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Inositol 1,3,4,5-Tetrakisphosphate Is Essential for Sustained Activation of the Ca^{2+}-Dependent K^+ Current in Single Internally Perfused Mouse Lacrimal Acinar Cells

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Summary. We have examined the effects of various inositol polyphosphates, alone and in combination, on the Ca^{2+}-activated K^+ current in internally perfused, single mouse lacrimal acinar cells. We used the patch-clamp technique for whole-cell current recording with a set-up allowing exchange of the pipette solution during individual experiments so that control and test periods could be directly compared in individual cells. Inositol 1,4,5-trisphosphate (Ins 1,4,5 P_3) (10–100 μM) evoked a transient increase in the Ca^{2+}-sensitive K^+ current that was independent of the presence of Ca^{2+} in the external solution. The transient nature of the Ins 1,4,5 P_3 effect was not due to rapid metabolic breakdown, as similar responses were obtained in the presence of 5 mM 2,3-diphosphoglyceric acid, that blocks the hydrolysis of Ins 1,4,5 P_3, as well as with the stable analogue DL-inositol 1,4,5-trisphosphorothioate (Ins 1,4,5 $P(S)_3$) (100 μM). Ins 1,3,4 P_3 (50 μM) had no effect, whereas 50 μM Ins 2,4,5 P_3 evoked responses similar to those obtained by 10 μM Ins 1,4,5 P_3. A sustained increase in Ca^{2+}-dependent K^+ current was only observed when inositol 1,3,4,5-tetrakisphosphate (Ins 1,3,4,5 P_4) (10 μM) was added to the Ins 1,4,5 P_3 (10 μM)-containing solution and this effect could be terminated by removal of external Ca^{2+}. The effect of Ins 1,3,4,5 P_4 was specifically dependent on the presence of Ins 1,4,5 P_3 as it was not found when 10 μM concentrations of Ins 1,3,4 P_3 or Ins 2,4,5 P_3 were used. Ins 2,4,5 P_3 (but not Ins 1,3,4 P_3) at the higher concentration of 50 μM did, however, support the Ins 1,3,4,5 P_4-evoked sustained current activation. Ins 1,3,4 P_3 could not evoke sustained responses in combination with Ins 1,4,5 P_3 excluding the possibility that the action of Ins 1,3,4,5 P_4 could be mediated by its breakdown product Ins 1,3,4 P_3. Ins 1,3,4,5 P_4 also evoked a sustained response when added to an Ins 1,4,5 $P(S)_3$-containing solution. Ins 1,3,4,5,6 P_5 (50 μM) did not evoke any effect when administered on top of Ins 1,4,5 P_3. In the absence of external Ca^{2+}, addition of Ins 1,3,4,5 P_4 to an Ins 1,4,5 P_3-containing internal solution evoked a second transient K^+ current activation. Readmitting external Ca^{2+} in the continued presence internally of Ins 1,4,5 P_3 and Ins 1,3,4,5 P_4 made the response reappear. We conclude that both Ins 1,4,5 P_3 and Ins 1,3,4,5 P_4 play crucial and specific roles in controlling intracellular Ca^{2+} homeostasis.

Key Words inositol trisphosphate · inositol tetrakisphosphate · Ca^{2+}-dependent K^+ current · lacrimal acinar cell

Introduction

Activation of many different surface membrane receptors evokes breakdown of phosphatidyl inositol bisphosphate (PIP_2), resulting in the generation of the two important intracellular messengers inositol 1,4,5-triphosphate (Ins 1,4,5 P_3) and diacylglycerol (Berridge & Irvine, 1984; Nishizuka, 1986). The role of Ins 1,4,5 P_3 in releasing intracellular Ca^{2+} (Streb et al., 1983, 1984) is now well established (Berridge, 1988), but it is less clear how Ca^{2+} entry from the extracellular fluid is regulated in cells that do not fire action potentials (Berridge, 1988). Irvine and Moor (1986) suggested, on the basis of microinjection experiments in oocytes, that inositol 1,3,4,5 P_4 (Ins 1,3,4,5 P_4), which is formed from Ins 1,4,5 P_3 by the Ca^{2+}-regulated enzyme Ins P_3-3 kinase (Biden & Wollheim, 1986; Irvine et al., 1986a) could play a role in controlling Ca^{2+} entry by acting together with Ins 1,4,5 P_3. Morris et al. (1987*c*) showed in experiments on single internally perfused lacrimal acinar cells that neither intracellular Ins 1,4,5 P_3 alone nor Ins 1,3,4,5 P_4 alone could qualitatively mimick the stimulatory effect of external ACh on Ca^{2+}-dependent K^+ current, whereas this could be achieved by the combination of Ins 1,4,5 P_3 and Ins 1,3,4,5 P_4. Morris et al. (1987*c*) showed that the sustained increase in Ca^{2+}-dependent K^+ current evoked by the combination of Ins 1,4,5 P_3 and Ins 1,3,4,5 P_4 is dependent on the presence of extracellular Ca^{2+} and suggested that Ca^{2+} enters the cell through an Ins 1,4,5 P_3-sensitive Ca^{2+} store with Ins 1,3,4,5 P_4 controlling entry into that store. Morris et al. (1987*c*), however, did not investigate the specificity of the action of the inositol polyphosphates and also did not test for possible effects of Ins 1,3,4,5 P_4 in the absence of external Ca^{2+}. The

