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Injection of inositol 1,3,4,5-tetrakisphosphate into *Xenopus* oocytes generates a chloride current dependent upon intracellular calcium

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Injection of inositol 1,3,4,5-tetrakisphosphate ($Ins(1,3,4,5)P_4$) into voltage-clamped oocytes of *Xenopus laevis* elicited an oscillatory chloride membrane current. This response did not depend upon extracellular calcium, because it could be produced in calcium-free solution and after addition of cobalt to block calcium channels in the surface membrane. However, it was abolished after intracellular loading with the calcium chelating agent EGTA, indicating a dependence upon intracellular calcium. The mean dose of $Ins(1,3,4,5)P_4$ required to elicit a threshold current was 4×10^{-14} mol. In comparison, inositol 1,4,5-trisphosphate ($Ins(1,4,5)P_3$) gave a similar oscillatory current with doses of about one twentieth as big. Hyperpolarization of the oocyte membrane during activation by $Ins(1,3,4,5)P_4$ elicited a transient inward current, as a result of the opening of calcium-dependent chloride channels subsequent to the entry of external calcium. In some oocytes the injection of $Ins(1,3,4,5)P_4$ was itself sufficient to allow the generation of the transient inward current, whereas in others a prior injection of $Ins(1,4,5)P_3$ was required. We conclude that $Ins(1,3,4,5)P_4$ causes the release of intracellular calcium from stores in the oocyte, albeit with less potency than $Ins(1,4,5)P_3$. In addition, $Ins(1,3,4,5)P_4$ activates voltage-sensitive calcium channels in the surface membrane, via a process that may require 'priming' by $Ins(1,4,5)P_3$.

Introduction

Cell signalling in a wide variety of tissues is regulated by calcium-mobilizing receptors, which function by using the hydrolysis of membrane phospholipid as part of a mechanism to generate second messengers. Specifically, the cleavage of phosphatidylinositol 4,5-bisphosphate yields diacylglycerol and inositol 1,4,5-trisphosphate ($Ins(1,4,5)P_3$), both of which seem to act as intracellular messengers (Berridge & Irvine 1984; Hokin 1985; Downes & Michell 1985). Diacylglycerol activates protein kinase C (Hirasawa & Nishizuka 1985), whereas $Ins(1,4,5)P_3$ is thought to function by causing the release of calcium ions from intracellular stores (Berridge & Irvine 1984; Berridge 1986). It was found recently that $Ins(1,4,5)P_3$is rapidly phosphorylated by a specific kinase in brain and other cell types to form inositol 1,3,4,5-tetrakisphosphate ($Ins(1,3,4,5)P_4$), which is

subsequently dephosphorylated to inositol 1,3,4-trisphosphate (Irvine *et al.* 1986*a*; Batty *et al.* 1985; Palmer *et al.* 1986). After receptor activation, intracellular levels of Ins(1,3,4,5)P_4 rise and subsequently fall quite rapidly (Batty *et al.* 1985; Palmer *et al.* 1986; Jackson *et al.* 1987). This, together with the presence of a specific synthetic enzyme, suggests that Ins(1,3,4,5)P_4 may act as an intracellular messenger, distinct from Ins(1,4,5)P_3. We have looked for possible messenger actions of Ins(1,3,4,5)P_4 by injecting it into oocytes of *Xenopus laevis* while recording membrane currents under voltage clamp control.

Xenopus oocytes have several advantages as a preparation to study the actions of inositol phosphates. The cells are large (over 1 mm in diameter), and are easy to microinject with various substances. In addition, the surface membrane contains many chloride channels, which are activated by intracellular calcium (Miledi 1982; Barish 1983; Miledi & Parker 1984). Measurement of the chloride membrane current in oocytes thus provides a convenient monitor of the free intracellular calcium level. Our results show that intracellular injections of Ins(1,3,4,5)P_4 cause the generation of oscillatory chloride currents, which appear to arise as a result of calcium liberation from internal stores.

