Relationship between hormonal, GTP and $\text{Ins}(1,4,5)\text{P}_3$ -stimulated Ca^{2+} uptake and release in pancreatic acinar cells

Shmuel MUALLEM*† and Timothy G. BEEKER†

*Department of Physiology, University of Texas Health Science Center at Dallas, TX 75235, and †The Laboratory of Membrane Biology, Research Institute, Cedars Sinai Medical Center, UCLA School of Medicine, Los Angeles, CA 90048, U.S.A.

Electrically permeabilized rat pancreatic acini were used to evaluate the contributions of GTP and $\text{Ins}(1,4,5)\text{P}_3$ to hormone-stimulated Ca^{2+} uptake and release from intracellular pools. Treatment of permeabilized acini with Ca^{2+} -mobilizing hormones, GTP or GTP[S] resulted in stimulation of an ATP-dependent, VO_4^{2-} -sensitive Ca^{2+} uptake into a non-mitochondrial intracellular pool. GTP and GTP[S] also augmented the hormone-mediated stimulation of Ca^{2+} uptake. Including oxalate in the uptake medium increased Ca^{2+} uptake into this pool but did not modify the stimulation of Ca^{2+} uptake induced by hormones or GTP. $\text{Ins}(1,4,5)\text{P}_3$ released all the extra Ca^{2+} accumulated as a result of hormone, GTP or GTP[S] stimulation. Hence, these stimuli activated the Ca^{2+} pump localized in the membrane of the hormone and $\text{Ins}(1,4,5)\text{P}_3$ -sensitive Ca^{2+} pool. Including 2,3-diphosphoglyceric acid (PGA) [an inhibitor of $\text{Ins}(1,4,5)\text{P}_3$ hydrolysis] in the incubation medium blunted the GTP and GTP[S]-stimulated Ca^{2+} uptake. In the presence of PGA, the hormones inhibited Ca^{2+} accumulation, and GTP and GTP[S] augmented this effect. Accordingly, PGA stabilized the $\text{Ins}(1,4,5)\text{P}_3$ -evoked Ca^{2+} release from intracellular pools. Only in the presence of PGA was it possible to demonstrate hormonally-evoked Ca^{2+} release from permeabilized cells. GTP, and more importantly GTP[S], augmented the hormone-evoked Ca^{2+} release. Hormones and $\text{Ins}(1,4,5)\text{P}_3$ in the presence or absence of GTP or GTP[S] released Ca^{2+} from the same intracellular pool. The extent of Ca^{2+} release caused by the combination of hormones and GTP or GTP[S] was similar to that evoked by $\text{Ins}(1,4,5)\text{P}_3$ alone. Taken together, these results suggest that GTP or GTP[S] facilitates stimulation of phospholipase C by hormones. Such stimulation results in stimulation of protein kinase C and increased levels of $\text{Ins}(1,4,5)\text{P}_3$ and is sufficient to explain the effects of GTP and GTP[S] on Ca^{2+} uptake and release from pancreatic acinar cells.

INTRODUCTION

Stimulation of pancreatic acinar cells with Ca^{2+} -mobilizing hormones results in Ca^{2+} release from intracellular stores [1–7] as well as stimulation of Ca^{2+} uptake into a non-mitochondrial intracellular organelle [8–10]. It is now widely accepted that $\text{Ins}(1,4,5)\text{P}_3$ mediates the Ca^{2+} release process. The ability of $\text{Ins}(1,4,5)\text{P}_3$ to mobilize Ca^{2+} from intracellular stores has been documented in many cell types [11]. Furthermore, in the original observations of Streb *et al.* [1] it was demonstrated that Ca^{2+} -mobilizing hormones and $\text{Ins}(1,4,5)\text{P}_3$ release Ca^{2+} from the same intracellular pool.

Another mediator of Ca^{2+} release from intracellular stores is GTP [12–15]. This process requires the hydrolysis of GTP and it is blocked by a non-hydrolysable analogues of GTP [12]. Conflicting reports exist as to the intracellular site from which GTP releases Ca^{2+} . In neuroblastoma and smooth muscle cell lines, GTP and $\text{Ins}(1,4,5)\text{P}_3$ appear to release Ca^{2+} from the same intracellular organelle [14], whereas in parotid gland acinar cells the two mediators release Ca^{2+} from different pools [15]. In the liver, GTP did not induce any Ca^{2+} release

from permeabilized cells or isolated vesicles but augmented the $\text{Ins}(1,4,5)\text{P}_3$ -mediated Ca^{2+} release [16,17].

There is virtually no information concerning the possible contribution of the GTP-mediated Ca^{2+} release to the overall process of hormone-mediated Ca^{2+} release. Such information does not exist largely because most permeabilized cells no longer respond to hormonal stimulation. In this respect, pancreatic acinar cells are among the few cells which still release Ca^{2+} from intracellular stores upon hormonal stimulation when permeabilized [1]. In the present studies we used this cell system to evaluate the contribution of $\text{Ins}(1,4,5)\text{P}_3$ and GTP to the hormone-evoked Ca^{2+} release from intracellular stores.

