

Inositol triphosphate produces different patterns of cytoplasmic Ca^{2+} spiking depending on its concentration

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In single mouse pancreatic acinar cells the effects of intracellular infusion of inositol 1,4,5-trisphosphate (InsP_3) or the non-metabolizable InsP_3 analogue inositol 1,4,5-trisphosphorothioate (InsPS_3) have been investigated using a wide range of concentrations. Different types of cytosolic Ca^{2+} fluctuation patterns (monitored as Ca^{2+} -dependent Cl^- current in patch-clamp whole-cell recording experiments) could be generated by InsP_3 or InsPS_3 , dependent on concentration, resembling those previously shown to be evoked by varying degrees of receptor activation in these cells. Low InsPS_3 concentrations evoked repetitive local Ca^{2+} spikes whereas at relatively high concentrations repetitive Ca^{2+} waves were produced. In the presence of intracellular citrate a much lower messenger level was sufficient to generate waves. The InsP_3 concentration determines whether the cytosolic Ca^{2+} signals are local or global.

Ca^{2+} spike; Ca^{2+} wave; Inositol triphosphate; Inositol triphosphorothioate

1. INTRODUCTION

Activation of receptors linked to the enzyme phospholipase C (PLC) evokes repetitive transient spikes in the cytoplasmic Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) [1]. Two quantitative models seem capable of explaining Ca^{2+} spike generation. In the inositol 1,4,5-trisphosphate (InsP_3)- Ca^{2+} crosscoupling (ICC) model the receptor-triggered formation of InsP_3 leads to a rise in $[\text{Ca}^{2+}]_i$ that accelerates InsP_3 formation via Ca^{2+} -activation of PLC, creating a positive feedback loop [2,3]. In the Ca^{2+} -induced Ca^{2+} -release (CICR) model the primary InsP_3 -evoked rise in $[\text{Ca}^{2+}]_i$ opens channels in an InsP_3 -insensitive store. Here positive feedback comes from Ca^{2+} catalyzing its own release [1,4,5].

The ICC model requires that the InsP_3 concentration like $[\text{Ca}^{2+}]_i$ undergoes spiking [2], but experimental evidence from pancreatic acinar cells shows that intracellular infusion of the non-metabolizable InsP_3 analogue inositol 1,4,5-trisphosphorothioate (InsPS_3) evokes regular Ca^{2+} spikes [6]. This appears to rule out the ICC model for these cells. Nevertheless Meyer and Stryer [2] have stressed that in the pancreatic experiments [6] the InsPS_3 -evoked spikes were of a much shorter duration than those often evoked by receptor activation. In the pancreatic acinar cells low concentrations of acetylcholine (ACh) as well as InsP_3 or InsPS_3 evoke local short-lasting Ca^{2+} spikes near the cell membrane whereas

higher ACh concentrations or cholecystokinin (CCK) can evoke longer transient Ca^{2+} signals that spread as waves throughout a cell or even a coupled acinar network. Each Ca^{2+} wave is triggered by a short-lasting local spike [7–10]. It is not known whether a constant level of InsP_3 can generate such repetitive global Ca^{2+} transients. We now show that intracellularly applied InsP_3 or InsPS_3 can evoke a number of different cytoplasmic Ca^{2+} signal patterns depending on the concentration of the messenger. A relatively high InsPS_3 concentration is required in order to evoke repetitive long Ca^{2+} transients, but in the presence of intracellular citrate a much lower InsPS_3 level is sufficient.

2. MATERIALS AND METHODS

Fragments of mouse pancreas were digested by pure collagenase, washed and pipetted to produce single acinar cells as previously described [4,6,7]. The tight-seal, whole-cell current configuration of the patch-clamp technique was used for the measurement of the transmembrane current from single cells as previously described in detail for pancreatic acinar cell studies [11]. Patch-clamp pipettes had resistances of 2–4 M Ω and the access resistance to the cell interior was about 5–10 M Ω [11]. Ca^{2+} -dependent Cl^- currents were measured with the two-voltage pulse protocol as described previously [9]. The extracellular solution contained (mM): NaCl 140, KCl 4.7, CaCl_2 1.0, MgCl_2 , HEPES 10 (pH 7.2) and glucose 10. The intracellular pipette solution contained (mM): KCl 140, Na_2ATP 1, MgCl_2 1.13, glucose 10 and HEPES 10 (pH 7.2). EGTA (10 μM) was also present.

