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The effect of inositol 1,3,4,5-tetrakisphosphate on inositol trisphosphate-induced Ca^{2+} mobilization in freshly isolated and cultured mouse lacrimal acinar cells

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Earlier reports have shown a remarkable synergism between InsP_4 and InsP_3 [either Ins(1,4,5)P_3 or Ins(2,4,5)P_3] in activating Ca^{2+}-dependent K^+ and Cl^- currents in mouse lacrimal cells [Changya, Gallacher, Irvine, Potter and Petersen (1989) J. Membr. Biol. **109**, 85–93; Smith (1992) Biochem. J. **283**, 27–30]. However, Bird, Rossier, Hughes, Shears, Armstrong and Putney [(1991) Nature (London) **352**, 162–165] reported that they could see no such synergism in the same cell type. A major experimental difference between the two laboratories lies in whether or not the cells were maintained in primary culture before use. Here we have compared directly the responses to inositol polyphosphates in freshly isolated cells versus cells cultured for 6–72 h. In the cultured cells, Ins(2,4,5)P_3 at 100 μM produced a robust stimulation of K^+ and Cl^- currents, as much as an order of magnitude greater than that observed in the freshly isolated cells. However, the freshly isolated cells could be restored to a sensitivity similar to cultured cells by the addition of InsP_4 at a concentration two orders of magnitude lower than that of Ins(2,4,5)P_3. We discuss the implications of this with respect to the actions of InsP_4, including the possibility that disruption of the cellular structure during the isolation of the cells exposes an extreme manifestation of a possible physiological role for InsP_4 in controlling calcium-store integrity.

Key words: Cl^- current, exocrine, InsP_4, K^+ current, patch-clamp.

INTRODUCTION

InsP_4 has had a long and chequered history as an intracellular second messenger [1,2] surrounded by controversy fuelled by confusing and contradictory results. Overall, InsP_4 has been shown to influence the kinetics of Ca^{2+} release from stores and Ca^{2+} entry stimulated by InsP_3. However, the effects of InsP_4 are highly variable between tissues and are undetectable in some systems [1,2]. Some of the most convincing and clear-cut evidence in favour of a role for InsP_4 as a second messenger has been obtained by measuring the amplification of the InsP_3-induced Ca^{2+}-dependent K^+ current in mouse lacrimal acinar cells [3–5]. However, many other cellular preparations do not show a similar synergism and, even in lacrimal cells, Bird et al. have published data [6] showing that an increased concentration of InsP_3 could produce maximal activation of the K^+ current with no requirement for InsP_4.

Despite further studies [7] that showed, by measuring the less Ca^{2+}-sensitive Cl^- current in addition to the Ca^{2+}-dependent K^+ current, that InsP_3/InsP_4 synergism did occur at very high InsP_3 concentrations, the differences between results from the two laboratories have remained unexplained. It may be in part because of this and other unresolved discrepancies [8,9] in the findings of these two laboratories that subsequent reports of InsP_4-dependent modulation of InsP_3-induced Ca^{2+} mobilization [10–13] have frequently been ignored [14].

One possible explanation for the conflicting reports of the role of InsP_4 in Ca^{2+} mobilization in lacrimal cells may be found by close examination of the methods used to isolate and prepare lacrimal cells for experimentation. Although not explicitly stated in [6], these data were obtained from cells maintained in primary culture for up to 24 h [8], whereas the experiments reported in [3–5] and [7] were performed using freshly isolated cells. In the experiments described here we have systematically compared the effects of a secretory agonist and of InsP_3 and InsP_4 on Ca^{2+} mobilization and activation of Ca^{2+}-dependent currents in freshly isolated and cultured mouse lacrimal acinar cells. Our intention was, in the first instance, to resolve the contradictions described above. Secondly, we hoped to encourage reappraisal of the effects of InsP_4 on InsP_3-induced Ca^{2+} mobilization and Ca^{2+} influx, most especially the separate mechanisms by which the InsP_3 and InsP_4 act and the absolute requirement for InsP_3 to be present with InsP_4 [3–5,7]. Finally, we believe that if we can clarify the conditions under which InsP_4 has such a pronounced synergism with InsP_3, we may significantly advance our understanding of the putative second-messenger action of this inositol phosphate.

EXPERIMENTAL

Adult male CD1 mice were killed by cervical dislocation and lacrimal cells were isolated by collagenase (Worthington Biochemical Corp., Lakewood, NJ, U.S.A.) digestion in extracellular medium containing 1 mM Ca^{2+} as described previously [7]. Following dispersal, cells were suspended in serum-free Dulbecco's minimal essential medium/F12 (1:1, v/v) medium containing antibiotics and antimycotics (Life Technologies, Paisley, Renfrewshire, Scotland, U.K.) and placed on circular glass coverslips (22 mm diameter). For primary culture, the coverslips were coated with a thin ($\approx$ 1 mm) layer of a basement-membrane

Abbreviations used: ACh, acetylcholine; $[Ca^{2+}]_i$, cytosolic free Ca^{2+} activity; fura-2/AM, fura-2 acetoxymethyl ester.

