Inositol 1,4,5-Trisphosphate and the Endoplasmic Reticulum Ca$^{2+}$ Cycle of a Rat Insulinoma Cell Line*

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Regulation of endoplasmic reticulum (ER) Ca$^{2+}$ cycling by inositol 1,4,5-trisphosphate (IP$_3$) was studied in saponin-permeabilized RINm5F insulinoma cells. Cells were incubated with mitochondrial inhibitors, and medium Ca$^{2+}$ concentration established by nonmitochondrial pools (presumably the ER) was monitored with a Ca$^{2+}$ electrode.

IP$_3$ degradation accounted for the transience of the Ca$^{2+}$ response induced by pulse additions of the molecule. To compensate for degradation, IP$_3$ was infused into the medium. This resulted in elevation of [Ca$^{2+}$] from about 0.2 µM to a new steady state between 0.3 and 1.0 µM, depending on both the rate of IP$_3$ infusion and the ER Ca$^{2+}$ content. The elevated steady state represented a bidirectional buffering of [Ca$^{2+}$] by the ER, as slight displacements in [Ca$^{2+}$], by small aliquots of Ca$_{2+}$ or the Ca$^{2+}$ chelator quin 2, resulted in net uptake or efflux of Ca$^{2+}$ to restore the previous steady state. When IP$_3$ infusion was stopped, [Ca$^{2+}$] returned to its original low level.

Ninety per cent of the Ca$^{2+}$ accumulated by the ER in permeabilized RINm5F cells was released by IP$_3$ when the total Ca$^{2+}$ content did not exceed 15 nmol/mg of cell protein. Above this high Ca$^{2+}$ content, Ca$^{2+}$ was accumulated in an IP$_3$-insensitive, A23187-releasable pool. The maximal amount of Ca$^{2+}$ that could be released from the ER by IP$_3$ was 13 nmol/mg of cell protein.

The data support the concept that in the physiological range of Ca$^{2+}$ contents, almost all the ER is an IP$_3$-sensitive Ca$^{2+}$ store that is capable of finely regulating [Ca$^{2+}$] through independent influx (Ca$^{2+}$-ATPase) and efflux (IP$_3$-modulated component) pathways of Ca$^{2+}$ transport. IP$_3$ may continuously modulate Ca$^{2+}$ cycling across the ER and play an important role in determining the ER Ca$^{2+}$ content and in regulating cytosolic Ca$^{2+}$ mobilization under both stimulated and possibly basal conditions.

In pancreatic β cells, an elevation in the cytosolic free Ca$^{2+}$ concentration is thought to play a central role in the induction of insulin release by physiological secretagogues such as glucose or acetylcholine (1, 2). Both external Ca$^{2+}$ and Ca$^{2+}$ mobilized from intracellular stores appear to contribute to this elevation in cytosolic Ca$^{2+}$ (1, 2). It is presumed, based on extensive evidence in several cell types, that IP$_3$, a newly discovered second messenger (3-9), mobilizes internal Ca$^{2+}$ in insulin-secreting cells in response to the nonfuel secretagogue carbamylcholine (2). Whether IP$_3$ is involved in mediating the elevation in cytosolic Ca$^{2+}$ in response to fuel secretagogues such as glucose is presently unknown. In a variety of cell types, IP$_3$ has been documented to transiently mobilize Ca$^{2+}$ from a nonmitochondrial pool (4-7), identified as the endoplasmic reticulum (7, 10). The reason for the transient nature of the Ca$^{2+}$ response (Ca$^{2+}$ release followed by Ca$^{2+}$ resequestration) is presently unclear, although several possibilities including IP$_3$ degradation (5,11), IP$_3$-insensitive compartments (7, 11, 12), or desensitization (7) have been proposed. Considerable differences in the pattern of IP$_3$-induced Ca$^{2+}$ responses or IP$_3$ degradation rates have been documented in different cell types or preparations. Thus, permeabilized insulinoma cells (11) are sensitive to a second challenge of IP$_3$, whereas permeabilized human neutrophils (13) and insulinoma (7) or liver (12) microsomes are marked less responsive to a second pulse addition of the molecule. In addition, microsomal preparations from both insulinoma (7) and liver cells (12) are poorly responsive to IP$_3$ in comparison to permeabilized cells (4, 5, 11, 10). Finally, the rate of Ca$^{2+}$ reuptake following IP$_3$ addition appears to correlate roughly with IP$_3$ degradation in permeabilized hepatocytes (5) and insulinoma cells (11), whereas in both permeabilized neutrophils and insulinoma microsomes (7), Ca$^{2+}$ reuptake occurs rapidly despite very slow degradation of IP$_3$.

