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Inositol 1,3,4,5-tetrakisphosphate and inositol 1,4,5-trisphosphate act by different mechanisms when controlling Ca^{2+} in mouse lacrimal acinar cells

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In internally perfused single lacrimal acinar cells the competitive inositol 1,4,5-trisphosphate (Ins 1,4,5-P_3)-antagonist heparin inhibits the ACh-evoked K^+ current response mediated by internal Ca^{2+} and also blocks both the Ins 1,4,5-P_3-evoked transient as well as the sustained K^+ current increase evoked by combined stimulation with internal Ins 1,4,5-P_3 and inositol 1,3,4,5-tetrakisphosphate (Ins 1,3,4,5-P_4). When, during sustained stimulation with both Ins 1,4,5-P_3 and Ins 1,3,4,5-P_4, one of the inositol polyphosphates is removed, the K^+ current declines; whereas removal of Ins 1,4,5-P_3 results in an immediate termination of the response, removal of Ins 1,3,4,5-P_4 only causes a very gradual and slow reduction in the current. Ins 1,3,4,5-P_4 is therefore not an acute controller of Ca^{2+} release from stores into the cytosol, but modulates the release of Ca^{2+} induced by Ins 1,4,5,P_3 by an unknown mechanism, perhaps by linking Ins 1,4,5 P_3-sensitive and insensitive Ca^{2+} stores.

Inositol trisphosphate; Inositol tetrakisphosphate; K^+ current; Ca^{2+} transport

1. INTRODUCTION

Acetylcholine (ACh) interacting with muscarinic receptors on mammalian exocrine acinar cells opens Ca^{2+}-dependent K^+ channels, via an increase in the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) [1]. In the mouse lacrimal acinar cells this effect can be mimicked by intracellular application of a mixture of inositol 1,4,5-trisphosphate (Ins 1,4,5-P_3) and inositol 1,3,4,5-tetrakisphosphate (Ins 1,3,4,5-P_4), but not by either one of the two inositol polyphosphates alone [2]. It has been suggested that while Ins 1,4,5-P_3 opens Ca^{2+} channels to intracellular Ca^{2+} stores, Ins 1,3,4,5-P_4 controls Ca^{2+} entry from the extracellular fluid together with Ins 1,4,5-P_3 [3]. Direct evidence for Ins 1,4,5-P_3-evoked opening of single Ca^{2+} channels in membranes from intracellular organelles has now been provided [4] although the exact nature and localization of the Ca^{2+} pool from which Ins 1,4,5-P_3 causes Ca^{2+} release is still unclear [5]. It is known that Ins 1,3,4,5-P_4 binds to intracellular sites different and separate from those accepting Ins 1,4,5-P_3 [6], but the localization and nature of the Ins 1,3,4,5-P_4 action is still completely unkown. Using internally perfused lacrimal acinar cells (patch-clamp whole-cell configuration [7]) in which the intracellular composition can be changed several times during individual experiments [8] and where measurement of Ca^{2+}-dependent K^+ current can be used to assess changes in $[Ca^{2+}]_i$ [2], we can now show that Ins 1,4,5-P_3 and Ins 1,3,4,5-P_4 act in very different ways.

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2. MATERIALS AND METHODS

Mouse lacrimal acinar cells were isolated by collagenase and trypsin treatment as previously described [9]. The patch-clamp technique was used to measure the transmembrane current of single dialyzed acinar cells [7,8]. Internal perfusion of the tip of the patch-clamp pipette via a second small capillary inserted into the main pipette allowed exchanges of the internal solution inside the cell during individual experiments [2,8]. The bath solution (extracellular) contained: 140 mM NaCl, 4.7 mM KCl, 1.2 mM $CaCl_2$, 1.13 mM $MgCl_2$, 10 mM glucose and 10 mM Hepes. pH was 7.2. The solution filling the patch pipettes (intracellular) contained: 140 mM KCl, 4 mM $MgCl_2$, 5 mM Na_2 ATP and 10 mM Hepes. pH was 7.2. Ins 1,4,5-P_3 and Ins 1,3,4,5-P_4 were added to the intracellular solution to achieve concentrations of 10 μM at the times indicated in the figures. Ins 1,4,5-P_3 and Ins 1,3,4,5-P_4 were prepared and checked for purity as previously described [10]. Heparin (Na^+ salt) (Sigma) was added to the intracellular solution to achieve a concentration of 50 μg/ml as indicated in the figures. The whole-cell current recording configuration was established with an intracellular solution in the patch pipette not containing any inositol polyphosphate. The holding potential was -50 mV and repetitive and alternating voltage jumps to 0 and -90 mV lasting 200 ms each were applied [2]. The Cl^- equilibrium potential (E_{Cl^-}) was about 0 mV, whereas E_{K+} was about -90 mV. The original current traces shown in figs 1-3 have been labelled is such a way that the introduction of new pipette solution is shown at the exact times when the reservoirs were reconnected. In these figures we have not corrected for the dead space in the internal perfusion system.