present investigation was designed to answer the following questions: (i) Is the effect of Ins 1,3,4,5 P_4 specifically dependent on Ins 1,4,5 P_3 or could other inositol trisphosphates fulfill the same function? (ii) Does Ins 1,3,4,5 P_4 itself act on Ca^{2+} entry or is the effect due to its breakdown product Ins 1,3,4 P_3? (iii) Is Ins 1,3,4,5 P_4 only concerned with Ca^{2+} entry from the extracellular fluid or does it also play a role in intracellular Ca^{2+} mobilization? and (iv) Can it be rigorously excluded (a) that the transient response to Ins 1,4,5 P_3 stimulation is due to rapid metabolism and (b) that the effect of Ins 1,3,4,5 P_4 is due to protection of Ins 1,4,5 P_3 against such metabolism by virtue of their sharing a common phosphatase (Conolly et al., 1987; Joseph, Hansen & Williamson, 1987).

Materials and Methods

All experiments were carried out on single acinar cells isolated from the exorbital lacrimal glands of mice by collagenase and trypsin treatment as previously described (Findlay, 1984). The patch-clamp technique was used to measure the transmembrane currents of single acinar cells under voltage-clamp conditions (Hamill et al., 1981; Jauch, Petersen & Läuger, 1986). As in a previous paper on this topic (Morris et al., 1987*c*), we used internal perfusion of the tip of the patch-pipette via a second small glass capillary inserted into the main pipette, in this way allowing exchanges of the internal solution inside the cell during individual experiments (Jauch et al., 1986).

The bath solution (extracellular) contained (mM): 140 NaCl, 4.7 KCl, 1.2 $CaCl_2$, 1.13 $MgCl_2$, 10 glucose, 10 HEPES, and the pH was 7.2. In Ca^{2+}-free solutions no $CaCl_2$ was added and 1 mM EGTA was present. The solution filling the patch pipettes (intracellular) contained (mM): 140 KCl, 4 $MgCl_2$, 5 Na_2ATP, 10 HEPES and the pH was 7.2. No Ca^{2+} was added and EGTA (0.5 mM) was always present. The inositol polyphosphates Ins 1,4,5 P_3, Ins 1,3,4 P_3, Ins 2,4,5 P_3 and Ins 1,3,4,5 P_4 (all D-configuration) were prepared as described by Irvine et al. (1986*b*). The Ins 1,4,5 P_3 and Ins 2,4,5 P_3 were checked for purity by ionophoretic analysis (Irvine et al., 1986*b*) and were >99% pure. The Ins 1,3,4,5 P_4 and Ins 1,3,4 P_3 were further purified by HPLC (Irvine et al., 1986*b*) and are estimated to be >99.9% free of Ins 1,4,5 P_3 contamination and contained <1% inorganic phosphate by ionophoretic analysis. Ins 1,3,4,5,6 P_5 was obtained from Boehringer, Mannheim. Ins 1,4,5 $P(S)_3$ was prepared as described by Cooke, Gigg and Potter (1987). As Ins 1,4,5 $P(S)_3$ is a racemic mixture of D- and L-enantiomers, the 100 μM concentration used (Fig. 3) in fact only contains 50 μM of the active D-species. The inositol polyphosphates were added to the intracellular solution alone or in various combinations in the concentrations indicated in the text.

The whole-cell current recording configuration was always established with an intracellular solution in the patch pipette not containing any inositol polyphosphates. The holding potential was −50 mV, and repetitive voltage jumps to 0 mV lasting 200 msec were applied. In some experiments such voltage jumps alternated with hyperpolarizing pulses (also of 200 msec duration) to −90 mV. In some situations a more complete characterization of the current-voltage relationship was obtained by applying a series of depolarizing and hyperpolarizing pulses (Fig. 1). The Cl^- equilibrium potential (E_{Cl^-}) was about 0 mV, and at this voltage therefore the K^+ current was not contaminated by Cl current. E_K was about −90 mV, and at this voltage there was therefore no K^+ current. The first exchange of solution in the patch pipette always involved introduction of fresh control fluid (without inositol polyphosphates) into the pipette tip. This was a control procedure to establish that the mechanics of fluid exchange did not evoke any current response. Thereafter the various test solutions were introduced as described in the Results section. In some cells introduction of Ins 1,4,5 P_3 evoked no effect (*see also* Llano, Marty & Tanguy, 1987). The results from these cells were not included in the material presented here. The original current traces reproduced in the figures have been labeled in such a way that the introduction of new pipette solutions is shown at the exact times when the reservoirs were reconnected. We have thus not made any corrections for the time it takes a new solution to begin to enter the perfused cell. As is clear from inspection of the figures, this took about 20–80 sec.