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matrix (Matrigel; Becton Dickinson) [15]. Survival rates for cells cultured on uncoated coverslips were poor compared with cells placed on Matrigel. Each coverslip was placed into one well of a six-well plate and covered with medium. Cells were maintained within an incubator for up to 72 h at 37 °C in a CO_2/air (1 : 19) atmosphere. For experimental purposes, the glass coverslips formed the base of a perfusion chamber which was placed on the stage of an inverted microscope. Identical methods were used to prepare freshly isolated cells, except that these cells were placed on uncoated glass coverslips and used within 2 h of preparation. Control experiments (results not shown) showed that Matrigel itself had no effect on the responses shown by freshly isolated cells.

The patch-clamp whole-cell configuration was achieved with single cells using 1.5–2.0 MΩ patch-clamp pipettes pulled from Assistant micro-haematocrit tubing (Karl Hecht, Sondheim/Rhön, Germany). Access resistance through the patch pipette was approx. 3 times that of the pipette itself. Cells were voltage-clamped to −40 mV using an Axopatch 200a patch-clamp amplifier (Axon Instruments, Foster City, CA, U.S.A.). K^+ and Cl^- currents were measured separately by pulsing to 0 mV and −80 mV respectively for 100 ms twice a second [16]. Currents were digitized using the CED 1401 interface (Cambridge Electronics Design, Cambridge, U.K.) and stored and analysed using a personal computer with custom-written software [17].

The patch-clamp pipette contained (in mM) 140 KCl, 1.13 $MgCl_2$, 10 glucose, 0.5 EGTA and 1 ATP, buffered to pH 7.4 with 10 mM Hepes and, where indicated in the text and Figure legends, InsP_3 or InsP_3 + InsP_4.

The extracellular bathing solution contained (in mM) 140 NaCl, 4.7 KCl, 1.13 $MgCl_2$, 1.2 $CaCl_2$ and 10 glucose, buffered to pH 7.4 with 10mM Hepes. Cells were superfused continuously at 0.5 ml/min from one of several parallel superfusion pipettes. The solution bathing the cell could be changed in 1–2 s. All experiments were carried out at 24 ± 2 °C. These experiments take advantage of the access to the cell interior offered by the patch-clamp whole-cell technique to infuse inositol phosphates into the cell. In all such experiments, inositol phosphates were added to the intracellular medium at the start of the experiment and thus entered the cell as soon as the breakthrough to the whole-cell condition was achieved.

Isolated acinar cells prepared by the methods given above were loaded with fura-2 by incubation for 10–20 min in the presence of fura-2 acetoxymethyl ester (fura-2/AM; Molecular Probes, Eugene, OR, U.S.A.). Simultaneous patch- clamp and microfluorimetry experiments were performed using the perfusion chamber, perfusion apparatus and extracellular bathing solution described above. The ratio of UV light emitted at 510 nm following excitation at 340 nm to that emitted following excitation at 380 nm was measured using a Cairn Research (Faversham, Kent, U.K.) spectrophotometer (excitation was at 96 Hz; data were averaged online and collected at 4 Hz.). Intracellular $[Ca^{2+}]$ was calculated from this ratio using the Grynkiewiez equation and custom-written software.

Ins(1,4,5)P_3 and Ins(2,4,5)P_3 were prepared by the methods given in [18], and HPLC-purified Ins(1,3,4,5)P_4 by those given in [19].

Means were tested for statistical significance using Student's *t* test.

RESULTS

Freshly isolated mouse lacrimal acinar cells respond to acetylcholine (ACh; 100–5000 nM) with a dose-dependent biphasic increase in both K^+ and Cl^- currents [20]. The ion channels that carry these currents are both Ca^{2+}-activated [21,22]. Whole-cell currents in exocrine acinar cells are used routinely to assess changes in cytosolic free Ca^{2+} activity ($[Ca^{2+}]_i$), and they have been shown to reflect accurately changes in $[Ca^{2+}]_i$ [23]. In contrast with studies using pancreatic [23], submandibular [24] or parotid [25] acinar cells, we have been previously unable to demonstrate oscillatory changes in Ca^{2+}-dependent currents in response to ACh in freshly isolated lacrimal acinar cells (Figure 1A; typical of 36 observations.) However, we could elicit damped sinusoidal oscillations in both the K^+ and the Cl^- current in cells maintained in culture for as little as 6 hours (Figure 1B, typical of 15 observations). Cholinergically induced sinusoidal oscillations in $[Ca^{2+}]_i$ have been previously reported in mouse lacrimal cells maintained in primary culture by Bird et al. [9]. One characteristic of these oscillations was that their frequency was largely independent of agonist concentration. Figure 2 shows that the oscillations in Ca^{2+}-dependent currents also demonstrated this trait. Furthermore, beyond a minimum period of 6 h in culture, the frequency of oscillations was also independent of the length of time spent in primary culture. Lacrimal cells maintained in primary culture responded with damped sinusoidal oscillations with a period of 7–10 s when stimulated with 50 or 500 nM ACh and following culture periods between 6 and 72 h. These observations demonstrate that there is a real difference in the response patterns to cholinergic agonists of lacrimal acinar cells maintained in culture and those freshly isolated.

