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THE EFFECT OF myo-INOSITOL 1,4,5-TRISPHOSPHOROTHIOATE ON Cl^- CURRENT PATTERN AND INTRACELLULAR Ca^{2+} IN THE *XENOPUS LAEVIS* OOCYTE

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Summary: Microinjection of myo-inositol 1,4,5-trisphosphate into voltage-clamped *Xenopus laevis* oocytes or the stimulation of the phosphatidylinositol cycle elicits a complex Ca^{2+}-dependent Cl^- current pattern. Microinjection of myo-inositol 1,3,4,5-tetrakisphosphate causes an immediate release of Ca^{2+}, but elicits a different Cl^- current pattern than myo-inositol 1,4,5-trisphosphate. We have studied the effects of myo-inositol 1,4,5-trisphosphorothioate, which can not be converted to myo-inositol 1,3,4,5-tetrakisphosphate. Myo-inositol 1,4,5-trisphosphorothioate caused an immediate release of intracellular Ca^{2+}, as measured by fura-2 imaging. Myo-inositol 1,4,5 -trisphosphorothioate generated a Cl^- current pattern similar to myo-inositol 1,3,4,5-tetrakisphosphate, not myo-inositol 1,4,5-trisphosphate.

Activation of the phosphatidylinositol (PI) cycle involves the generation of inositol (1,4,5) trisphosphate ($Ins(1,4,5)P_3$), which triggers the release of Ca^{2+} from internal stores (1). The $Ins(1,4,5)P_3$ is then either broken down to $Ins(1,4)P_2$ or converted to $Ins(1,3,4,5)P_4$. Activation of receptors coupled to the PI cycle or the injection of either $Ins(1,4,5)P_3$ or $Ins(2,4,5)P_3$ initiates a complex opening and closing of Ca^{2+}-dependent Cl^- channels. Thus, the Cl^- currents are often used as indirect indicators of PI cycle activation. The current pattern is quite dependent on the amount of $Ins(1,4,5)P_3$ injected into the cell (3, 4, 5, 6). Injection of a small amount (between 8×10^{-17} moles and 3.0×10^{-14} moles) of $Ins(1,4,5)P_3$ into the oocyte triggers a single immediate Cl^- current pulse without any subsequent Cl^- current oscillations. When greater amounts (1×10^{-12} moles) of $Ins(1,4,5)P_3$ are injected the fast Cl^- current pulse is followed by a quiescent period which in turn is followed by oscillating Cl^- currents. The amplitude of the oscillating Cl^- currents is at first small but becomes progressively larger and is superimposed on top of a slow Cl^- current conductance. The current pattern which is induced by large amounts of $Ins(1,4,5)P_3$ is similar to the current pattern induced by activation of receptors coupled to the PI cycle.

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Abbreviations: $Ins(1,4,5)P_3$, myo-inositol 1,4,5-trisphosphate; $Ins(1,3,4,5)P_4$, myo-inositol 1,3,4,5-tetrakisphosphate; $Ins(1,4,5)P_3[S]_3$, myo-inositol 1,4,5-trisphosphorothioate.

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Recently it has been reported that Ins(1,3,4,5)P_4 initiates a Cl^- current pattern that differs from Ins(1,4,5)P_3 (4, 5, 6, 7). Ins(1,3,4,5)P_4 injection is followed by a quiescent period of up to several minutes which is followed by oscillating Cl^- currents. Ins(1,3,4,5)P_4 does not trigger the immediate Cl^- current pulse, even at high concentrations. We have recently reported that Ins(1,3,4,5)P_4 releases Ca^{2+} from intracellular stores in less than 0.5 sec. (5, 7). It is not clear why both Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 cause an immediate rise in intracellular Ca^{2+} but have different effects on Cl^- current pattern. The lag time between Ca^{2+} elevation and the appearance of currents suggests that the Cl^- currents are not accurate indicators of changes in intracellular Ca^{2+}.

