration of glucose metabolism increases the ATP/ADP ratio, which closes ATP-sensitive K^+ channels (K_{ATP} channels) in the plasma membrane (3). This closure decreases the K^+ conductance, which allows a yet unknown current to depolarize the plasma membrane, leading to opening of voltage-dependent Ca^{2+} channels, stimulation of Ca^{2+} influx, and eventually a rise in $[\text{Ca}^{2+}]_c$. In the presence of 10–15 mM glucose, B-cells display $[\text{Ca}^{2+}]_c$ oscillations that result mainly from intermittent Ca^{2+} influx (4, 5). However, it has been speculated that Ca^{2+} - or inositol 1,4,5-trisphosphate (IP_3)-induced Ca^{2+} release might contribute to each $[\text{Ca}^{2+}]_c$ oscillation induced by glucose (6–10).

The aim of the present study was to investigate the possible role of intracellular Ca^{2+} stores in $[\text{Ca}^{2+}]_c$ oscillations induced by Ca^{2+} influx in normal pancreatic B-cells. Strategies using targeted Ca^{2+} -sensitive proteins (11, 12) or trapped fluorescent low-affinity Ca^{2+} indicators (13–15) have recently been developed to measure directly the free Ca^{2+} concentration within intracellular organelles. However, these techniques suffer from drawbacks such as difficult transfection procedures of photo-proteins, very low light emission and Ca^{2+} -induced degradation of aequorin, and contamination of the trapped fluorescence of low-affinity Ca^{2+} indicators by the cytosolic signal, which severely limit their use in intact primary cells. We, therefore, used the classical technique of Ca^{2+} measurement within the cytosol, which is not invasive and is applicable to single or electrically coupled B-cells. The results demonstrate that $[\text{Ca}^{2+}]_c$ oscillations occurring spontaneously during stimulation by glucose, or artificially induced by pulses of high K^+ , are accompanied by cycles of rapid uptake and subsequent slow

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¹ The abbreviations used are: IP_3 , inositol 1,4,5-trisphosphate; ACh, acetylcholine; CPA, cyclopiazonic acid; ER, endoplasmic reticulum; I_{SOC} , store-operated current; PMCA, plasma membrane Ca^{2+} -ATPase; SERCA, sarco-endoplasmic reticulum Ca^{2+} -ATPase; TG, thapsigargin.

release of Ca²⁺ by the endoplasmic reticulum (ER). Thapsigargin-sensitive Ca²⁺-ATPases (SERCA pumps) are responsible for the sequestration process during the upstroke of the [Ca²⁺]_c transient, whereas the subsequent phase of release does not involve depolarization-, Ca²⁺- or IP₃-mediated processes and likely results from leakage from the ER. This suggests that the Ca²⁺ concentration within the endoplasmic reticulum ([Ca²⁺]_{ER}) oscillates. As the filling state in Ca²⁺ of the ER may modulate the membrane potential of B-cells (16), it is possible that [Ca²⁺]_{ER} oscillations play a role in the control of the oscillations of the membrane potential.

EXPERIMENTAL PROCEDURES

Solutions and Drugs

Except for patch-clamp measurements and the experiments illustrated in Fig. 4D (see below), the medium used was a bicarbonate-buffered solution that contained 120 mM NaCl, 4.8 mM KCl, 0.5–10 mM CaCl₂, 1.2 mM MgCl₂, 24 mM NaHCO₃ and 0–20 mM glucose as indicated. When the concentration of KCl was increased, that of NaCl was decreased accordingly to keep the osmolarity of the medium unchanged. Ca²⁺-free solutions were prepared by substituting MgCl₂ for CaCl₂ and were supplemented with 0.5 or 2 mM EGTA as indicated in the legends to Figs. 2, 3, and 5.

In the experiments illustrated in Fig. 4D, it was important to minimize changes in the activity of the Na⁺/Ca²⁺ exchange between solutions containing various K⁺ concentrations. Therefore, KCl was not replaced with NaCl but with choline chloride to keep a similar Na⁺ concentration in all solutions. The low K⁺ solution contained: 79.8 mM NaCl, 4.8 mM KCl, 40.2 mM choline chloride, 2.5 mM CaCl₂, 1.2 mM MgCl₂, 24 mM NaHCO₃, and 0.01 mM atropine, which prevented activation of muscarinic receptors by choline. The solutions containing higher K⁺ concentrations were prepared by substituting KCl for choline chloride.

All solutions were gassed with O₂/CO₂ (94:6) to maintain a pH of 7.4 at 37 °C. Except for electrophysiological recordings, they were supplemented with 1 mg/ml bovine serum albumin (fraction V; Roche Molecular Biochemicals).

Thapsigargin was obtained from Sigma or from Alomone Laboratories (Jerusalem, Israel). Ryanodine was from RBI (Natick, MA) or from Alomone Laboratories, diazoxide was from Schering-Plough Avondale (Rathdrum, Ireland), caffeine was from Merck A.G. (Darmstadt Germany), and ruthenium red was from Alexis Corp. (San Diego, CA). All other chemicals were from Sigma.

Preparation of Islets and Cells

All experiments were performed with tissue from fed female NMRI mice (25–30 g). Pancreatic islets were isolated aseptically after collagenase digestion of the pancreas, and when needed, they were dispersed into cells as described previously (17). Cells were allowed to attach to 22-mm circular coverslips and cultured for 2–3 days. Intact islets were maintained in culture for 1–3 days. When the membrane potential of B-cells was to be measured with an intracellular microelectrode, the islets were allowed to attach to the coverslip by a culture period of at least 2 days. The culture medium was RPMI 1640 medium containing 10 mM glucose, 10% heat-inactivated fetal calf serum, 100 IU/ml penicillin, and 100 μg/ml streptomycin.

Measurements of [Ca²⁺]_i

Cultured islets were loaded with 2 μM fura-PE3/AM (Teflabs, Austin, TX) for 90–120 min at 37 °C in a bicarbonate-buffered solution containing 10 mM glucose. Cultured cells were loaded with 1 μM fura-2/AM (Molecular Probes, Eugene, OR) for 60 min in a similar bicarbonate-buffered medium. The tissue was then transferred into a temperature-controlled (37 °C) perfusion chamber of ~1 ml (Intracell, Royston, Herts, United Kingdom) with a bottom made of a glass coverslip and mounted on the stage of an inverted microscope. The flow rate of the perfusion was approximately 2 ml/min. When rapid exchange of solutions was required, a ~250-μl chamber was used and solutions were changed by Iso-Latch valves (Parker Hannifin, Fairfield, NY). [Ca²⁺]_i was directly measured in cells attached to the coverslip or in islets held in place close to the coverslip by gentle suction with a glass micropipette. In some experiments, cultured cells were pressure-injected with an 5242 Eppendorf microinjector (Hamburg, Germany). The injected solution contained either 6–10 mM fura-2 K⁺ salt or 10 mM fura-dextran K⁺ salt (molecular weight, 3000) (Molecular Probes) dissolved in H₂O,

and it was supplemented or not with test substances. The techniques used to monitor [Ca²⁺]_c have been described previously (4).

Flash-Photolysis

Clusters of B-cells were incubated with 5 μM nitrophenyl-EGTA AM and 1.5 μM fura-2 AM (Molecular Probes) for 60 min at 37 °C. Photolysis of nitrophenyl-EGTA was performed by two or three consecutive 1-ms UV flashes of 240 J (Xenon flashlamp system XF-10, Hi-Tech, Hamburg, Germany).

Electrophysiology

Membrane Potential Recordings—The islets were mounted in a perfusion chamber (7 ml/min at 37 °C) following attachment to glass coverslips. The membrane potential of a single cell within the islet was continuously measured with a high resistance microelectrode.

Patch-Clamp Recordings—Voltage-clamp experiments were performed on single B-cells using the perforated patch-whole cell configuration and an EPC-7 patch-clamp amplifier (List Elektronik, Darmstadt, Germany). The holding potential was –70 mV, and the cells were submitted either to 100-ms depolarizations to 0 mV or to bursts of 100-ms depolarizations (2 Hz) from –50 mV to –10 mV for 12 s. The associated changes in [Ca²⁺]_i were measured using an IonOptix fluorescence imaging system (IonOptix, Inc., Milton, MA). The extracellular solution contained 138 mM NaCl, 5.6 mM KCl, 1.2 mM MgCl₂, 2.6 mM CaCl₂, 5 mM HEPES (pH 7.4 with NaOH), and 10 mM glucose. The pipette solution contained 76 mM Cs₂SO₄, 10 mM NaCl, 10 mM KCl, 1 mM MgCl₂, and 5 mM HEPES (pH 7.35 with CsOH). Electrical contact with the cell interior was established by adding 0.24 mg/ml amphotericin B to the pipette solution, and the voltage-clamp was considered satisfactory when the series conductance (G_{series}) was >35–40 nano Siemens. All experiments were performed at 33 °C, and the zero-current potential of the pipette was adjusted with the pipette in the bath solution.

