Rapid increases in inositol 1,4,5-trisphosphate, inositol 1,3,4,5-tetrakisphosphate and cytosolic free Ca^{2+} in agonist-stimulated pancreatic acini of the rat

Effect of carbachol, caerulein and secretin

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We compared the time course of increases in isomers of inositol trisphosphate [$\text{Ins}(1,4,5)\text{P}_3$] and $\text{Ins}(1,3,4)\text{P}_3$] and the tetrakisphosphate [$\text{Ins}(1,3,4,5)\text{P}_4$] with changes in cytosolic free Ca^{2+} ($[\text{Ca}^{2+}]_i$) in dispersed pancreatic acini of the rat. There were rapid (5 s) increases in $\text{Ins}(1,4,5)\text{P}_3$ and $\text{Ins}(1,3,4,5)\text{P}_4$ in response to carbachol, caerulein and secretin, whereas $\text{Ins}(1,3,4)\text{P}_3$ increased more slowly. All three secretagogues induced increases in $[\text{Ca}^{2+}]_i$, which reached a peak at 15–20 s. Our results indicate that the very rapid formation of $\text{Ins}(1,4,5)\text{P}_3$ is compatible with its second-messenger role in the initial elevation of $[\text{Ca}^{2+}]_i$.

INTRODUCTION

The hydrolysis of $\text{PtdIns}(4,5)\text{P}_2$ is considered to be a key event for cellular activation upon ligand binding to a wide variety of cell-surface receptors [1,2]. In particular, it has been suggested that $\text{Ins}(1,4,5)\text{P}_3$, one of the products of $\text{PtdIns}(4,5)\text{P}_2$ hydrolysis, is intimately involved in the rise in $[\text{Ca}^{2+}]_i$ also seen upon occupation of these receptors. Briefly, the evidence is that $\text{Ins}(1,4,5)\text{P}_3$ releases Ca^{2+} from an intracellular store, thought to be the endoplasmic reticulum [3–6], that InsP_3 is rapidly accumulated in a Ca^{2+} -independent manner after agonist binding [7,8], and that the same agonists promote rises of $[\text{Ca}^{2+}]_i$ in intact cells, which are at least initially independent of extracellular Ca^{2+} [9,10].

However, interpretation of some of the above findings has been compromised by the realization that InsP_3 fractions, as commonly measured, contain not only $\text{Ins}(1,4,5)\text{P}_3$ but also $\text{Ins}(1,3,4,5)\text{P}_4$ and $\text{Ins}(1,3,4)\text{P}_3$ [11–14]. The last two compounds are sequentially formed as metabolites of $\text{Ins}(1,4,5)\text{P}_3$ [15], but are of as yet unknown physiological function. Therefore, time-course studies of the rise in $[\text{Ca}^{2+}]_i$ after receptor occupation need to be compared with the kinetics of $\text{Ins}(1,4,5)\text{P}_3$ accumulation, not those of ‘total InsP_3 ’; at present such data are only available for the muscarinic stimulation of clonal insulin-secreting cells [16]. In the current study we show that carbachol, secretin and caerulein increase $\text{Ins}(1,4,5)\text{P}_3$ in isolated pancreatic acini, with time courses consistent with the proposed role of this compound in the elevation of $[\text{Ca}^{2+}]_i$.

MATERIALS AND METHODS

Dispersed pancreatic acini were prepared from male Wistar rats (180–200 g), fed *ad libitum*, by collagenase digestion as detailed previously [17].

For measurements of $[\text{Ca}^{2+}]_i$, acini were suspended (approx. 10 mg of acinar protein/ml) in RPMI 1640 medium, buffered to pH 7.4 with 25 mM-Hepes, containing 0.5% human serum albumin. $[\text{Ca}^{2+}]_i$ was determined by loading acini with quin2/AM (50 μM final concn.) for 30–35 min as previously described in detail [18]. The fluorescence signal was calibrated at the end of each trace, and finally $[\text{Ca}^{2+}]_i$ was estimated in accordance with eqn. (1) of Tsien *et al.* [19].