This study had three main objectives. First, to understand the reason for the transient nature of the IP$_3$-induced Ca$^{2+}$ response; second, to quantitate in insulinoma RINm5F cells the IP$_3$-sensitive and -insensitive nonmitochondrial Ca$^{2+}$ compartments; and third, to evaluate the role of IP$_3$ in regulating Ca$^{2+}$ cycling across the endoplasmic reticulum.

**EXPERIMENTAL PROCEDURES**

RINm5F cells were maintained in culture as described previously (14, 15). Cells (8 × 10$^6$) were detached from culture flasks using EDTA and trypsin (15) and were subsequently washed three times in a Ca$^{2+}$- and Mg$^{2+}$-free Hank's basal salt solution (4°C; pH 7.2) by sedimenting them at 100 × g for 8 min. The final cell pellet was resuspended in 0.3 ml of the same medium to give a concentration of about 2 × 10$^7$ cells/ml and kept on ice until use. Incubations were carried out at 30°C, pH 7.0, in 0.2 ml of a medium containing KCl (110 mM), NaCl (10 mM), KH$_2$PO$_4$ (2 mM), MgCl$_2$ (1 mM), Hepes (25 mM), oligomycin (1 µg/ml), antimycin A (0.2 µM), MgATP (1 mM),

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1 The abbreviations used are: IP$_3$, inositol 1,4,5-trisphosphate; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; ER, endoplasmic reticulum.

2 T. J. Biden, M. Prentki, C. B. Wollheim, and P. D. Lew, unpublished observation.

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and an ATP-regenerating system consisting of phosphocreatine (5 mM) and creatine kinase (20 units/ml). Cells were added at a final concentration of 5 x 10^6 cells/ml. Cell permeabilization was initiated by adding saponin at a final concentration of 50 μg/ml. This concentration of saponin corresponded to the minimum amount of detergent necessary to optimally permeabilize the cells, i.e. the fastest Ca^{2+}-sequestering activity by intracellular structures and the optimal IP3-induced Ca^{2+} response. The preparation and calibration of the Ca^{2+}-specific minielectrodes have been previously described (16). None of the compounds tested interfered with the Ca^{2+} electrode. IP3 was infused into the electrode chamber using a 25-μl Hamilton syringe and a small length (15 cm) of polyvinyl chloride tubing (0.5 mm of internal diameter). The rate of IP3 infusion was varied as described in the figures using a multipulsed transmission infusion pump (Harvard Apparatus Co., Dover, MA, model 600-910/920) connected to the syringe. The traces shown in the figures are taken from representative experiments which have been repeated at least three times. IP3 was produced by the alkaline hydrolysis of ox brain phosphatidylinositol 4,5-bisphosphate and purified by preparative paper chromatography (17).

**RESULTS**

**Pulse Additions of IP3 and the Ambient [Ca^{2+}] Established by Nonmitochondrial Compartments**—The purpose of this investigation was to gain insight into the role of IP3 in the regulation of Ca^{2+} cycling across the endoplasmic reticulum and to quantitate the IP3-sensitive and -insensitive nonmitochondrial Ca^{2+} pools, using permeabilized insulinoma RINm5F cells as a model. Thus, in all the experiments reported, cells were incubated in the absence of mitochondrial substrates and in the presence of the mitochondrial poisons oligomycin and antimycin A to ensure that only nonmitochondrial Ca^{2+} transporting activity was studied. It has been shown previously that the nonmitochondrial Ca^{2+} pool of permeabilized RINm5F insulinoma cells displayed similar characteristics to those observed with endoplasmic reticulum-enriched fractions of insulinoma cells (11, 18, 19). Thus, the ATP-dependent, vanadate-inhibitable, antimycin- and oligomycin-insensitive nonmitochondrial Ca^{2+} transporting activity is most probably attributable to the endoplasmic reticulum. It will henceforth be referred to as “ER” Ca^{2+} pool.