Methods

Experiments were done on fully grown oocytes of *Xenopus laevis*, which were treated with collagenase to remove the enveloping cells layers (Miledi & Parker 1984) and so facilitate impalement with micropipettes. For recording, oocytes were penetrated with two KCl-filled microelectrodes used for voltage clamping, and with one or two additional micropipettes to allow intracellular injections of inositol polyphosphates, EGTA and calcium. Procedures for electrophysiological recording and pneumatic pressure injection were as described before (Parker & Miledi 1986; Miledi & Parker 1984). Unless otherwise noted, all recordings were made at a clamp potential of -50 mV. During recording, oocytes were continuously superfused with frog Ringer solution at room temperature (*ca.* 23 °C). Normal Ringer contained (in millimoles per litre) NaCl 120, KCl 2, $CaCl_2$ 1.8 and HEPES 5, at pH about 7.0. Zero-calcium Ringer contained no added calcium, and additionally 2 mM $MgCl_2$ and 1 mM EGTA. *Myo*-inositol-1,3,4,5-tetrakisphosphate (tetrapotassium salt) and *myo*-inositol-1,4,5-trisphosphate (trilithium salt) were obtained from Calbiochem (La Jolla, California), and were injected at concentrations of 100–1000 μM and 1–100 μM respectively in an aqueous solution including 5 mM HEPES (at pH 7.0) and 50 μM EDTA. The EDTA was present to chelate any contaminating calcium in the injection solution. As a further precaution to prevent calcium entering the pipette from the Ringer solution, several pressure pulses were applied to expel fluid from the tip immediately before penetrating the oocyte.

The volumes of fluids ejected from the injection pipettes were estimated by measuring the diameters of fluid droplets expelled by pressure pulses applied with the pipette in the air. Pipettes were tested before insertion and after withdrawal from the oocyte to ensure that they had not become plugged during recording. Injections of inositol polyphosphates and calcium were always made into the animal (black) hemisphere of the oocyte, because the distribution of calcium-

activated chloride channels varied across the oocyte surface and was greatest in this hemisphere (Miledi & Parker 1984). In those experiments where comparison was made between the effects of different inositol phosphates, the two injection pipettes were inserted at about the same 'latitude' in the oocyte. Loading of oocytes with EGTA was accomplished by pressure injection from pipettes containing 50 mM EGTA, brought to pH 7.0 with KOH. Calcium injections were made from pipettes containing 10 or 50 mM $CaCl_2$.

Results

Membrane currents elicited by Ins(1,3,4,5)P_4

Injections of Ins(1,3,4,5)P_4 into the animal hemisphere of *Xenopus* oocytes elicited an oscillatory membrane current, which was inward at a potential of −50 mV, and persisted for many seconds or minutes after the injection (figures 1, 2*b* and 3*a*, *c*). Responses like those illustrated were seen after injecting Ins(1,3,4,5)P_4 in more than 40 oocytes from 6 donors, and were always present in cases where the injection pipette was confirmed to be functioning correctly after removal from the oocyte. The oscillatory current inverted direction when the oocyte membrane was clamped at potentials more positive than about −25 mV (figure 1), and is thus probably carried largely by chloride ions, because this reversal potential corresponds to the chloride equilibrium potential in the oocyte (Kusano *et al.* 1977, 1982; Barish 1983). Moreover, the oscillatory current activated by Ins(1,3,4,5)P_4 showed a rectification at negative membrane potentials, similar to the chloride currents activated in the oocyte by acetylcholine and serotonin (Kusano *et al.* 1982; Miledi *et al.* 1987) and by intracellular calcium (Miledi & Parker 1984). This can be seen in figure 1, where the current at −60 mV was no larger than at −10 mV, despite the greater driving force for Cl^- movement (i.e. about −35 mV from the equilibrium potential, compared with +15 mV).

Injections of small amounts of Ins(1,3,4,5)P_4 usually gave responses consisting of only one or two oscillations of current, beginning after a latency of between a few seconds and up to 2 min (e.g. the first trace in figure 2*b*). Larger quantities gave a more prolonged series of oscillations, starting a few seconds after injection, and then building up and decaying over several minutes (figures 2*b* and 3*a*, *c*). Towards the end of each response the durations of individual current oscillations became longer. Responses to repeated injections of Ins(1,3,4,5)P_4 applied a few minutes after the end of an initial response remained of similar size, except after injections that gave very large currents (several hundred nanoamps), when a maintained level of spontaneous oscillations persisted for many minutes after the large response and the currents to subsequent injections of Ins(1,3,4,5)P_4 were depressed.