For measurements of inositol phosphates, acini were suspended (approx. 10 mg of acinar protein/ml) in a modified Krebs–Ringer solution, buffered to pH 7.4 with 12.5 mM-Hepes, containing 0.1% human serum albumin. Acini were preincubated in the presence of 2 μM - myo -[2- ^3H]inositol (sp. radioactivity 16.5 Ci/mmol) for 2 h as described previously [20]. Acini were washed, resuspended in fresh buffer (approx. 2.5 mg of acinar protein/ml) and incubated for the times indicated in the Results section. Incubations (1 ml final volume) were started by addition of the agonists and were terminated by acidification with ice-cold trichloroacetic acid (final concn. 10%, w/v). Extracts were washed with ether, desalted with Dowex 50W (H^+ form), neutralized with Tris and freeze-dried [16]. Samples were reconstituted with 0.4 ml of 1 mM-EDTA, pH 7.0, and isomers of InsP_3 were then separated with a discontinuous gradient (0–1.7 M) of ammonium formate, as described previously [14,21]. Radioactivity in the fractions was determined by liquid-scintillation spectrometry. Baseline (background) radioactivity was subtracted from the easily identifiable peaks of both control and stimulated samples. The background values varied by a s.d. of ± 2 c.p.m. (in 12 randomly chosen elution profiles). Retention times of the ^3H -containing peaks and resolution of $\text{Ins}(1,4,5)\text{P}_3$ from $\text{Ins}(1,3,4)\text{P}_3$ were similar to those previously reported [21].

Abbreviations used: $\text{PtdIns}(4,5)\text{P}_2$, phosphatidylinositol 4,5-bisphosphate; $\text{Ins}(1,4,5)\text{P}_3$, inositol 1,4,5-trisphosphate; $\text{Ins}(1,3,4)\text{P}_3$, inositol 1,3,4-trisphosphate; $\text{Ins}(1,3,4,5)\text{P}_4$, inositol 1,3,4,5-tetrakisphosphate; $[\text{Ca}^{2+}]_i$, cytosolic free Ca^{2+} concentration.

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Fig. 1. Time course of the effect of $1\mu\text{M}$ -carbachol on $\text{Ins}(1,4,5)\text{P}_3$, $\text{Ins}(1,3,4,5)\text{P}_4$ and $\text{Ins}(1,3,4)\text{P}_3$ accumulation in dispersed rat pancreatic acini

Acini were labelled as described in the Materials and methods section, and then incubated in the presence of carbachol for the time indicated. Results are shown as means $\pm$ S.E.M. for eight determinations ($n = 4$ at 600 s) and are expressed as percentages of control values, which averaged 62 ± 8 , 28 ± 5 and 16 ± 2 c.p.m. for $\text{Ins}(1,4,5)\text{P}_3$, $\text{Ins}(1,3,4,5)\text{P}_4$ and $\text{Ins}(1,3,4)\text{P}_3$ respectively. $[\text{H}]\text{Ins}(1,4,5)\text{P}_3$, $[\text{H}]\text{Ins}(1,3,4,5)\text{P}_4$ and $[\text{H}]\text{Ins}(1,3,4)\text{P}_3$ were all significantly increased above control values at 5, 15, 60 and 600 s. At the consecutive time points the significance values for $[\text{H}]\text{Ins}(1,4,5)\text{P}_3$ were $P < 0.01$, $P < 0.05$, $P < 0.005$ and $P < 0.005$ respectively. For $[\text{H}]\text{Ins}(1,3,4,5)\text{P}_4$ the significance values were $P < 0.01$, $P < 0.05$, $P < 0.05$ and $P < 0.001$, and for the same time points the significance values for $[\text{H}]\text{Ins}(1,3,4)\text{P}_3$ were $P < 0.02$, $P < 0.05$, $P < 0.005$ and $P < 0.005$ respectively.

All materials and their sources were as previously indicated [18,20,21].

Results are presented as means $\pm$ S.E.M., and statistical analyses were performed by the paired Student's t test.

RESULTS

Carbachol ($1\mu\text{M}$) caused a rapid increase in $[\text{H}]\text{Ins}(1,4,5)\text{P}_3$. The maximum was found at 5 s, when the values were 260% of basal (Fig. 1). Thereafter, in the constant presence of the agonist, there was a tendency for $[\text{H}]\text{Ins}(1,4,5)\text{P}_3$ to decrease, but at 10 min the values were still greater than 200% of basal. $[\text{H}]\text{Ins}(1,3,4,5)\text{P}_4$ also

Table 1. Time course of the effect of 1 nM-caerulein on $\text{Ins}(1,4,5)\text{P}_3$, $\text{Ins}(1,3,4,5)\text{P}_4$ and $\text{Ins}(1,3,4)\text{P}_3$ accumulation in dispersed pancreatic acini

Acini were labelled as described in the Materials and methods section and then incubated in the presence of caerulein for the time indicated. Results are shown as means $\pm$ S.E.M. for five to seven determinations, and are expressed as percentages of control values, which averaged 36 ± 2 , 16 ± 1 and 12 ± 1 c.p.m. for $\text{Ins}(1,4,5)\text{P}_3$, $\text{Ins}(1,3,4,5)\text{P}_4$ and $\text{Ins}(1,3,4)\text{P}_3$ respectively. P values refer to stimulated versus control (100%); NS, not significant.