Results

Transient and Sustained Responses

Figure 1 shows a typical experiment on a single internally perfused cell. The membrane potential is held at −50 mV and repetitive depolarizing voltage jumps to 0 mV, lasting 200 msec each, were applied. Such depolarizations evoke activation of high-conductance Ca^{2+}-sensitive K^+ channels, giving rise to an increased outward K^+ current (Maruyama et al., 1983; Findlay, 1984; Trautmann & Marty, 1984). When the tip of the recording micropipette is perfused with a solution containing Ins 1,4,5 P_3 (10 μM), a transient (<2 min) increase in the voltage-activated K^+ current was observed (Fig. 1). The intracellular perfusion solutions only contained a low concentration of the Ca^{2+} buffer EGTA (0.5 mM) as it is well known that higher concentrations (5–10 mM) block the increase in K^+ current evoked by Ca^{2+} mobilizing agonists (Maruyama & Petersen, 1984; Trautmann & Marty, 1984; Morris et al., 1987*c*). When Ins 1,3,4,5 P_4 was introduced on top of Ins 1,4,5 P_3 a renewed increase in the voltage-activated K^+ current was seen, but this time the response was sustained (in all 21 experiments) until Ca^{2+} was removed from the extracellular solution, resulting in a reduction of the magnitude of the K^+ current to or slightly below the original control value (in all seven experiments where this part of the protocol was carried out).

Is the Transient Response to Ins 1,4,5 P_3 Stimulation Due to Rapid Metabolism?

The responses to Ins 1,4,5 P_3 were always transient (Fig. 1) and since Ins 1,3,4,5 P_4 might compete with Ins 1,4,5 P_3 for the enzymes responsible for break-