Presentation of Results

The experiments are illustrated by recordings that are averaged or representative traces of results obtained with the indicated number of cells or islets from at least three different cultures. The statistical significance of differences between means was assessed by unpaired Student's *t* test.

RESULTS

[Ca²⁺]_c Oscillations Induced by Glucose Are Followed by Ca²⁺ Release from the ER—B-cells within intact islets display a rhythmic electrical activity when perfused with a medium containing an insulin-releasing glucose concentration (10 mM) and 10 mM Ca²⁺ (Fig. 1A). These bursts of electrical activity consist of sharp depolarizing waves of the membrane potential with superimposed spikes reflecting Ca²⁺ influx through voltage-dependent Ca²⁺ channels (18). Under these conditions, [Ca²⁺]_c also oscillates, but, in contrast to the fast, monophasic repolarization of the oscillations of membrane potential, the descending phase of each Ca²⁺ oscillation clearly displays two components (Fig. 1B). Whereas the initial fast one appears to coincide with the closure of voltage-dependent Ca²⁺ channels following rapid repolarization of the plasma membrane, the second, much slower phase appears to occur during the repolarized intervals.

Previous experiments have shown that intracellular Ca²⁺ stores of whole islets are efficiently emptied by thapsigargin (TG), a specific inhibitor of the SERCA pump (19), but that this emptying requires preincubation of the islets with the drug (17). In islets pretreated with 1 μM TG, the amplitude of [Ca²⁺]_c oscillations was much larger than in control islets, and the descending phase of each [Ca²⁺]_c oscillation was surprisingly very fast with no slow second phase (Fig. 1C). This suggests that the slow phase observed in control islets results from a release of Ca²⁺ from the ER, rather than from a slow Ca²⁺ extrusion from the cytosol.

The effect of intracellular Ca²⁺ store depletion on [Ca²⁺]_c oscillations was also investigated in clusters of islet cells, a preparation in which the SERCA pump can be blocked by an

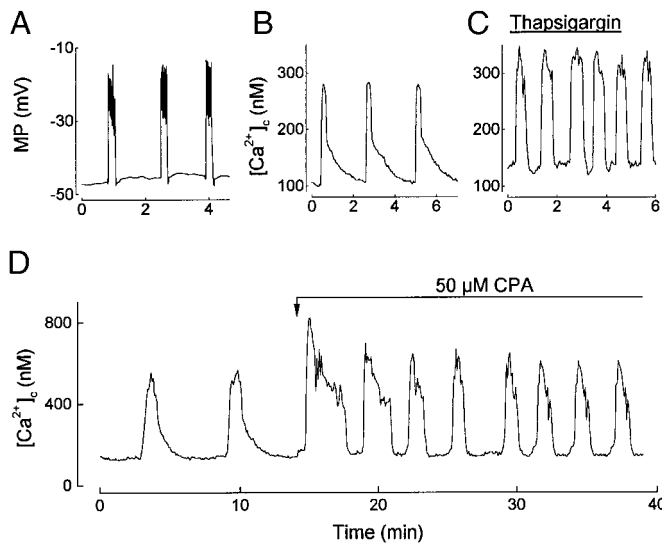


FIG. 1. Glucose induces sharp oscillations of the membrane potential (A), whereas the accompanying oscillations in $[Ca^{2+}]_c$ (B–D) display a slow recovery phase that is abolished by SERCA pump inhibitors in pancreatic B-cells. A–C, whole islets were perfused with a medium containing 10 mM glucose and 10 mM Ca^{2+} throughout. In the experiment shown in C, the medium used for the loading with fura-PE3 was supplemented with 1 μ M TG. D, a cluster of islet cells was perfused with 15 mM glucose and 2.5 mM Ca^{2+} throughout. CPA was added to the perfusion medium when indicated. The traces are representative of results obtained in 13 (A), 36 (B), and 18 (C) islets and 15 clusters of cells (D).

acute addition of TG or cyclopiazonic acid (CPA). CPA is an inhibitor structurally unrelated to TG (19) and has also been shown to empty the ER of Ca^{2+} in pancreatic B-cells (20). In the presence of 15 mM glucose and 2.5 mM Ca^{2+} , $[Ca^{2+}]_c$ oscillated slowly and regularly (Fig. 1D). Addition of 50 μ M CPA to the medium accelerated the oscillations, which increased in amplitude and frequency and became sharper mainly because of the disappearance of the slow recovery phase. Similar results were obtained in clusters of islet cells treated by TG (not shown).

Ca^{2+} Release from the ER Can be Detected after Pulses of High K^+ —In this series of experiments, glucose-induced $[Ca^{2+}]_c$ oscillations were inhibited by diazoxide, which, by opening K_{ATP} channels, clamps the membrane potential at a hyperpolarized level. $[Ca^{2+}]_c$ oscillations were then re-induced by rhythmically depolarizing the plasma membrane with high K^+ .

Raising the K^+ concentration of the perfusion medium from 4.8 to 45 mM rapidly depolarized the plasma membrane from -70 ± 2 mV to -22 ± 3 mV in control islets ($n = 4$; Fig. 2A, dotted line). The amplitude of this depolarization was not affected by TG pretreatment of the islets (-71 ± 3 to -22 ± 3 mV, $n = 4$). The time required to clamp the plasma membrane at a new, stable potential was also similar in both groups, as follows. Controls: 31 ± 2 s from 4.8 to 45 mM K^+ ($t_{1/2} = 4 \pm 0$ s) and 34 ± 1 s from 45 to 4.8 mM K^+ ($t_{1/2} = 5.7 \pm 0.5$ s), $n = 4$; TG-treated islets: 31 ± 1 s from 4.8 to 45 mM K^+ ($t_{1/2} = 4.5 \pm 0.3$ s) and 34 ± 1 s from 45 to 4.8 mM K^+ ($t_{1/2} = 5.7 \pm 0.5$ s), $n = 4$.

In control islets, high K^+ pulses (for 30 s) induced $[Ca^{2+}]_c$ oscillations characterized by a descending phase that displayed an initial fast component concomitant with rapid repolarization of the plasma membrane, followed by a slow decline (Fig. 2A, solid line). In TG-pretreated islets, $[Ca^{2+}]_c$ oscillations were higher than in control islets (467 ± 21 versus 352 ± 11 nM, $n = 10$, $p < 0.01$) and devoid of a slow recovery phase. Similar results were obtained after pretreatment of the islets with 50 μ M CPA.

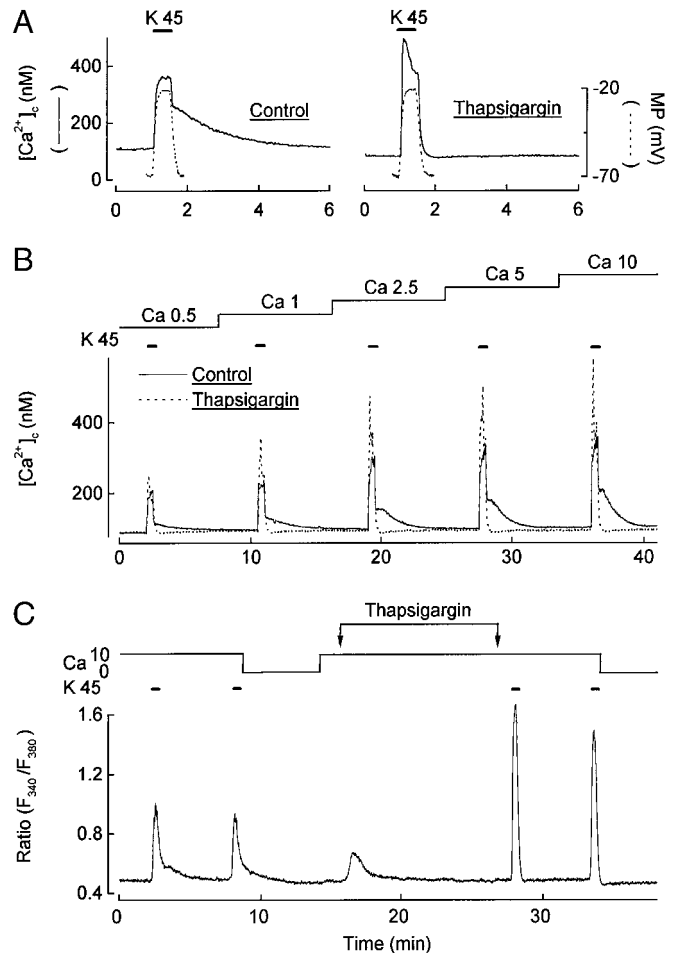


FIG. 2. Pulses of 45 mM K^+ induce $[Ca^{2+}]_c$ oscillations with a slow recovery phase that is prevented by SERCA pump inhibition in pancreatic B-cells. The medium contained 10 mM glucose and 250 μ M diazoxide in all experiments. 30-s pulses of 45 mM K^+ were applied as shown by bars. The Ca^{2+} concentration of the perfusion medium was either 10 mM throughout (A) or was changed as indicated (B–C). A and B, whole islets were used. In A, solid lines show changes in $[Ca^{2+}]_c$, whereas the dotted lines illustrate associated changes of the membrane potential recorded in separate islets. For the traces labeled thapsigargin in A and B, the islets were incubated with 1 μ M TG during the loading procedure with fura-PE3 ($[Ca^{2+}]_c$ measurements) or during a 90–120-min preincubation in the culture medium prior to the experiments (membrane potential measurements). C, a single pancreatic B-cell was used; it was injected with fura-2 dextran. TG was applied when indicated. Ca^{2+} -free solutions were supplemented with 2 mM EGTA. The traces are representative of results obtained in 12 (A) ($[Ca^{2+}]_c$), 4 (A) (MP), and 7 (B) islets and 4 single cells (C).