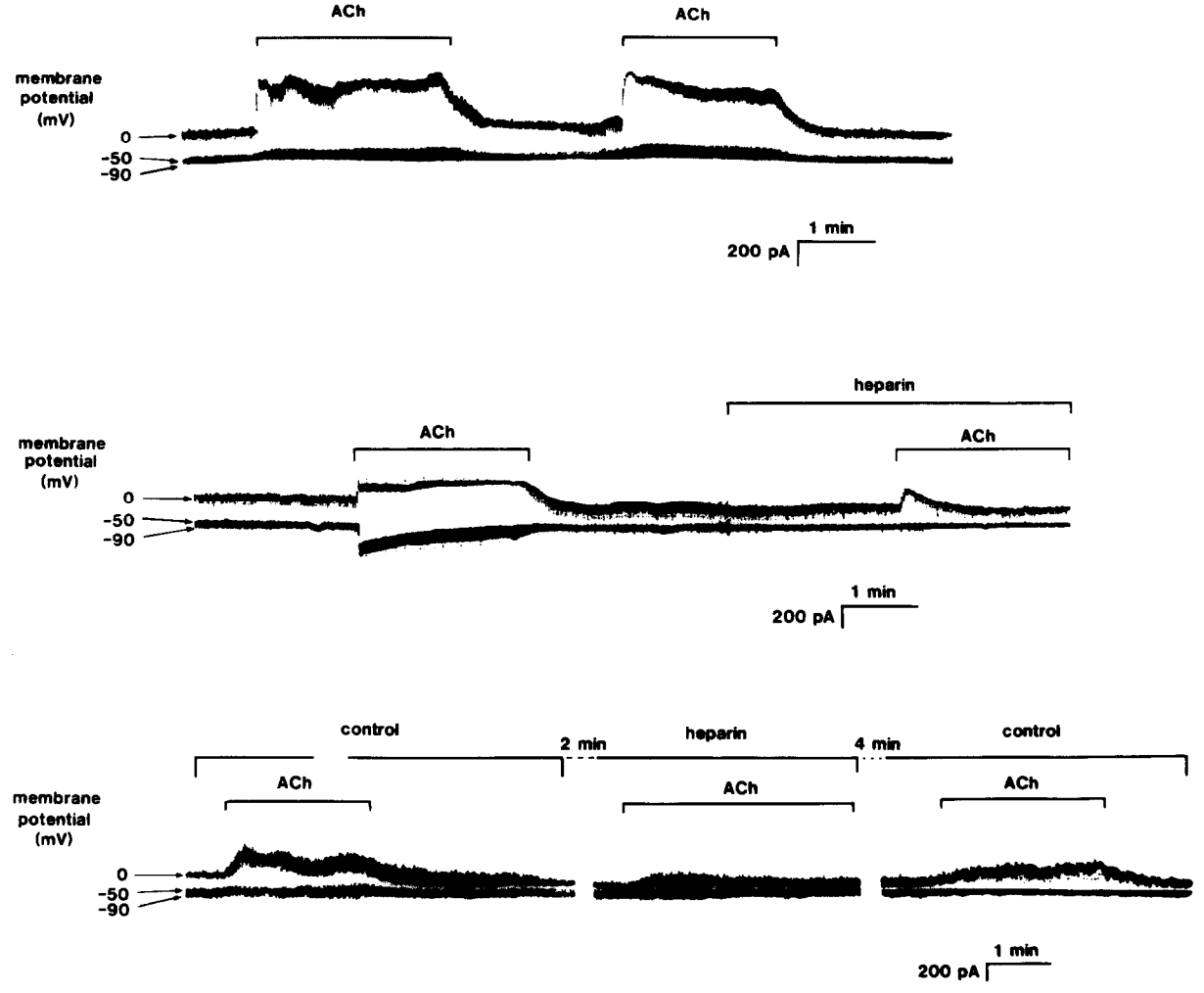

Fig.1. The effect of ACh on transmembrane current in internally perfused voltage-clamped single mouse lacrimal acinar cells. The upper trace shows that ACh (10 μM) can repeatedly evoke a sustained outward (K^+) current at a membrane potential of 0 mV. In some cases, as shown in the middle trace, ACh (10 μM) also evokes a transient inward (Cl^-) current at -90 mV, but in this preparation this effect is mostly absent [2]. The middle and lower traces show that internal perfusion with heparin (50 μg/ml) virtually abolishes the ACh (10 μM) response and that the heparin effect is at least qualitatively reversible.

3. RESULTS

Fig.1 shows the marked and sustained increase in outward K^+ current (at 0 mV) evoked by ACh (10 μM). Heparin has been reported to be a specific antagonist of Ins 1,4,5-P_3-evoked Ca^{2+} release from microsomes and also competes with Ins 1,4,5-P_3 for its specific binding sites [11–13]. More recently, it has been shown that heparin also inhibits Ins 1,4,5-P_3-gated Ca^{2+} channel activity in planar lipid bilayers into which vesicles from smooth muscle sarcoplasmic reticulum were incorporated [4]. Fig.1 shows that heparin (50 μg/ml) virtually abolishes the ACh response (n = 12) and that this effect is reversible (n = 5). The initial small transient response evoked by ACh in the presence of heparin probably indicates that heparin, applied via the patch pipette, does not reach all the functionally important Ins 1,4,5-P_3-binding sites in the cells. Heparin reversibly abolishes all effects of intracellular Ins 1,4,5-P_3 application (n = 5) (fig.2) and when applied during the sustained phase of K^+ current activation evoked by a mixture of Ins 1,4,5-P_3 and Ins 1,3,4,5-P_4 also, after some delay (probably due to the time it takes for the heparin to reach all the relevant Ins 1,4,5-P_3-binding sites), abolishes the response (n = 6) (fig.2).

Fig.3 demonstrates the results of experiments in which simultaneous application of 10 μM Ins 1,4,5-P_3 and 10 μM Ins 1,3,4,5-P_4 evoked sustained