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In experiments where 10 mM citrate was present the KCl concentration was reduced so as to keep the osmolarity at the control level. D-Ins 1,4,5-PS₃ was synthesized by a method identical to that previously described for the racemate [12] except that optically pure L-1,2,4-tri-*O*-benzyl-*myo*-inositol [13] was employed for phosphorylation. After deblocking of protecting groups, crude D-Ins 1,4,5-PS₃ was purified on a column of DEAE Sephadex A-25 resin using a gradient of triethylammonium bicarbonate, pH 8. After elution the pure phosphorothioate was quantified by quantitative phosphate analysis and stored as the triethylammonium salt.

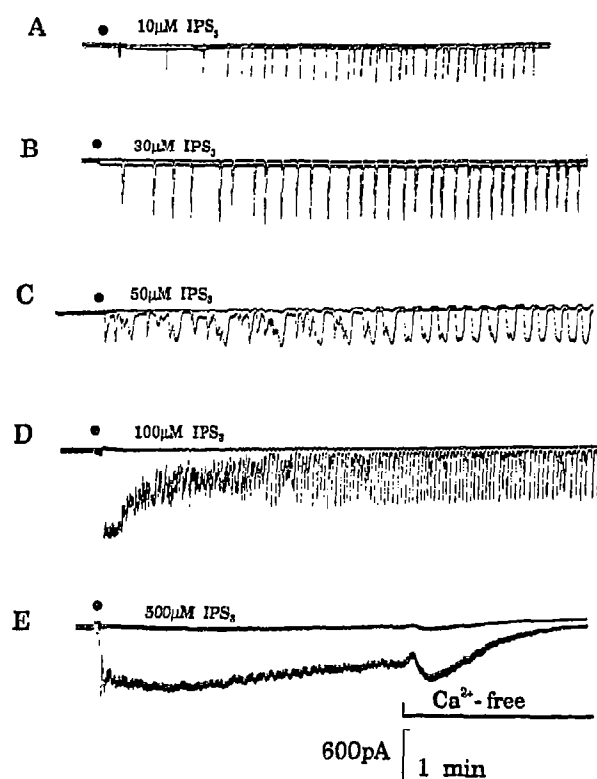


Fig. 1. Different patterns of cytoplasmic Ca²⁺ fluctuations monitored as Ca²⁺-dependent Cl⁻ current evoked by internal application of InsPS₃ (IPS₃) in single mouse pancreatic acinar cells. The cells were voltage-clamped at a holding potential of -30 mV and depolarizing voltage jumps of 150 ms duration to a membrane potential of 0 mV were repetitively applied throughout all experiments. Because of the compression of the current traces the records seem to show currents at -30 mV (bottom traces) and 0 mV (top traces) simultaneously. At 0 mV there are only very small current fluctuations as the Cl⁻ equilibrium potential (E_{Cl^-}) is close to zero. At -30 mV there is a large electrical gradient favouring Cl⁻ efflux and when the Ca²⁺-dependent Cl⁻ channels open, due to increases in [Ca²⁺]_i, inward currents (downward deflections) are seen. The panels A-E represent individual experiments in which the internal (pipette) solution contained 10, 30, 50, 100, or 500 μM IPS₃, respectively. The filled circle at the beginning of each panel indicates the time of establishment of the whole-cell recording configuration (start of IPS₃ diffusion into cell interior). In (E) the bar labelled Ca²⁺-free indicates that the external solution did not contain Ca²⁺ and that EGTA (1 mM) was present.

3. RESULTS

Fig. 1 shows examples of the various patterns evoked by InsPS₃. The traces shown for 10, 30 and 500 μM InsPS₃ are typical, but in the experiments with 50 and 100 μM InsPS₃ a variety of patterns ranging from repetitive short-lasting spikes to sustained responses were obtained. Table I summarizes all the results. The dose-response curve in the concentration range 30–100 μM is very steep, but the important point is that 50 or 100 μM InsPS₃ can evoke a pattern of repetitive long transients whereas at 10 or 30 μM repetitive short-lasting spikes are invariably produced. We used InsPS₃ rather than InsP₃ since the natural messenger is rapidly metabolized [1,14]. The synthetic analogue in which the phosphate groups have been replaced by phosphorothioate groups [12] confers resistance to phosphatase and kinase-mediated metabolism [14]. InsPS₃ is a full agonist at releasing intracellular Ca²⁺ and is only 3- to 6-fold less potent than the natural messenger [14]. InsPS₃ displaces [³H]InsP₃ from cerebellar membranes with an IC₅₀ value that is about 5 times higher than InsP₃ [15].