Another goal of the present studies was to determine the possible effect of a GTP-dependent process in the hormone-mediated activation of an intracellular Ca^{2+} pump. Stimulation of pancreatic acini with Ca^{2+} -mobilizing hormones increased the ATP-dependent and VO_4^{2-} -sensitive Ca^{2+} uptake into permeabilized cells [10] and isolated intracellular vesicles [8,9]. Two possible GTP-dependent mechanisms can account for the reported stimulation of the Ca^{2+} pump by hormones. Studies in some cell systems show that activation of

Abbreviations used: CCK-OP, cholecystokinin octapeptide; DAG, diacylglycerol; PLC, phospholipase C; PKC, protein kinase C; PGA, 2,3-diphosphoglyceric acid; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; GTP[S], guanosine 5'-[γ -thio]triphosphate; ER, endoplasmic reticulum.

† To whom correspondence should be addressed.

protein kinase C (PKC) by phorbol esters results in stimulation of an intracellular Ca^{2+} pump thought to be located in the membrane of the endoplasmic reticulum [18]. Stimulation of PKC requires the action of phospholipase C (PLC), a GTP-activated enzyme [19–22]. Hence it is possible that GTP and its analogues could indirectly stimulate the intracellular Ca^{2+} pump through the activation of PLC.

Another mode by which GTP can stimulate Ca^{2+} uptake into intracellular organelles is that reported for neuroblastoma and smooth muscle cell lines [14,23] and macrophages [13]. In these cells, GTP stimulates ATP-dependent Ca^{2+} uptake in the presence of oxalate. It was suggested that GTP stimulates the redistribution of Ca^{2+} between intracellular pools [14,23]. Whether such a mechanism is common to all cells and is related to the hormone-mediated Ca^{2+} -pump activation is not yet clear.

In the present studies we used electrically permeabilized pancreatic acini to evaluate the possible contribution of these mechanisms to the stimulation of the Ca^{2+} pump by Ca^{2+} -mobilizing hormones. In pancreatic acini, no evidence could be obtained for hormone- or GTP-mediated Ca^{2+} transfer between intracellular pools. We also show that the Ca^{2+} pump stimulated by hormones and GTP is located in the membrane of the $\text{Ins}(1,4,5)\text{P}_3$ -mobilizable Ca^{2+} pool.

MATERIALS AND METHODS

Materials

Cholecystokinin-octapeptide (CCK-OP) was a gift from the Squibb Institute for Medical Research, Princeton, NJ, U.S.A. Soybean trypsin inhibitor, carbachol, atropine and 2,3-diphosphoglyceric acid (PGA) were obtained from Sigma. $\text{Ins}(1,4,5)\text{P}_3$ was from Calbiochem. GTP and guanosine 5'-[γ -thio]triphosphate (GTP[S]) were from Boehringer-Mannheim. Purified collagenase was obtained from Worthington Biochemical. Fura 2-tetra K^+ salt was from Molecular Probes.

Preparation of acini

Dispersed pancreatic acini were prepared from Sprague-Dawley rats using a collagenase digestion procedure previously described [24] with some modification as previously cited [25]. Acini prepared from the pancreas of one rat were suspended in an incubation solution containing (in mM) HEPES (pH 7.4) 10; NaCl 140; KCl 5; MgCl_2 1; CaCl_2 1.5; glucose 10; sodium pyruvate 10; soybean trypsin inhibitor 0.01 % (w/v) and bovine serum albumin 0.1 % (w/v).

Cell permeabilization

Acini from the pancreas of one rat were washed twice and resuspended in 2.5–3 ml of a solution containing (in mM): KCl 140; MgCl_2 5; HEPES-Na 10 (pH 7.2 at 37 °C); CaCl_2 0.01 (uptake medium). To adjust the free Ca^{2+} concentrations in all uptake media, 0.2 μM of the tetra K^+ form of Fura 2 was added to a sample of medium maintained at 37 °C. Increasing concentrations of EGTA were added and free Ca^{2+} was calculated from F , $F_{\min}$, and $F_{\max}$. (F = fluorescence) [26]. Acini washed and suspended in uptake medium containing the required adjusted ionized Ca^{2+} concentration were incubated for 30–45 min at 0 °C prior to permeabilization. Then the acini were permeabilized by two exposures to an electric field of 0.75 kV through a capacitance of 25 μF using a

Gene Pulser (BioRad). Cell permeabilization was then estimated from the measurements of ethidium bromide fluorescence as previously described [10]. Routinely, more than 90 % of the cells were permeabilized by the applied electric field.