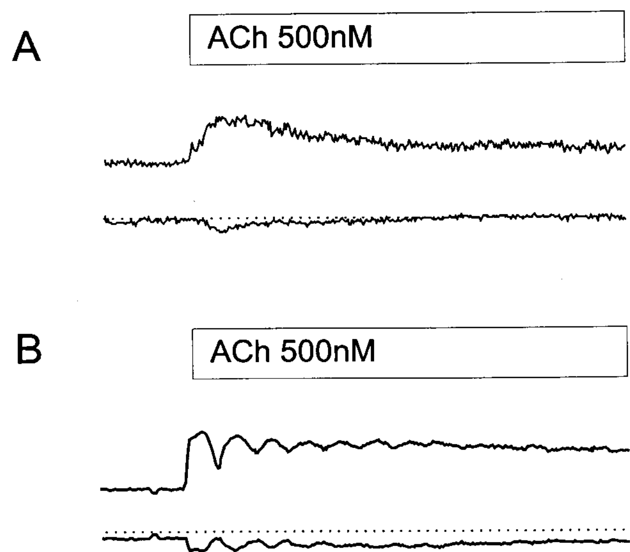

Figure 1 Sinusoidal oscillations in the K^+ and Cl^- currents stimulated by ACh in cells maintained in primary culture

K^+ (upper trace) and Cl^- (lower trace) currents in response to 500 nM ACh measured in a single mouse lacrimal cell. (**A**) Freshly isolated; (**B**) following 6 h in primary culture. The dotted line indicates zero current. Results are typical of 15 observations.

Lacrimal acinar cells are known to be relatively insensitive to internal perfusion by inositol polyphosphates, and high concentrations have previously been used to elicit significant activation of K^+ and Cl^- currents [6,7]. Figure 3(B) shows the effect of perfusion with 1 mM Ins(2,4,5)P_3 compared with that seen in cells perfused with a control K^+ Hepes solution (Figure 3A). In these and all subsequent traces the bar shows where breakthrough from the cell-attached configuration to the whole-cell configuration was achieved. Upon breakthrough in the presence of 1 mM Ins(2,4,5)P_3 there was significant activation of the K^+

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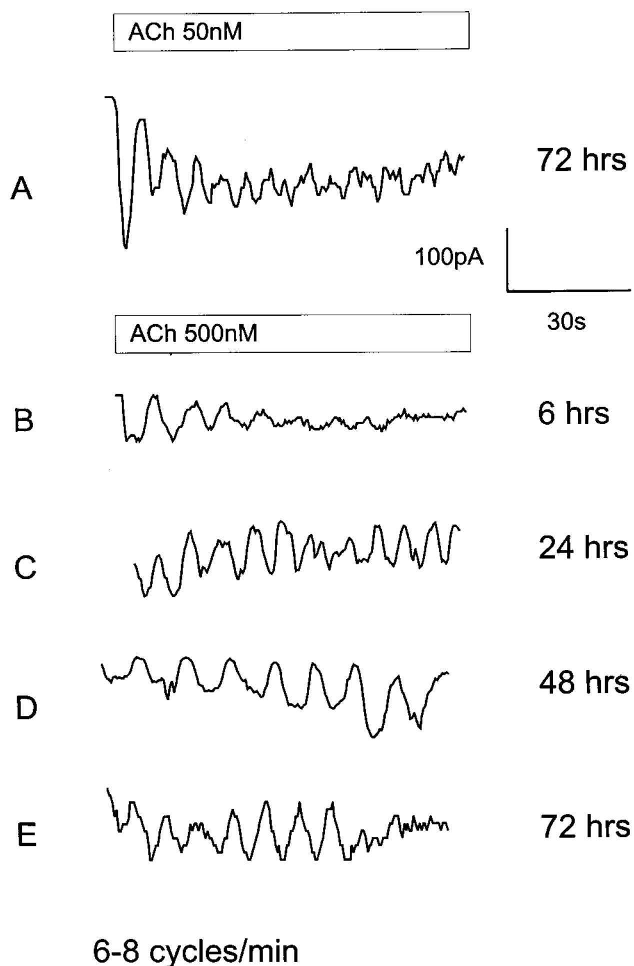

Figure 2 Effects of time in culture on ACh-induced sinusoidal oscillations in the K^+ current

K^+ currents measured in a single mouse lacrimal cell in response to ACh. (**A**) 50 nM ACh following 72 h in culture; (**B–E**) 500 nM ACh following 6, 24, 48 and 72 h in culture respectively.