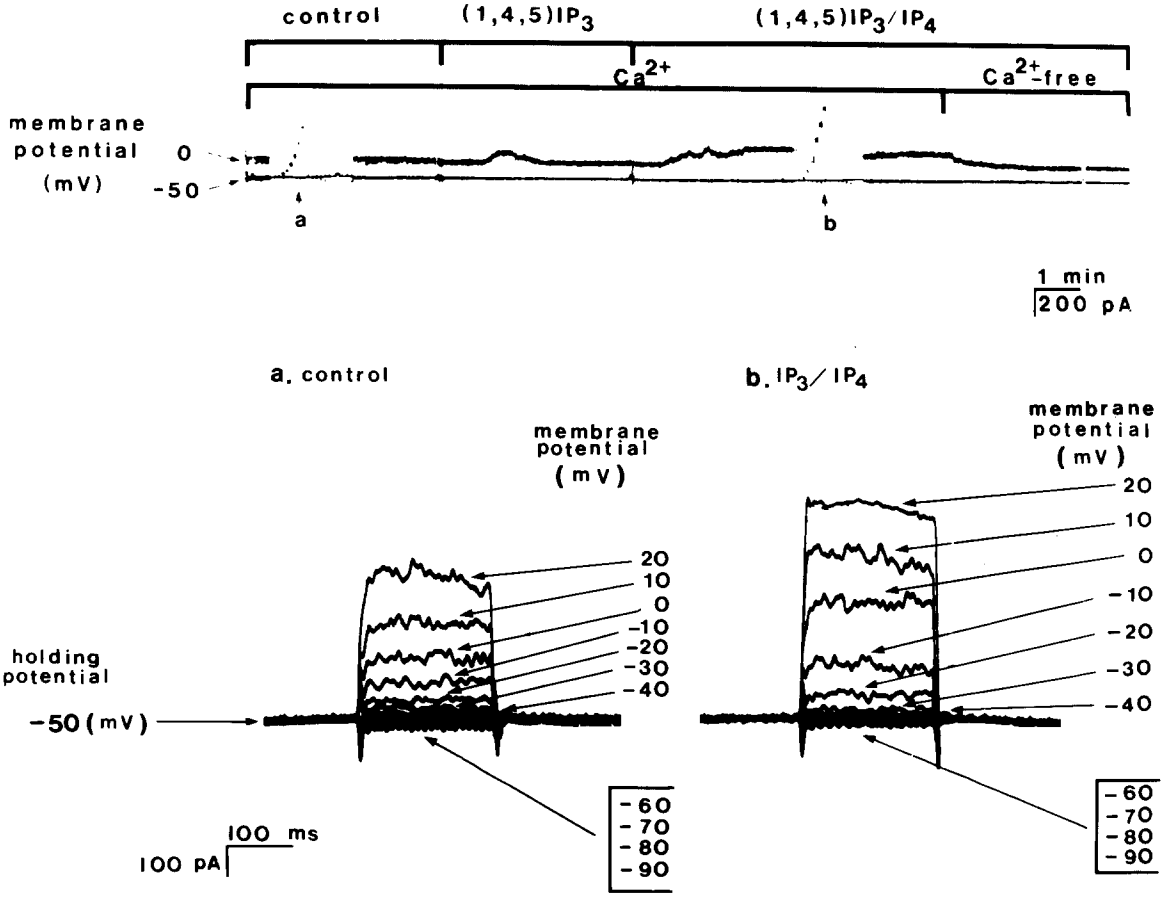

Fig. 1. Whole-cell current recording from single mouse lacrimal acinar cell. The holding potential was −50 mV and from that level repetitive depolarizing voltage steps to 0 mV lasting 200 msec each were applied. In the upper part of the figure is shown a pen-recording trace in which the currents resulting from individual voltage steps cannot be distinguished, giving rise to a record apparently representing the transmembrane current simultaneously at the membrane potentials of −50 and 0 mV, respectively. During the intervals labeled *a* and *b* the repetitive depolarizing pulses were discontinued and, instead, a series of first depolarizing and then hyperpolarizing voltage steps of increasing magnitude were applied. The oscilloscope photographs, seen in the lower part of the figure, show time courses of the individual current responses to the different voltage steps. The hyperpolarizing pulses evoked very small inward currents, and different magnitudes of these currents due to varying sizes of the current steps cannot be distinguished in *a* and *b*. The inward currents evoked by the hyperpolarizing steps to −60, −70, −80 and −90 mV have therefore been bracketed together. In the upper pen-recording trace the top line labeled *control, (1,4,5) IP_3*, etc., indicates the type of solution being perfused into the tip of the recording patch-pipette, whereas the second line from the top labeled *Ca^{2+}* and *Ca^{2+}-free* gives information on the solution present in the bath outside the isolated cell. *(1,4,5)P_3* or *IP_3* means Ins 1,4,5 P_3 (10 μM) and *IP_4* means Ins 1,3,4,5 P_4 (10 μM)

down (*see* Introduction), this could explain the prolonged response to the combination of Ins 1,4,5 P_3 and Ins 1,3,4,5 P_4. It was therefore necessary to investigate the possibility that breakdown of Ins 1,4,5 P_3 limited the duration of its effect.

In one series of experiments Ins 1,4,5 P_3 was used at the very high concentration of 100 μM. As seen in Fig. 2, which shows a typical experiment (four other experiments were very similar), 10 μM Ins 1,4,5 P_3 gave a clear but only transient response. When the concentration of the inositol trisphosphate was subsequently increased to 100 μM there was a tiny, very short-lasting second transient, but still no sustained increase in the Ca^{2+}-dependent K^+ current. A sustained response was, however, obtained when 10 μM Ins 1,3,4,5 P_4 was added on top of the 100 μM Ins 1,4,5 P_3.

In another series 2,3-diphosphoglyceric acid(2,3 DPG), which blocks the hydrolysis of Ins 1,4,5 P_3 (Downes, Mussat & Michell, 1982) was used. 2,3 DPG (5 mM) by itself caused a very small increase in Ca^{2+}-dependent K^+ current, but when Ins 1,4,5 P_3 was subsequently added to the pipette solution only the usual transient response was seen. In other experiments Ins 1,4,5 P_3 (10 μM) was introduced first, giving a transient response, and thereafter 2,3 DPG was added on top. 2,3 DPG in these cases evoked a small transient response, but again

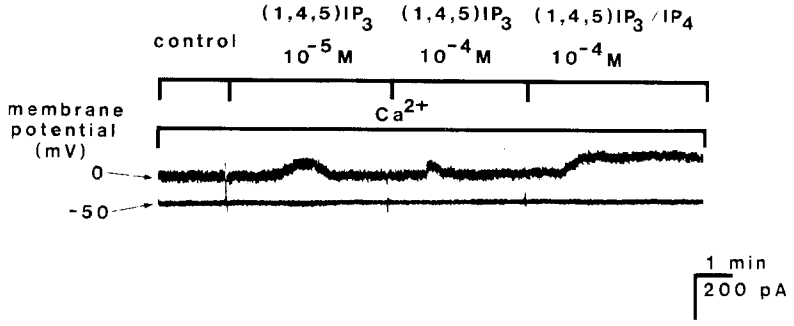

Fig. 2. The effects of 10 and 100 μM Ins 1,4,5 P_3 (*(1,4,5)IP*$_3$) as well as the combination 100 μM Ins 1,4,5 P_3 and 10 μM Ins 1,3,4,5 P_4 (*IP*$_4$) on the Ca^{2+}- and voltage-activated K^+ current

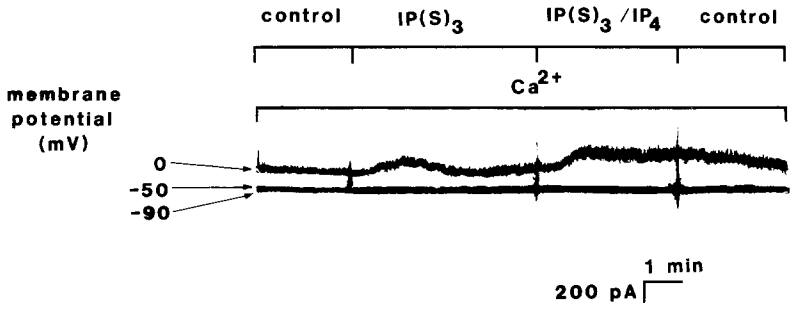

Fig. 3. The effects of Ins 1,4,5 $P(S)_3$ (100 μM) alone and together with Ins 1,3,4,5 P_4 (10 μM) on transmembrane current