Measurement of Ca^{2+} uptake

The suspension of permeabilized acini was warmed to 37 °C by a 1 min incubation with continuous shaking. Ca^{2+} uptake was initiated within 3 min of cell permeabilization. To initiate Ca^{2+} uptake, samples of permeabilized acini were added to uptake medium also containing the following (final concentrations): 10 μM -antimycin A; 5 μM -oligomycin; ^{45}Ca (approx. 10^6 cpm/ml), with or without 3 mM-ATP and 10 mM-PGA as specified. The pH of all solutions was verified to be 7.2 at 37 °C prior to addition of ^{45}Ca and initiation of uptake.

Fig. 1. CCK-OP-stimulated Ca^{2+} uptake into permeabilized pancreatic acini

Permeabilized pancreatic acini were added to uptake medium containing 0.25 μM - Ca^{2+} with (□, ■, ○, ●) or without (△, ▲) 3 mM-ATP. As indicated, the medium also contained 3 mM- VO_4^{2-} (▲, ●, ■) and 10^{-8} M-CCK-OP (□, ■). At the indicated times, samples were removed to measure Ca^{2+} content of acini as described in the Materials and methods section. Carbachol (0.1 mM) or a mixture of 0.1 mM-carbachol and 10^{-8} M-CCK-OP stimulated Ca^{2+} uptake similar to that by CCK-OP alone. Ca^{2+} uptake in the absence of ATP was measured in three experiments giving similar results to those in the Fig.

Fig. 2. Stimulation of VO₄²⁻-inhibitable Ca²⁺ uptake by GTP and GTP[S]

Ca²⁺ uptake into permeabilized pancreatic acini was measured as described in the Materials and methods section and in the legend to Fig. 1. The uptake medium contained 0.25 μM-Ca²⁺ alone (○) or with 50 μM-GTP[S] (Δ) or 100 μM-GTP (□). The Fig. shows the fraction of VO₄²⁻-inhibitable Ca²⁺ uptake under all conditions. Ca²⁺ uptake in the presence of 3 mM-VO₄²⁻ was subtracted from the total Ca²⁺ uptake in the absence of VO₄²⁻.

At the indicated times, 0.2 ml portions of cell suspension were transferred to 8 ml of ice-cold uptake medium containing 1 mM-LaCl₃. The acini were washed three times with this medium, dissolved in 1 ml of 1 M-NaOH and ⁴⁵Ca was counted by a standard scintillation counting method.

RESULTS

In previous experiments to study Ca²⁺-pump stimulation, acini were permeabilized by incubation with saponin for 3 min at 37 °C [10]. Using the saponin procedure it was not possible to consistently demonstrate hormonal stimulation of Ca²⁺ uptake or release subsequent to cell permeabilization. Since cell permeabilization by electrical discharge was shown to be superior to that by detergent in retaining cellular responses [27], we tested the applicability of this technique to pancreatic acini. Fig. 1 shows the time course of Ca²⁺ uptake into electrically permeabilized rat pancreatic acini. In the presence of 3 mM-VO₄²⁻, there was a rapid ATP-independent Ca²⁺ incorporation into the acini. In the absence of added ATP, a VO₄²⁻-sensitive Ca²⁺ uptake could be detected. This Ca²⁺ uptake decayed with time and could be abolished by incubation of the acini at 37 °C for 5–7 min prior to initiation of ⁴⁵Ca uptake. Hence it is likely to represent Ca²⁺ uptake supported by endogenous ATP. When ATP was included in the incubation medium, Ca²⁺ uptake increased for approx. 2–2.5 min and then

Table 1. Effects of hormone stimulation, GTP and GTP[S] alone or in combination on VO₄²⁻-inhibitable Ca²⁺ uptake

ATP-dependent and VO₄²⁻-inhibitable Ca²⁺ uptake was measured as described in the legends to Figs. 1 and 2. The rate of Ca²⁺ uptake was calculated from the linear portion of Ca²⁺ uptake at the specific indicated conditions. When present, hormone concentrations were 10⁻⁸ M-CCK-OP and 0.1 mM-carbachol. GTP and GTP[S] concentrations were 100 and 50 μM respectively. Values are means ± S.E.M. for the indicated number of experiments (n).

Conditions	Ca ²⁺ uptake (nmol/min per mg of protein)	n
Control	1.21 ± 0.04	12
Hormones	1.88 ± 0.05	10
GTP	2.78 ± 0.09	9
GTP[S]	2.03 ± 0.07	7
Hormones + GTP	3.15 ± 0.07	4
Hormones + GTP[S]	2.90 ± 0.11	4
GTP + GTP[S]	2.81 ± 0.08	4

stabilized. Ca²⁺ uptake in the presence of ATP could be blocked by VO₄²⁻. Thus, pump-mediated Ca²⁺ uptake was considered as the difference between Ca²⁺ uptake in the presence and absence of VO₄²⁻. The fraction of Ca²⁺ uptake that is inhibited by VO₄²⁻ was linear for approx. 1 min. Fig. 1 also shows that stimulation of permeabilized acinar cells with 10⁻⁸ M-CCK-OP increased the rate and extent of the VO₄²⁻-inhibitable Ca²⁺ uptake. Similar results were obtained with another Ca²⁺-mobilizing hormone, carbachol (results not shown). To ensure maximal stimulation, in subsequent experiments the acini were stimulated with 10⁻⁸ M-CCK-OP and 0.1 mM-carbachol.