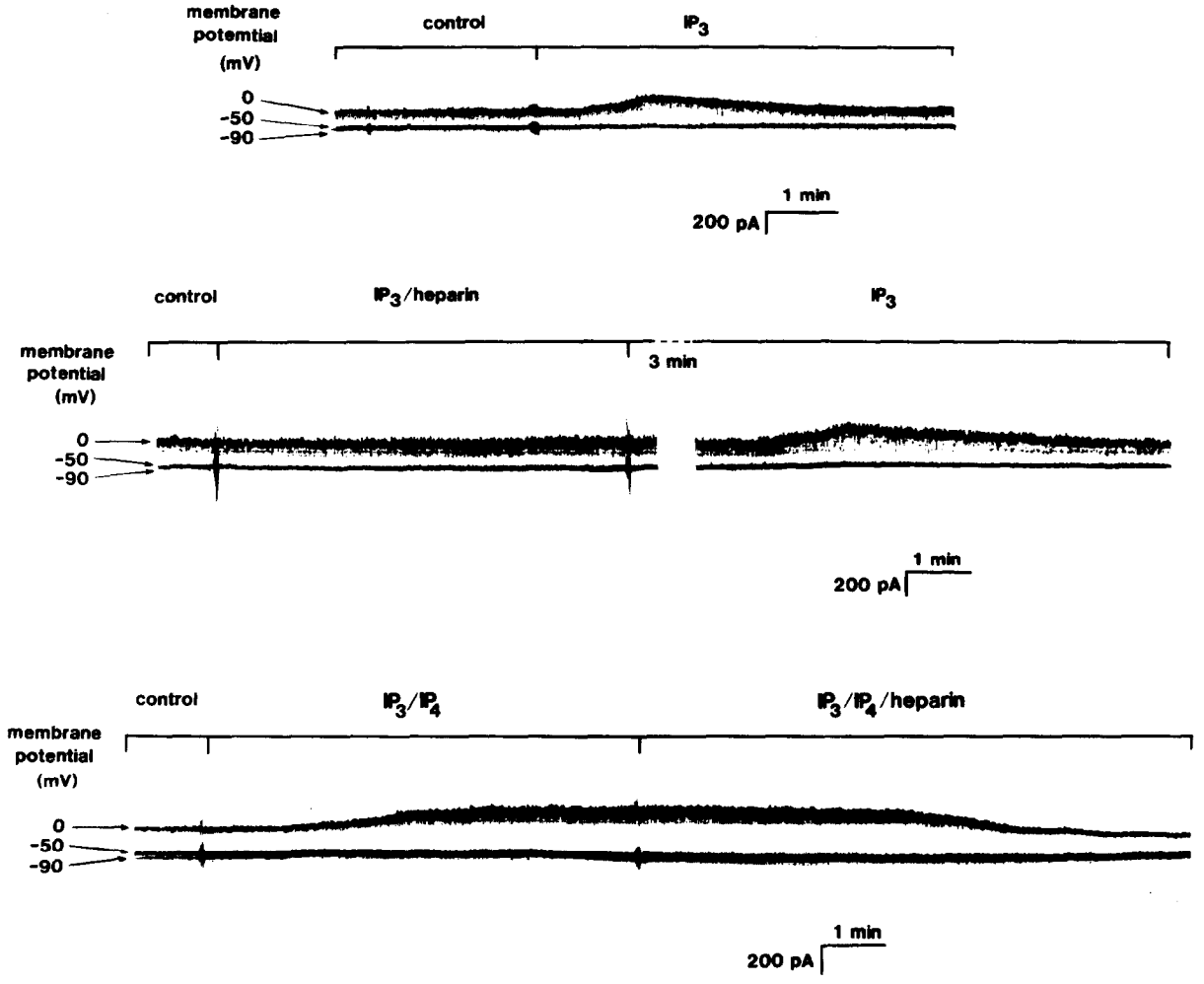

Fig.2. The effects of internal heparin (50 μg/ml) on the K^+ current response evoked by internal Ins 1,4,5-P_3 (IP_3) (10 μM) or the combination of 10 μM Ins 1,4,5-P_3 and 10 μM Ins 1,3,4,5-P_4 (IP_4).