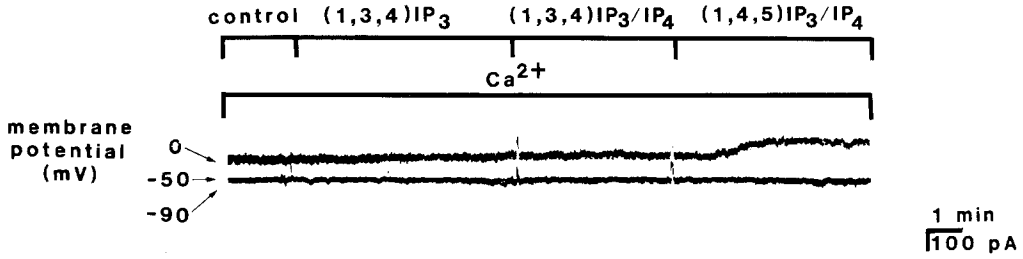

Fig. 4. The effects first of Ins 1,3,4 P_3 (*(1,3,4) IP*$_3$) (10 μM) alone and together with Ins 1,3,4,5 P_4 (10 μM) and thereafter the effect of the combination Ins 1,4,5 P_3 (10 μM) and Ins 1,3,4,5 P_4 (10 μM) on the transmembrane current

failed to transform the response to Ins 1,4,5 P_3 into a sustained one. Five experiments with 2,3 DPG (5 mM) were carried out, and in none of them did 10 μM Ins 1,4,5 P_3 evoke sustained effects.

Finally, the phosphatase-resistant analogue DL-inositol 1,4,5-trisphosphorothioate (Ins 1,4,5 $P(S)_3$) was used (Cooke et al., 1987; Hamblin, Flora & Potter, 1987; Taylor et al., 1988). This analogue, as well as being a potent releaser of calcium, is a much more effective 5-phosphatase inhibitor than 2,3 DPG and has a K_i of 6 μM (Cooke, Nahorski & Potter, 1989). Ins 1,4,5 $P(S)_3$ (100 μM, corresponding to 50 μM of the D-form) evoked a transient response very similar to that seen when using Ins 1,4,5 P_3 (Fig. 3). Ins 1,4,5 $P(S)_3$ together with Ins 1,3,4,5 P_4 evoked sustained responses (six experiments all gave similar results).

Is the Effect of Ins 1,3,4,5 P_4 Specific and Specifically Dependent on the Presence of Ins 1,4,5 P_3 and Could Ins 1,3,4,5 P_4 Act via Its Breakdown Product Ins 1,3,4 P_3?

Figure 4 shows the results of an experiment in which Ins 1,3,4 P_3 (10 μM) had no effect. When 10 μM Ins 1,3,4,5 P_4 was added on top of the Ins 1,3,4 P_3 there was still no response, but a sustained activation of the Ca^{2+}-dependent K^+ current was observed when Ins 1,3,4 P_3 was subsequently replaced by Ins 1,4,5 P_3 in the continued presence of Ins 1,3,4,5 P_4. Four experiments of the type shown in Fig. 4 were carried out, all giving similar results. Two further experiments in which Ins 1,3,4 P_3 was used at the higher concentration of 50 μM confirmed

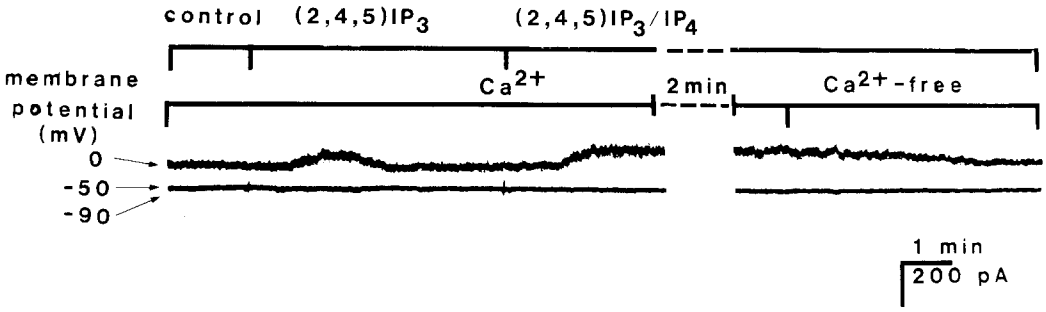

Fig. 5. The effects of first Ins 2,4,5 P_3 (50 μM) alone and then together with Ins 1,3,4,5 P_4 (10 μM) on the transmembrane current. Towards the end of the experiment extracellular Ca^{2+} is removed, resulting in a loss of the response