In an attempt to learn more about the underlying mechanism behind the Ins(1,4,5)P_3-induced Cl^- current pattern versus that involved in Ins(1,3,4,5)P_4-induced Cl^- current pattern, we studied the effects of a new $InsP_3$ analogue, myo-inositol 1,4,5-trisphosphorothioate, (Ins(1,4,5)P_3[S]$_3$) [see 8 for review]. In this analogue each phosphate is replaced by a phosphorothioate group. Since it is 5-phosphatase and 3-kinase resistant, it cannot be readily broken down to Ins(1,4)P_2 or converted to Ins(1,3,4,5)P_4 (8, 9, 10). Ins(1,4,5)P_3[S]$_3$ has been shown to release Ca^{2+} from a variety of permeabilized (8, 10, 12, 13) cells and has been reported to cause membrane potential oscillations in the *Xenopus* oocyte (11). We have studied the effect of Ins(1,4,5)P_3[S]$_3$ on the Cl^- current pattern in voltage-clamped *Xenopus* oocytes and have directly measured changes in intracellular Ca^{2+} using fura-2 imaging. A preliminary report of these data has been presented (14).

Material and Methods

OOCYTE PREPARATION: Ovaries were surgically removed from gravid *Xenopus* females. Stage VI oocytes were isolated and the follicle cells removed with forceps. Albino *Xenopus* oocytes were used in the fura-2 experiments to minimize autofluorescence.
SOLUTIONS: OR2: 82.5 mM NaCl; 2.5 mM KCl; 1.0 mM $MgCl_2$; 2.5 mM $NaHC0_3$; 1.0 mM $CaCl_2$; 5 mM Hepes pH 7.4. O-Ca^{2+}-high-Mg^{2+}: 82.5 mM NaCl, 2.0 mM KCl; 20 mM $MgCl_2$, 1.0 mM EGTA; 5 mM Hepes pH 7.4.
ELECTROPHYSIOLOGY: Cells were voltage-clamped at -60 mV with a two-electrode clamp (Dagan model 8500, Minneapolis, Minn.). The current was measured through a virtual ground circuit. Cells were injected by pressure or iontophoresis. Injections were made in the animal hemisphere because the plasma membrane in that hemisphere contains more Cl^- channels (15, 16) and more calcium storage sites (17, 18, 19). For pressure injections, the width of the injection pipette tip was 3 μm. For iontophoresis, the amount injected was determined by the formula q= -nI/ zF. The transport number was assumed to be 0.5. Each phosphate on the inositol was assumed to have a charge of -2. Amounts of polyphosphoinositols are reported in coulombs and the theoretical calculated amounts of moles are in parentheses. All injections were done with theta tubing and each barrel was filled with a different inositol. The two electrode tips' close proximity insures that effects of the different inositol phosphates or analogues were examined on the same cell area. Ins(1,4,5)P_3[S]$_3$ injections were done at the same depth as Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 injections.

Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 were generously provided by Dr. Robin Irvine. DL-Ins(1,4,5)P_3[S]$_3$, D-Ins(1,4,5)P_3[S]$_3$, and L-Ins(1,4,5)P_3 were synthesized similarly using resolved precursor (20). The inositol phosphates were prepared as 1.0 mM stock solutions in 0.1 mM Hepes and adjusted to pH 7.8 with KOH. DL-Ins(1,4,5)P_3[S]$_3$ and D-Ins(1,4,5)P_3[S]$_3$ were both used in the voltage-clamp experiments. The fura-2 imaging studies were done with D-Ins(1,4,5)P_3[S]$_3$.
FURA-2 IMAGING: The optical system used for fura-2 imaging was described previously (5). In each image, the Ca^{2+} was measured in a circular spot of a specific diameter, centered at the site of injection. Each data point represents the average Ca^{2+} level in the circular spot during a specific time interval. The cells were first prepared with a 50 nl injection of 1 mM fura-2. The 1 mM fura-2 stock was made in a physiological intracellular buffer: 19 mM NaCl, 52 mM KCl, and

10 mM HEPES, adjusted to pH 7.3 with KOH. 0.5 mM $CaCl_2$ was also added so that the fura-2 was half-saturated, generating a free Ca^{2+} concentration of 240 nM. The final intracellular concentration of fura-2 was always 55 μM, based on a cytoplasmic volume of 0.91μl. The fura-2 was allowed to diffuse throughout the oocyte for approximately 30 minutes at 20 °C.

Results

Ins(1,4,5)P_3[S]$_3$-Induced Cl^- Current Pattern

Figure 1A shows the typical Ins(1,4,5)P_3-induced Cl^- current pattern when large amounts are injected into a voltage-clamped oocyte. The pattern consists of a fast, immediate Cl^- current pulse, followed by a quiescent period, followed by oscillating Cl^- currents (2). When an oocyte is injected with Ins(1,3,4,5)P_4, the current pattern is quite different as shown in Fig.1B (4, 5, 6). Thus, Ins(1,3,4,5)P_4 only initiates the latter part of the Ins(1,4,5)P_3-induced Cl^- current pattern.

