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Inositol 1,3,4,5-tetrakisphosphate increases the duration of the inositol 1,4,5-trisphosphate-mediated Ca^{2+} transient

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The effect of Ins 1,3,4,5-P_4 on the intracellular Ca^{2+} mobilization produced by Ins 1,4,5-P_3 has been examined in permeabilized hepatocytes. Ins 1,3,4,5-P_4 did not affect the magnitude of the Ins 1,4,5-P_3-mediated Ca^{2+} release but did inhibit re-accumulation of the released Ca^{2+} back into intracellular stores. This effect was not mimicked by Ins 1,3,4-P_3. In hepatocytes, the re-uptake phase of the response results from Ins 1,4,5-P_3 hydrolysis. Measurements using labeled substrates indicate that Ins 1,3,4,5-P_4 inhibits the hydrolysis of Ins 1,4,5-P_3 and vice versa. Since the removal of the 5-phosphate on Ins 1,4,5-P_3 and Ins 1,3,4,5-P_4 is a common step in the disposal of both compounds, it is suggested that one of the biological effects of Ins 1,3,4,5-P_4 may be to slow hydrolysis of Ins 1,4,5-P_3 and thereby prolong the duration of a Ca^{2+} transient.

Inositol tetrakisphosphate; Inositol trisphosphate; Phosphatase; Ca^{2+}; (Liver cell)

1. INTRODUCTION

Inositol trisphosphate (Ins 1,4,5-P_3) is the messenger molecule that mediates the intracellular Ca^{2+} mobilization that occurs in response to a wide variety of cell surface stimuli [1,2]. The metabolic pathways involved in its disposal have recently been clarified with the discovery that, in addition to being dephosphorylated to Ins 1,4-P_2, it is also phosphorylated by a specific 3-kinase to form inositol tetrakisphosphate (Ins 1,3,4,5-P_4) [3–5]. This compound can then be further dephosphorylated to form Ins 1,3,4-P_3 [3–5]. Although Ins 1,3,4,5-P_4 and Ins 1,3,4-P_3 accumulate in many stimulated cells, they are inactive in mobilizing intracellular Ca^{2+} at low concentrations [6,7] and their biological functions are presently unknown. It has been proposed that Ins 1,3,4,5-P_4 may function to regulate Ca^{2+} entry across the plasma membrane [5,8]. Here, we have examined the effects of Ins 1,3,4,5-P_4 on the intracellular Ca^{2+} mobilization produced by Ins 1,4,5-P_3. We find that the re-uptake phase of the trisphosphatase-mediated ca^{2+} transient is inhibited by the tetrakisphosphate when added to saponin-permeabilized hepatocytes. Measurements indicate that Ins 1,3,4,5-P_4 inhibits the hydrolysis of Ins 1,4,5-P_3 under these conditions. These data suggest that Ins 1,3,4,5-P_4 accumulation in an intact stimulated cell may slow the hydrolysis of Ins 1,4,5-P_3 and modulate the duration of a cytosolic free Ca^{2+} transient.

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2. MATERIALS AND METHODS

The procedures used for isolation and incubation of saponin-permeabilized hepatocytes and the measurement of free Ca^{2+} concentration changes with Ca^{2+} electrodes were as described in [9]. The incubation buffer contained (mM): KCl (120), NaHepes (20), pH 7.2, $MgCl_2$ (0.3), MgATP (1), creatine phosphate (10), together with creatine kinase (10 mU/ml), saponin (300 μg/ml) and 1 μM each of ruthenium red and antimycin A. Liver cells

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were added to this buffer at a final concentration of 6 mg dry wt/ml. Metabolites of ^{3}H-labeled Ins 1,4,5-P_3 (New England Nuclear) were measured by an HPLC method [5]. Ins 1,3,4,5-P_4 labeled with ^{32}P in the 3-position was prepared using [γ-^{32}P]ATP, Ins 1,4,5-P_3 and Ins 1,4,5-P_3 3-kinase from rat brain as in [10]. Metabolites formed from ^{32}P-labeled Ins 1,3,4,5-P_4 were measured in neutralized perchloric acid extracts using small columns of Dowex AG-1X8 (formate form) anion-exchange resins. The elution buffers all contained 0.1 M formic acid and inositol bis-, tris- and tetrakisphosphates were selectively eluted with 0.4 M (6 ml), 0.8 M (6 ml) and 1.5 M (3 ml) ammonium formate, respectively.