The ability to stimulate Ca²⁺ uptake by the intracellular Ca²⁺ pump after cell permeabilization allowed us to explore several aspects of the mechanism of pump stimulation. First we tested the effect of GTP and its non-hydrolysable analogue GTP[S] on Ca²⁺ uptake. Fig. 2 shows the VO₄²⁻-inhibitable fraction of Ca²⁺ uptake into permeabilized pancreatic acini. Both GTP[S] and GTP stimulated this Ca²⁺ uptake. The degree of pump stimulation by GTP[S] was similar to that induced by hormone stimulation. In all experiments tested, GTP stimulated Ca²⁺ uptake better than did the hormones or GTP[S].

Table 1 summarizes the effects of hormones, GTP and GTP[S] alone and in combination on ATP-dependent, VO₄²⁻-sensitive Ca²⁺ uptake. Hormones and GTP[S] stimulated the pump to a similar extent. This degree of pump stimulation was substantially lower than that observed when the acini were stimulated prior to permeabilization [10]. Exposure of the acini to GTP or the combination of hormones and GTP, hormones and GTP[S] or GTP plus GTP[S] resulted in the maximal observed pump stimulation which was similar to that measured in our previous studies [10]. These results indicate that pump stimulation by hormones and guanine nucleotides was not additive. Furthermore, GTP[S] did not inhibit either GTP- or hormone-mediated stimulation of Ca²⁺ uptake.

The effect of the Ca²⁺-chelating anion, oxalate, on Ca²⁺ uptake by intracellular organelles is shown in Fig. 3. Oxalate has a small effect on the initial rate of Ca²⁺

Fig. 3. Effect of oxalate on Ca^{2+} uptake of control and stimulated cells

The composition of the uptake media was as specified in the Materials and methods section (open symbols) except that 10 mM-oxalate was included in some conditions (closed symbols). Ca^{2+} concentration in the presence or absence of oxalate was adjusted to $0.25 \mu\text{M}$ prior to initiation of Ca^{2+} uptake. Oxalate augmented Ca^{2+} uptake when the Ca^{2+} concentration was increased to $10 \mu\text{M}$ (not shown). Permeabilized acini were added to the corresponding uptake medium containing 3 mM-ATP in the absence of further additions ($\circ$, $\bullet$), or in the presence of 10^{-8} M-CCK-OP and 0.1 mM-carbachol ($\triangle$, $\blacktriangle$), 100 μM -GTP ($\square$, $\blacksquare$), or 3 mM- VO_4^{2-} ($\diamond$, $\blacklozenge$). At the indicated times, samples were removed to measure Ca^{2+} content of acini. The Fig. shows the means $\pm$ S.E.M. of three experiments. The error bars for Ca^{2+} uptake in the absence of oxalate were not included for clarity but they were in the same range as those shown in Table 1.

uptake but allowed Ca^{2+} uptake to continue for an incubation period of at least 15 min. The oxalate-supported Ca^{2+} uptake could be blocked by VO_4^{2-} as was found for Ca^{2+} uptake in the absence of oxalate. Oxalate augmented Ca^{2+} uptake into control and stimulated acini to the same extent. Hence, unlike Ca^{2+} uptake into permeabilized neuronal and smooth muscle cells [14,23] or macrophages [13], oxalate did not augment Ca^{2+} uptake into stimulated cells beyond its effect on Ca^{2+} uptake into control cells.

To identify the intracellular site of the Ca^{2+} pump which is stimulated by hormones and GTP, we next measured the effect of $\text{Ins}(1,4,5)\text{P}_3$ on the Ca^{2+} content of the acini (Fig. 4). It can be seen that $\text{Ins}(1,4,5)\text{P}_3$ evoked

Fig. 4. $\text{Ins}(1,4,5)\text{P}_3$ -evoked Ca^{2+} release from intracellular stores

Ca^{2+} uptake in the absence (control; $\circ$, $\bullet$), or in the presence of 10^{-8} M-CCK-OP and 0.1 mM-carbachol ($\triangle$, $\blacktriangle$) or 100 μM -GTP ($\square$, $\blacksquare$) was measured as described in the legend to Fig. 1. Where indicated, portions of acini from each condition were transferred to tubes containing $\text{Ins}(1,4,5)\text{P}_3$ to give a final concentration of 5 μM (closed symbols). The Fig. shows the fraction of VO_4^{2-} -inhibitable Ca^{2+} uptake of one experiment representative of two others with similar results.

a transient release of Ca^{2+} from control, hormone- and GTP-stimulated acini. More importantly, the extent of the $\text{Ins}(1,4,5)\text{P}_3$ -evoked Ca^{2+} release increased as the pump was stimulated. Hence, the experiments in Fig. 4 indicate that the hormones, GTP and GTP[S] stimulated Ca^{2+} uptake into the same $\text{Ins}(1,4,5)\text{P}_3$ -sensitive pool.