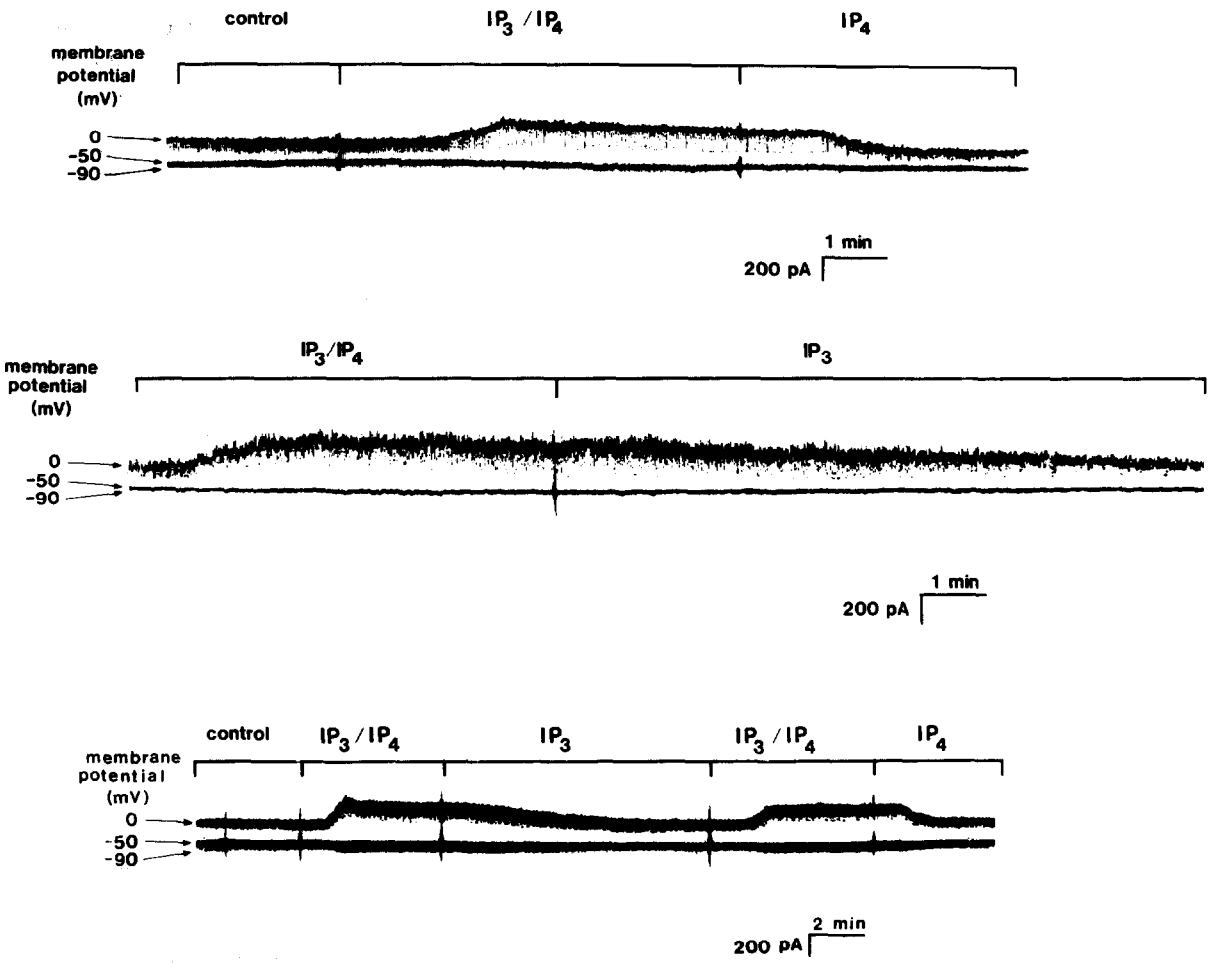

Fig.3. The effect of stimulating with internal Ins 1,4,5-P_3 (10 μM) and Ins 1,3,4,5-P_4 (10 μM) and then removing either Ins 1,4,5-P_3 or Ins 1,3,4,5-P_4 from the mixture.

K^+ current responses. Removal of Ins 1,4,5-P_3 from the inositol polyphosphate mixture resulted in an abrupt loss of the response, whereas removal of Ins 1,3,4,5-P_4, in the continuing presence of Ins 1,4,5-P_3, only caused a gradual and slow return to the prestimulation baseline current. It took on average 5.4 min ± 0.4 (SE) (n = 5) after removing Ins 1,3,4,5-P_4 from the Ins 1,4,5-P_3/Ins 1,3,4,5-P_4 mixture in the internal perfusion fluid before the K^+ current had been reduced to 50% of the sustained level measured in the presence of both inositol polyphosphates. In contrast, it took on average only 1.9 ± 0.2 min (n = 7) for the reduction of the K^+ current to the 50% level to occur after removal of Ins 1,4,5-P_3 from the same mixture. These numbers underestimate the difference between the results obtained with the two protocols since there is a considerable dead-space and therefore lag time between reconnection of reservoirs and the actual appearance of new solution inside the cell. This delay can be estimated by inspection of the traces and the data recalculated to correct for this. Using this method it took 0.6 min ± 0.1 (n = 7) for the response to decline to 50% of the sustained stimulated level after removal of Ins 1,4,5-P_3, whereas it took 1.9 min ± 0.1 (n = 5) after removal of Ins 1,3,4,5-P_4. In one cell we were able to carry out both experimental protocols and the result is shown in the bottom trace of fig.3. In this case there was also a very clear difference between