The effects of TG on voltage-dependent Ca^{2+} current were evaluated in single B-cells using the perforated patch configuration. Under control conditions, a 100-ms voltage-step from -70 to 0 mV elicited a peak Ca^{2+} current of 52 ± 6 pA ($n = 8$) that was not significantly affected by a 5-min exposure to 1 μ M TG (48 ± 3 pA). The integrated whole-cell Ca^{2+} current was similarly unaffected by TG (data not shown). This excludes the possibility that the larger rise in $[Ca^{2+}]_c$ induced by high K^+ in TG-treated islets results from an increased Ca^{2+} current.

We also verified that the slow $[Ca^{2+}]_c$ recovery is not a peculiarity observed only in a medium containing 10 mM Ca^{2+} . To this end, 30-s pulses of 45 mM K^+ were applied in the presence of various concentrations of external Ca^{2+} (0.5–10 mM) (Fig. 2B). The amplitude of the resulting $[Ca^{2+}]_c$ peaks clearly depended on the Ca^{2+} concentration of the medium, but a slow decaying phase, prevented by TG pretreatment, was observed at all external Ca^{2+} concentrations tested. The observation that TG suppresses the slow recovery after $[Ca^{2+}]_c$ os-

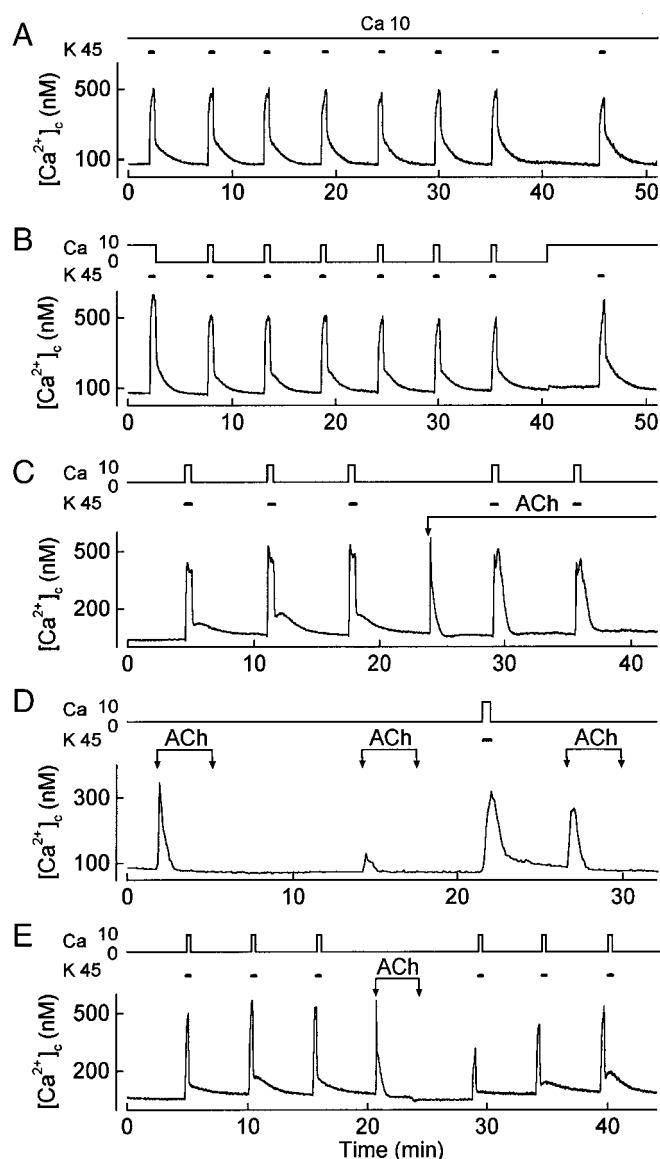


FIG. 3. Ca^{2+} is rapidly taken up by the ER during the upstroke of each $[Ca^{2+}]_c$ oscillation and is released at the end of each oscillation. All solutions contained 10 mM glucose and 250 μ M diazoxide. The Ca^{2+} concentration of the perfusion medium was changed when indicated. Ca^{2+} -free solutions were supplemented with 2 mM EGTA, 100 μ M ACh and 30-s (A–D) or 20-s (E) pulses of 45 mM K^+ were applied when indicated. The traces are representative of results obtained in 11 (A), 12 (B), 4 (C), 8 (D), and 4 (E) islets.

cillations of various amplitude also excludes the possibility that TG might increase the rate of Ca^{2+} extrusion from the cytosol due to a high Ca^{2+} signal.

The slow $[Ca^{2+}]_c$ recovery phase could be artifactual and reflect changes in the Ca^{2+} concentration within the ER if fura-PE3 is compartmentalized. To exclude this possibility, single B-cells were microinjected with fura-dextran, a Ca^{2+} probe that is exclusively localized in the cytosol (21). High K^+ pulses induced $[Ca^{2+}]_c$ oscillations with a slow recovery phase (Fig. 2C). Addition of TG to the medium induced a transient increase in $[Ca^{2+}]_c$ reflecting intracellular Ca^{2+} pool emptying. Subsequent depolarization by pulses of high K^+ triggered $[Ca^{2+}]_c$ oscillations that were of much larger amplitude than before addition of the SERCA pump inhibitor and that lacked a slow $[Ca^{2+}]_c$ recovery phase. These observations strongly support the conclusion that the slow decaying $[Ca^{2+}]_c$ phase results from release of Ca^{2+} from the ER.

Characteristics and Kinetics of Ca^{2+} Exchanges between the ER and the Cytosol—Application of high K^+ pulses every 5 min triggered a train of $[Ca^{2+}]_c$ oscillations with a slow decaying phase, which indicates that the phenomenon is not a transient one (Fig. 3A). The experiments depicted in Fig. 3B were designed to explore the temporal requirements for refilling the intracellular Ca^{2+} stores responsible for the slow decay in $[Ca^{2+}]_c$. The islets were repetitively depolarized by 30-s pulses of high K^+ . Extracellular Ca^{2+} (10 mM) was present before and during the depolarization (first and last pulses) or only during the depolarization (second to seventh pulses). The slow recovery phase was present and not attenuated by Ca^{2+} omission during the repolarization phases (compare Fig. 3, A and B). This shows first that it does not result from Ca^{2+} influx, and second that Ca^{2+} entry during depolarization is sufficient to refill the pools from which Ca^{2+} is slowly released.

However, no slow recovery phase was observed when high K^+ pulses were applied in the continuous presence of acetylcholine (ACh), a potent IP_3 -producing agent in pancreatic B-cells (Fig. 3C). This is likely due to the fact that Ca^{2+} cannot accumulate into the ER because it immediately exits from the ER into the cytosol through IP_3 receptors that are maintained opened by the continuous presence of ACh.

The ability of the ER to take up Ca^{2+} rapidly was next tested (Fig. 3D). Islets perfused with a Ca^{2+} -free medium were submitted to three pulses of 100 μ M ACh applied at 12.5-min intervals. A 30-s pulse of high K^+ /high Ca^{2+} was applied between the second and the third pulses of ACh. Whereas the first application of ACh triggered a large $[Ca^{2+}]_c$ peak, the second one induced only a small rise in $[Ca^{2+}]_c$ suggesting that intracellular Ca^{2+} stores were nearly completely emptied already by the first application of ACh. However, the third application of ACh in a Ca^{2+} -free medium after the short pulse with high K^+ /high Ca^{2+} induced a transient rise in $[Ca^{2+}]_c$ that was much larger than that seen after the second application of ACh. This indicates further that intracellular Ca^{2+} pools rapidly refill during the large $[Ca^{2+}]_c$ rises triggered by high K^+ pulses.