Fig. 4 shows that $\text{Ins}(1,4,5)\text{P}_3$ -evoked Ca^{2+} release from intracellular stores is a transient process. This is likely to result from the rapid hydrolysis of $\text{Ins}(1,4,5)\text{P}_3$. It was previously shown that Ca^{2+} -mobilizing hormones, GTP and GTP[S] can stimulate the production of $\text{Ins}(1,4,5)\text{P}_3$ by permeabilized pancreatic acinar cells [22]. We therefore reason that our ability to measure pump stimulation is due to a rapid hydrolysis of $\text{Ins}(1,4,5)\text{P}_3$ which is generated during the incubation of the acini with hormones, GTP or GTP[S]. To test this it was necessary to inhibit $\text{Ins}(1,4,5)\text{P}_3$ hydrolysis during incubation with the stimulants. It was previously shown that PGA is a very effective inhibitor of $\text{Ins}(1,4,5)\text{P}_3$ hydrolysis. Therefore, we first measured the effect of PGA on VO_4^{2-} -sensitive Ca^{2+} uptake in the presence or absence of stimulants. Fig. 5 shows that in the presence of 10 mM-PGA, GTP increased Ca^{2+} uptake by approx. 18 % in this experiment (mean $\pm$ S.E.M. $24 \pm 6\%$, $n = 6$). In the presence of GTP[S], Ca^{2+} uptake was similar to that measured under control conditions. With the hor-

Fig. 5. Effect of PGA on Ca²⁺ uptake into permeabilized acini

Uptake medium containing 0.25 μM-Ca²⁺ was supplemented with 10 mM-PGA in the absence of other additions (control, ○), or in the presence of 50 μM-GTP[S] (△), 100 μM-GTP (□), 10⁻⁸ M-CCK-OP and 0.1 mM-carbachol (◇), the hormones and GTP[S] (▲) or the hormones and GTP (■). At the indicated times, samples were removed to determine ⁴⁵Ca content of acini. The Fig. shows the fraction of VO₄²⁻-inhibitable Ca²⁺ uptake of a single experiment. The summary of results from other experiments for each specific experimental condition is given in the text.

mones, Ca²⁺ uptake was higher during the first 30 s of incubation, but then Ca²⁺ content in the cells was reduced by approx. 21 % (18 ± 4 %, *n* = 6). The combination of hormones with GTP[S] reduced Ca²⁺ content by 45 % (51 ± 6 %, *n* = 3) and the combination of hormones with GTP reduced Ca²⁺ content by 49 % (53 ± 9 %, *n* = 6). The effect of GTP, GTP[S] and the hormones, alone or in combination, on Ca²⁺ uptake in the presence of PGA further supports the notion that all stimulants act on the same organelle to stimulate Ca²⁺ uptake and release.

The ability to inhibit Ins(1,4,5)P₃ hydrolysis with PGA allowed us to study the relationship between hormones and Ins(1,4,5)P₃-evoked Ca²⁺ release from intracellular pools and the effects of GTP and GTP[S] on this process. Accordingly, Fig. 6 shows that 5 μM-Ins(1,4,5)P₃ released approx. 1.5 nmol of Ca²⁺/mg of protein. As expected, in the presence of PGA, the Ins(1,4,5)P₃-evoked Ca²⁺ release was not transient. Stimulation of the acini with hormones resulted in an initial increase in Ca²⁺ uptake which is followed by a slow but sustained Ca²⁺ release. The hormones released approx. 50 % of the Ins(1,4,5)P₃-mobilizable Ca²⁺. Furthermore, when the acini were exposed to both hormones and Ins(1,4,5)P₃, Ca²⁺ release was identical to that induced by Ins(1,4,5)P₃ alone.