Ca^{2+} sequestration by RINm5F cells incubated in the presence of a low concentration of saponin (for plasma membrane permeabilization) started within 1-2 min after addition of cells to the medium and resulted in a decrease in ambient [Ca^{2+}] (Fig. 1A). Within 15 min, the ER compartment lowered ambient [Ca^{2+}] from 1.25 μM to about 0.25 μM (from -log [Ca^{2+}] = 5.9 to -log [Ca^{2+}] = 6.6) (Fig. 1). A pulse addition of IP3 then promoted a rapid and dramatic Ca^{2+} release from the ER. Thus, ambient [Ca^{2+}] increased within 30 s from 0.25 μM to a peak value of 1.0 μM. The released Ca^{2+} was then slowly resequestered into the store (Fig. 1A). The response to subsequent IP3 additions was barely attenuated, and each Ca^{2+} release was followed by Ca^{2+} reuptake into the store. When cells were incubated in the presence of mitochondrial substrates without mitochondrial inhibitors, the Ca^{2+} response was much less pronounced (about 60% reduced), suggesting that mitochondria dampen the Ca^{2+} response by taking up a fraction of the Ca^{2+} released from the ER. Fig. 1A further shows that most of the Ca^{2+} accumulated by the ER was rapidly released by a maximal concentration of IP3 despite the presence of an operative Ca^{2+} uptake system. When successive IP3 additions were made at short time intervals, [Ca^{2+}] remained markedly elevated and the small amount of Ca^{2+} taken up between pulses was released by the subsequent IP3 addition (Fig. 1B). This observation did not favor the concept that either desensitization to IP3 or IP3-insensitive compart-

3 M. Prentki, unpublished observation.
not attributable to Ca\textsuperscript{2+} contamination of the compound.

The new Ca\textsuperscript{2+} steady state during IP\textsubscript{3} infusion presumably reflected a balance between Ca\textsuperscript{2+} influx (via the Ca\textsuperscript{2+}-ATPase) and a separate IP\textsubscript{3}-sensitive Ca\textsuperscript{2+} efflux component (13) (see also Fig. 5). To investigate this hypothesis, experiments were carried out to determine whether bidirectional buffering of ambient [Ca\textsuperscript{2+}] occurred at steady state. Thus, when a Ca\textsuperscript{2+} steady state was reached during IP\textsubscript{3} infusion, alternate small additions of either Ca\textsuperscript{2+} or the Ca\textsuperscript{2+} chelator quin 2 (free acid form) were made to slightly increase or decrease [Ca\textsuperscript{2+}]. In both cases, the ER tended to restore the previous Ca\textsuperscript{2+} steady state (Fig. 3A). This indicates that Ca\textsuperscript{2+} cycling across the ER occurred at steady state and that the steady state reflects the balance between the influx and efflux components of Ca\textsuperscript{2+} transport. It should be noted that the first quin 2 addition lowered [Ca\textsuperscript{2+}] less than subsequent additions (Fig. 3). This observation was made consistently and was attributed to some nonspecific quin 2-binding sites in the preparation. It was of interest to determine whether bidirectional buffering of ambient [Ca\textsuperscript{2+}] occurs without IP\textsubscript{3} infusion. In order to obtain a Ca\textsuperscript{2+} steady state in the absence of IP\textsubscript{3} infusion and in the range of Ca\textsuperscript{2+} concentrations (above 0.2 \mu M Ca\textsuperscript{2+}) where the electrode is optimally sensitive, cells were incubated in the absence of an ATP-regenerating system (Fig. 3B). Indeed, it has been shown previously that in the presence of an ATP-regenerating system, endoplasmic reticulum vesicles are capable of decreasing ambient [Ca\textsuperscript{2+}] to lower values than in the absence of an ATP-regenerating system, due to the fact that ADP markedly influences the extramicrosomal Ca\textsuperscript{2+} steady state (18). As shown in Fig. 3B, the ER was also capable of similar bidirectional buffering of [Ca\textsuperscript{2+}] without IP\textsubscript{3} infusion, although it occurred at lower [Ca\textsuperscript{2+}] levels. This suggests that IP\textsubscript{3} may not be absolutely necessary for Ca\textsuperscript{2+} cycling to occur across the ER and that other possible route(s) of Ca\textsuperscript{2+} efflux (such as pump reversal (18, 20)) may operate. It is also possible that the bidirectional buffering of ambient [Ca\textsuperscript{2+}] observed in the absence of IP\textsubscript{3} infusion may occur due to some IP\textsubscript{3} production by the cells during incubation. Although the ER buffered ambient [Ca\textsuperscript{2+}] in a bidirectional manner, the previous [Ca\textsuperscript{2+}] steady state was not “exactly” restored after the small additions of Ca\textsuperscript{2+} or quin 2. Ambient [Ca\textsuperscript{2+}] remained slightly elevated after a Ca\textsuperscript{2+} addition and slightly decreased after quin 2 addition (Fig. 3). Thus, we investigated whether the Ca\textsuperscript{2+} steady state obtained during IP\textsubscript{3} infusion depended on the ER Ca\textsuperscript{2+} content.