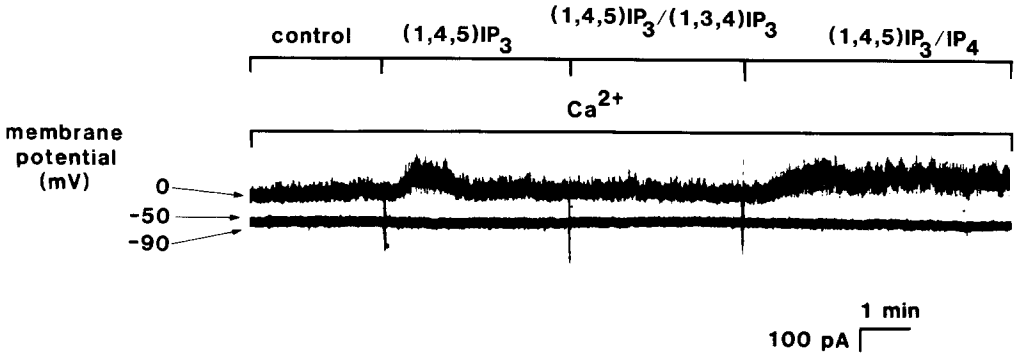

Fig. 6. The effects of Ins 1,4,5 P_3 (10 μM), the combination Ins 1,4,5 P_3 (10 μM) and Ins 1,3,4 P_3 (10 μM) and finally the combination Ins 1,4,5 P_3 (10 μM) and Ins 1,3,4,5 P_4 (10 μM) on the transmembrane current

that this substance did not have any effect alone or in combination with Ins 1,3,4,5 P_4.

Ins 2,4,5 P_3, when used in a concentration of 50 μM, evoked a transient response and was able to support Ins 1,3,4,5 P_4-evoked sustained activation of the K^+ current (four experiments) (Fig. 5). When Ins 2,4,5 P_3 was used at the lower concentration of 10 μM no effects were obtained (five experiments).

Although Ins 1,3,4 P_3 did not elicit any response alone or in combination with Ins 1,3,4,5 P_4 (Fig. 4), it was still necessary to test whether it might work together with Ins 1,4,5 P_3. Figure 6 shows an experiment in which Ins 1,4,5 P_3 evoked its usual response, but where the addition of Ins 1,3,4 P_3 had no further effect. When subsequently Ins 1,3,4 P_3 was replaced by Ins 1,3,4,5 P_4 in the continued presence of Ins 1,4,5 P_3, the usual sustained response was obtained. Four experiments of this type were carried out, all giving similar results.

In five experiments the possible effects of Ins 1,3,4,5,6 P_5 (50 μM) on top of 10 μM Ins 1,4,5 P_3 were investigated. In none of these cases did Ins 1,3,4,5,6 P_5 evoke any response. This demonstrates that the Ins 1,3,4,5 P_4 response is not an artifact caused by a more highly charged inositol polyphosphate. From the biological activity of Ins 1,3,4,5,6 P_5 in sea urchin eggs (Irvine & Moor, 1987) and Ins 1,3,4,5 P_4-binding assays (Bradford & Irvine 1987; Enyedi & Williams, 1988), we might expect this compound to mimick Ins 1,3,4,5 P_4 had the concentration been increased further, but problems of solubility in our intracellular perfusion solution precluded a systematic exploration of this possibility.

The Table presents a summary of the specificity of the inositol polyphosphate actions on the Ca^{2+}-dependent K^+ current.

Does the Combination Ins 1,4,5 P_3 and Ins 1,3,4,5 P_4 Have Any Effect in the Absence of External Ca^{2+}?

Figure 7 shows that Ins 1,4,5 P_3, as expected, evokes a normal transient response also in a situation where the isolated lacrimal acinar cell is exposed to a Ca^{2+}-free external solution containing 1 mM EGTA. When Ins 1,3,4,5 P_4 is added to the Ins 1,4,5 P_3-containing pipette solution, still in the absence of external Ca^{2+}, a second transient response is obtained. After readmission of external Ca^{2+} there is again an increase in the Ca^{2+}-dependent K^+ current, but removal of external Ca^{2+} leads to loss

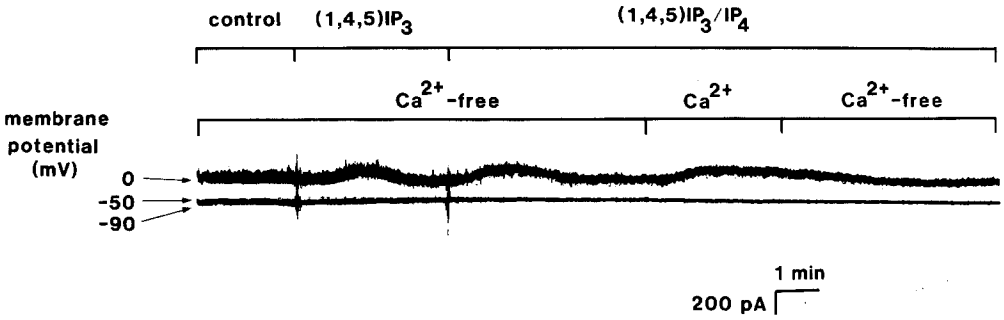

Fig. 7. The effect of Ins 1,4,5 P_3 (10 μM) in the absence of extracellular Ca^{2+} and thereafter the effect of the combination Ins 1,4,5 P_3 (10 μM) and Ins 1,3,4,5 P_4 (10 μM) in the absence and presence of extracellular Ca^{2+}