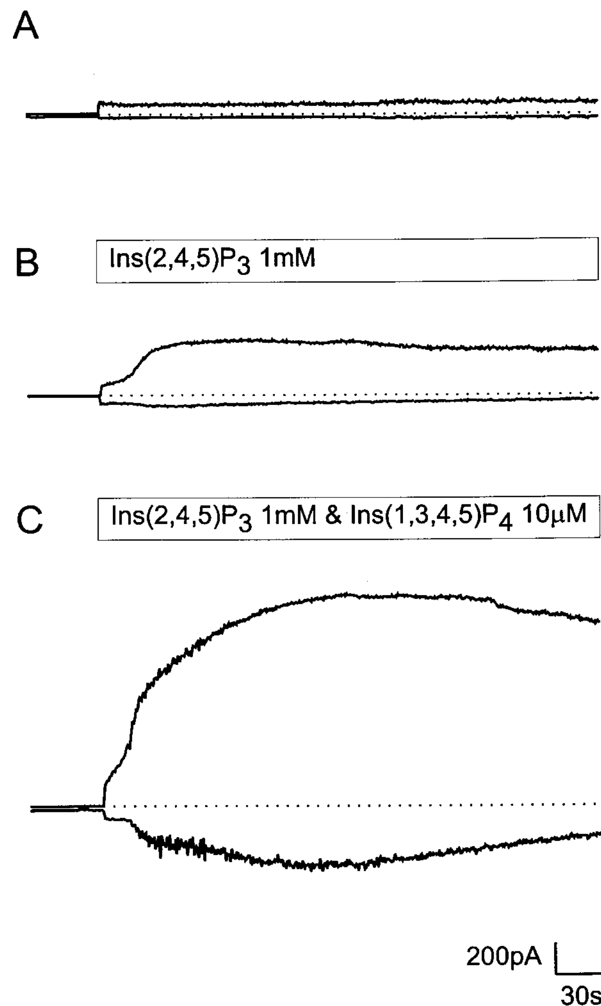

Figure 3 Effects of Ins(2,4,5)P_3 and InsP_4 on freshly isolated cells

K^+ (upper trace) and Cl^- (lower trace) currents measured in a single freshly isolated mouse lacrimal cell. (**A**) Under control conditions; (**B**) with 1 mM Ins(2,4,5,)P_3 in the pipette; (**C**) with 1 mM Ins(2,4,5)P_3 and 10 μM InsP_4 in the pipette. The dotted line indicates zero current. (**A**), (**B**) and (**C**) are typical of eight, five and three observations respectively.

current and a small increase in Cl^- current compared with the control trace. These traces are comparable with those previously obtained using 100 μM Ins(1,4,5)P_3 in the patch pipette [7]. Figure 3(C) shows activation of the K^+ and Cl^- currents when 10 μM InsP_4 as well as 1 mM Ins(2,4,5)P_3 is present in the pipette. These traces are typical of eight, five and three experiments for Figures 3(A), 3(B) and 3(C) respectively. On average, 1 mM Ins(2,4,5)P_3 generated a peak K^+ current of 541 ± 221 pA and a peak Cl^- current of 123 ± 31 pA ($n = 5$). Ins(2,4,5)P_3 at 1 mM and InsP_4 at 10 μM together generated a peak K^+ current of 1383 ± 109 pA and a peak Cl^- current of 350 ± 29pA ($n = 3$). The mean K^+ and Cl^- currents in the presence of 1 mM Ins(2,4,5)P_3 and 10 μM InsP_4 together are significantly greater, $P < 0.05$ and $P < 0.01$ respectively, than those measured in the presence of Ins(2,4,5)P_3 alone. The presence of Ins(2,4,5)P_3 at 100 times the Ins(1,3,4,5)P_4 concentration eliminates any possibility that these data result from conversion of Ins(1,3,4,5)P_4 into Ins(1,4,5)P_3. Furthermore, as Ins(1,3,4)P_3 has been shown to be completely ineffective as a Ca^{2+}-mobilizing agent [5], these data cannot result from conversion of Ins(1,3,4,5)P_4 into Ins(1,3,4)P_3. When the whole-cell configuration was established under control conditions in the absence of inositol polyphosphates, K^+ currents averaged 114 ± 19 pA and Cl^- currents 59 ± 11 pA ($n = 8$).

Perfusion of InsP_4 at 1% of the Ins(2,4,5)P_3 concentration also elevated both K^+ and Cl^- currents in conjunction with an Ins(2,4,5)P_3 concentration that by itself had little effect (Figure 4). Averaged K^+ and Cl^- currents in the presence of 100 μM Ins(2,4,5)P_3 were 162 ± 24 pA and 43 ± 3 pA ($n = 4$) respectively, little different from those measured in the absence of Ins(2,4,5)P_3. However, the addition of 1 μM InsP_4 was sufficient to raise the peak K^+ current to 679 ± 181 pA [$P < 0.05$ compared with the K^+ current stimulated by Ins(2,4,5)P_3 alone], although there was no significant alteration to the less-Ca^{2+}-sensitive Cl^- current (68 ± 15 pA, $n = 5$).

Figure 5 shows one of the few stable whole cells we were able to obtain using patch pipettes containing 1 mM Ins(2,4,5)P_3 in cells maintained in culture for 24 h. In over 90% of experiments the whole-cell configuration was lost within a few seconds. The sustained activation of the K^+ and Cl^- currents shown in this Figure is equivalent to the maximal response evoked in freshly isolated cells by 1 mM Ins(2,4,5)P_3 and 10 μM Ins(1,3,4,5)P_4

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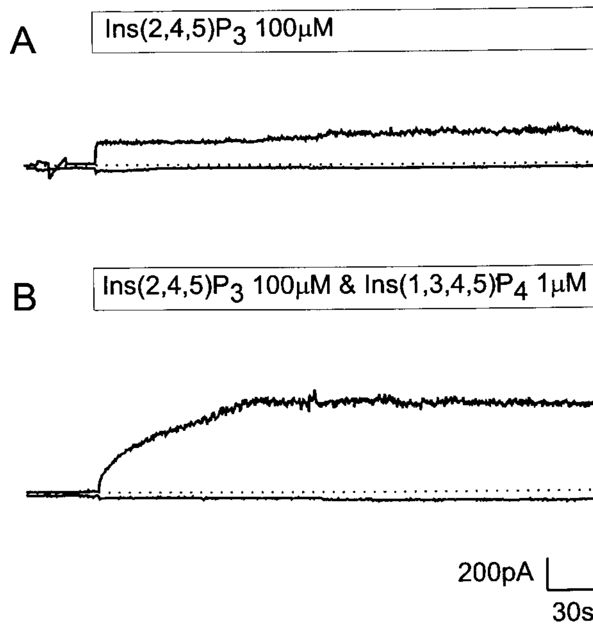