As shown in Fig. 4, both the Ca\textsuperscript{2+} level reached before and during IP\textsubscript{3} infusion depended markedly on ER Ca\textsuperscript{2+} content. This was demonstrated by allowing cells to accumulate Ca\textsuperscript{2+} from various starting ambient Ca\textsuperscript{2+} concentrations and finding that the higher the Ca\textsuperscript{2+} content of the ER, the higher the IP\textsubscript{3}-induced Ca\textsuperscript{2+} steady state (Fig. 4).

**IP\textsubscript{3}-sensitive and -insensitive Nonmitochondrial Compartments of RINm5F Cells**—We carried out experiments to better characterize and quantify the nonmitochondrial pools of RINm5F cells. Cells were allowed to accumulate Ca\textsuperscript{2+} from the medium until a lower ambient [Ca\textsuperscript{2+}] was reached (Fig. 5A). At this point vanadate (1 mM) was added. Vanadate was used at a maximal concentration to completely block ATP-dependent nonmitochondrial Ca\textsuperscript{2+} influx (not shown). This resulted in a progressive increase in [Ca\textsuperscript{2+}] until a steady state was obtained. A further addition of vanadate had no effect, indicating that Ca\textsuperscript{2+} contamination did not account for the response. Subsequent addition of IP\textsubscript{3} rapidly released the remaining Ca\textsuperscript{2+} previously accumulated by the ER. As seen in Fig. 5A, vanadate released a small fraction of the Ca\textsuperscript{2+} that had been accumulated until a new steady state was reached, but was not capable of releasing all the accumulated Ca\textsuperscript{2+}. One interpretation of this effect of vanadate is that only a small fraction of Ca\textsuperscript{2+} can be released from the store through
The presence or in the absence of vanadate) all the Ca\textsuperscript{2+} accumulation of an excess of glucose plus hexokinase (data not shown). The trace shown in the figure  were performed with cells from the same preparation. Where noted, IP\textsubscript{3} was infused into the medium in the amount indicated for each trace.

**FIG. 4.** Maintenance of elevated Ca\textsuperscript{2+} steady state following IP\textsubscript{3} infusion: effect of the Ca\textsuperscript{2+} load. Cells (10\textsuperscript{6}) were incubated as described in the legend to Fig. 1, but in a medium containing the Ca\textsuperscript{2+} chelator quin 2 (1.6 nmol/0.2 ml of incubation medium), a, b, and c denote the traces in which, respectively, 0.0, 0.4, and 0.8 nmol of Ca\textsuperscript{2+} were added to the medium before cell addition. The three traces shown in the figure were made with the same cell preparation. Where noted, IP\textsubscript{3} was infused into the medium in the amount indicated for each trace.

pump reversal due to the very low medium ADP concentration in the presence of the ATP-regenerating system. Consistent with this interpretation was the observation that (in the absence of vanadate) the Ca\textsuperscript{2+} accumulated was releasable when the ATP-regenerating system was overcome (ATP transformed into ADP), following the addition of glucose plus hexokinase (data not shown). The observation that all the Ca\textsuperscript{2+} did not leak out from the ER when the Ca\textsuperscript{2+} influx component was blocked by vanadate indicates that the ER of permeabilized cells is not "leaky" to Ca\textsuperscript{2+}.