Quantitative estimates of the minimal amount of Ins(1,3,4,5)P_4 required to give a detectable current were made by injecting known volumes from a pipette filled with 100 μM Ins(1,3,4,5)P_4. The threshold was quite sharply defined in each oocyte, because although a particular dose might fail to give any response, a dose twice as large would give one or more distinct oscillations of 10–20 nA. The mean amount of Ins(1,3,4,5)P_4 required to elicit a threshold response, measured in nine oocytes from three donors, was 4.4×10^{-14} mol ($\pm 1.3 \times 10^{-14}$ mol standard error of

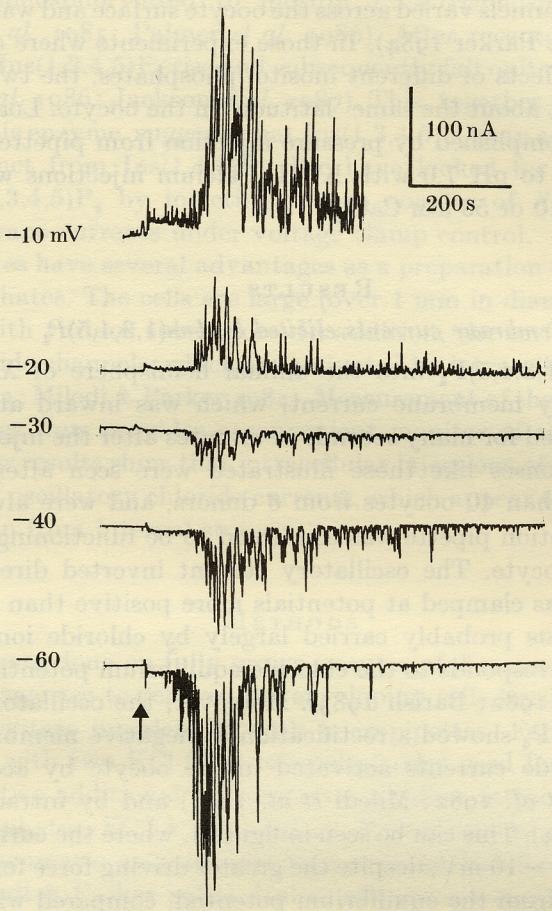

FIGURE 1. Reversal potential of membrane currents elicited by intracellular injection of Ins-(1,3,4,5)P_4. The oocyte was clamped at the potentials indicated next to each trace, and clamp currents were recorded in response to a constant pressure pulse (400 kPa for 400 ms) applied to an intracellular pipette containing 100 μM Ins(1,3,4,5)P_4. Pulses were applied at the times marked by the arrow, and intervals of several minutes were allowed between each trial. In this and other figures, downward deflections correspond to inward membrane currents.

mean). Considerable variation was seen between different oocytes; the most sensitive responded to 3×10^{-15} mol and the least sensitive required 10^{-13} mol. Although some variation probably arose from errors in measuring the volumes of fluid ejected, and from differences in the depth to which the injection pipettes were inserted into the oocytes, there also appeared to be consistent differences in sensitivity between oocytes from different donors.

Comparison of responses to Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4

Injection of Ins(1,4,5)P_3 into oocytes gives rise to oscillatory chloride currents that closely resemble those induced by Ins(1,3,4,5)P_4 (figure 2*a*) (see Oron *et al.* 1985; Parker & Miledi 1986, 1987; Miledi *et al.* 1987). However, there were