Figure 4 Effects of Ins(2,4,5)P_3 and InsP_4 on freshly isolated cells

K^+ (upper trace) and Cl^- (lower trace) currents measured in a single mouse lacrimal cell. (**A**) With 100 μM Ins(2,4,5)P_3 in the pipette and (**B**) with 100 μM Ins(2,4,5)P_3 and 1 μM InsP_4 in the pipette. The dotted line indicates zero current. (**A**) and (**B**) are typical of four and five observations respectively

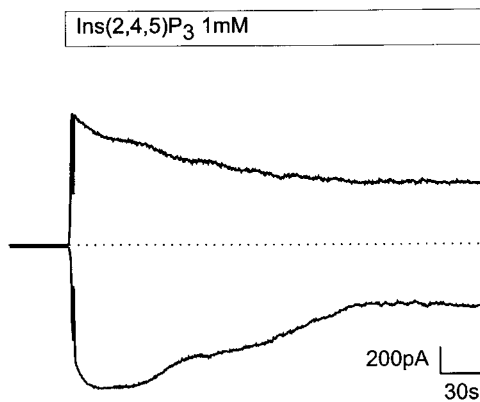

Figure 5 Effects of 1 mM Ins(2,4,5)P_3 on cells maintained in primary culture

K^+ (upper trace) and Cl^- (lower trace) currents stimulated by 1 mM Ins(2,4,5)P_3 measured in a single mouse lacrimal cell following 24 h in primary culture. The dotted line indicates zero current.

(Figure 3) or by 500 μM Ins(1,4,5)P_3 and 100 μM Ins(1,3,4,5)P_4 [7]. Compared with Figure 1(B), this trace indicates that there was a significant increase in the sensitivity of the acinar cells to Ins(2,4,5)P_3 following 24 h in primary culture. This increase in sensitivity could also account for the instability of these experiments, as the large elevation of $[Ca^{2+}]_i$ necessary to produce these currents would likely cause substantial cell-volume changes, which could destroy the whole-cell configuration or even lead to cell death. Loss of the whole-cell configuration following rapid volume change can be observed in freshly isolated cells as well as in cells that have been maintained in primary culture (P. M. Smith, unpublished work).

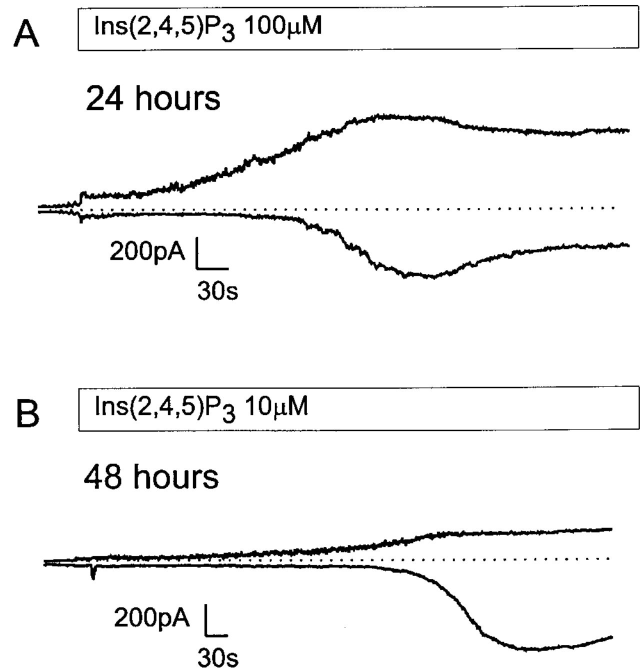

Figure 6 Effects of Ins(2,4,5)P_3 on cells maintained in primary culture

K^+ (upper trace) and Cl^- (lower trace) currents stimulated by (**A**) 100 μM and (**B**) 10 μM Ins(2,4,5)P_3 measured in single mouse lacrimal cells maintained in primary culture for (**A**) 24 h and (**B**) 48 h. The dotted line indicates zero current. (**A**) and (**B**) are typical of five and seven observations respectively.