Unlabeled Ins 1,4,5-P_3 (free of contaminating Ins 2,4,5-P_3) was from Behring Diagnostics (La Jolla, CA). Ins 1,3,4,5-P_4 and Ins 1,3,4-P_3 were prepared as described [7,10] and the purity of these compounds was ascertained by NMR spectroscopy [10].

3. RESULTS

Fig.1A shows the time course of Ca^{2+} release produced by a maximal concentration of Ins 1,4,5-P_3 added to saponin-permeabilized hepatocytes. As observed in many systems [9,11,12] the rapid release of Ca^{2+} is followed by a slower phase of Ca^{2+} re-uptake. In agreement with previous reports [6,7], Ins 1,3,4,5-P_4 alone at concentrations up to 10 μM did not alter Ca^{2+} fluxes (not shown), nor did it significantly affect the amplitude or initial rate of the Ca^{2+} transient produced by Ins 1,4,5-P_3. However, the presence of Ins 1,3,4,5-P_4 slowed the re-uptake phase of the Ins 1,4,5-P_3 response and consequently increased the duration of the Ca^{2+} transient. This effect was most marked when Ins 1,3,4,5-P_4 was present in 20-fold excess over Ins 1,4,5-P_3, but effects could still be observed at equimolar concentrations of both compounds. In another series of experiments, the effect of combining suboptimal concentrations of Ins 1,4,5-P_3 with Ins 1,3,4,5-P_4 was tested (fig.2). The lower concentration of Ins 1,4,5-P_3 (0.15 μM) produced a more transient response than a maximal concentration (fig.2A and [9]). When an equal concentration of Ins 1,3,4,5-P_4 was included, the duration of the Ca^{2+} transient was again increased. In this case Ins 1,3,4,5-P_4 predominantly affected the later stages of the re-uptake phase. Higher concentrations of Ins 1,3,4,5-P_4 inhibited over the entire period of re-uptake and produced a larger increase in the duration of the transient. In three separate experiments, addition of 0.15 μM Ins 1,3,4,5-P_4 produced a 56.1 ± 8.7% increase in the time taken for the Ca^{2+} transient generated by an equal concentration of Ins 1,4,5-P_3 to return to baseline.

A 20-fold excess of Ins 1,3,4-P_3 was without ef-

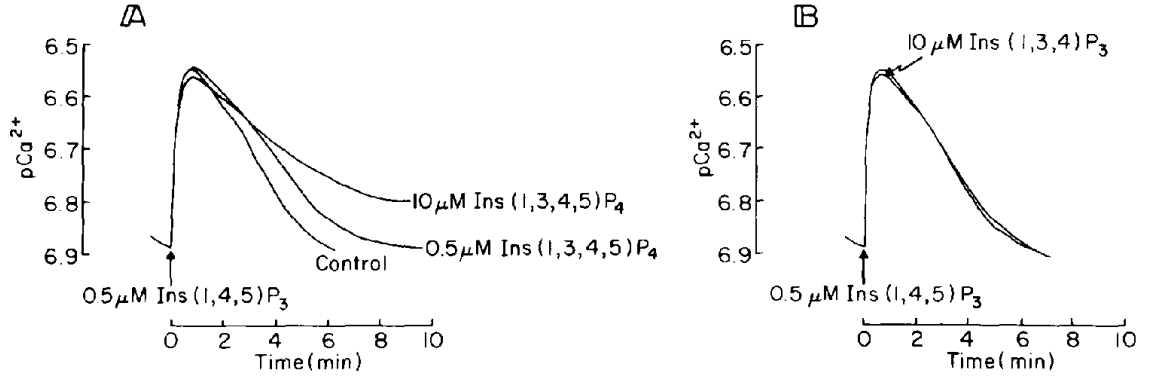

Fig.1. Effect of Ins 1,3,4,5-P_4 and Ins 1,3,4-P_3 on intracellular Ca^{2+} release induced by a maximal concentration of Ins 1,4,5-P_3. Liver cells (6 mg dry wt/ml) were incubated in a Ca^{2+} electrode chamber with saponin in the presence of MgATP and an ATP-regenerating system (see section 2). Ca^{2+} accumulation was followed until the system approached a steady state (approx. 10 min). At this point, Ins 1,4,5-P_3 (0.5 μM) was added and Ca^{2+} release was measured. When present, Ins 1,3,4,5-P_4 (A) or Ins 1,3,4-P_3 (B) was added simultaneously with Ins 1,4,5-P_3.