Although InsP₃ is most likely metabolized when infused into cells we carried out a few experiments to see if different concentrations of the natural messenger could also evoke different cytoplasmic Ca²⁺ fluctuation patterns. In the two experiments with 10 μM InsP₃ in the pipette solution repetitive short-lasting spikes were seen (Fig. 2A) confirming the results of the 12 experiments previously reported by us [4]. In the 4 experiments with 50 or 100 μM InsP₃ there was initially a quasi-sustained response followed by a somewhat irre-

Table I
Cytoplasmic Ca²⁺ fluctuation patterns evoked by stimulation with D-Ins 1,4,5-PS₃ (InsPS₃)

Stimulus	Number of cells investigated	Response	Number of cells showing response
10 μM InsPS ₃	11	Short-lasting spikes	11
10 μM InsPS ₃ in the presence of 10 mM citrate	4	Long transients + spikes	4
30 μM InsPS ₃	9	Short-lasting spikes	9
50 μM InsPS ₃	17	Short-lasting spikes	5
		Long transients + spikes	3
		Sustained	9
100 μM InsPS ₃	13	Short-lasting spikes	3
		Long transients + spikes	5
		Sustained	5
500 μM InsPS ₃	7	Sustained	7

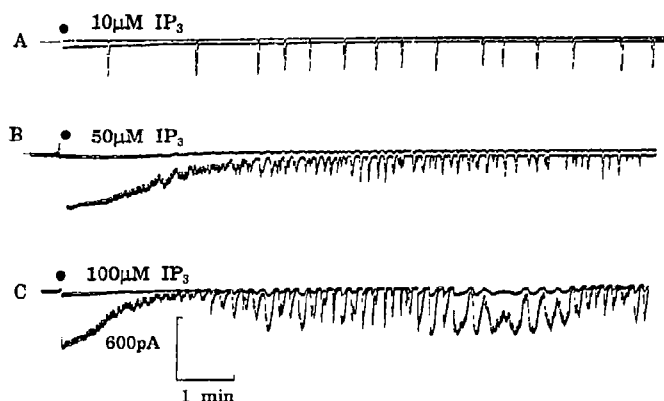


Fig. 2. Different Ca^{2+} fluctuation patterns evoked by different concentrations of InsP_3 (IP_3). Panels A–C represent individual experiments in which the internal (pipette) solution contained 10, 50 and 100 μM IP_3 , respectively.

gular mixed pattern of short spikes and longer transients (Fig. 2B and C).

The repetitive short-lasting spikes evoked by low concentrations of InsPS_3 or InsP_3 are insensitive to removal of extracellular Ca^{2+} during the initial 10-min period of stimulation [6] although later an extracellular Ca^{2+} dependency develops [16]. Removal of external Ca^{2+} results, after a brief transient increase, in a gradual and severe reduction of the sustained responses (Fig. 1E). When a high concentration (1 mM) of the Ca^{2+} chelator EGTA was present in the external Ca^{2+} -free solution (as in Fig. 1E) the response could not be brought back after Ca^{2+} re-admission, but when the EGTA concentration was reduced to 0.2 mM the effect was reversible.

The probability of ACh-evoked short-lasting Ca^{2+} spikes triggering longer transients is dramatically increased by intracellular perfusion with solutions containing a high concentration of the mobile low-affinity Ca^{2+} buffer citrate [9]. Since the InsPS_3 concentration of 50 μM (corresponding to 10 μM InsP_3) required to produce long transients (Fig. 1C) is relatively high (in permeabilized pancreatic acinar cells 5 μM InsP_3 appears to be a maximal dose [17]) we investigated the effect of a low InsPS_3 concentration in the presence of citrate. Fig. 3 compares the effects of InsPS_3 in control and citrate pipette solutions and shows that with 10 mM citrate in the perfusion solution 10 μM InsPS_3 (corresponding to an InsP_3 concentration of 2 μM) evokes a pattern of free-standing slim spikes mixed with spikes followed by much longer transients (Table I). In the absence of citrate such an effect can be induced by addition of CCK (Fig. 3A) [9]. Irrespective of the mechanism by which citrate acts these results show that the precise InsP_3 concentration required to produce a particular cytoplasmic Ca^{2+} fluctuation pattern may depend on soluble cytosolic components that could be subject to wash-out during whole-cell recording.