The increased sensitivity of the cells to Ins(2,4,5)P_3 was not restricted to high concentrations, as Figure 6 shows activation of K^+ and Cl^- currents by 100 μM Ins(2,4,5)P_3 in cells maintained in culture for 24 h (cf. Figure 4A) and by 10 μM Ins(2,4,5)P_3 in cells maintained in culture for 48 h. The increased lag between reaching the whole-cell configuration and activation of the currents was typical of these experiments, although the duration of the lag phase varied from cell to cell. The increase in sensitivity to InsP_3 was also not restricted to the non-phosphorylatable isomer. Figure 7 shows a scatter plot of the peak Cl^- current elicited by 100 μM Ins(2,4,5)P_3 (Figure 7A) and Ins(1,4,5)P_3 (Figure 7B) against time in culture. The magnitude of the current elicited by Ins(1,4,5)P_3 was greater than that produced by Ins(2,4,5)P_3 at all time points; however, the trend towards greater sensitivity is clearly evident in both cases. The inability of Ins(1,4,5)P_3 to produce a full response, when one would superficially expect it to be phosphorylated to produce some InsP_4, is initially surprising, although consistent with our earlier data [3–5]. However, part of the explanation may lie in the pronounced Ca^{2+} requirement for the B isoform of InsP_3 kinase [26], the isoform we now know to be the most likely one to be present in peripheral tissues [27]. Under patch-clamp conditions, 500 μM EGTA present in the pipette may damp phosphorylation of Ins(1,4,5)P_3 sufficiently to prevent the formation of sufficient InsP_4 to satisfy the InsP_4-dependent component of the response.

One outcome of the increased sensitivity of the cells to Ins(2,4,5)P_3 following primary culture was that maximal activation of the K^+ and Cl^- currents could be obtained using Ins(2,4,5)P_3 alone (Figure 5). However, InsP_4, added at 1 % of the Ins(2,4,5)P_3 concentration, potentiated activation of the

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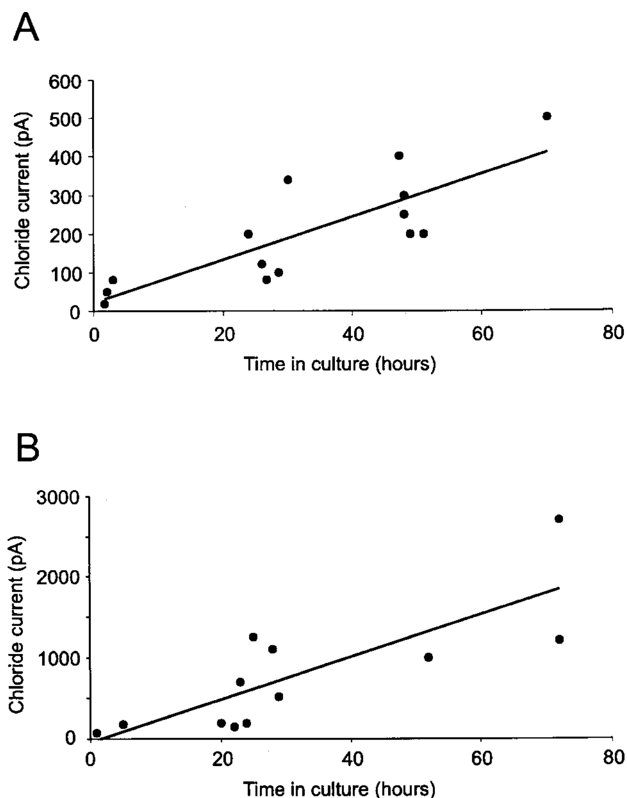

Figure 7 Effects of time in culture on the increase in Cl^- current stimulated by InsP_3

Peak Cl^- current stimulated by (**A**) 100 μM Ins(2,4,5)P_3 and (**B**) 100 μM Ins(1,4,5)P_3 plotted against time in culture.

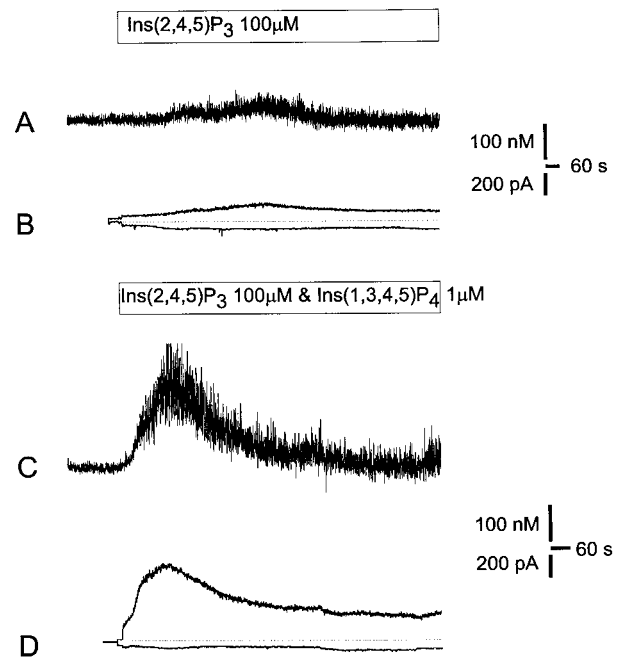

Figure 8 Simultaneous measurement of $[Ca^{2+}]_i$ (A and C) and K^+ and Cl^- currents (B and D) stimulated by 100 µM Ins(2,4,5)P_3 and 100 µM Ins(2,4,5)P_3 plus 1µM InsP_4

Changes in $[Ca^{2+}]_i$ measured by fura-2 fluorescence following intracellular perfusion with (A) 100 μM Ins(2,4,5)P_3 or (C) 100 μM Ins(2,4,5)P_3 plus 1μM InsP_4. Changes in K^+ (upper trace) and Cl^- (lower trace) currents measured following intracellular perfusion with (**B**) 100μM Ins(2,4,5,)P_3 or (**D**) 100 μM Ins(2,4,5,)P_3 plus 1 μM Ins(1,3,4,5,)P_4. The dotted line indicates zero current. Calcium and patch-clamp measurements were made simultaneously in a single mouse lacrimal cell following 24 h in primary culture.