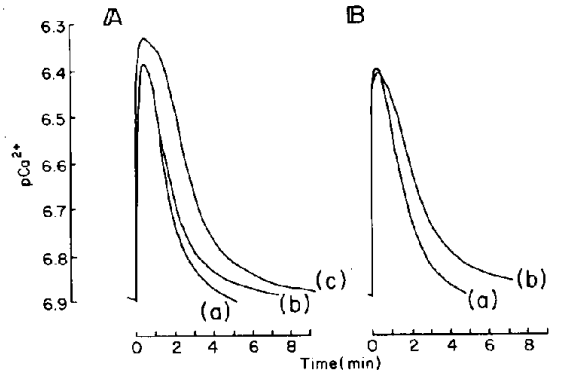

Fig.2. Effect of Ins 1,3,4,5-P_4 on intracellular Ca^{2+} release induced by a submaximal dose of Ins 1,4,5-P_3. The experimental conditions used were as described in fig.1 except that a lower concentration of Ins 1,4,5-P_3 (0.15 μM) was tested. The traces were obtained using a different cell preparation from that in fig.1 and illustrates that the shape of the Ca^{2+} transient varies somewhat between different batches of cells.

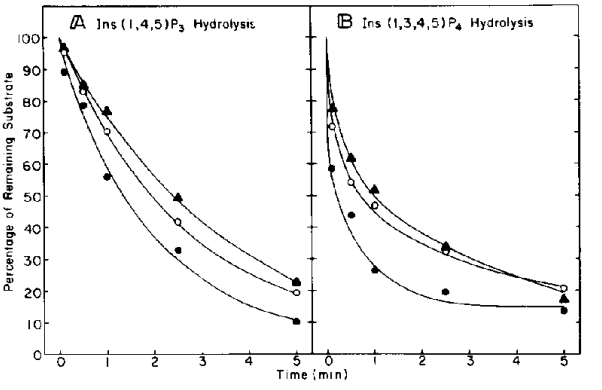

Fig.3. Hydrolysis of labeled inositol polyphosphates by saponin-permeabilized hepatocytes. Liver cells were treated at a final concentration of 6 mg dry wt/ml with 240 μg saponin for 10 min at 30°C in a final volume of 0.8 ml using incubation conditions identical to those used for the Ca^{2+} electrode studies. At this time 0.5 μM of either [^{3}H]Ins 1,4,5-P_3 (0.1 Ci/mmol) or [3-^{32}P]Ins 1,3,4,5-P_4 (10000 cpm/pmol) was added. The appropriate unlabeled inositol phosphates were added at the same time as the labeled compounds. Samples (0.15 ml) were withdrawn at the indicated times and quenched in 0.4 ml of 5% (w/v) perchloric acid containing 1.25 mM Tris EDTA and 0.375 mM diethylenetriaminepentaacetic acid. After removal of precipitated proteins, the extract was neutralized and analyzed by HPLC or Dowex anion-exchange chromatography as described in section 2. (A) Hydrolysis of 0.5 μM [^{3}H]Ins 1,4,5-P_3 was measured in the absence (●—●) or presence of 0.5 μM (○—○) or 10 μM (△—△) Ins 1,3,4,5-P_4. (B) Hydrolysis of [^{32}P]Ins 1,3,4,5-P_4 was measured in the absence (●—●) or presence of 0.5 μM (○—○) or 10 μM (△—△) Ins 1,4,5-P_3.

fect on the Ins 1,4,5-P_3-mediated Ca^{2+} transient (fig.1B). This suggests that Ins 1,3,4,5-P_4 itself, rather than its dephosphorylation product, is responsible for the observed effects on the Ca^{2+} transient. Experimental evidence is available to support the view that the re-uptake phase of the Ca^{2+} response observed in the permeabilized cell results from the hydrolysis of Ins 1,4,5-P_3, by a phosphatase that catalyses the removal of phosphate at the 5-position [1,9]. Competition between Ins 1,4,5-P_3 and Ins 1,3,4,5-P_4 for this enzyme [13] would account for the experimental data. To test this possibility, the hydrolysis of 0.5 μM ^{3}H-labeled Ins 1,4,5-P_3 was measured in the presence and absence of Ins 1,3,4,5-P_4. Fig.3A shows that when present at equal concentrations Ins 1,3,4,5-P_4 substantially decreased the initial rate of hydrolysis of Ins 1,4,5-P_3, while a 20-fold excess of Ins 1,3,4,5-P_4 had little further effect. The major [^{3}H]inositol phosphate product detected by HPLC was Ins 1,4-P_2. Approx. 10% of the label appeared as Ins 1,3,4-P_3 and Ins 3,4-P_2 isomers in the 5 min incubation period (not shown) indicating a small but significant flux through Ins 1,4,5-P_3 3-kinase in the permeabilized cell. Fig.3B shows that the initial rate of hydrolysis of 0.5 μM [^{32}P]Ins 1,3,4,5-P_4 was almost 6-fold faster than an equal concentration of [^{3}H]Ins 1,4,5-P_3. Ins 1,3,4,5-P_4 hydrolysis was markedly inhibited upon addition of 0.5 or 10 μM unlabeled Ins 1,4,5-P_3.