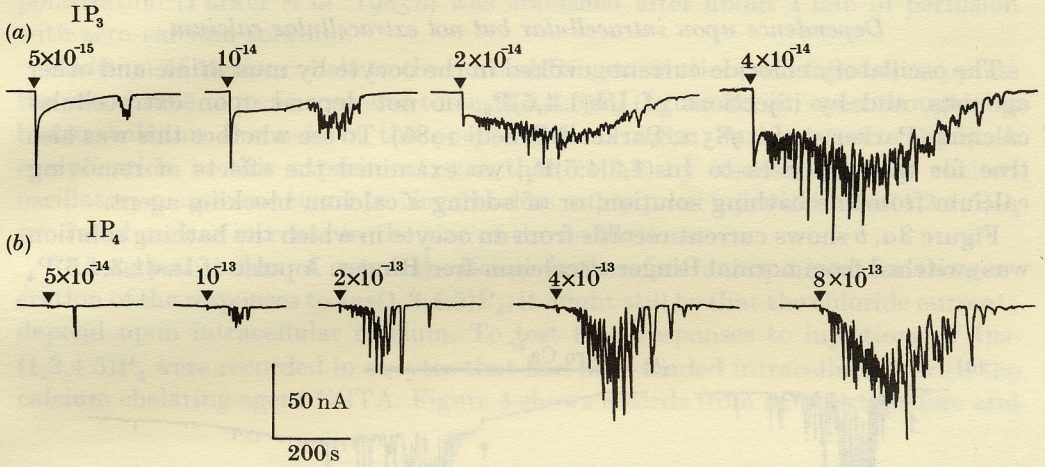

FIGURE 2. Membrane currents recorded in an oocyte in response to intracellular injections of different amounts of (*a*) Ins(1,4,5)P_3 (IP_3) and (*b*) Ins(1,3,4,5)P_4 (IP_4). The oocyte was voltage clamped at a potential of −50 mV. Injections of Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 were made by pressure pulses, applied to pipettes containing solutions of, respectively, 100 μM and 1 mM. The times of the injections are marked by arrowheads, and the amounts injected are indicated in moles.

quantitative differences in the sensitivity of oocytes to these two compounds, as well as qualitative differences in the nature of the membrane currents evoked.

Membrane currents elicited by Ins(1,4,5)P_3 usually showed an initial 'spike' beginning coincident with the pressure pulse, followed by a smooth maintained current upon which oscillations were superimposed (figure 2*a*) (see also Parker & Miledi 1986). This sequence of currents resembles the responses seen after activation of native muscarinic receptors in the oocyte (Kusano *et al.* 1982) and of exogenous serotonin receptors expressed by foreign messenger RNA (Gundersen *et al.* 1983). In contrast, Ins(1,3,4,5)P_4 usually produced an almost pure oscillatory current (e.g. figures 1, 2*b*, 3*a*, *c* and 4*a*), although a spike and maintained component were sometimes seen (e.g. figure 5*a*).

Oscillatory currents were evoked by doses of Ins(1,4,5)P_3 smaller than those required for Ins(1,3,4,5)P_4. The most sensitive oocyte responded to injection of as little as 5×10^{-17} mol of Ins(1,4,5)P_3 (50 pl of a 1 μM solution). Because of variations in sensitivity between oocytes to both Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4, comparisons were restricted to four oocytes that were each injected with both compounds. In these oocytes (e.g. figure 2), the amount of Ins(1,3,4,5)P_4 required to elicit a threshold oscillatory response, or to give a small oscillatory response of a certain size, was from 15 to 30 (mean 21) times greater than the corresponding amount of Ins(1,4,5)P_3. Some (but not all) oocytes showed small 'spike' and smooth currents with doses of Ins(1,4,5)P_3 smaller than those used to elicit an appreciable oscillatory current. In these cases the potency ratio would have been greater than indicated above.

Dependence upon intracellular but not extracellular calcium

The oscillatory chloride currents evoked in the oocyte by muscarinic and other agonists, and by injections of Ins(1,4,5)P_3, do not depend upon extracellular calcium (Parker *et al.* 1985*a*; Parker & Miledi 1986). To see whether this was also true for the responses to Ins(1,3,4,5)P_4, we examined the effects of removing calcium from the bathing solution, or of adding a calcium blocking agent.

Figure 3*a*, *b* shows current records from an oocyte in which the bathing solution was switched from normal Ringer to calcium-free Ringer. A pulse of Ins(1,3,4,5)P_4