Fig. 7 shows the effects of GTP and GTP[S] on

Fig. 6. Hormone- and Ins(1,4,5)P₃-evoked Ca²⁺ release in the presence of PGA

Ca²⁺ uptake in the presence of 10 mM-PGA was measured as described in the legend to Fig. 5. After 2 min of incubation at 37 °C, samples of control acini (○) were transferred to tubes containing CCK-OP and carbachol (■), Ins(1,4,5)P₃ (●) or Ins(1,4,5)P₃ plus hormones (▲). Where indicated, 5 μM-Ins(1,4,5)P₃ was added to acini stimulated with the hormones. Final concentrations were 10⁻⁸ M-CCK-OP, 0.1 mM-carbachol and 5 μM-Ins(1,4,5)P₃. The Fig. shows the means ± S.E.M. of four experiments.

hormone- and Ins(1,4,5)P₃-evoked Ca²⁺ release. Ins(1,4,5)P₃ alone and in combination with the hormones induced a similar Ca²⁺ release from acini incubated with GTP (Fig. 7a). GTP also modified the effect of the hormones on Ca²⁺ content of the acini. In the presence of GTP, a hormone-mediated increase in Ca²⁺ uptake was not detected and the hormones were more effective in releasing Ca²⁺ from intracellular stores. A significant observation is shown in Fig. 7(b) in which GTP[S] affected hormone-evoked Ca²⁺ release in a manner similar to that of GTP (Fig. 7a). Thus, GTP[S], which inhibits GTP-mediated Ca²⁺ release [12], did not inhibit but augmented the hormone-evoked release from the hormone- and Ins(1,4,5)P₃-sensitive intracellular Ca²⁺ pool.

DISCUSSION

The purpose of the present study was to evaluate the possible role of GTP or a GTP-mediated process in hormone-stimulated Ca²⁺ uptake and release from intracellular stores of pancreatic acinar cells. Our data are most compatible with GTP-dependent activation of PLC which leads to the hydrolysis of PtdIns(4,5)P₂ and the generation of Ins(1,4,5)P₃ and diacylglycerol (DAG). DAG stimulates PKC which, in turn, stimulates the Ca²⁺ pumps located in the membrane of the intracellular organelle containing the hormone- and Ins(1,4,5)P₃-mobilizable Ca²⁺. The Ins(1,4,5)P₃ generated as a result of GTP stimulation of PLC triggers Ca²⁺ release from intracellular stores.

Stimulation of pancreatic acinar cells with Ca²⁺-

Fig. 7. Effects of GTP and GTP[S] on hormone- and Ins(1,4,5) P_3 -evoked Ca^{2+} release

Experimental protocol for Ca^{2+} uptake and release in the presence of 10 mM-PGA, the hormones and Ins(1,4,5) P_3 concentration are identical to those in Fig. 6 except that the uptake medium contained either 100 μ M-GTP (a) or 50 μ M-GTP[S] (b). The Fig. shows the means $\pm$ S.E.M. of three experiments. $\circ$, Control; $\blacksquare$, CCK-OP plus carbachol; $\bullet$, Ins(1,4,5) P_3 ; $\blacktriangle$, Ins(1,4,5) P_3 plus hormones.

mobilizing hormones resulted in stimulation of Ca^{2+} uptake into a non-mitochondrial pool in permeabilized cells [10] and isolated endoplasmic reticulum (ER) [8,9]. In those studies, the acini were stimulated prior to cell permeabilization or isolation of the ER. Here we show that in electrically permeabilized cells it is possible to stimulate Ca^{2+} uptake with hormones, GTP or GTP[S] after cell permeabilization. The properties of Ca^{2+} uptake into permeabilized cells are identical to those reported for Ca^{2+} uptake into isolated ER [28–31]. Thus Ca^{2+} uptake was not sensitive to blockers of mitochondrial Ca^{2+} uptake. Ca^{2+} uptake was inhibited by a high concentration of VO_4^{2-} and was increased by the Ca^{2+} -chelating anion oxalate. Part of the Ca^{2+} incorporated by control acini and all of the extra Ca^{2+} incorporated into acini incubated with hormones, GTP[S] or GTP could be released by Ins(1,4,5) P_3 (Fig. 4). In addition, including PGA in the incubation medium resulted in inhibition of Ca^{2+} uptake measured in the presence of hormones, GTP or GTP[S] (Fig. 5). These findings suggest that hormones, GTP and GTP[S] specifically stimulate the Ca^{2+} pumps located in the membrane of the organelles storing the hormone- and Ins(1,4,5) P_3 -mobilizable Ca^{2+} . The nature of these organelles is not clear at present. Subcellular fractionation of pancreatic acinar cells suggested that this organelle is the rough ER [2]. However, recently an organelle containing a Ca^{2+} -binding protein immunologically related to calsequestrin has been identified and termed a 'calciosome' [32]. It was suggested that the calciosomes contain the hormone- and Ins(1,4,5) P_3 -mobilizable Ca^{2+} .