The injection of Ins(1,4,5)P_3[S]$_3$ triggered only oscillating Cl^- currents. These currents were identified as Cl^- currents due to a reversal potential of approximately of -25 mV, which is the expected reversal potential for an internal [Cl^-] of 33.4 mM and an external [Cl^-] of 89 mM (21). Injection of Ins(1,4,5)P_3[S]$_3$ was followed first by a quiescent period, which was followed by oscillating Cl^- currents. The quiescent period varied from many seconds to minutes, however usually the quiescent period was on the order of seconds. Ins(1,4,5)P_3[S]$_3$ did not trigger the fast immediate Cl^- current pulse even at high concentrations (Fig 2, Fig 3). Thus, Ins(1,4,5)P_3[S]$_3$ gives a current pattern similar to the Ins(1,3,4,5)P_4-induced pattern.

The initial voltage-clamp experiments were done with DL-Ins(1,4,5)P_3[S]$_3$,which is a racemic mixture. Therefore, as a control we injected L-Ins(1,4,5)P_3 (also identified as D-Ins(3,5,6)P_3), and found it had no effect on conductance even at 12 μC (1 x 10^{-11} moles) (data not shown). However, in most of the experiments we used D-Ins(1,4,5)P_3[S]$_3$, which gave the same current response as the DL- Ins(1,4,5)P_3[S]$_3$. Injection by either iontophoresis or pressure injection gave

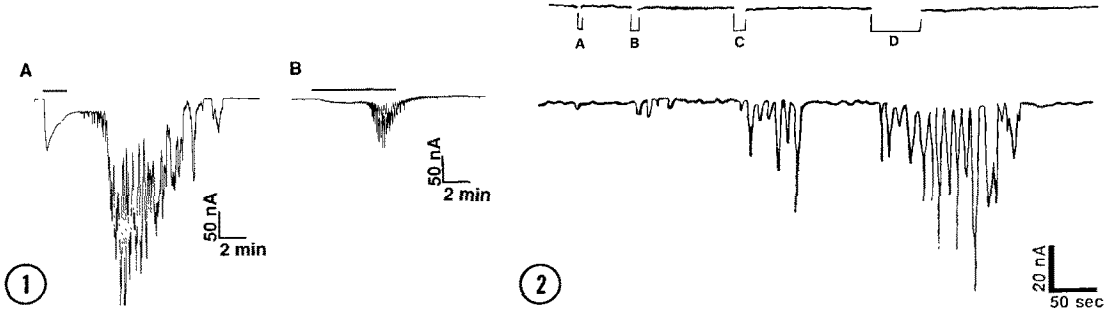


Fig. 1. The typical Cl^- current patterns initiated by large injections of Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4, shown in two different cells. (A) Injection of 1.5 μC (1.3 x 10^{-12} moles) of Ins(1,4,5)P_3. (B) Injection of 12.9 μC (8.3 x 10^{-12} moles) of Ins(1,3,4,5)P_4. The bars represent the time the iontophoresis injection current was on.

Fig 2. Response of a voltaged-clamped oocyte to iontophoresis of D-Ins(1,4,5)P_3[S]$_3$. The upper trace of the figure is the iontophoresis current and the lower trace represents the current crossing the oocyte membrane. Inward current is downward. The cell was injected 4 times. (A) 0.058 μC (5 x 10^{-14} moles), (B) 0.29 μC (2.5 x 10^{-13} moles), (C) 0.58 μC (5 x 10^{-13} moles), (D) 2.9 μC.(2.5 x 10^{-12} moles).