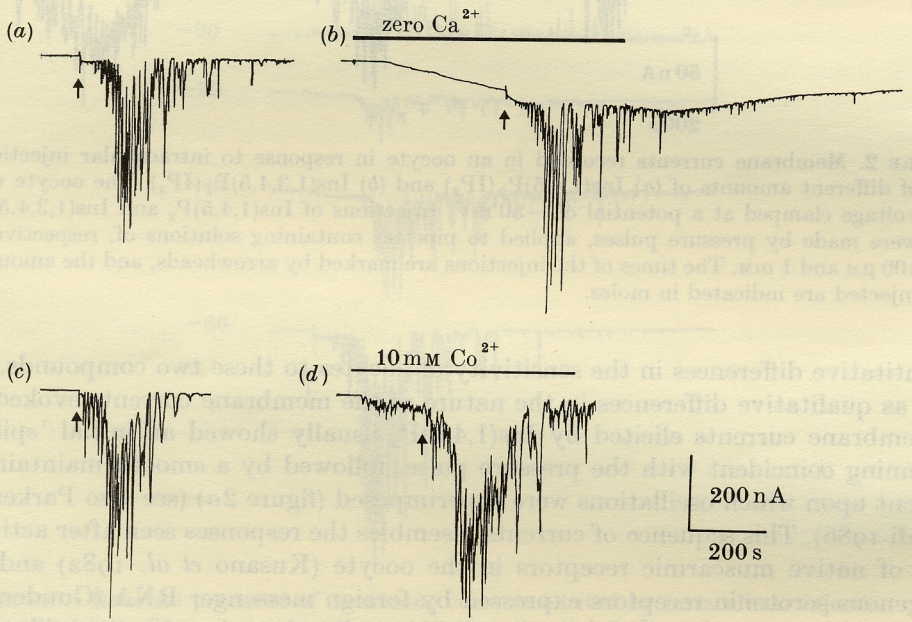

FIGURE 3. Oscillatory currents activated by Ins(1,3,4,5)P_4 are not abolished in calcium-free solution (*b*), or by addition of 10 mM cobalt to the bathing medium (*d*). Records in (*a*, *b*) and (*c*, *d*) are from two different oocytes. In each case the left-hand trace shows a control response to Ins(1,3,4,5)P_4 obtained in normal Ringer, and the right-hand trace shows the response to a repeated injection made after changing the bathing solution. Injections were made as shown by the arrows, and the bars indicate when the solution was changed. Clamp potential was −50 mV. The amounts of Ins(1,3,4,5)P_4 injected were not measured.

applied about 5 min after changing to the calcium-free solution still gave an oscillatory response of similar size to that obtained in normal Ringer, and results like those illustrated were obtained in three other oocytes. In these experiments the input resistance of the oocytes declined in the calcium-free Ringer, and it was usually necessary to test the response to Ins(1,3,4,5)P_4 within a few minutes of changing the solution. However, one oocyte remained stable for 20 min in calcium-free solution, and still gave a large oscillatory response. In any case, the level of free calcium around the oocyte appeared to fall rapidly after the solution change, because the calcium-dependent transient inward current activated by hyper-

polarization (Parker *et al.* 1985*b*) was abolished after about 1 min of perfusion with zero-calcium solution.

Addition of 10 mM cobalt to the normal Ringer solution also failed to suppress the membrane current response to $Ins(1,3,4,5)P_4$ (figure 3*c*, *d*), even though this blocks calcium channels in the oocyte (Miledi 1982; Parker *et al.* 1985*b*). A complication in this experiment was that cobalt by itself frequently elicited oscillatory membrane currents. Nevertheless, subsequent injection of $Ins(1,3,4,5)P_4$ produced a clear increase in the size of the oscillations.

Although an influx of external calcium is therefore unimportant for the generation of the responses to $Ins(1,3,4,5)P_4$, it might still be that the chloride currents depend upon intracellular calcium. To test this, responses to injections of $Ins(1,3,4,5)P_4$ were recorded in oocytes that had been loaded intracellularly with the calcium chelating agent EGTA. Figure 4 shows records from an oocyte before and