The observation that IP\textsubscript{3} releases Ca\textsuperscript{2+} after total blockade of Ca\textsuperscript{2+} influx with vanadate suggests, as observed in permeabilized human neutrophils (13), that IP\textsubscript{3} acts by stimulating an independent efflux component of the ER and not by inhibiting the influx component of Ca\textsuperscript{2+} transport. The presence of an additional nonmitochondrial compartment was detected since the Ca\textsuperscript{2+} ionophore A23187 released further Ca\textsuperscript{2+} from the cells (Fig. 5A). Fig. 5B shows that the Ca\textsuperscript{2+} that could be released by vanadate alone (Fig. 5A) was also IP\textsubscript{3}-releasable. Indeed, the final amount of Ca\textsuperscript{2+} rapidly released by IP\textsubscript{3} (in Fig. 5B) was the same as that obtained in Fig. 5A when IP\textsubscript{3} was added after a plateau Ca\textsuperscript{2+} level had already been obtained with vanadate. Thus, in the experiments reported in Figs. 6 and 7, the IP\textsubscript{3}-releasable Ca\textsuperscript{2+} is the amount of Ca\textsuperscript{2+} rapidly released from the cells after the simultaneous addition of vanadate plus IP\textsubscript{3}. Vanadate was added to eliminate the problem of Ca\textsuperscript{2+} resequestration by the ER during IP\textsubscript{3}-induced Ca\textsuperscript{2+} release.

The amount of Ca\textsuperscript{2+} released by IP\textsubscript{3} was measured as a function of ATP-dependent Ca\textsuperscript{2+} accumulation by the ER (Fig. 6). It was observed that the IP\textsubscript{3}-releasable Ca\textsuperscript{2+} was linearly related to the amount of Ca\textsuperscript{2+} taken up by the ER. Fig. 6 further shows that about 90% of the accumulated Ca\textsuperscript{2+} was IP\textsubscript{3}-releasable when cells were allowed to accumulate relatively small amounts of Ca\textsuperscript{2+} (less than 15 nmol/mg of cell protein). In another series of experiments, the amount of Ca\textsuperscript{2+} released by IP\textsubscript{3} was evaluated as a function of total releasable Ca\textsuperscript{2+} (IP\textsubscript{3}+ A23187) (Fig. 7). We observed that the IP\textsubscript{3}-releasable Ca\textsuperscript{2+} was linearly related to the increase in total cell Ca\textsuperscript{2+} content until the total releasable Ca\textsuperscript{2+} was about 16 nmol/mg of cell protein. Above this point, the additional Ca\textsuperscript{2+} taken up was accumulated in an IP\textsubscript{3}-insensitive, A23187-sensitive compartment (Fig. 7). Thus, the maximal amount of Ca\textsuperscript{2+} that could be released from the ER by IP\textsubscript{3} was about 13 nmol/mg of cell protein. In marked contrast, the A23187-releasable compart-

**FIG. 5.** IP\textsubscript{3}-induced stimulation of a Ca\textsuperscript{2+} efflux component of the nonmitochondrial pool. Cells (10\textsuperscript{6}) were incubated as described in the legend to Fig. 1, at the various times noted, MgATP (1 mM), vanadate (1 mM), the Ca\textsuperscript{2+} ionophore A23187 (1 μg/ml), IP\textsubscript{3} (5 μM), or CaC\textsubscript{12} (1 nmol/0.2 ml) were added. The three traces shown in the figure were performed with cells from the same preparation. The trace shown in panel C was used to calibrate Ca\textsuperscript{2+} release by various agents in traces A and B. Note the difference in time scale in the traces A, B, and C.