the slow and very gradual return to the baseline after Ins 1,3,4,5-P_4 removal and the sharp and quick reduction to the prestimulation current level occurring after Ins 1,4,5-P_3 removal from the inositol polyphosphate mixture.

4. DISCUSSION

The experiments presented here clearly demonstrate that Ins 1,3,4,5-P_4 alone is incapable of sustaining Ca^{2+} mobilization into the cytosol even after a period when Ins 1,4,5-P_3 has been present. Ins 1,3,4,5-P_4 only has an effect in the continued presence of Ins 1,4,5-P_3. This result is in agreement with previous findings made in the same system in which it was shown that Ins 1,3,4,5-P_4 had no effect alone [2] and also had no effect together with the inactive compound Ins 1,3,4-P_3 or low concentrations of Ins 2,4,5-P_3 which are incapable of causing Ca^{2+} release in these cells under our experimental conditions [14]. The effects of heparin, an apparently specific and competitive inhibitor of Ins 1,4,5-P_3 action [4,11–13], also fit in well with this picture since the effects of ACh, Ins 1,4,5-P_3 and Ins 1,4,5-P_3 plus Ins 1,3,4,5-P_4 were blocked by this compound again showing that any stimulant-evoked rise in $[Ca^{2+}]_i$ is acutely dependent on Ins 1,4,5-P_3-evoked Ca^{2+} channel opening. All these pieces of evidence point very clearly to the conclusion that Ins 1,3,4,5-P_4 does not open up pathways connecting Ca^{2+} stores with the cytosol.