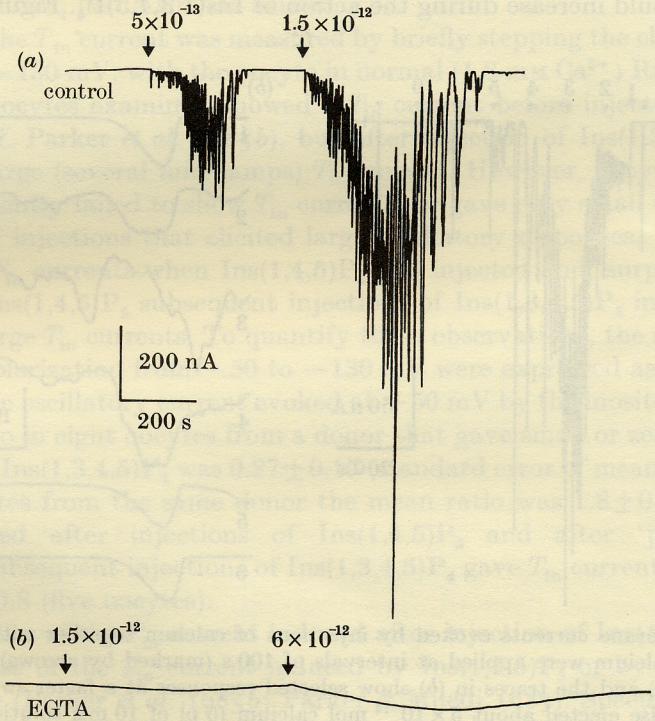

FIGURE 4. Abolition of oscillatory responses to $Ins(1,3,4,5)P_4$ after intracellular loading with EGTA. (*a*) Control responses to injections of two different amounts of $Ins(1,3,4,5)P_4$, as indicated in moles; (*b*) records obtained beginning 15 min after loading the oocyte with about 10^{-10} mol of EGTA.

after loading with about 10^{-10} mol of EGTA, an amount that would have given a final intracellular concentration of about 100 μM assuming an even distribution of EGTA throughout the cell volume. Fifteen minutes after injection of EGTA, the oscillatory responses to $Ins(1,3,4,5)P_4$ were completely abolished, leaving a small, smooth current. A dose of $Ins(1,3,4,5)P_4$ that gave a control response of

over 700 nA produced a smooth current of only about 10 nA after loading with EGTA. Further increasing the dose fourfold still failed to elicit oscillations, although the smooth current became larger (figure 4*b*). Results like those illustrated were obtained in three other oocytes loaded with similar, or slightly smaller, amounts of EGTA.

Ins(1,3,4,5)P_3 *does not enhance sensitivity to calcium*

The most likely explanation for the abolition of the oscillatory response by intracellular EGTA is that it chelates calcium released from intracellular stores by Ins(1,3,4,5)P_4, and thus prevents activation of calcium-dependent chloride channels. However, an alternative possibility could be that Ins(1,3,4,5)P_4 increases the sensitivity of the channels to calcium in such a way that an oscillatory current is seen even though the intracellular free calcium remains at a steady resting level. If this is so, the currents elicited by injections of a fixed amount of calcium into the oocyte should increase during the action of Ins(1,3,4,5)P_4. Figure 5 illustrates

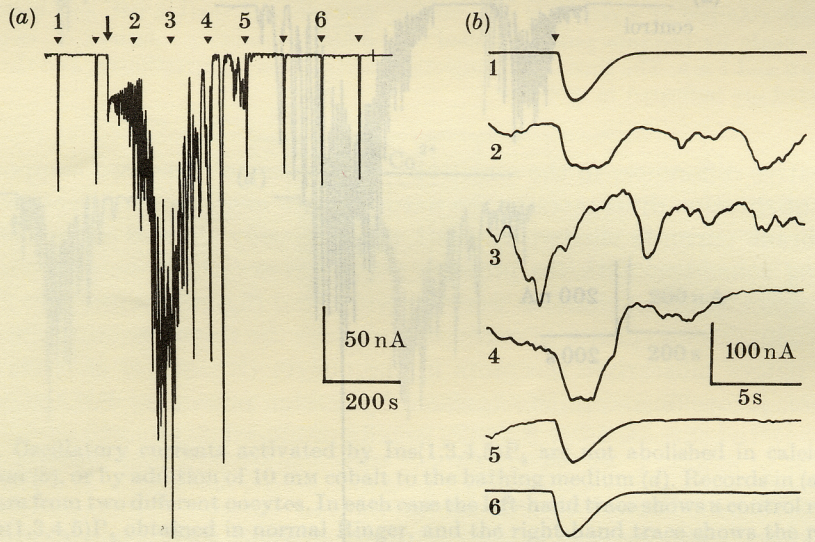

FIGURE 5. Membrane currents evoked by injections of calcium together with Ins(1,3,4,5)P_4. Pulses of calcium were applied at intervals of 100 s (marked by arrows) throughout the record in (*a*) and the traces in (*b*) show selected responses at a faster sweep speed. Each pressure pulse ejected about 5×10^{-14} mol calcium (5 pl of 10 mM solution). A single injection of about 8×10^{-14} mol Ins(1,3,4,5)P_4 was applied at the time indicated by the arrow. Injections were made from two pipettes inserted about 75 μm apart in the animal hemisphere of the oocyte. Clamp potential was −50 mV.