4. DISCUSSION

Removal of the phosphate at the 5-position of the inositol ring is a common step in the metabolism of both Ins 1,4,5-P_3 and Ins 1,3,4,5-P_4 and may be catalyzed by the same enzyme [13,14]. Therefore, while Ins 1,3,4,5-P_4 is itself inactive in mobilizing intracellular Ca^{2+}, it may indirectly affect this process by decreasing the rate of Ins 1,4,5-P_3 hydrolysis by the 5′-phosphomonoester-

ase. Such an effect has been observed in this study using permeabilized hepatocytes. The extent to which such interactions occur in the intact stimulated cell is unknown, but would presumably depend on factors such as the relative amounts of Ins 1,4,5-P_3 and Ins 1,3,4,5-P_4 formed, as well as the kinetic parameters of the enzyme(s) involved in metabolizing these compounds.

In any one experimental system the relative amounts of ^{3}H-labeled Ins 1,4,5-P_3 and Ins 1,3,4,5-P_4 formed appear to vary according to the stimuli. For the rat hepatocyte, vasopressin, at maximal concentrations, produces approx. 3-times more Ins 1,4,5-P_3 than Ins 1,3,4,5-P_4 over a 30 s interval [5]. Under similar conditions α-adrenergic stimulation resulted in approximately equal increases of both inositol phosphates, while in the case of glucagon, the increase of Ins 1,3,4,5-P_4 greatly exceeded that of Ins 1,4,5-P_3 [15]. The data in figs 1 and 2 suggest that significant decreases in the rate of Ca^{2+} re-uptake can be observed when Ins 1,4,5-P_3 and Ins 1,3,4,5-P_4 are present at equal concentrations. The relative potency of Ins 1,3,4,5-P_4 in inhibiting the hydrolysis of Ins 1,4,5-P_3 by the 5′-phosphomonoesterase is further emphasized from studies on the kinetic properties of the purified soluble enzyme from human platelets [13] and rat brain [14]. In both cases it was found that the apparent K_m and V_{max} for Ins 1,3,4,5-P_4 hydrolysis (K_m = 0.5–1.0 μM) was lower than for Ins 1,4,5-P_3 (K_m = 3–10 μM). In liver [16–18] this enzyme is primarily membrane bound and, although a systematic kinetic analysis was not undertaken in this study, it is clear that Ins 1,3,4,5-P_4 is hydrolyzed more rapidly than Ins 1,4,5-P_3 (fig.3). Thus, available information on the relative amounts of Ins 1,3,4,5-P_4 and Ins 1,4,5-P_3 formed in stimulated cells, as well as the kinetic properties of purified 5′-phosphomonoesterase, raises the possibility that one of the biological effects of Ins 1,3,4,5-P_4 formed in a stimulated cell may be to prolong the Ca^{2+} signal mediated by Ins 1,4,5-P_3.

Potentially, competition between substrates for the 5′-phosphomonoesterase may also play a role in prolonging the effects of Ins 1,3,4,5-P_4 on cell function. It has recently been shown that microinjection of Ins 2,4,5-P_3 together with Ins 1,3,4,5-P_4 activates sea urchin eggs whereas microinjection of these compounds separately does not [8]. The necessity for co-injection of Ins 2,4,5-P_3 was attributed to its ability to mobilize intracellular Ca^{2+} without an attendant phosphorylation catalysed by 3-kinase. It was further proposed that a full activation of the egg requires intracellular Ca^{2+} mobilization as well as an accelerated entry of Ca^{2+} across the plasma membrane and that the latter process could be activated by Ins 1,3,4,5-P_4 [8]. An additional possibility arising from the present data which needs to be considered is that Ins 2,4,5-P_3 may, like Ins 1,4,5-P_3 (fig.3B), inhibit the rapid hydrolysis of Ins 1,3,4,5-P_4 and thereby facilitate the expression of the biological effects of this molecule on the sea urchin egg.

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