If the slow recovery phase reflects release of Ca^{2+} from the ER, its characteristics should depend on the filling state of the ER. This was tested by emptying the ER with ACh between two series of 3 pulses of high K^+ /high Ca^{2+} of 20 s duration (Fig. 3E). The first three $[Ca^{2+}]_c$ oscillations were all characterized by a slow recovery phase. In contrast, the first two oscillations following intracellular Ca^{2+} pool depletion by ACh were of lower amplitude and displayed a much smaller slow recovery phase than before ACh application. Because the pulses were of constant duration, the lower amplitude of $[Ca^{2+}]_c$ oscillations post-ACh is unlikely to result from a decreased Ca^{2+} influx. It may rather be explained by a more avid sequestration of Ca^{2+} into an emptied than into a filled ER. Because the first high K^+ /high Ca^{2+} pulse did not carry enough Ca^{2+} to fully refill the ER, no slow recovery phase could be seen, and three pulses were needed to refill the ER enough to see a slow recovery phase of an amplitude similar to that observed at the end of the first series of $[Ca^{2+}]_c$ oscillations. These data demonstrate that the buffering capacity of the ER permits a rapid control of $[Ca^{2+}]_c$ and that its ability to release Ca^{2+} is affected by its filling state.

Comparison of the $[Ca^{2+}]_c$ changes induced by a pulse of high K^+ in control and TG-treated islets permits estimation of the kinetics of Ca^{2+} uptake and release from the ER (Fig. 4A). After normalization of resting $[Ca^{2+}]_c$ before each $[Ca^{2+}]_c$ oscillation the averaged $[Ca^{2+}]_c$ oscillation of TG-treated islets was subtracted from the averaged $[Ca^{2+}]_c$ oscillation of control

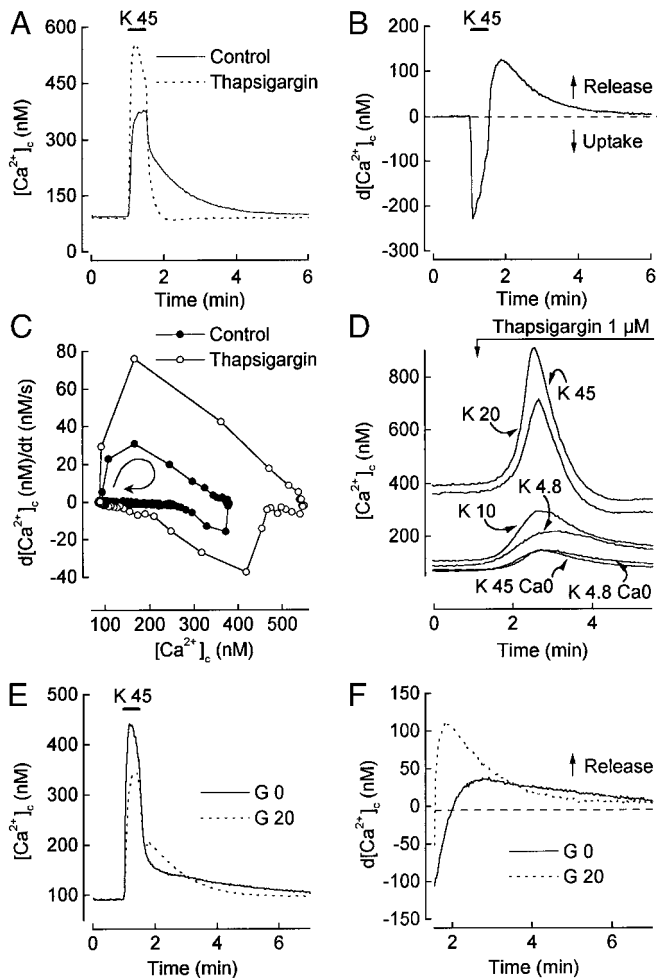


FIG. 4. A and B, kinetics of uptake and release of Ca^{2+} by the ER during and after high K^+ -induced $[Ca^{2+}]_c$ oscillations. All solutions contained 10 mM Ca^{2+} , 10 mM glucose and 250 μ M diazoxide throughout. A 30-s pulse of 45 mM K^+ was applied when indicated by the bar. A illustrates averaged traces from 10 $[Ca^{2+}]_c$ oscillations recorded in control islets (solid line) or islets treated with 1 μ M TG during the period of loading with fura-PE3 (dotted line). The trace in B was obtained by subtracting the average TG trace from the average control trace. C, rate of $[Ca^{2+}]_c$ changes during the $[Ca^{2+}]_c$ oscillations. D, the Ca^{2+} concentration of the ER depends on $[Ca^{2+}]_c$. Clusters of islet cells were perfused with a medium containing 10 mM glucose, 250 μ M diazoxide, and various concentrations of K^+ (4.8 to 45 mM). The Ca^{2+} concentration of the perfusion medium was 2.5 mM except in two sets of experiments for which the medium was a Ca^{2+} -free medium supplemented with 500 μ M EGTA (Ca0). The Na^+ concentration was kept constant between the different media tested (see under "Experimental Procedures"). TG was added as indicated. The traces are representative of results obtained in 30–57 clusters of cells. E and F, glucose buffers the $[Ca^{2+}]_c$ rise during a pulse of high K^+ and enhances the amplitude of the subsequent slow $[Ca^{2+}]_c$ recovery phase. All solutions contained 10 mM Ca^{2+} and 250 μ M diazoxide throughout, and no glucose (G 0) or 20 mM of the sugar (G 20). A 30-s pulse of 45 mM K^+ was applied when indicated by the bar. E illustrates averaged traces from $[Ca^{2+}]_c$ oscillations recorded in control islets perfused without glucose ($n = 11$) or with 20 mM of the sugar ($n = 8$). The traces in F were obtained by subtracting, at each glucose concentration, the average TG trace (obtained in islets preincubated with 1 μ M TG during the period of time they were loaded with fura-PE3) from the average control trace. Only the fragment of the traces reflecting release of Ca^{2+} is represented.

islets (Fig. 4B). The downward deflection of the curve reflects Ca^{2+} uptake by the ER, whereas the upward deflection reflects Ca^{2+} release from the ER. This shows that the uptake is very

fast, whereas the release is comparably slow and lasts several minutes.

The role of the ER during the whole $[Ca^{2+}]_c$ oscillation is best demonstrated by the comparison of the rates of $[Ca^{2+}]_c$ changes as a function of $[Ca^{2+}]_c$ in control and TG-treated islets (Fig. 4C). It clearly shows that the ER strongly buffers the rate of $[Ca^{2+}]_c$ changes during the whole $[Ca^{2+}]_c$ oscillation, thereby preventing any abrupt large change in $[Ca^{2+}]_c$.

Modulation of Ca^{2+} Exchanges between the ER and the Cytosol—The above results suggest that the amount of Ca^{2+} that is taken up by the ER is directly proportional to $[Ca^{2+}]_c$. This was indirectly verified by measuring the amplitude of the $[Ca^{2+}]_c$ peak that occurred upon addition of TG to clusters of cells in which $[Ca^{2+}]_c$ was clamped artificially at different levels with various concentrations of K^+ (4.8–45 mM). The amplitude of the $[Ca^{2+}]_c$ peak directly depended on the steady-state level of $[Ca^{2+}]_c$ before TG addition (Fig. 4D), suggesting that the Ca^{2+} loading of the ER is directly proportional to the level of $[Ca^{2+}]_c$. This did not result from a K^+ effect, as the amplitude of the rise in $[Ca^{2+}]_c$ was similar in clusters perfused with a Ca^{2+} -free medium containing 4.8 or 45 mM K^+ . The large rise in $[Ca^{2+}]_c$ produced by TG in the presence of high K^+ and Ca^{2+} is in agreement with the large slow $[Ca^{2+}]_c$ decay observed after depolarizing pulses with high K^+ .

The effect of glucose was also tested. 30-s pulses of high K^+ induced a larger $[Ca^{2+}]_c$ rise in the absence of glucose than in the presence of 20 mM glucose (Fig. 4E). By contrast, the slow $[Ca^{2+}]_c$ recovery phase was more pronounced in a glucose-containing than in a glucose-free medium. It was prevented by TG pretreatment (not shown). To estimate the amplitude and the kinetics of Ca^{2+} release from the ER in glucose-containing and glucose-free medium, the averaged $[Ca^{2+}]_c$ oscillation of TG-treated islets was subtracted from the averaged $[Ca^{2+}]_c$ oscillation of control islets in the presence and in the absence of glucose (Fig. 4F). This revealed a much larger $[Ca^{2+}]_c$ release phase in the presence of 20 mM glucose than in its absence.

Mechanisms of the Slow Ca^{2+} Release Process from the ER—In skeletal muscle cells, depolarization of the plasma membrane alone can trigger release of Ca^{2+} from intracellular stores (22). However, this process does not seem to be operative in pancreatic B-cells, as no $[Ca^{2+}]_c$ increase could be detected when the islets were depolarized by pulses of high K^+ in a Ca^{2+} -free medium supplemented with 2 mM EGTA (Fig. 5A). This lack of effect of high K^+ did not result from exhaustion of intracellular Ca^{2+} pools by EGTA present in the medium because ACh could still trigger Ca^{2+} mobilization after 2 inefficient pulses of high K^+ .