an experiment to test this point. Two micropipettes for injection of calcium and Ins(1,3,4,5)P_4 were inserted close (less than 100 μm) to each other, so that Ins(1,3,4,5)P_4 would diffuse in the cytoplasm to the area of membrane around the calcium pipette. Calcium pulses were applied at regular intervals, at a dose that gave fairly consistent responses of about 100 nA. After injection of Ins(1,3,4,5)P_4 it was sometimes difficult to discern the extra current elicited by the calcium

pulses from the Ins(1,3,4,5)P_4-induced oscillations in current (e.g. trace 3 in figure 5*b*), but it was clear that the responses to the calcium were not appreciably greater than the control level. Results like those in figure 5 were obtained in a total of seven trials (four oocytes), in which the mean size of the response to the calcium pulse during the response to Ins(1,3,4,5)P_4 was 100% ($\pm$4% standard error of mean) of that seen before and after the oscillations.

Transient inward current

Hyperpolarization of the oocyte after injection of Ins(1,4,5)P_3, or activation of serotonin and other receptors, generates a transient inward (T_{in}) current, which is carried by chloride ions but depends upon an influx of calcium (Parker *et al.* 1985*b*; Parker & Miledi 1987). This current appears to arise because Ins(1,4,5)P_3 modulates the activity of a class of calcium channel in the oocyte membrane, so as to allow a calcium influx on hyperpolarization. We were therefore interested to see if Ins(1,3,4,5)P_4 had a similar action.

The size of the T_{in} current was measured by briefly stepping the clamp potential from -50 to -130 mV, with the oocyte in normal (1.8 mM Ca^{2+}) Ringer solution. None of the oocytes examined showed a T_{in} current before injection of inositol phosphates (cf. Parker *et al.* 1985*b*), but after injection of Ins(1,3,4,5)P_4 many developed a large (several microamps) T_{in} current. However, oocytes from some donors consistently failed to show T_{in} currents, or gave only small currents, even after repeated injections that elicited large oscillatory responses. These oocytes did generate T_{in} currents when Ins(1,4,5)P_3 was injected and, surprisingly, after exposure to Ins(1,4,5)P_3 subsequent injections of Ins(1,3,4,5)P_4 induced the appearance of large T_{in} currents. To quantify these observations, the sizes of the T_{in} currents on polarization from -50 to -130 mV were expressed as a ratio of the peak size of the oscillatory current evoked at -50 mV by the inositol phosphates. The mean ratio in eight oocytes from a donor that gave small or zero T_{in} currents in response to Ins(1,3,4,5)P_4 was 0.27 ± 0.15 (standard error of mean). In contrast, in seven oocytes from the same donor the mean ratio was 1.8 ± 0.26 for the T_{in} current elicited after injections of Ins(1,4,5)P_3 and after 'priming' with Ins(1,4,5)P_3 subsequent injections of Ins(1,3,4,5)P_4 gave T_{in} currents with a mean ratio of 3.6 ± 0.8 (five oocytes).

The properties of the T_{in} current induced after injection of Ins(1,3,4,5)P_4 were similar to those of the T_{in} current induced by Ins(1,4,5)P_3 or by serotonin and other agonists (Parker *et al.* 1985*b*; Parker & Miledi 1987). Specifically, the tail currents recorded when the potential was stepped to different levels at the peak of the T_{in} current inverted direction at about the chloride equilibrium potential, and the T_{in} current was abolished by removal of calcium in the bathing solution or by addition of 10 mM cobalt.

Discussion

Injections of Ins(1,3,4,5)P_4 into *Xenopus* oocytes consistently evoked an inward oscillatory membrane current. This response was present in oocytes that had been treated with collagenase to remove epithelial and follicular cell layers. Thus it

probably arises in the oocyte proper, unlike, for example, the currents evoked by catecholamines and gonadotropins, which involve the follicular cells (Kusano *et al.* 1982; Woodward & Miledi 1987).