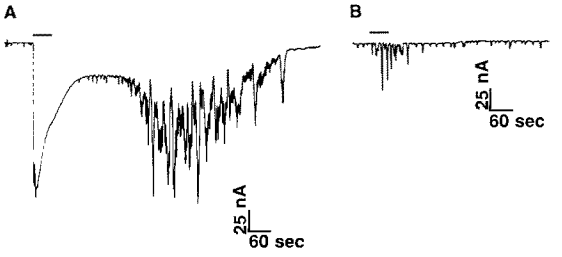


Fig. 3. Comparison of the effects of Ins(1,4,5)P_3 and D-Ins(1,4,5)P_3[S]$_3$ on the Cl^- current pattern in the same cell, voltaged-clamped at -60 mV. (A) 1.5 μC (1.3 x 10^{-12} moles) of Ins(1,4,5)P_3 was injected during the time period indicated by the bar. (B) inject 1.5 μC (1.3 x 10^{-12} moles) of Ins(1,4,5)P_3[S]$_3$.

the same current pattern. Figure 3 demonstrates the effects of similar amounts of Ins(1,4,5)P_3 and D-Ins(1,4,5)P_3[S]$_3$ on Cl^- current pattern in the same oocyte.

Intracellular Ca^{2+} Measurement

Internal Ca^{2+} was measured directly with a fura-2 imaging system. One barrel of the theta tubing contained Ins(1,4,5)P_3 and the other barrel contained D-Ins(1,4,5)P_3[S]$_3$. Figure 4

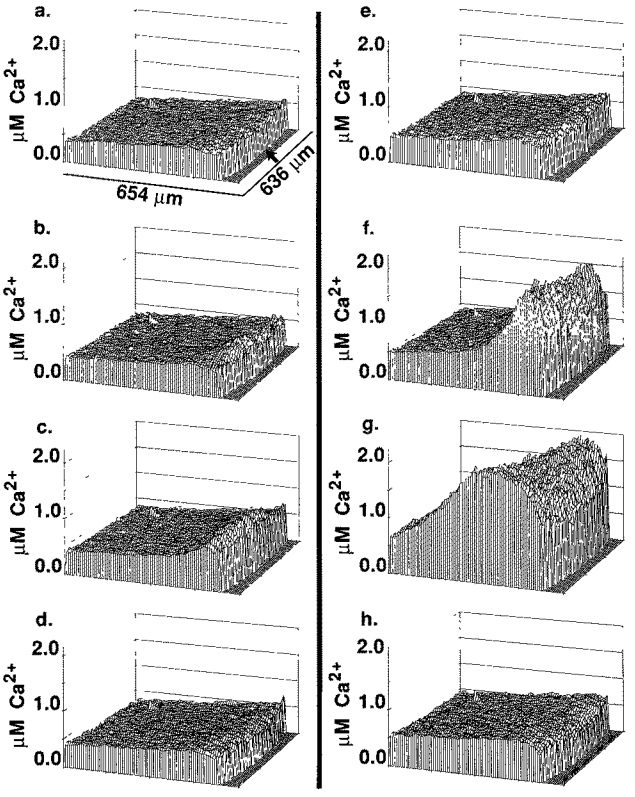
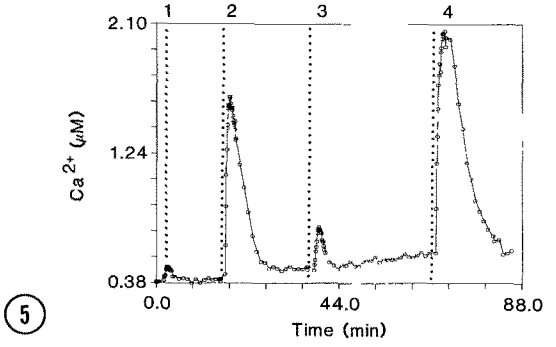


Fig 4. Three dimensional, spatial graph of intracellular [Ca^{2+}]. The graph shows half of the cell. The arrow in panel "a" represents the placement of the double-barrel injection pipette. Panels a-d: 1.6 μC (1.38 x 10^{-12} moles) of D-Ins(1,4,5)P_3[S]$_3$ was injected: "a" before injection, "b" 10 sec. after injection,"c" maximal response is at 52 sec., "d" recovery at 3 min and 10 sec. e-h: 1.6 μC (1.38 x 10^{-12} moles) of Ins(1,4,5)P_3 was injected into the same oocyte at the same location through the other barrel of the injection pipette; "e" before injection, "f" 10 sec. after injection, "g" maximal response is at 1 min and 25 sec., "h" recovery at 25 min and 46 sec.

shows that both Ins(1,4,5)P_3 and Ins(1,4,5)P_3[S]$_3$ caused a localized release in Ca^{2+}, but there is a difference in their Ca^{2+} releasing efficacy. Injections of large amounts of these inositol polyphosphates or analogues will cause a rise of Ca^{2+} which spreads from the point of injection. The maximal release, as shown in figure 4, was determined by measuring the Ca^{2+} in a circular spot 10 μm in diameter at the edge of the cell. At later time points the Ca^{2+} rose further inside the cell.