**FIG. 6.** The relationship between the IP\textsubscript{3}-releasable Ca\textsuperscript{2+} and the Ca\textsuperscript{2+} accumulated into the nonmitochondrial compartment. Cells (10\textsuperscript{6}) were incubated as described in the legend to Fig. 1 and with an experimental design similar to that shown in Fig. 5A. The points shown in the figure were obtained from separate traces with three different cell preparations. In order to have various amounts of Ca\textsuperscript{2+} accumulated in the nonmitochondrial pool, cells were incubated for various times before the addition of vanadate plus IP\textsubscript{3} and at various starting ambient Ca\textsuperscript{2+} concentrations. From Fig. 6A, it was observed that the IP\textsubscript{3}-releasable Ca\textsuperscript{2+} was calculated from the increase in ambient [Ca\textsuperscript{2+}] following the addition of ATP to the medium (not including the small immediate chelation of medium Ca\textsuperscript{2+}). The ATP-dependent Ca\textsuperscript{2+} uptake was calculated from the lowering of ambient [Ca\textsuperscript{2+}] following the addition of ATP to the medium (not including the small immediate chelation of medium Ca\textsuperscript{2+} by ATP) until vanadate (1 mM) and IP\textsubscript{3} (10 μM) were simultaneously added to the medium (see also Fig. 5A). The IP\textsubscript{3}-releasable Ca\textsuperscript{2+} was calculated from the increase in ambient [Ca\textsuperscript{2+}] following the simultaneous addition of vanadate plus IP\textsubscript{3} until a plateau was reached after about 3 min (see also Fig. 5B). Both uptake and release of Ca\textsuperscript{2+} were calculated using separate Ca\textsuperscript{2+} calibration curves similar to the one shown in Fig. 5C. The amount of Ca\textsuperscript{2+} that was released by IP\textsubscript{3} plus vanadate before cells were allowed to take up Ca\textsuperscript{2+} was 4.1 ± 0.4 nmol of Ca\textsuperscript{2+}/mg of cell protein (mean ± S.E. of three separate experiments). This "endogenous" IP\textsubscript{3}-releasable Ca\textsuperscript{2+} was subtracted from all the experimental values to give the points shown in the figure.
FIG. 7. IP$_3$ and A23187-releasable Ca$^{2+}$ from nonmitochondrial pools. The left panel illustrates the experimental design used to obtain the points shown in the right panel. Cells were incubated as described in the legend to Fig. 1, but in a medium containing also the Ca$^{2+}$ chelator quin 2 (5 μM) and various amounts of added Ca$^{2+}$. Left panel, where indicated, cells (10$^6$) and the Ca$^{2+}$ ionophore A23187 (1 μg/ml) were added. IP$_3$ denotes the simultaneous addition of IP$_3$ (5 μM) plus vanadate (1 mM). Right panel, the points and corresponding triangles were obtained from separate traces with four different cell preparations. The values for the IP$_3$-releasable Ca$^{2+}$ were obtained from the increase in ambient [Ca$^{2+}$] following the simultaneous addition of IP$_3$ plus vanadate. The A23187-releasable Ca$^{2+}$ was calculated from the increase in ambient Ca$^{2+}$ following A23187 addition, after the release of Ca$^{2+}$ from the IP$_3$-sensitive Ca$^{2+}$ pool. The total releasable Ca$^{2+}$ is the sum of the IP$_3$- and A23187-releasable Ca$^{2+}$.

**Discussion**

The results demonstrate that the transient nature of the IP$_3$-induced Ca$^{2+}$ response that has been observed in a variety of cell types (4-8) is due to degradation of IP$_3$ in RINm5F insulinoma cells, not to Ca$^{2+}$ reaccumulation by an IP$_3$-insensitive nonmitochondrial compartment or desensitization to the molecule. We ruled out desensitization by showing that high Ca$^{2+}$ levels could be maintained by the ER if several pulse additions of IP$_3$ were made in rapid succession. Furthermore, if the molecule was infused, a Ca$^{2+}$ steady state was maintained by the ER and Ca$^{2+}$ reuptake occurred only when infusion was stopped. The possibility that Ca$^{2+}$ reuptake following IP$_3$ addition was due to an IP$_3$-insensitive ER compartment was discarded by showing that at relatively low Ca$^{2+}$ contents, almost all (about 90%) of the ER pool of RINm5F cells was IP$_3$-releasable. It should be added that the conclusion that the IP$_3$-induced Ca$^{2+}$ response is transient due to IP$_3$ degradation is in accord with previous studies carried out in insulinoma RINm5F cells (11) and hepatocytes (5) showing that [32P]IP$_3$ degradation correlated approximately with the time course of Ca$^{2+}$ reuptake. Thus, assuming a similar mode of action of IP$_3$ in other cell types or preparations, it is likely that the lack of a "second IP$_3$-induced Ca$^{2+}$ response" observed in permeabilized human neutrophils (13) or rat insulinoma (7) and liver (12) microsomes is due to an IP$_3$-insensitive nonmitochondrial compartment present in these preparations.