Table. Specificity of inositol polyphosphates in activating Ca^{2+}-dependent K^+ current

Stimulus	Response
Ins 1,4,5 P_3 (10 or 100 μM)	Transient
Ins 1,3,4 P_3 (10 or 50 μM)	None
Ins 2,4,5 P_3 (10 μM)	None
Ins 2,4,5 P_3 (50 μM)	Transient
Ins 1,4,5 $P(S)_3$ (100 μM)	Transient
Ins 1,3,4,5 P_4 (10 μM) on top of Ins 1,4,5 P_3 (10 or 100 μM)	Sustained
Ins 1,3,4,5 P_4 (10 μM) on top of Ins 1,3,4 P_3 (10 or 50 μM)	None
Ins 1,3,4,5 P_4 (10 μM) on top of Ins 2,4,5 P_3 (10 μM)	None
Ins 1,3,4,5 P_4 (10 μM) on top of Ins 2,4,5 P_3 (50 μM)	Sustained
Ins 1,3,4,5 P_4 (10 μM) on top of Ins 1,4,5 $P(S)_3$ (100 μM)	Sustained
Ins 1,3,4, P_3 (10 μM) on top of Ins 1,4,5 P_3 (10 μM)	None
Ins 1,3,4,5,6 P_5 (50 μM) on top of Ins 1,4,5 P_3 (10 μM)	None

of the response. Transient responses evoked by Ins 1,3,4,5 P_4 addition to an Ins 1,4,5 P_3-containing pipette solution in the absence of external Ca^{2+} were observed in all five experiments of this type carried out and were also seen when Ins 2,4,5 P_3 (50 μM) was used instead of Ins 1,4,5 P_3 (10 μM) (six experiments).

Discussion

Activation of receptors coupled to phosphatidylinositol bisphosphate (PIP_2) breakdown (Berridge & Irvine, 1984) evokes opening of Ca^{2+}-dependent K^+ channels in the acinar cells from a variety of exocrine glands (Maruyama et al., 1983; Findlay, 1984; Maruyama & Petersen, 1984; Trautmann & Marty, 1984; Petersen et al., 1985; Suzuki, Petersen & Petersen, 1985; Gallacher & Morris, 1986; Morris et al., 1987*b*; Suzuki & Petersen, 1988) explaining the classical phenomenon of stimulant-evoked K^+ release (Burgen, 1956; Petersen & Maruyama, 1984; Petersen, 1986). There is good evidence showing that this response is mediated by a rise in $[Ca^{2+}]_i$ and that the initial response is independent of external Ca^{2+}, whereas the sustained response requires the presence of Ca^{2+} in the extracellular solution (Maruyama et al., 1983; Maruyama & Petersen, 1984; Gallacher & Morris, 1987; Morris et al., 1987*a*,*c*; Nauntofte & Dissing, 1987). Morris et al. (1987*c*) showed that continued internal cell perfusion with Ins 1,4,5 P_3, in a relatively high concentration (10 μM), never evoked a sustained increase in Ca^{2+}-dependent K^+ current, whereas such a response could be obtained by using a combination of Ins 1,4,5 P_3 and Ins 1,3,4,5 P_4.

We have now provided fresh evidence for the synergism of Ins 1,4,5 P_3 and Ins 1,3,4,5 P_4 in acinar cell activation (Fig. 1), excluded the possibility that the transient response to Ins 1,4,5 P_3 alone can be explained by rapid metabolic breakdown (Figs. 2 & 3) and demonstrated (Fig. 6) that the effect of Ins 1,3,4,5 P_4 is not mediated via its breakdown product Ins 1,3,4 P_3 as suggested by Ohya et al. (1988). We have also shown that the effect of Ins 1,3,4,5 P_4 requires specifically the presence of Ins 1,4,5 P_3 and cannot be sustained by Ins 1,3,4 P_3 (Fig. 4) although Ins 2,4,5 P_3 at 50 μM concentration can perform the same function as 10 μM Ins 1,4,5 P_3 (Fig. 5) (*compare also* Irvine & Moor, 1986). It is therefore clear that Ins 1,3,4,5 P_4, which is rapidly formed from Ins 1,4,5 P_3 during receptor activation (Batty, Nahorski & Irvine, 1985; Hawkins, Stephens & Downes, 1986; Trimble et al., 1987), has a dramatic effect on cellular Ca^{2+} handling since it transforms the transient Ca^{2+}-activated K^+ current response evoked by Ins 1,4,5 P_3 alone into a long sustained current increase (Fig. 1). This effect of Ins 1,3,4,5 P_4 cannot be explained by interference with the metabolism of

Ins 1,4,5 P_3, since it is also seen when the trisphosphate is present in great excess of the tetrakisphosphate (Fig. 2) as well as when the stable analogue Ins 1,4,5 $P(S)_3$ (Cooke et al., 1987; Taylor et al., 1988) is used (Fig. 3).