Depolarization is not sufficient and even not necessary, as shown by the following experiment. Pancreatic B-cells were loaded with the caged Ca^{2+} compound, nitrophenyl-EGTA, whereas $[Ca^{2+}]_c$ was kept at basal levels by the presence of 4.8 mM K^+ and 250 μ M diazoxide in the medium. Flashes of UV light induced a large rise in $[Ca^{2+}]_c$, which then decreased in two phases, an initial fast one followed by a slow recovery to basal levels (Fig. 5B). Brief depolarization with 45 mM K^+ was followed by a similar biphasic response. After addition of TG, a second series of UV flashes triggered a new increase in $[Ca^{2+}]_c$ followed by a rapid decrease that now lacked the slow recovery phase. These experiments clearly demonstrate that the rise in $[Ca^{2+}]_c$ is sufficient to induce a slow recovery phase, even in the absence of membrane depolarization.

Two well characterized mechanisms can trigger Ca^{2+} release from intracellular Ca^{2+} stores: Ca^{2+} -induced and IP_3 -induced Ca^{2+} release (2, 23). High concentrations (5–20 mM) of caffeine are known to induce or to potentiate Ca^{2+} -induced Ca^{2+} release and to inhibit IP_3 -induced Ca^{2+} -release (24). However, 10

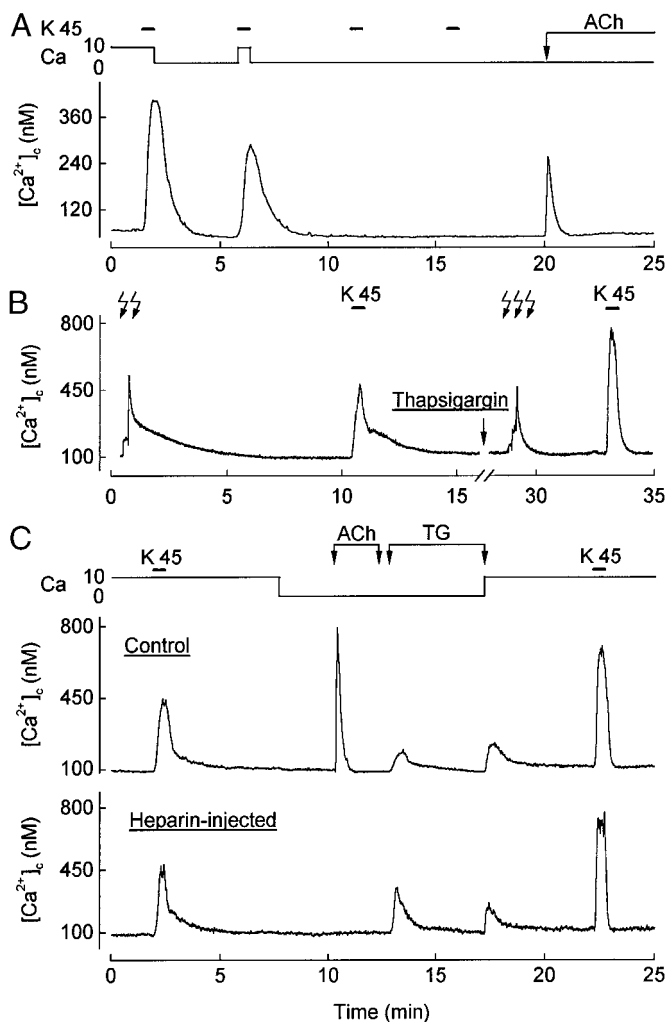


FIG. 5. The slow [Ca²⁺]_c recovery phase following high K⁺-induced [Ca²⁺]_c oscillations does not involve depolarization- or IP₃-induced Ca²⁺ release and a similar slow decay is observed after an abrupt rise in [Ca²⁺]_c triggered by uncaging Ca²⁺ by flashes of UV light. *A*, whole islets loaded with fura-PE3. *B*, clusters of islet cells loaded with fura-2 and nitrophenyl-EGTA. *C*, single cells injected with fura-2, with or without heparin. All perfusion solutions contained 10 mM glucose and 250 μ M diazoxide throughout. The Ca²⁺ concentration of the medium was either constant throughout the whole experiment (*B*), or it was changed and test agents (100 μ M ACh or 1 μ M TG) were added when indicated (*A* and *C*). [Ca²⁺]_c was increased by uncaging Ca²⁺ from nitrophenyl-EGTA with two or three flashes of UV light (arrows in *B*) or by 30-s pulses of 45 mM K⁺ (bars in *A–C*). In *A* and *C*, Ca²⁺-free solutions were supplemented with 2 mM EGTA. In *B*, interruption of the trace corresponds to a period during which 1 μ M TG was applied. The traces are representative of results obtained in 10 islets (*A*), 3 clusters of cells (*B*), and 4 (control) and 6 (heparin) single cells (*C*).

mm caffeine was without effect on the slow recovery phase observed at the end of a 30-s pulse of 45 mM K⁺ (data not shown). Ryanodine is a potent modulator of Ca²⁺-induced Ca²⁺ release. It activates this process at low concentrations (≤ 1 μ M) but blocks it at high concentrations (≥ 10 μ M) (24, 25). Here, the islets were treated acutely, preincubated, or cultured with different concentrations of ryanodine (1–100 μ M). In no case did we observe an effect of ryanodine on basal [Ca²⁺]_c, on peak [Ca²⁺]_c-induced by high K⁺ pulses, or on the subsequent slow recovery phase (data not shown). Microinjection of B-cells with 10 mM ryanodine was also ineffective ($n = 3$, data not shown).

In many tissues, including pancreatic B-cells, phospholipase C can be activated directly by a rise in [Ca²⁺]_c or by depolarization of the plasma membrane (8, 26). It is therefore possible

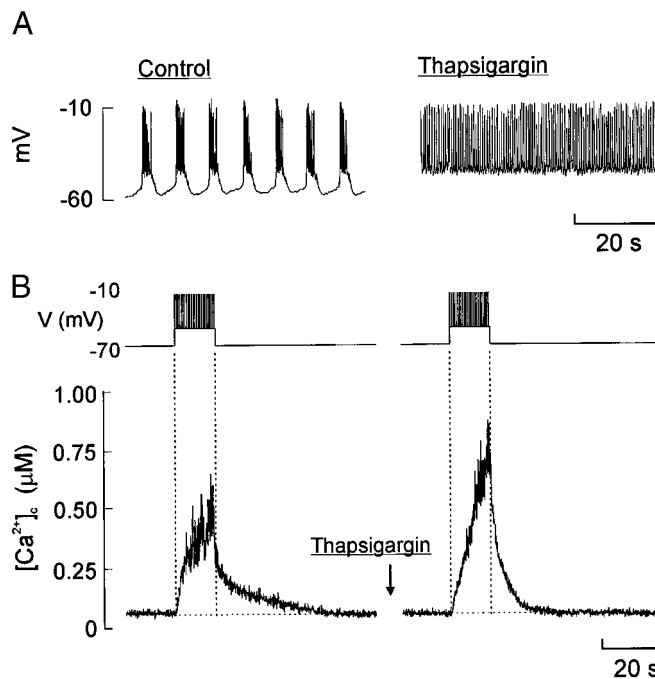


FIG. 6. *A*, inhibition of the SERCA pump by TG transforms oscillations of the membrane potential into a sustained depolarization with spikes in pancreatic B-cells perfused with 10 mM glucose and 2.5 mM Ca²⁺. In the *right panel*, the islet was pretreated for 90 min with 1 μ M TG in the culture medium. The traces are representative of results obtained in 29 (control) and 11 (TG) islets. *B*, TG increases the amplitude of the [Ca²⁺]_c rise and suppresses the slow phase of [Ca²⁺]_c recovery in voltage-clamped pancreatic B-cells submitted to trains of 100-ms depolarizations designed to mimic glucose-induced bursts of action potentials. The traces are representative of results obtained in eight cells.

that a [Ca²⁺]_c rise increases IP₃ levels, which in turn trigger Ca²⁺ release. To test this hypothesis, pancreatic B-cells were microinjected with heparin, a blocker of the IP₃ receptor in various tissues, including pancreatic B-cells (27). In B-cells microinjected with fura-2 free acid alone, a pulse of high K⁺ induced a large [Ca²⁺]_c oscillation characterized by a slow recovery phase (Fig. 5C). Subsequent addition of 100 μ M ACh triggered a large and transient increase in [Ca²⁺]_c. Emptying the ER with TG induced a further rise in [Ca²⁺]_c and prevented the slow recovery phase of the [Ca²⁺]_c oscillation induced by a subsequent pulse of high K⁺. Heparin microinjection (molecular weight 6000; 200 mg/ml) completely prevented the ACh-induced [Ca²⁺]_c rise without affecting the response to TG (Fig. 5C). As heparin did not affect the slow recovery phase of the [Ca²⁺]_c oscillation induced by high K⁺, it is clear that this phase is not induced by an IP₃-mediated Ca²⁺ release.