It has been suggested that within the intact cell only a part of the ER is sensitive to IP$_3$ (8) and that the molecule releases only a small fraction of the ER Ca$^{2+}$ content from a specialized region of the ER (21). The view that the ER possesses IP$_3$-sensitive and -insensitive regions which are noncommunicating Ca$^{2+}$ compartments or that only a small fraction of the ER Ca$^{2+}$ content is IP$_3$-sensitive does not seem to apply to insulinoma cells. Indeed, three separate arguments contradict this view. First, a pulse addition of a maximal concentration of IP$_3$ released almost all the Ca$^{2+}$ accumulated by the ER, even in the presence of an operative Ca$^{2+}$ influx component (Fig. 1). It is of interest to note that a similar experiment performed on permeabilized neutrophils (13) or hepatocytes (21) released only 25-50% of the Ca$^{2+}$ accumulated into the nonmitochondrial compartment. Second, the maintenance of a Ca$^{2+}$ steady state by the ER during IP$_3$ infusion (Figs. 2-4) suggests that it is unlikely that an IP$_3$-insensitive ER compartment is of major importance to Ca$^{2+}$ buffering in RINm5F cells. Indeed, were a nonmitochondrial IP$_3$-insensitive compartment important, the Ca$^{2+}$ released by the IP$_3$-sensitive pool could be resequestered by the IP$_3$-insensitive ER, and consequently, the Ca$^{2+}$ response would be transient even during IP$_3$ infusion. Third, we have shown (Figs. 6 and 7) that at reasonable Ca$^{2+}$ contents (less than 15 nmol of Ca$^{2+}$/mg cell protein) almost all the Ca$^{2+}$ accumulated by the ER was released by IP$_3$; for comparison, most cells have a total Ca$^{2+}$ content of about 10 nmol/mg of protein (22). When the total nonmitochondrial cellular Ca$^{2+}$ content exceeded 16 nmol/mg of cell protein, Ca$^{2+}$ was accumulated exclusively in an IP$_3$-insensitive compartment. The nature of this IP$_3$-insensitive compartment which sequesters Ca$^{2+}$ at high Ca$^{2+}$ loadings is presently unknown. It is possible that at high and unphysiological Ca$^{2+}$ contents, an IP$_3$-insensitive ER is formed due to a deleterious action of such large amounts of Ca$^{2+}$. Thus, the ER might be fragmented into IP$_3$-sensitive and -insensitive regions. Alternatively, at high Ca$^{2+}$ concentrations and following saturation of the ER, Ca$^{2+}$ might be accumulated in other structures (such as Golgi elements, lysosomes, coated vesicles, etc.). A third possibility is that the releasing mechanism induced by IP$_3$ is inhibited at high Ca$^{2+}$ concentrations (23). Further studies are required to evaluate these possibilities. It can be supposed that the IP$_3$-insensitive, A23187-releasable pool (which remains constant at about 3 nmol Ca$^{2+}$/mg protein) over quite a wide range of Ca$^{2+}$ contents consists of nonmitochondrial compartments such as secretory granules, lysosomes, and coated vesicles. Indeed, insulin secretory granules are known to contain large amounts of A23187-releasable Ca$^{2+}$ (18), despite the fact that they do not appear to play a role in short-term regulation of cytosolic Ca$^{2+}$ (18). Hence, we favor the concept that in intact insulinoma cells containing reasonable amounts of Ca$^{2+}$, the ER is primarily a single Ca$^{2+}$ compartment with all regions communicating in such a way that virtually all the ER Ca$^{2+}$ content is IP$_3$-releasable. This does not exclude the possibility that some regions of the ER contain more IP$_3$-"receptors" than others or even that some regions are devoid of IP$_3$ receptors. Indeed, such possibilities could explain the heterogeneity of microsomal vesicles generated by the fragmentation of the ER when cells are homogenized (8). The view that the ER is a single IP$_3$-sensitive Ca$^{2+}$ compartment is compatible with morphological observations carried out in a variety of tissues showing that the ER membrane is continuous throughout the cell and that it encloses a single lumen (24, 25).