Incubation time with caerulein	Value (% of control)		
	$\text{Ins}(1,4,5)\text{P}_3$	$\text{Ins}(1,3,4,5)\text{P}_4$	$\text{Ins}(1,3,4)\text{P}_3$
5 s	194 ± 9 ($P < 0.001$)	127 ± 6 ($P < 0.01$)	120 ± 15 NS
30 s	206 ± 21 ($P < 0.005$)	184 ± 24 ($P < 0.02$)	409 ± 45 ($P < 0.001$)
300 s	172 ± 10 ($P < 0.001$)	168 ± 21 ($P < 0.02$)	469 ± 54 ($P < 0.001$)

increased rapidly when the acinar tissue was exposed to carbachol. Maximum values of approx. 300% compared with controls were found at 15 s, and at 10 min the values were still greater than 250% of controls. The fold increase in $[\text{H}]\text{Ins}(1,3,4)\text{P}_3$ was greater than that for either $[\text{H}]\text{Ins}(1,4,5)\text{P}_3$ or $[\text{H}]\text{Ins}(1,3,4,5)\text{P}_4$, but, in contrast with these compounds, the maximum value recorded for $[\text{H}]\text{Ins}(1,3,4)\text{P}_3$ was found at a late time point, i.e. 10 min. Similar results have been recorded after stimulation with 1 nM-caerulein (Table 1).

We have previously shown that secretin, a hormone previously thought to act on exocrine tissue only through the adenylate cyclase system, also causes hydrolysis of $\text{PtdIns}(4,5)\text{P}_2$ [20]. Here we show that, as for carbachol and caerulein, there is a rapid increase in both $[\text{H}]\text{Ins}(1,4,5)\text{P}_3$ and $[\text{H}]\text{Ins}(1,3,4,5)\text{P}_4$, both of which are maximum by 15 s. The highest recorded increase in $[\text{H}]\text{Ins}(1,3,4)\text{P}_3$ was found at 1 min. Both $[\text{H}]\text{Ins}(1,4,5)\text{P}_3$ and $[\text{H}]\text{Ins}(1,3,4)\text{P}_3$ showed marked decreased by 10 min (Fig. 2).

The increases in $[\text{Ca}^{2+}]_i$ induced by carbachol and secretin are shown in Fig. 3. These rises reached a peak at 15–20 s and were transient, since $[\text{Ca}^{2+}]_i$ had regained baseline values within 2–3 min.

DISCUSSION

The kinetics of both $[\text{H}]\text{Ins}(1,4,5)\text{P}_3$ accumulation and the rises in $[\text{Ca}^{2+}]_i$ after stimulation with any of the agonists used in this study are consistent, in each case, with a role for $\text{Ins}(1,4,5)\text{P}_3$ in Ca^{2+} mobilization in pancreatic exocrine tissue. Our results with respect to formation of $[\text{H}]\text{Ins}(1,4,5)\text{P}_3$ are somewhat at variance with a report [22] where, in isolated pancreatic exocrine cells, no increase was found in $[\text{H}]\text{Ins}(1,4,5)\text{P}_3$ on stimulation with 1 mM-carbachol, despite the use of a concentration 3 orders of magnitude greater than that used in our experiments and also 2–3 orders greater than that which gives maximum amylase secretion [18,23–25]. This is most surprising, since it has been shown that concentrations of carbachol of $1\mu\text{M}$ and greater increase the total InsP_3 fraction in acini [20,25,26], and with these

Fig. 2. Time course of the effect of $0.2 \mu\text{M}$ -secretin on $\text{Ins}(1,4,5)\text{P}_3$, $\text{Ins}(1,3,4,5)\text{P}_4$ and $\text{Ins}(1,3,4)\text{P}_3$ accumulation in dispersed rat pancreatic acini

Acini were labelled as described in the Materials and methods section, and then incubated in the presence of secretin for the time indicated. Results are shown as means $\pm$ S.E.M. for eight determinations ($n = 4$ at 600 s) and are expressed as percentages of control [see legend to Fig. 1 for average values (c.p.m.)]. $[\text{^3H}]\text{Ins}(1,4,5)\text{P}_3$ was significantly increased above control values at 5 s ($P < 0.05$), 15 s ($P < 0.01$) and 60 s ($P < 0.025$), $[\text{^3H}]\text{Ins}(1,3,4,5)\text{P}_4$ was significantly increased at 15 s ($P < 0.02$) and 60 s ($P < 0.005$), and $[\text{^3H}]\text{Ins}(1,3,4)\text{P}_3$ was increased above control values at 15 s ($P < 0.005$).