4. DISCUSSION

Our results show that a constant concentration of the Ca^{2+} -releasing messenger InsP_3 can generate a number of different cytosolic Ca^{2+} spike and wave patterns, depending on its concentration, resembling those previously shown to be evoked by varying degrees of receptor activation [7,9,10]. The spreading of the local short-lasting Ca^{2+} spikes can be prevented by non-mobile cytosolic buffers, uptake into intracellular organelles and/or extrusion through the cell membrane [2,9,18]. Mobile Ca^{2+} buffers can facilitate Ca^{2+} diffusion by picking up Ca^{2+} at the high end of a steady gradient and then diffuse with its bound Ca^{2+} to the low end where Ca^{2+} will be released [9] and this process may explain the result shown in Fig. 3, but how can an increase in InsP_3 concentration promote spreading and thereby account for the generation of the longer Ca^{2+} transients (Figs. 1 and 2)?

The simplest explanation is provided by the quantal nature of InsP_3 -evoked intracellular Ca^{2+} release [17] in conjunction with the recent finding that an elevation of cytoplasmic Ca^{2+} concentration can evoke further Ca^{2+} release not only through InsP_3 -insensitive caffeine-sensitive channels [4,5,19] but also via InsP_3 -sensitive pores [19] in the endoplasmic reticulum. In pancreatic cells a small steady Ca^{2+} release evoked by a low InsP_3 concentration generates local repetitive Ca^{2+} -induced Ca^{2+} spikes according to the two-pool model [1,4,16,20] and mainly via InsP_3 -insensitive channels since intracellular Ca^{2+} infusion can mimic this effect and the Ca^{2+} -evoked Ca^{2+} spikes cannot be blocked by infusion of the InsP_3 antagonist heparin [4]. A higher InsP_3 concentration would release Ca^{2+} from pools not previously accessible [17] and effectively allow further Ca^{2+} -induced Ca^{2+} release also via InsP_3 -sensitive channels [19] thereby explaining the spreading of the Ca^{2+} signal.

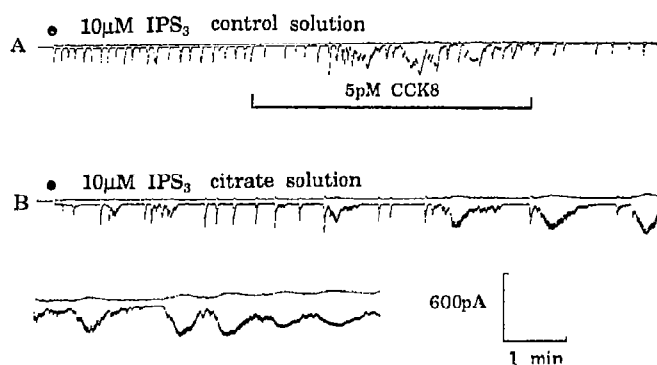


Fig. 3. Comparison of the effects of 10 μM InsPS_3 (IPS_3) in a control pipette solution (A) and a pipette solution containing 10 mM citrate (B). In the control experiment (A) IPS_3 (10 μM) evokes repetitive slim spikes, but addition of 5 pM CCK8 externally evoked longer transients whereas in the citrate experiment (B) the same concentration of IPS_3 by itself is able to induce longer Ca^{2+} transients. The bottom part of panel B is a direct continuation of the upper part.

Certain types of receptor activation may in addition to generating InsP_3 also control Ca^{2+} spreading by regulation of Ca^{2+} extrusion and/or Ca^{2+} re-uptake into organelles or by the production of a caffeine-like substance (spreading factor) promoting Ca^{2+} -induced Ca^{2+} release [9]. Our results indicate that InsP_3 is itself a spreading factor, but additional receptor-mediated controls of Ca^{2+} transport are most likely required to fully account for the different shapes of Ca^{2+} transients evoked by different agonists [9,21,22].

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