A delayed, slow return of [Ca²⁺]_c to basal level after a depolarization-induced [Ca²⁺]_c rise has been observed in neurons and chromaffin cells (28–31) and attributed to a slow release of Ca²⁺ from mitochondria. No similar process seems to be operative in B-cells. Thus, microinjection of B-cells with 1 mM ruthenium red (giving a final cytosolic concentration > 1 μ M) did not affect the slow recovery [Ca²⁺]_c phase after a pulse of high K⁺ ($n = 3$, data not shown), although the drug is regarded as a potent and selective inhibitor of the mitochondrial Ca²⁺ uniporter at ~ 1 μ M (32).

These experiments indicate that the release of Ca²⁺ observed after a rise in [Ca²⁺]_c originates from the ER; that it is not triggered by depolarization-, IP₃-, or Ca²⁺-induced Ca²⁺ release; and that none of these three mechanisms contribute to the [Ca²⁺]_c rise induced by high K⁺.

Effect of Intracellular Ca²⁺ Pool Depletion under More

Physiological Conditions—Ideally, release of Ca^{2+} at the end of $[Ca^{2+}]_c$ oscillations induced by glucose in a physiological medium containing 2.5 mM Ca^{2+} should now be sought for. Unfortunately, this was not possible because emptying of intracellular Ca^{2+} pools by TG transformed oscillations of the membrane potential of whole islets induced by 10 mM glucose into a sustained depolarization with continuous spike activity (Fig. 6A).

We therefore tested the effect of intracellular Ca^{2+} pool depletion in voltage-clamped single B-cells subjected to trains of 100-ms depolarizations (2 Hz for 12 s) from -50 to -10 mV (holding potential, -70 mV) designed to mimic glucose-induced bursts of action potentials (Fig. 6B). This induced a large rise in $[Ca^{2+}]_c$ that was followed by a slow recovery to basal levels upon repolarization to -70 mV. Once $[Ca^{2+}]_c$ had returned to basal levels, TG was applied for 5 min, and the cell was again subjected to a burst of depolarizations. This raised $[Ca^{2+}]_c$ to a higher level than before TG addition (776 ± 64 versus 577 ± 53 nM, respectively; $p < 0.05$; $n = 8$). Importantly, the time constant of the falling phase was much shorter (3.1 ± 2.1 versus 13 ± 2.4 s, respectively; $p < 0.01$). This suggests that intracellular Ca^{2+} stores play a role in the oscillations in $[Ca^{2+}]_c$ during bursts of action potentials induced by glucose.

DISCUSSION

The present study demonstrates that rapid uptake and release of Ca^{2+} by the ER contributes to $[Ca^{2+}]_c$ oscillations induced by Ca^{2+} influx through voltage-dependent Ca^{2+} channels in pancreatic B-cells.

Nature of the Intracellular Ca^{2+} Store Taking up and Releasing Ca^{2+} in Response to a Rise in $[Ca^{2+}]_c$ Triggered by Ca^{2+} Influx—A fast and strong $[Ca^{2+}]_c$ buffering has been documented in patch-clamped B-cells (33). Two observations in the present study ascribe this property to the ER. First, a 30-s pulse of high K^+ could replenish nearly completely emptied ACh-sensitive stores. Second, the rise in $[Ca^{2+}]_c$ induced by depolarization was faster and larger in TG and CPA-treated islets than in controls, although the Ca^{2+} current was not increased. This is in agreement with the recent report that 20 mM K^+ raises $[Ca^{2+}]_{ER}$ in INS-1 rat insulinoma cells expressing aequorin in the ER (34). The ER is also the source of Ca^{2+} that is released into the cytoplasm after a rise in $[Ca^{2+}]_c$, because inhibition of the SERCA pump by TG or CPA or opening of IP_3 receptors by ACh completely abolished the slow $[Ca^{2+}]_c$ recovery phase. Furthermore, the filling state of the ER profoundly affected the characteristics of the slow recovery phase.

Uptake of Ca^{2+} by mitochondria during a rapid rise in $[Ca^{2+}]_c$ has been documented in various cell types, including neurons, chromaffin cells, and insulin-secreting cells (28–31, 35, 36). However, we found that the slow decay of $[Ca^{2+}]_c$ following an abrupt rise was unaffected by ruthenium red, a blocker of the mitochondrial Ca^{2+} uniporter (32). These observations clearly establish that mitochondria are not primarily responsible for the slow $[Ca^{2+}]_c$ decay.

Regulation of Ca^{2+} Uptake—Uptake of Ca^{2+} by the ER directly depends on $[Ca^{2+}]_c$. Indeed, the amount of Ca^{2+} that was released from the ER by TG was proportional to the steady-state level of $[Ca^{2+}]_c$ in clusters of cells depolarized with various concentrations of K^+ . Such a Ca^{2+} dependence of the uptake has been clearly demonstrated in various cells (14, 37, 38). Pancreatic B-cells express SERCA-2B and SERCA-3 isoforms (39), SERCA-2B being the most sensitive to Ca^{2+} among all SERCA isoforms (40).

Uptake of Ca^{2+} by the ER is also modulated by the glucose concentration of the medium. A smaller influx of Ca^{2+} through voltage-dependent Ca^{2+} channels cannot explain why high K^+ -

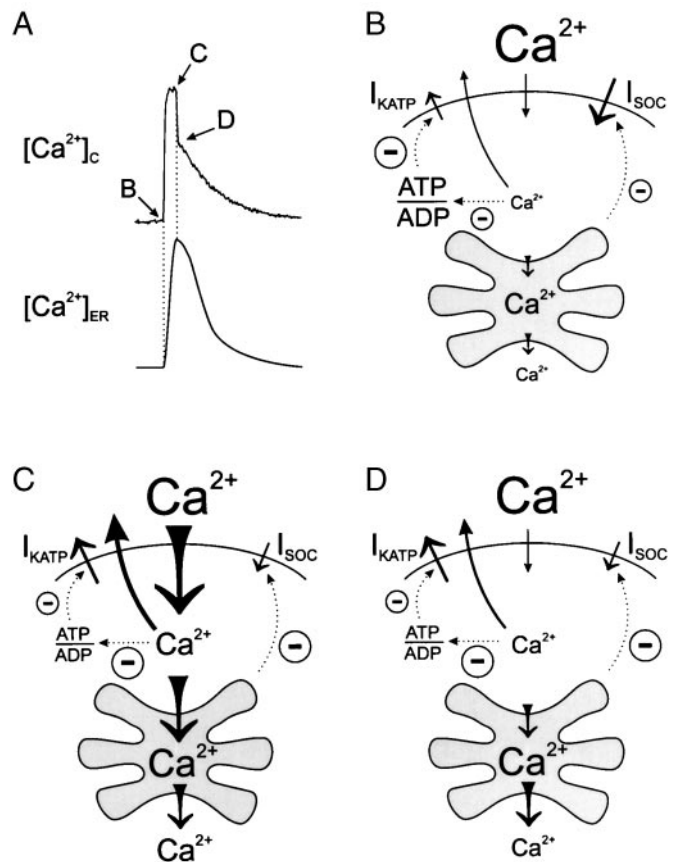


FIG. 7. Model of the interplay between $[Ca^{2+}]_c$ and $[Ca^{2+}]_{ER}$, and of the role of $[Ca^{2+}]_{ER}$ in the control of the membrane potential during $[Ca^{2+}]_c$ oscillations induced by glucose. A illustrates a typical $[Ca^{2+}]_c$ oscillation induced by 10 mM glucose in a medium containing 10 mM Ca^{2+} . Below are the expected changes of $[Ca^{2+}]_{ER}$. B–D schematize Ca^{2+} movements and major ionic currents at the times indicated by the corresponding arrows in A. The thickness of the arrows shown with solid lines is proportional to the rate of pumping or intensity of the current. The size of the symbols is proportional to the concentration of Ca^{2+} or the ATP/ADP ratio. The size of the negative sign is proportional to the amplitude of the inhibitory effect of the modulatory pathways. See under “Discussion” for explanation.

induced $[Ca^{2+}]_c$ oscillations were smaller in the presence of 20 mM glucose than 0 mM glucose because glucose enhances voltage-dependent Ca^{2+} currents in B-cells (41). The difference rather results from an increased buffering capacity of the ER in the presence of glucose. This is in agreement with the observation that stimulation of B-cells with glucose causes an initial drop in $[Ca^{2+}]_c$ that is blocked by TG (42). Other studies have shown that the amount of Ca^{2+} taken up by the ER of permeabilized RINm5F insulinoma cells depends on the ATP/ADP ratio (37). The longer $[Ca^{2+}]_c$ recovery phase observed in the presence of glucose therefore reflects a larger release of Ca^{2+} from the ER into which glucose has promoted Ca^{2+} sequestration during influx of the ion.