The very slow effect of Ins 1,3,4,5-P_4 removal from a mixture of Ins 1,4,5-P_3 and Ins 1,3,4,5-P_4 also suggests that Ins 1,3,4,5-P_4 is not controlling the gating of an ion channel. Ins 1,3,4,5-P_4 does, however, have a very significant effect on Ca^{2+} homeostatis in the internally perfused lacrimal acinar cells (fig.2) [2,14]. We have also recently shown that addition of Ins 1,3,4,5-P_4 to an internal perfusion fluid containing Ins 1,4,5-P_3 always causes a further transient rise in $[Ca^{2+}]_i$ even in the complete absence of extracellular Ca^{2+} [14] indicating that Ins 1,3,4,5-P_4 is not exclusively concerned with the regulation of Ca^{2+} uptake from the external medium. It would appear that the role of Ins 1,3,4,5-P_4 is to make more Ca^{2+} available to be released by the action of Ins 1,4,5-P_3 and one of several possible simple models to illustrate this concept is presented in fig.4. The effect of Ins 1,3,4,5-P_4 may therefore to be to link up Ins

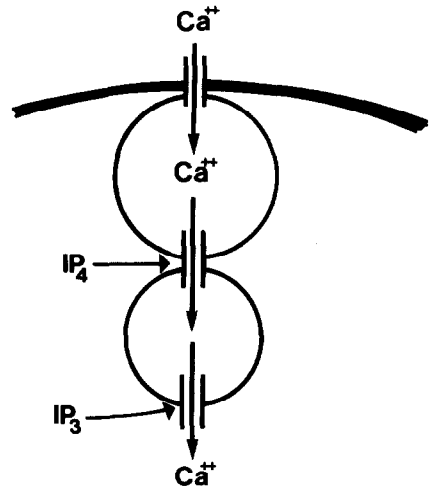

Fig.4. Simple model concept showing that whereas IP_3 opens channels allowing Ca^{2+} to move from within stores to the cytosol, IP_4 connects different Ca^{2+} stores thereby also establishing a connection to extra-cellular compartment.

1,4,5-P_3-sensitive and insensitive Ca^{2+} pools thereby also establishing a connection to the extracellular Ca^{2+} pool. Recently, evidence has been provided for the existence of two separate non-mitochondrial Ca^{2+} pools in permeabilized pancreatic acinar cells with different Ca^{2+} uptake mechanisms [15]. It is possible that Ins 1,3,4,5-P_4 could act to link up these two pools. The relationship between the effects of Ins 1,3,4,5-P_4 discussed here and the ability of this compound to induce Ca^{2+} sequestration in liver cells [16] is not clear, but passage of Ca^{2+} between pools with different types of Ca^{2+} pumps having different capacities and affinities could most likely explain both findings.

The transient Ins 1,4,5-P_3 response described here is very much shorter than that found in other exocrine acinar cells, where a protocol in which Ins 1,4,5-P_3 is present in the pipette solution at the moment of cell penetrations has been used [17,18]. Our finding that the action of Ins 1,3,4,5-P_4 is only slowly reversible (fig.3) may help to explain these different results obtained by various investigators. In the intact cell the connections between the Ca^{2+} pools shown in fig.4 may be functioning, or they may be activated artefactually as a consequence of patch rupture. When Ins 1,4,5-P_3 is introduced into the cell at the moment of establishing the whole-cell recording configuration, Ins 1,4,5-P_3 will con-

tinue to be active as long as these links are stable. Since the Ins 1,3,4,5-P_4-sensitive links remain stable for many minutes in the complete absence of Ins 1,3,4,5-P_4, the relatively long-lasting Ins 1,4,5-P_3-evoked Ca^{2+} transients [17], often seen as repetitive oscillations [18] can easily be explained. In contrast, in the present experiments, Ins 1,4,5-P_3 was introduced many minutes after the start of intracellular wash-out and the links between the Ca^{2+} pools might have disappeared within this period. Thus, Ins 1,4,5-P_3 only has a very short-lasting effect and it is possible therefore to demonstrate a clear-cut effect of Ins 1,3,4,5-P_4 in prolonging the response.

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