Ca^{2+}-dependent currents at all Ins(2,4,5)P_3 concentrations that elicited submaximal responses. Figure 8 shows an example of the synergism of Ins(2,4,5)P_3 and InsP_4 in a cell maintained in primary culture for 24 h. In this experiment, intracellular $[Ca^{2+}]$ was measured using fura-2 spectrophotometry simultaneously with the K^+ and Cl^- currents. These data are typical of over 30 experiments in which simultaneous measurements of $[Ca^{2+}]_i$ and Ca^{2+}-dependent whole-cell currents were made and show a precise correspondence between changes in $[Ca^{2+}]_i$ and changes in whole-cell currents.

DISCUSSION

The most immediate conclusion from the present study is that we believe that we have resolved the long-standing controversy between two groups who have consistently obtained profoundly different data on the same cell type [3–6,7]. By moving our experimental protocols more closely towards those used by Bird et al. [6] we have been able to reproduce some of their findings, such as the oscillatory patterns of Ca^{2+} signalling in response to muscarinic agonists that may only be observed in cells maintained in primary culture. Moreover, we have shown that the sensitivity of the cells to InsP_3 increases as a function of the time spent in culture. This would serve to obscure the synergism between InsP_3 and InsP_4 in cultured cells because these cells would respond maximally to lower doses of InsP_3 than freshly isolated cells. Notwithstanding the increase in InsP_3-sensitivity, we can, even in the cultured lacrimal cells, see effects of InsP_4, [for example the amplification of the response to a submaximal Ins(2,4,5)P_3 dose by InsP_4 at 1 % of that of Ins(2,4,5)P_3; see Figure 8], that are difficult to account for by metabolic effects such as protection of InsP_3 against hydrolysis. These data, in common with previous data from lacrimal cells and with data from other systems that show a clear response to InsP_4 [10–13], indicate that, in all cases, more Ca^{2+} may be mobilized by Ins(1,4,5)P_3 when InsP_4 is also present. Although the original interpretation of this response in lacrimal cells emphasized the effect of InsP_4 on InsP_3-induced Ca^{2+} entry [3], subsequent data [4,5,7], including those obtained in the present study, have indicated that this is more likely to be an indirect consequence of increased Ca^{2+} mobilization.

The implications of the culture-induced change in cellular responses go further than providing a resolution to incompatible data from different laboratories. These data demonstrate a means whereby the 'visibility' of the InsP_4 response may be varied and thus may offer new insight into what InsP_4 is doing within the cell. It has for a long time been evident that mouse lacrimal acinar cells, specifically when freshly isolated, are something of a 'freak' preparation in which the effects of InsP_4 are so pronounced as to be clearly obvious. Furthermore, it is also clear that this is not the case in most other experimental systems [1,2]. However, the clear isomeric specificity of the requirement for InsP_4 [4] has always argued [28] for this being a quantitative rather than a qualitative artefact; in short, an exaggeration of a physiological event rather than a creation of an entirely new one.

A phenomenon which may be related to the change in InsP_3-sensitivity described here has been previously described in hepatocytes by Renard-Rooney et al. [29], who compared InsP_3-

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dependent Ca^{2+} mobilization in freshly isolated cells with that of cultured cells. In the former they found that, after permeabilization, the InsP_3-mobilizable Ca^{2+} pools appeared to be 'fragmented', and a suboptimal InsP_3 concentration could only access a fraction of the total InsP_3-mobilizable Ca^{2+}. In the latter they seemed to be 'contiguous', and fractional InsP_3 receptor activation could access all the mobilizable Ca^{2+}. These functional differences were supported by evident structural changes seen under the electron microscope. A similar sort of phenomenon is an intriguing possibility that would account for what we describe here, and indeed, Putney [30] has discussed the possibility that Ca^{2+} pool integrity could be different in freshly isolated versus cultured lacrimal acinar cells (see also [31,32]).

The physiological correlate of this possible change in endomembrane structure is not obvious, but it may be the fragmentation of the endoplasmic reticulum that has been reported in response to Ca^{2+} [33] or phorbol esters [34]. Fragmentation of the intracellular Ca^{2+} store could account not only for the insensitivity of the lacrimal cells to InsP_3 but also, assuming a requirement for a minimal endomembrane structural integrity [35], for the lack of Ca^{2+} oscillations in freshly isolated cells (Figure 1). Although it is possible that the action of InsP_4 is unconnected with these events, which serve rather to simply emphasize InsP_4-dependent Ca^{2+} mobilization, an attractive alternative is that the physiological role of InsP_4 may be to contribute a controlling influence on endomembrane integrity.

Our extensive studies using permeabilized L-1210 cells, in which we have shown that responses to InsP_4 are entirely distinct from the action of InsP_3 on its receptor [13], are also consistent with this hypothesis. We have suggested previously that the action of InsP_4 in L-1210 cells might be to regulate the access of InsP_3 to mobilizable Ca^{2+}, perhaps via control of the linking of Ca^{2+} pools [36,37] and that this phenomenon may be mediated by the putative InsP_4 receptor GAP1^{IP_4BP} [38,39]. We hope to exploit this convergence of mechanisms in seeking a further understanding of the possible second-messenger function of InsP_4.