The surface membrane of *Xenopus* oocytes contains many chloride channels, which are activated by intracellular calcium (Miledi & Parker 1984). It is likely that these mediate the oscillatory response to Ins(1,3,4,5)P_4, because this current inverted direction at about the chloride equilibrium potential, showed properties resembling those of chloride currents activated by calcium mobilizing agonists (Kusano *et al.* 1982; Parker *et al.* 1985*a*), and was abolished by intracellular injection of the calcium chelating agent EGTA. Moreover, the current persisted after removal of calcium from the bathing medium and after addition of cobalt to block calcium influx. The simplest interpretation of our results is therefore that Ins(1,3,4,5)P_4 causes the liberation of calcium from intracellular stores, and that the resulting rise in intracellular free calcium activates calcium-dependent chloride channels in the oocyte membrane (Miledi & Parker 1984). The alternative possibility, that Ins(1,3,4,5)P_4 acts on the chloride channels to increase their sensitivity to calcium so as to produce an oscillatory current in the absence of any change in the resting level of intracellular free calcium, is unlikely because chloride currents elicited by injections of calcium into the oocyte were not enhanced during responses to Ins(1,3,4,5)P_4. A calcium liberating action has previously been described for Ins(1,4,5)P_3 injected into *Xenopus* oocytes (Busa *et al.* 1985; Parker & Miledi 1986), and into many other cell types (Berridge & Irvine 1984). In the present experiments the potency of Ins(1,3,4,5)P_4 to elicit current oscillations was about one twentieth that of Ins(1,4,5)P_3.

Previous studies of the actions of Ins(1,3,4,5)P_4 have indicated that it is almost completely ineffective in mobilizing calcium from intracellular stores in a variety of cell types (Irvine & Moor 1986; Higashida & Brown 1986; Irvine *et al.* 1986*b*), including the *Xenopus* oocyte (W. Busa, R. Nucitelli, M. J. Berridge & R. F. Irvine, unpublished work cited in Irvine & Moor 1986). Reasons for the discrepancy between those findings and the present results are unclear. A possibility that must be considered is that the samples of Ins(1,3,4,5)P_4 used here were impure; for example, a 5% contamination by Ins(1,4,5)P_3 could be sufficient to give responses of the observed sizes. However, the Ins(1,3,4,5)P_4 was purified during manufacture by HPLC and is specified by the manufacturer as being a pure isomer, with no Ins(1,4,5)P_3 detectable by ^{31}P NMR. Also, there were qualitative differences between the membrane currents evoked by Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4, which would suggest that the responses to the tetrakisphosphate did not arise because of contamination by the trisphosphate. Another possibility is that some metabolite (inositol phosphate or otherwise) is present in the oocyte which, in conjunction with Ins(1,3,4,5)P_4, acts to cause intracellular release of calcium. Alternatively, it may simply be that the calcium-activated chloride channels in the oocyte provide an unusually sensitive monitor of intracellular calcium release.

As well as causing the release of calcium from intracellular stores, Ins(1,3,4,5)P_4 also induced an influx of extracellular calcium, as monitored by the appearance of a calcium-dependent T_{in} current on hyperpolarization. In some oocytes this

current was induced by $Ins(1,3,4,5)P_4$ alone, whereas in oocytes from other donors it was initially absent but could be 'primed' by injection of $Ins(1,4,5)P_3$ so that a T_{in} current was induced by subsequent injections of $Ins(1,3,4,5)P_4$. The reason for this variability is not clear, but it might arise if some oocytes had a resting level of $Ins(1,4,5)P_3$ sufficiently high to 'prime' the response to $Ins(1,3,4,5)P_4$. The observation that a T_{in} current can sometimes be evoked in control (non-injected) oocytes (Parker *et al.* 1985*b*) lends support to this view.

Irvine & Moor (1986) recently presented evidence suggesting that $Ins(1,3,4,5)P_4$ may be the messenger that opens calcium channels in the membrane of sea urchin eggs, and further showed that this action occurred only if the $Ins(1,3,4,5)P_4$ was injected together with $Ins(2,4,5)_3$, a trisphosphate isomer believed to cause liberation of intracellular calcium. Our observation of the priming by $Ins(1,4,5)P_3$ of the T_{in} current induced by $Ins(1,3,4,5)P_4$ appears similar to those results. However, the fact that injection of $Ins(1,3,4,5)P_4$ sometimes gave large oscillatory currents (and hence presumably release of intracellular calcium) without inducing a T_{in} current suggests that intracellular calcium mobilization *per se* is not sufficient to prime the plasma membrane calcium channels so that they can be opened by $Ins(1,3,4,5)P_4$. Instead, the trisphosphate may have some specific action on the calcium channels, in addition to mobilizing intracellular calcium.

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