To compare the Ca^{2+} releasing characteristics of Ins(1,4,5)P_3 and Ins(1,4,5)P_3[S]$_3$, we measured the difference in the height of the maximal release of Ca^{2+} at two different concentrations (Fig.5). The change in Ca^{2+} was measured in both a 70 μm circle around the injection site and in a 30 μm circle around the injection site. When 320 nC (2.76×10^{-13} moles) of Ins(1,4,5)P_3 and Ins(1,4,5)P_3[S]$_3$ was injected it was found that Ins(1,4,5)P_3 was 11.7 ± 3.8 (s.d., n=9) times more potent when the measurement were made using a 70 μm circle. If the Ca^{2+} was measured in a 30 μm circle Ins(1,4,5)P_3 was found to be 11.5 ± 2.3 (n=6) times more potent. When 1.6 μC (1.38×10^{-12} moles) was injected and the Ca^{2+} measured in a 70 μm circle, Ins(1,4,5)P_3 was found to be 7.0 ± 1.7 (n=2) times more potent. If the Ca^{2+} was measured in a 30 μm circle, Ins(1,4,5)P_3 was found to be 7.7 ± 5.3 (n=6) times more potent. Ins(1,4,5)P_3[S]$_3$ did not cause the intracellular Ca^{2+} to stay elevated for a long period, even though Ins(1,4,5)P_3[S]$_3$ cannot be rapidly degraded. Fluctuations in $[Ca^{2+}]$ were not observed when the Ca^{2+} was measured in a 70 μm circle every 6 sec. If the Ca^{2+} was measured in a 10 μm circle just beneath the plasma membrane, every 3 seconds a few Ca^{2+} fluctuations were observed (data not shown).



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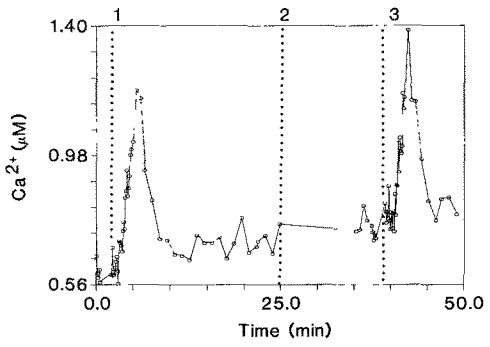

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Fig. 5. Time course of Ca^{2+} release in an immature oocyte measured with the fura-2 technique in response to similar amounts of Ins(1,4,5)P_3 and D-Ins(1,4,5)P_3[S]$_3$. The Ca^{2+} release was monitored by imaging the oocyte, which had been injected with fura-2. A circular spot 70 μm in diameter was positioned at the edge of each image, at the site of injection. Each data point represents the average Ca^{2+} level in the circular spot every 6 seconds. At the dotted line marked "1", 0.32 μC (2.76×10^{-13} moles) of D-Ins(1,4,5)P_3[S]$_3$ was injected. "2", 0.31 μC (2.68×10^{-13} moles) of Ins(1,4,5)P_3 was injected. "3", 1.6 μC (1.38×10^{-12} moles) of D-Ins(145)P_3[S]$_3$ was injected. "4", 1.6 μC (1.38×10^{-12} moles) of Ins(1,4,5)P_3 was injected.

Fig. 6. Fura-2 imaging measurement of Ca^{2+} release which demonstrates that the intracellular Ca^{2+} rise caused by D-Ins(145)P_3[S]$_3$ in the oocyte is not dependent on extracellular Ca^{2+}. The Ca^{2+} release was monitored in the same manner as figure 5, except that the Ca^{2+} was measured in a 30 μm circular spot. The cell was initially in OR2 buffer. At the dotted line marked "1", 3.2 μC (2.76×10^{-12} moles) of D-Ins(1,4,5)P_3[S]$_3$ was injected, "2", the cell was transferred to 0-Ca^{2+}-high Mg^{2+} buffer, "3", 3.2 μC (2.76×10^{-12} moles) of Ins(1,4,5)P_3[S]$_3$ was injected into the oocyte.