Stimulation of an intracellular Ca^{2+} pump by Ca^{2+} -mobilizing hormones has been previously reported [8,18]. On the basis of the effect of the phorbol ester 12-*O*-tetradecanoylphorbol 13-acetate (TPA), it has been suggested that hormone-stimulated PKC results in the observed

stimulation of Ca^{2+} uptake into intracellular organelles. It was also shown that PLC is a GTP-dependent enzyme where GTP stimulates PLC-mediated hydrolysis of Ptd-Ins(4,5) P_2 [11,19–22]. Thus, one mechanism by which GTP can activate intracellular Ca^{2+} pumps is through the activation of PLC. A second mechanism of GTP-mediated stimulation of Ca^{2+} uptake into intracellular organelles has been described in several cells [13,14]. It was suggested that GTP promotes the fusion of intracellular vesicles containing both oxalate-permeating pathways and Ca^{2+} pumps with vesicles solely containing Ca^{2+} pumps [23]. This phenomenon has been termed a GTP-promoted 'conveyance' of intracellular membranes [23].

In pancreatic acini, stimulation of the intracellular Ca^{2+} pump by hormones and GTP was observed in the absence of oxalate. Further, including oxalate in the incubation medium did not augment the hormone- or GTP-stimulated Ca^{2+} uptake (Fig. 3). Finally, GTP[S], which inhibited the GTP-promoted conveyance phenomenon [23], induced Ca^{2+} -pump stimulation similar to that seen with hormones, and augmented the hormone-mediated Ca^{2+} -pump stimulation. When taken together, these observations argue that the increased Ca^{2+} uptake observed when the cells are stimulated with hormones, GTP or GTP[S] results from activation of the intracellular Ca^{2+} pump of pancreatic acinar cells rather than initiation of membrane conveyance.

The biochemical pathway responsible for Ca^{2+} -pump stimulation by the various stimuli may involve activation of PKC. This is suggested by the effect of PGA on Ca^{2+} uptake and release from intracellular stores. PGA was shown to inhibit Ins(1,4,5) P_3 hydrolysis in permeabilized cells [33]. In agreement with these findings, the Ins(1,4,5) P_3 -evoked Ca^{2+} release from permeabilized pancreatic acini was transient in the absence of PGA but

sustained in the presence of PGA. Further, we could demonstrate a hormone-mediated Ca²⁺ release from intracellular stores only in the presence of PGA. This Ca²⁺ release was augmented by GTP and GTP[S]. These results are consistent with stimulation of PLC by the hormones, GTP and GTP[S] to generate Ins(1,4,5)P₃ and DAG. Indeed, GTP and GTP[S] were shown to stimulate the release of [³H]Ins(1,4,5)P₃ from permeabilized pancreatic acinar cells labelled with [³H]inositol [22]. The increased levels of DAG should result in activation of PKC. Such can account for the stimulation of the Ca²⁺ pump by hormones, GTP and GTP[S] in the permeabilized pancreatic acini.

Hormone-evoked Ca²⁺ release from intracellular stores has been documented in many cell types. In all systems studied it has been established that binding of hormones to specific receptors initiates a cascade of events leading to the generation of mediators of Ca²⁺ release from intracellular pools. While the role of Ins(1,4,5)P₃ is well documented, the possible contribution of GTP-triggered Ca²⁺ release to the overall hormone-evoked Ca²⁺ release has not been previously investigated in detail.

The results presented here suggest that GTP augments hormone-evoked Ca²⁺ release by facilitating PLC stimulation. A direct GTP-triggered Ca²⁺ release or mobilization from intracellular pools does not appear to contribute to the hormone-mediated process in pancreatic acinar cells. Similar conclusions can be reached from measurements of the effect of GTP on mitogen-mediated Ca²⁺ release from permeabilized fibroblast [34]. In pancreatic acini, this conclusion is supported by the findings that in the absence of oxalate, we could not detect a GTP-triggered Ca²⁺ release from permeabilized cells, whereas Ins(1,4,5)P₃ released Ca²⁺ from control and GTP-stimulated cells. Furthermore, it was shown that GTP[S] inhibited the GTP-triggered Ca²⁺ release [12]. In agreement with previous results [22], GTP[S] augmented, rather than inhibited, the hormone-evoked Ca²⁺ release from permeabilized pancreatic acini (Fig. 7). Significantly, the amount of Ca²⁺ mobilized by GTP[S] in combination with the hormones was very similar to that mobilized by Ins(1,4,5)P₃. The latter findings provide the most compelling evidence that a GTP-triggered Ca²⁺ release does not contribute to the hormone-evoked Ca²⁺ release from intracellular pools in pancreatic acinar cells. It is important to note that as in hepatocytes [16,17], a GTP-mediated Ca²⁺ release could not be demonstrated in pancreatic acinar cells. Therefore, our data do not exclude the possibility that such a mechanism may contribute to the hormone-mediated Ca²⁺ release from internal stores of cells in which a GTP-mediated Ca²⁺ release was documented. A similar approach to that used here for pancreatic acinar cells should provide the answer to this critical question.

We thank Rita Kaz and Jackie Williams for their invaluable assistance in the preparation of this manuscript. This work was supported by NIH Grant DK-38938. This work was done during the tenure of an established investigatorship from the American Heart Association to S. M.