Our finding that elevated Ca$^{2+}$ steady states can be maintained by the ER when IP$_3$ is constantly infused into the permeabilized cells is of particular interest. We demonstrated that bidirectional buffering of ambient [Ca$^{2+}$] occurred at steady state, since the ER tended to restore the previous Ca$^{2+}$ level when small additions of either Ca$^{2+}$ or the Ca$^{2+}$ chelator quin 2 were added to the system. However, this Ca$^{2+}$ steady state established by the ER is not strictly a "set point" as it is for the "extramitochondrial Ca$^{2+}$ steady state" (16, 26, 27).
Thus, unlike the mitochondria which are known to maintain an extramitochondrial Ca\(^{2+}\) steady state that is independent of the Ca\(^{2+}\) load over quite a wide range (16, 26, 27), the "extra ER Ca\(^{2+}\) steady state" was markedly dependent on the Ca\(^{2+}\) load. The results suggest that when a constant IP\(_3\) level is achieved during IP\(_3\) infusion, the ER maintains a Ca\(^{2+}\) steady state at which Ca\(^{2+}\) influx via the Ca\(^{2+}\)-ATPase equals Ca\(^{2+}\) efflux through an independent IP\(_3\)-regulated component. Extended to the intact cell, such Ca\(^{2+}\) cycling through independent influx and efflux pathways would allow very precise regulation of cytosolic Ca\(^{2+}\) concentration. Thus, it can be hypothesized that IP\(_3\) may not be a molecule which is the driving force of the Ca\(^{2+}\)-ATPase. The second mode of control is likely to be of less importance in most cells since the phosphorylation potential of cells is in general remarkably stable. Under pathophysiological conditions where the phosphorylation potential is lowered and consequently Ca\(^{2+}\) influx is decreased, this could be a means by which Ca\(^{2+}\) escapes the ER. In pancreatic islets, however, such modulation of the influx component might be of physiological significance. Indeed, it can be hypothesized that when \(\beta\) cell glucose phosphorylation by glucokinase (28) is acutely stimulated by a stepwise elevation in glucose concentration, the pancreatic islet phosphorylation potential might be transiently lowered, leading to a transient mobilization of Ca\(^{2+}\) from the ER. This would be a very simple mechanism for linking metabolic changes induced by glucose to the early Ca\(^{2+}\) mobilization (2). However, this does not preclude the possibility that fuel secretagogues also mobilize Ca\(^{2+}\) from the ER through enhanced IP\(_3\) production.

The exact concentrations of IP\(_3\) that occur in cells under basal or stimulated conditions are at present unknown. Rough estimates based on isotopic methods give values in the micromolar range (8, 21, 29) which are also the levels active in permeabilized cells (8). It should be added that in some tissues both the cytosolic Ca\(^{2+}\) concentration (21, 30, 31) and the IP\(_3\) level (3, 21, 29, 32) remain significantly elevated over basal values as long as a high concentration of the agonist is present. Thus, it can be hypothesized that IP\(_3\) may not be a molecule that only transiently stimulates Ca\(^{2+}\) efflux from the ER. Our IP\(_3\) infusion experiments suggest that the IP\(_3\)-sensitive independent efflux component of the ER could be continuously regulated by this molecule. Consequently, the size of the ER Ca\(^{2+}\) pool is likely to be continuously determined by the concentration of IP\(_3\) in the cytosol. Studies carried out in several tissues have suggested that the ER, together with the plasma membrane, may be involved in establishing the cytosolic free Ca\(^{2+}\) concentration (18, 21, 22, 33). Hence, when extended to the in vivo situation, our data suggest that IP\(_3\) by finely modulating the rate of Ca\(^{2+}\) cycling across the ER may play a major role in regulating the level of cytosolic Ca\(^{2+}\) under both basal and stimulated conditions.

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