concentrations an increase in $[\text{Ca}^{2+}]_i$ has also been observed [18,20,23,27]. To our knowledge all substances known to mobilize Ca^{2+} from intracellular stores have also been shown to increase $\text{Ins}(1,4,5)\text{P}_3$, when measured by h.p.l.c. The reasons for the differences between our present results and those of Merritt *et al.* [22] are not immediately obvious, but could be related to the differences in cell preparations used; we have used acini, which are more sensitive to agonists than are single cells.

We have previously shown that secretin also causes hydrolysis of $\text{PtdIns}(4,5)\text{P}_2$ [20]. The present results show that, at early time points, the relative rates of formation of $[\text{^3H}]\text{Ins}(1,4,5)\text{P}_3$, $[\text{^3H}]\text{Ins}(1,3,4,5)\text{P}_4$ and $[\text{^3H}]\text{Ins}(1,3,4)\text{P}_3$ upon stimulation with secretin are similar to those found with carbachol and caerulein, agonists well known to cause $\text{PtdIns}(4,5)\text{P}_2$ hydrolysis [2]. There has been no previous investigation of $\text{Ins}(1,3,4,5)\text{P}_4$ in the exocrine pancreas, but its kinetics of

Fig. 3. Effect of carbachol (CCh), caerulein and secretin on $[\text{Ca}^{2+}]_i$ in dispersed pancreatic acini

Acini were loaded with quin2/AM as described in the Materials and methods section. The traces are representative of three experiments. Arrows indicate the time of addition of the secretagogues.

formation after stimulation are similar to those described in other cells [14,21,28,29]. Moreover, the delayed accumulation of $[\text{^3H}]\text{Ins}(1,3,4)\text{P}_3$ in response to each agonist is consistent with previous suggestions [14,15] that this compound is a metabolite of $\text{Ins}(1,4,5)\text{P}_3$ and $\text{Ins}(1,3,4,5)\text{P}_4$.

Another point arising out of these results is that, during stimulation with carbachol and caerulein, $[\text{Ca}^{2+}]_i$ returned again to basal values within 2–3 min, whereas $[\text{^3H}]\text{Ins}(1,4,5)\text{P}_3$ remained increased for longer periods. This could be due to desensitization to the effect of $\text{Ins}(1,4,5)\text{P}_3$, to emptying of the $\text{Ins}(1,4,5)\text{P}_3$ -sensitive Ca^{2+} pool, or to compensatory mechanisms which prevent continued elevation of $[\text{Ca}^{2+}]_i$ by rapid redistribution of released Ca^{2+} . It has been shown that the intracellular $\text{Ins}(1,4,5)\text{P}_3$ -sensitive Ca^{2+} pool is discrete [30] and can be emptied [30,31], and that, at least in GH_3 cells, Ca^{2+} can be transferred from this store into the mitochondria [30]. On the other hand, studies in which permeabilized RINm5F cells were continuously infused with $\text{Ins}(1,4,5)\text{P}_3$ would tend to argue against a desensitization phenomenon [31]. It should also be pointed out that in acini, although some authors have found only transient increases in $[\text{Ca}^{2+}]_i$ in response to caerulein and carbachol [18,24], others have described an

early peak, followed by a prolonged plateau phase which is dependent on extracellular Ca^{2+} [32]. However, it is difficult to envisage a direct role for $\text{Ins}(1,4,5)\text{P}_3$ in this later response, since there have been no reports of $\text{Ins}(1,4,5)\text{P}_3$ acting on plasma-membrane channels.

In conclusion, these results show that in pancreatic acini, as in other tissues, the very rapid formation of $\text{Ins}(1,4,5)\text{P}_3$ is compatible with its second-messenger role in the elevation of $[\text{Ca}^{2+}]_i$. Our results are therefore inconsistent with the hypothesis that increases in $\text{Ins}(1,4,5)\text{P}_3$ in the exocrine pancreas are compartmentalized [22]. Compartmentalization may play a role in the second-messenger function of $\text{Ins}(1,4,5)\text{P}_3$, but available evidence would suggest that this is due more to compartmentalization of the $\text{Ins}(1,4,5)\text{P}_3$ -sensitive Ca^{2+} pool [30] than to localized increases in $\text{Ins}(1,4,5)\text{P}_3$ itself.

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