Mechanism of Ca^{2+} Release—Three mechanisms of Ca^{2+} release from intracellular Ca^{2+} stores have been described: depolarization-, Ca^{2+} -, and IP_3 -induced Ca^{2+} release (2, 22, 23). We did not detect any depolarization-induced Ca^{2+} release from filled Ca^{2+} stores in mouse pancreatic B-cells, but we showed that a rise in $[Ca^{2+}]_c$ is sufficient to induce slow release of the ion from the ER when $[Ca^{2+}]_c$ decreases. Ca^{2+} - and/or IP_3 -induced Ca^{2+} release has been suggested to play a role in glucose-induced $[Ca^{2+}]_c$ changes (6–10). Pancreatic B-cells express very low levels of type 2 ryanodine receptors (9) responsible for Ca^{2+} -induced Ca^{2+} release (2) and high amounts of IP_3

receptors. We used heparin, caffeine, and ryanodine, three established modulators of Ca²⁺- or IP₃-induced Ca²⁺ release (24, 25, 27), to investigate the possible contribution of these processes to [Ca²⁺]_c oscillations induced by high K⁺ pulses. None of these compounds affected the oscillations. Moreover, if a depolarization-, Ca²⁺-, or IP₃-induced Ca²⁺ release participated in high K⁺-induced [Ca²⁺]_c rise in pancreatic B-cells, the latter would be reduced by depletion of intracellular pools with TG. The results show exactly the opposite. Taken together, our experiments suggest that the release of Ca²⁺ observed after a rise in [Ca²⁺]_c is not triggered by depolarization-, IP₃- or Ca²⁺-induced Ca²⁺ release and that none of these three mechanisms contribute to the [Ca²⁺]_c rise induced by high K⁺.

Ca²⁺ release from the ER at the end of [Ca²⁺]_c oscillations more likely corresponds to a slow release of Ca²⁺ from the organelle, which slowly adapts its Ca²⁺ concentration to [Ca²⁺]_c. Release of Ca²⁺ through the same pathway may explain the rise in [Ca²⁺]_c that occurs upon blockade of the SERCA pump with TG. Because of its high Ca²⁺ permeability, this pathway has often been referred to as leak from the ER. Although it has been observed in many cell types, its exact nature has not been determined (14, 43).

Sequence of Events—Many processes regulating [Ca²⁺]_c are directly influenced by [Ca²⁺]_c and [Ca²⁺]_{ER}. The plasma membrane Ca²⁺-ATPase (PMCA) is strongly stimulated when [Ca²⁺]_c increases (38, 43). The Ca²⁺-ATPase of the ER is proportionally more stimulated by cytosolic Ca²⁺ than the PMCA (38), but it is inhibited by luminal Ca²⁺ (14). Ca²⁺ leakage through the ER seems only mildly stimulated by luminal Ca²⁺ (14). On the other hand, there is an interplay between the rate at which Ca²⁺-ATPases work and the available energy. It is indeed possible that changes in the pumping rate of Ca²⁺-ATPases during [Ca²⁺]_c oscillations modulate the ATP/ADP ratio, which in turn modulates the activity of Ca²⁺-ATPases (44). We have recently shown that the ATP/ADP ratio drops rapidly when [Ca²⁺]_c is raised and increases when [Ca²⁺]_c falls (45). Periodic release and reuptake of Ca²⁺ from the ER of permeabilized RINm5F cells supplemented with an oscillating glycolytic cell-free muscle extract have been reported (46).

Basal [Ca²⁺]_c is set by a balance between processes that increase [Ca²⁺]_c (leak entry of Ca²⁺ from the extracellular space and leak release of Ca²⁺ from the ER) and processes that decrease [Ca²⁺]_c (extrusion mechanisms that remove Ca²⁺ from the cytosol) (Fig. 7B). When [Ca²⁺]_c increases abruptly as a result of Ca²⁺ influx through voltage-dependent Ca²⁺ channels, Ca²⁺ extrusion out of the cell by the PMCA is stimulated, and Ca²⁺ uptake by the ER occurs at a higher rate than Ca²⁺ release (Fig. 7C). As [Ca²⁺]_c rises, the ATP/ADP ratio decreases (45), perhaps because of ATP consumption by these ATPases. When repolarization of the plasma membrane closes voltage-dependent Ca²⁺ channels, Ca²⁺ influx abruptly stops. This, together with rapid uptake by the ER and extrusion out of the cell, produces a fast drop in [Ca²⁺]_c that corresponds to the first phase of [Ca²⁺]_c decrease (Fig. 7D). As [Ca²⁺]_c decreases, the PMCA is less stimulated. In the ER, Ca²⁺ release predominates over Ca²⁺ uptake because the latter is inhibited by high luminal [Ca²⁺] (14) and by the lowering of the ATP/ADP ratio. This corresponds to the beginning of the slow phase of [Ca²⁺]_c decrease that lasts until release and uptake reach a new equilibrium. As the PMCA extrudes Ca²⁺ less efficiently than the SERCA pumps it into the ER (38), Ca²⁺ could even cycle between the cytosol and the ER, which would prolong the slow [Ca²⁺]_c decrease.

Previous studies have clearly demonstrated that glucose-induced [Ca²⁺]_c oscillations of single pancreatic B-cells or clusters of islet cells do not require the participation of the ER (17,

47, 48). Our present data do not contradict these observations, but they strongly support the hypothesis that [Ca²⁺]_{ER} oscillations occur synchronously with and in parallel to glucose-induced [Ca²⁺]_c oscillations. The parallel nature of these oscillations strikingly contrasts with the antiparallel changes of IP₃-induced [Ca²⁺]_c and [Ca²⁺]_{ER} oscillations (13, 49, 50).

Physiological Implications—In many cell types, emptying of intracellular Ca²⁺ pools activates various types of currents with distinct ion selectivity (Ca²⁺, K⁺, or Na⁺) through a family of channels that have been termed store-operated channels (51–54). It has recently been demonstrated that the magnitude of the current activated by intracellular pool emptying correlates with the extent of store depletion and that it can be activated even by small decreases in [Ca²⁺]_{ER} (55). In pancreatic B-cells, intracellular Ca²⁺ pool depletion activates a small Ca²⁺ entry and induces a depolarizing current, possibly carried by Na⁺, that potentiates the activation of voltage-dependent Ca²⁺ channels (17, 56). This was evidenced in the present study by the observation that in the presence of 2.5 mM Ca²⁺ in the medium, TG transformed oscillations of the membrane potential into a sustained depolarization. It is therefore likely that oscillations of the Ca²⁺ concentration within the ER rhythmically activate a depolarizing current.

The model that we suggest is presented in Fig. 7. The membrane potential of B-cells is controlled by several conductances, among which the K_{ATP} current (I_{KATP}) and the store-operated current (I_{SOC}) play major roles. In the presence of a nonstimulating concentration of glucose, I_{SOC} is too small to counteract the overwhelming repolarizing current through K_{ATP} channels. At stimulating glucose concentrations, I_{KATP} is much reduced and can be counteracted by I_{SOC} (Fig. 7B). When [Ca²⁺]_c increases, the ER fills with Ca²⁺, leading to decrease of I_{SOC} and repolarization of the plasma membrane (Fig. 7C). Ca²⁺ influx through voltage-dependent Ca²⁺ channels then stops, [Ca²⁺]_c decreases, and the ER starts to release more Ca²⁺ than it takes up (Fig. 7D). The subsequent slow emptying of intracellular Ca²⁺ pools reactivates I_{SOC} and the plasma membrane depolarizes again (Fig. 7B). This increases [Ca²⁺]_c, and a new cycle starts again. The model depicted in Fig. 7 thus suggests that glucose controls the membrane potential of B-cells partly indirectly, by modulating the buffering capacity of the ER. It is fully compatible with our recent model (45) indicating that I_{KATP} might also oscillate during [Ca²⁺]_c oscillations. Before the onset of [Ca²⁺]_c oscillations, I_{KATP} would be small, when I_{SOC} is large. At the end of [Ca²⁺]_c oscillations, it would be large, when I_{SOC} is small. Some mathematical models of the bursting electrical activity of the pancreatic B-cell (57) also include a mechanism by which oscillations of the Ca²⁺ concentration within the ER concomitant with [Ca²⁺]_c oscillations control slow waves of the membrane potential. In contrast, our model differs from that proposed by Dukes *et al.* (58), which involves a depolarization- or IP₃-induced emptying of intracellular Ca²⁺ stores during the upstroke of [Ca²⁺]_c oscillation.