REFERENCES

1. Streb, H., Irvine, R. F., Berridge, M. J. & Schulz, I. (1983) *Nature (London)* **306**, 67–69
2. Streb, H., Bayerdorffer, E., Hasse, W., Irvine, R. F. & Schulz, I. (1984) *J. Membr. Biol.* **81**, 241–253
3. Ochs, D. L., Korenbrot, J. I. & Williams, J. A. (1985) *Am. J. Physiol.* **249**, G389–G398
4. Bruzzone, R., Pozzan, T. & Wolheim, C. B. (1986) *Biochem. J.* **235**, 139–143
5. Pandol, S. J., Schoeffield, M. S., Fimmel, C. J. & Muallem, S. (1987) *J. Biol. Chem.* **262**, 16963–16968
6. Muallem, S., Schoeffield, M. S., Fimmel, C. J. & Pandol, S. (1988) *Am. J. Physiol.* **255**, G221–G228
7. Muallem, S., Schoeffield, M. S., Fimmel, C. J. & Pandol, S. (1988) *Am. J. Physiol.* **255**, G229–G235
8. Ponappa, B. C. & Williams, J. A. (1981) *Life Sci.* **28**, 2395–2402
9. Richardson, A. E. & Dormer, R. L. (1984) *Biochem. J.* **219**, 679–685
10. Muallem, S., Beeker, T. G. & Fimmel, C. J. (1987) *Biochem. Biophys. Res. Commun.* **149**, 213–220
11. Berridge, M. J. (1987) *Annu. Rev. Biochem.* **56**, 159–193
12. Gill, D. L., Ueda, T., Chueh, S. H. & Noel, M. W. (1986) *Nature (London)* **320**, 461–464
13. Hamachi, T., Hirata, M., Kimura, Y., Ikebe, T., Ishimatsu, T., Yamaguchi, K. & Koga, T. (1987) *Biochem. J.* **242**, 253–260
14. Mullaney, J., Yu, M., Ghosh, T. K. & Gill, D. L. (1988) *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2499–2503
15. Henne, V., Piiper, A. & Soling, H.-D. (1987) *FEBS Lett.* **218**, 153–158
16. Dawson, A. P., Comerford, J. G. & Fulton, D. V. (1986) *Biochem. J.* **234**, 311–315
17. Thomas, A. P. (1988) *J. Biol. Chem.* **263**, 2704–2711
18. Yoshida, K. & Nachmias, V. T. (1987) *J. Biol. Chem.* **262**, 16048–16054
19. Taylor, S. J. & Exton, J. H. (1987) *Biochem. J.* **248**, 791–799
20. Orellana, S., Solski, P. A. & Brown, J. H. (1987) *J. Biol. Chem.* **262**, 1638–1643
21. Smith, C. D., Uhing, R. J. & Snyderman, R. (1987) *J. Biol. Chem.* **262**, 6121–6127
22. Schulz, I., Schnefel, S., Banfic, H., Thevenod, F., Kemmer, T. & Lickhardt, L. (1986) *Soc. Gen. Physiol. Ser.* **42**, 117–131
23. Mullaney, J. M., Chueh, S.-H., Ghosh, T. K. & Gill, D. L. (1987) *J. Biol. Chem.* **262**, 13865–13872
24. Bruzzone, R., Halban, P. A., Gjinovci, A. & Trimble, E. R. (1985) *Biochem. J.* **226**, 621–624
25. Muallem, S., Beeker, T. G. & Pandol, S. J. (1988) *J. Membr. Biol.* **102**, 153–162
26. Gryniewicz, G., Poenie, M. & Tsien, R. Y. (1985) *J. Biol. Chem.* **260**, 3440–3450
27. Knight, D. E. & Scrutton, M. C. (1986) *Biochem. J.* **234**, 497–506
28. Wakasugi, H., Kimura, T., Haase, W., Kribben, A., Kaufmann, R. & Schulz, I. (1982) *J. Membr. Biol.* **65**, 205–220
29. Preissler, M. & Williams, J. A. (1983) *J. Membr. Biol.* **73**, 137–144
30. Bayerdorffer, E., Streb, H., Eckhardt, L., Haase, W. & Schulz, I. (1984) *J. Membr. Biol.* **81**, 69–82
31. Carafoli, E. (1987) *Annu. Rev. Biochem.* **56**, 395–433
32. Volpe, P., Krause, K.-H., Hashimoto, S., Zorzato, F., Pozzan, T., Meldolesi, J. & Lew, D. P. (1988) *Proc. Natl. Acad. Sci. U.S.A.* **85**, 1091–1095
33. Leslie, B. A., Burgess, G. M. & Putney, J. W., Jr. (1988) *Cell Calcium* **9**, 9–16
34. Muldoon, L. L., Jamieson, G. A., Jr. & Villereal, M. L. (1987) *J. Cell Physiol.* **130**, 29–36