It has been suggested that Ins 1,3,4,5 P_4 may act at the plasma membrane by opening up a pore permeable to Ca^{2+} (Berridge, 1988; Gallacher, 1988; Petersen & Gallacher, 1988), but results so far obtained do not, strictly speaking, permit any conclusions about the precise site of action. The situation is not very different for Ins 1,4,5 P_3 since there is also in this case debate concerning the precise localization and nature of the intracellular Ca^{2+} pool (Volpe et al., 1988; Berridge, 1988).

Our result showing that Ins 1,3,4,5 P_4 evokes transient Ca^{2+} release when added to an Ins 1,4,5 P_3-containing solution in the absence of external Ca^{2+} (Fig. 7) indicates that Ins 1,3,4,5 P_4 may not solely participate in controlling Ca^{2+} entry from the outside of the cell, but can also play a role in the regulation of intracellular Ca^{2+} mobilization. The function of Ins 1,3,4,5 P_4 may in this respect be somewhat similar to the previously proposed role for GTP (Mullaney et al., 1987) in controlling the passage of Ca^{2+} between different intracellular compartments (Irvine et al., 1988). Hill, Dean and Boynton (1988) have recently shown that in permeabilized hepatoma cells Ins 1,3,4,5 P_4 can stimulate Ca^{2+} sequestration; this phenomenon could also be due to passage of Ca^{2+} between pools (with different Ca^{2+} pumping capacities), and, indeed, such a concept is the simplest explanation consistent with both the data of Hill et al. (1988) and our present data.

In those systems where inositol polyphosphates are the main regulators of Ca^{2+} influx, it is possible that there could be differences in the relative importance of Ins 1,4,5 P_3 and Ins 1,3,4,5 P_4. If an influx pathway is normally controlled at two different points by two messengers, respectively, situations could arise where one of these gates were permanently open and the regulation only occurred at the other point. This may especially be true during experimental manipulations and might explain why in one smooth muscle preparation effects of Ins 1,3,4,5 P_4 alone were found in a particular situation, whereas in other situations only effects of Ins 1,4,5 P_3 alone were observed (Ohya et al., 1988). Similar considerations might apply to mast cells (Penner, Matthews & Neher, 1988) where Ins 1,4,5 P_3, apparently on its own (some phosphorylation to Ins 1,3,4,5 P_4 cannot be excluded), evokes a Ca^{2+} entry and also to rat lacrimal acinar cells (Llano et al., 1987) where the experimental protocol is different from that used here, in particular, with regard to the presence of Ins 1,4,5 P_3 in the pipette at the moment of cell penetration.

The situation is also complicated in the oocyte. Irvine and Moor (1986) originally showed that Ins 1,3,4,5 P_4 was effective in raising a visible fertilization envelope in sea urchin eggs if Ins 2,4,5 P_3 was co-injected and if Ca^{2+} was present in the bathing solution. However, as discussed by Irvine et al. (1988) and Crossley et al. (1988), this is not a universal feature of all sea urchin eggs. In *Xenopus* oocytes Parker and Miledi (1987) showed that Ins 1,3,4,5 P_4 activated voltage-sensitive Ca^{2+} channels in the surface membrane via a process that seemed to require priming by Ins 1,4,5 P_3. On the other hand, Snyder, Krause and Welsh (1988) in a study of the same cell type found that Ins 1,3,4,5 P_4 alone evoked activation of a Ca^{2+}-dependent Cl^- current, but this response was independent of external Ca^{2+} and therefore presumably due to release of Ca^{2+} from an intracellular store. They also found a sustained Ca^{2+} entry phase caused by Ins 1,4,5 P_3, apparently independently of Ins 1,3,4,5 P_4. These results are clearly very different from the ones presented here on the mouse lacrimal acinar cells, where known concentrations of inositol polyphosphates are applied directly to the cell interior under conditions where control and test periods can be compared in individual experiments and where we find an invariable requirement for the presence of both Ins 1,4,5 P_3 and Ins 1,3,4,5 P_4 in order to obtain sustained K^+ current responses dependent on external Ca^{2+}. Such Ca^{2+}-dependent sustained current responses can also be evoked by extracellular ACh application (Morris et al., 1987*c*) suggesting that the effect of the neurotransmitter is mediated by both Ins 1,4,5 P_3 and Ins 1,3,4,5 P_4. Since Ins 1,4,5 P_3 has a transient effect on its own, whereas Ins 1,3,4,5 P_4 has no effect when applied alone (Morris et al., 1987*c*), the immediate controller of Ca^{2+} flux into the cytosol is undoubtedly Ins 1,4,5 P_3. Because Ins 1,3,4,5 P_4 enables Ins 1,4,5 P_3 to mobilize more Ca^{2+} also in the absence of external Ca^{2+}, the most economical hypothesis may be to propose that Ins 1,3,4,5 P_4 helps to establish a functional link between Ins 1,4,5 P_3-sensitive and insensitive Ca^{2+} pools. Such a link-up would appear also to involve the establishment of a connection to the extracellular compartment. The existence of two separate intracellular Ca^{2+} pools, with different Ca^{2+} uptake mechanisms, one sensitive to Ins 1,4,5 P_3 the other not, has recently been demonstrated in pancreatic acinar cells (Thévenod et al., 1989).

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