Conclusions—The present study demonstrated that in pancreatic B-cells, the ER plays a dual role during [Ca²⁺]_c oscillations. It attenuates [Ca²⁺]_c rises by rapidly taking up Ca²⁺ and delays [Ca²⁺]_c decreases by slowly releasing Ca²⁺. Buffering the fast rise in [Ca²⁺]_c could protect the cell from Ca²⁺ overload, whereas buffering the decrease in [Ca²⁺]_c prolongs the Ca²⁺ signal in the cytosol. It is likely that oscillations of [Ca²⁺]_{ER} occur synchronously with [Ca²⁺]_c oscillations. They may have a major impact on the control of the oscillations of the membrane potential and consequently on [Ca²⁺]_c oscillations themselves.

REFERENCES

- Clapham, D. E. (1995) *Cell* **80**, 259–268
- Berridge, M. J. (1997) *J. Physiol. Lond.* **499**, 290–306
- Ashcroft, F. M., and Rorsman, P. (1989) *Prog. Biophys. Mol. Biol.* **54**, 87–143
- Gilon, P., and Henquin, J. C. (1992) *J. Biol. Chem.* **267**, 20713–20720
- Santos, R. M., Rosario, L. M., Nadal, A., Garcia Sancho, J., Soria, B., and Valdeolmillos, M. (1991) *Pfluegers Arch.* **418**, 417–422
- Ämmälä, C., Larsson, O., Berggren, P. O., Bokvist, K., Juntti-Berggren, L., Kindmark, H., and Rorsman, P. (1991) *Nature* **353**, 849–852
- Barker, C. J., Nilsson, T., Kirk, C. J., Michell, R. H., and Berggren, P. O. (1994) *Biochem. J.* **297**, 265–268
- Gromada, J., Frokjaer-Jensen, J., and Dissing, S. (1996) *Biochem. J.* **314**, 339–345
- Islam, M. S., Leibiger, I., Leibiger, B., Rossi, D., Sorrentino, V., Ekstrom, T. J., Westerblad, H., Andrade, F. H., and Berggren, P. O. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95**, 6145–6150
- Liu, Y. J., Grapengiesser, E., Gylfe, E., and Hellman, B. (1996) *Arch. Biochem. Biophys.* **334**, 295–302
- Rizzuto, R., Brini, M., Murgia, M., and Pozzan, T. (1993) *Science* **262**, 744–747
- Miyawaki, A., Llopis, J., Heim, R., McCaffery, J. M., Adams, J. A., Ikura, M., and Tsien, R. Y. (1997) *Nature* **388**, 882–887
- Chatton, J.-Y., Liu, H., and Stucki, J. W. (1995) *FEBS Lett.* **368**, 165–168
- Mogami, H., Tepikin, A. V., and Petersen, O. H. (1998) *EMBO J.* **17**, 435–442
- Golovina, V. A., and Blaustein, M. P. (1997) *Science* **275**, 1643–1648
- Worley, J. F., III, McIntyre, M. S., Spencer, B., Mertz, R. J., Roe, M. W., and Dukes, I. D. (1994) *J. Biol. Chem.* **269**, 14359–14362
- Miura, Y., Henquin, J. C., and Gilon, P. (1997) *J. Physiol. Lond.* **503**, 387–398
- Henquin, J. C., and Meissner, H. P. (1984) *Experientia* **40**, 1043–1052
- Inesi, G., and Sagara, Y. (1994) *J. Membr. Biol.* **141**, 1–6
- Miura, Y., Gilon, P., and Henquin, J. C. (1996) *Biochem. Biophys. Res. Commun.* **224**, 67–73
- Schlatterer, C., Knoll, G., and Malchow, D. (1992) *Eur. J. Cell Biol.* **58**, 172–181
- Rios, E., and Pizzaro, G. (1988) *News Physiol. Sci.* **3**, 223–227
- Endo, M. (1977) *Physiol. Rev.* **57**, 71–108
- Ehrlich, B. E., Kaftan, E., Bezprozvannaya, S., and Bezprozvanny, I. (1994) *Trends Pharmacol. Sci.* **15**, 145–149
- Sutko, J. L., and Airey, J. A. (1996) *Physiol. Rev.* **76**, 1027–1071
- Biden, T. J., Peter-Riesch, B., Schlegel, W., and Wollheim, C. B. (1987) *J. Biol. Chem.* **262**, 3567–3571
- Nilsson, T., Zwiller, J., Boynton, A. L., and Berggren, P. O. (1988) *FEBS Lett.* **229**, 211–214
- Park, Y. B., Herrington, J., Babcock, D. F., and Hille, B. (1996) *J. Physiol. Lond.* **492**, 329–346
- Babcock, D. F., Herrington, J., Goodwin, P. C., Park, Y. B., and Hille, B. (1997) *J. Cell Biol.* **136**, 833–844
- Friel, D. D., and Tsien, R. W. (1994) *J. Neurosci.* **14**, 4007–4024
- Herrington, J., Park, Y. B., Babcock, D. F., and Hille, B. (1996) *Neuron* **16**, 219–228
- Csordás, G., Thomas, A. P., and Hajnóczky, G. (1999) *EMBO J.* **18**, 96–108
- Rorsman, P., Ämmälä, C., Berggren, P. O., Bokvist, K., and Larsson, O. (1992) *EMBO J.* **11**, 2877–2884
- Maechler, P., Kennedy, E. D., Sebo, E., Valeva, A., Pozzan, T., and Wollheim, C. B. (1999) *J. Biol. Chem.* **274**, 12583–12592
- Rizzuto, R., Bastianutto, C., Brini, M., Murgia, M., and Pozzan, T. (1994) *J. Cell Biol.* **126**, 1183–1194
- Rutter, G. A., Theler, J. M., Murgia, M., Wollheim, C. B., Pozzan, T., and Rizzuto, R. (1993) *J. Biol. Chem.* **268**, 22385–22390
- Corkey, B. E., Deeney, J. T., Glennon, M. C., Matschinsky, F. M., and Prentki, M. (1988) *J. Biol. Chem.* **263**, 4247–4253
- Camello, P., Gardner, J., Petersen, O. H., and Tepikin, A. V. (1996) *J. Physiol. Lond.* **490**, 585–593
- Varadi, A., Molnar, E., Ostenson, C. G., and Ashcroft, S. J. (1996) *Biochem. J.* **319**, 521–527
- Lytton, J., Westlin, M., Burk, S. E., Shull, G. E., and MacLennan, D. H. (1992) *J. Biol. Chem.* **267**, 14483–14489
- Smith, P. A., Rorsman, P., and Ashcroft, F. M. (1989) *Nature* **342**, 550–553
- Roe, M. W., Mertz, R. J., Lancaster, M. E., Worley, J. F., III, and Dukes, I. D. (1994) *Am. J. Physiol. Endocrinol. Metab.* **266**, E852–E862
- Missiaen, L., Wuytack, F., Raeymaekers, L., De Smedt, H., Droogmans, G., Declercq, I., and Casteels, R. (1991) *Pharmacol. Ther.* **50**, 191–232
- Landolfi, B., Curci, S., Debellis, L., Pozzan, T., and Hofer, A. M. (1998) *J. Cell Biol.* **142**, 1235–1243
- Detimary, P., Gilon, P., and Henquin, J. C. (1998) *Biochem. J.* **333**, 269–274
- Corkey, B. E., Tornheim, K., Deeney, J. T., Glennon, M. C., Parker, J. C., Matschinsky, F. M., Ruderman, N. B., and Prentki, M. (1988) *J. Biol. Chem.* **263**, 4254–4258
- Liu, Y. J., Grapengiesser, E., Gylfe, E., and Hellman, B. (1994) *Arch. Biochem. Biophys.* **315**, 387–392
- Liu, Y. J., Grapengiesser, E., Gylfe, E., and Hellman, B. (1995) *Cell Calcium* **18**, 165–173
- Hofer, A. M., Landolfi, B., Debellis, L., Pozzan, T., and Curci, S. (1998) *EMBO J.* **17**, 1986–1995
- Hajnóczky, G., and Thomas, A. P. (1997) *EMBO J.* **16**, 3533–3543
- Hoth, M. (1996) *FEBS Lett.* **390**, 285–288
- Parekh, A. B., Terlau, H., and Stuhmer, W. (1993) *Nature* **364**, 814–818
- Parekh, A. B., and Penner, R. (1997) *Physiol. Rev.* **77**, 901–930
- Putney, J. W., Jr., and Bird, G. S. (1993) *Endocr. Rev.* **14**, 610–631
- Hofer, A. M., Fasolato, C., and Pozzan, T. (1998) *J. Cell Biol.* **140**, 325–334
- Worley, J. F., III, McIntyre, M. S., Spencer, B., and Dukes, I. D. (1994) *J. Biol. Chem.* **269**, 32055–32058
- Chay, T. R. (1997) *Biophys. J.* **73**, 1673–1688
- Dukes, I. D., Roe, M. W., Worley, J. F., III, and Philipson, L. H. (1997) *Curr. Opin. Endocrinol. Diabetes* **4**, 262–271