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REFERENCES

1 Irvine, R. F. (1991) Bioessays **13**, 419–427
2 Putney, Jr., J. W. and Bird, G. S. (1993) Endocr. Rev. **14**, 610–631
3 Morris, A. P., Gallacher, D. V., Irvine, R. F. and Petersen, O. H. (1987) Nature (London) **330**, 653–655
4 Changya, L., Gallacher, D. V., Irvine, R. F. and Petersen, O. H. (1989) FEBS Lett. **251**, 43–48
5 Changya, L., Gallacher, D.V., Irvine, R. F., Potter, B. V. and Petersen, O. H. (1989) J. Membr. Biol. **109**, 85–93
6 Bird, G. S., Rossier, M. F., Hughes, A. R., Shears, S. B., Armstrong, D. L. and Putney, Jr., J. W. (1991) Nature (London) **352**, 162–165
7 Smith, P. M. (1992) Biochem. J. **283**, 27–30
8 Bird, G. S. and Putney, Jr., J. W. (1996) J. Biol. Chem. **271**, 6766–6770
9 Bird, G. S., Rossier, M. F., Obie, J. F. and Putney, Jr., J. W. (1993) J. Biol. Chem. **268**, 8425–8428
10 Gawler, D. J., Potter, B. V., Gigg, R. and Nahorski, S. R. (1991) Biochem. J. **276**, 163–167
11 Maruyama, Y. (1993) J. Physiol. (London) **463**, 729–746
12 Van der Zee, L., Sipma, H., Nelemans, A. and Den Hertog, A. (1995) Eur. J. Pharmacol. **289**, 463–469
13 Loomis-Husselbee, J. W., Cullen, P. J., Dreikausen, U. E., Irvine, R. F. and Dawson, A. P. (1996) Biochem. J. **314**, 811–816
14 MacKrill, J. J. (1999) Biochem. J. **337**, 345–361
15 Hann, L. E., Kelleher, R. S. and Sullivan, D. A. (1991) Invest. Ophthalmol. Vis. Sci. **32**, 2610–2621
16 Smith, P. M. and Gallacher, D. V. (1992) J. Physiol. (London) **449**, 109–120
17 Smith, P. M. (1992) J. Physiol. (London) **446**, 72P
18 Irvine, R. F., Brown, K. D. and Berridge, M. J. (1984) Biochem. J. **222**, 269–272
19 Irvine, R. F., Letcher, A. J., Lander, D. J. and Berridge, M. J. (1986) Biochem. J. **240**, 301–304
20 Smith, P. M. and Gallacher, D. V. (1994) Biochem. J. **299**, 37–40
21 Trautman, A. and Marty, A. (1984) Proc. Natl. Acad. Sci. U.S.A. **81**, 611–615
22 Findlay, I. and Petersen, O. H. (1985) Eur. J. Physiol. **403**, 328–330
23 Osipchuk, Y. V., Wakui, M., Yule, D. I., Gallacher, D. V. and Petersen, O. H. (1990) EMBO J. **9**, 697–704
24 Smith, P. M. and Gallacher, D. V. (1992) J. Physiol. (London) **449**, 109–120
25 Liu, P., Scott, J. and Smith, P. M. (1998) Biochem. J. **330**, 847–852
26 Communi, D., Vanweyenberg, V. and Erneux, C. (1994) Biochem. J. **298**, 669–673
27 Vanweyenberg, V., Communi, D., D'Santos, C. S. and Erneux, C. (1995) Biochem. J. **306**, 429–435
28 Irvine, R. F. (1991) Nature (London) **352**, 115
29 Renard-Rooney, D. C., Hajnóczky, G., Seitz, M. B., Schneider, T. G. and Thomas, A. P. (1993) J. Biol. Chem. **268**, 23601–23610
30 Putney, Jr., J. W. (1997) Capacitative Calcium Entry, R. G. Landes and Co./Chapman and Hall, Austin
31 Kwan, C. Y., Takemura, H., Obie, J. F., Thastrup, O. and Putney, Jr., J. W. (1990) Am. J. Physiol. **258**, C1006–C1015
32 Bird, G. J., Obie, J. F. and Putney, Jr., J. W. (1992) J. Biol. Chem. **267**, 18382–18386
33 Subramanian, K. and Meyer, T. (1997) Cell **89**, 963–971
34 Ribeiro, C. M. P. and Putney, J. W. (1996) J. Biol. Chem. **271**, 21522–21528
35 Stricker, S. A., Silva, R. and Smythe, T. (1998) Dev. Biol. **203**, 305–322
36 Irvine, R. F. (1989) Biochem. Soc. Trans. **17**, 6–9
37 Soriano, S. and Banting, G. (1997) FEBS Lett. **403**, 1–4
38 Cullen, P. J., Hsuan, J. J., Truong, O., Letcher, A. J., Jackson, T. R., Dawson, A. P. and Irvine, R. F. (1995) Nature (London) **376**, 527–530
39 Loomis-Husselbee, J. W., Walker, C. D., Bottomley, J. R., Cullen, P. J., Irvine, R. F. and Dawson, A. P. (1998) Biochem. J. **331**, 947–952

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