Figure 6 demonstrates that D-Ins(1,4,5)P_3[S]$_3$ released Ca^{2+} from intracellular stores. The Ins(1,4,5)P_3[S]$_3$-induced Ca^{2+} release was similar whether the cell was bathed in a 1 mM Ca^{2+} buffer or 0-Ca^{2+}/high Mg^{2+} buffer.

Discussion

Ins(1,4,5)P_3[S]$_3$ unexpectedly elicited a Cl^- current pattern similar to the Ins(1,3,4,5)P_4-induced Cl^- current pattern, rather than that of Ins(1,4,5)P_3. The only previous study of the effects of Ins(1,4,5)P_3[S]$_3$ on *Xenopus* oocytes examined membrane potential oscillations rather than current oscillations, therefore distinct differences between Ins(1,4,5)P_3 and Ins(1,4,5)P_3[S]$_3$ were not seen (11). The underlying cause for the difference in current pattern generated by Ins(1,4,5)P_3 compared to Ins(1,3,4,5)P_4 and Ins(1,4,5)P_3[S]$_3$ is not yet known. One possible explanation is that the Ins(1,4,5)P_3[S]$_3$ binds to the Ins(1,3,4,5)P_4 receptor rather than the Ins(1,4,5)P_3 receptor. We have previously reported that Ins(1,3,4,5)P_4 directly triggers an immediate release of Ca^{2+} when injected into oocytes (5, 7). The Ins(1,3,4,5)P_4-induced release of Ca^{2+} is not due to its conversion back to Ins(1,4,5)P_3 (7). Both Ins(1,3,4,5)P_4 and Ins(1,4,5)P_3[S]$_3$ cause an immediate release of Ca^{2+} but there is a lag time before any currents are observed. This discrepancy between Ca^{2+} release and Cl^- current activation should make investigators cautious about using Cl^- currents as exact indicators of changes in intracellular Ca^{2+}. The different current patterns cannot be explained only in terms of the different Ca^{2+} releasing potency of these inositol polyphosphates, because very small amounts of Ins(1,4,5)P_3 (1×10^{-17} moles) will only initiate a fast Cl^- pulse with no oscillating currents. As the amount of Ins(1,4,5)P_3 injected into an oocyte is increased, oscillating currents will eventually follow the immediate Cl^- current pulse. Ins(1,3,4,5)P_4 and Ins(1,4,5)P_3[S]$_3$, regardless of concentration, can only trigger oscillating Cl^- currents and these currents only occur after a quiescent or latent period. The length of the quiescent time is much more variable and shorter following Ins(1,4,5)P_3[S]$_3$ than Ins(1,3,4,5)P_4 injections. The quiescent period suggests that some type of biochemical step may be involved in the regulation of the oscillating Cl^- current. The evidence also suggests that there may be either two different classes of Cl^- channels, or one type which can be altered by some Ca^{2+}-dependent biochemical event. Since Ins(1,4,5)P_3[S]$_3$ and Ins(1,3,4,5)P_4 injections were made at the same electrode depth as Ins(1,4,5)P_3 injections, depth is not responsible for the current pattern differences.

Ins(2,4,5)P_3 is another $InsP_3$ analogue which is not readily converted to Ins(1,3,4,5)P_4 (22). We and another group have previously studied the effects of Ins(2,4,5)P_3 (5, 6, 7) and found that Ins(2,4,5)P_3 generated the same current pattern as Ins(1,4,5)P_3. We reported that the only difference between Ins(1,4,5)P_3 and Ins(2,4,5)P_3 was that the latter was approximately 4 times less effective in eliciting the fast Cl^- current pulse (5,7). Thus, two $InsP_3$ analogues, Ins(2,4,5)P_3 and Ins(1,4,5)P_3[S]$_3$, which cannot be converted to Ins(1,3,4,5)P_4, each have unexpectedly different effects on Cl^- current pattern.

We also showed directly with fura-2 imaging that Ins(1,4,5)P_3[S]$_3$ releases Ca^{2+} from an intact, non-permeabilized cell. Both Ins(1,4,5)P_3 and Ins(1,4,5)P_3[S]$_3$ triggered a localized release and spread of Ca^{2+}. However it took a large amount of Ins(1,4,5)P_3 or Ins(1,4,5)P_3[S]$_3$ to initiate a spread of free Ca^{2+} across the cell. This is in contrast to results in the mature egg,

where a very small amount of Ins(1,4,5)P_3 triggers a localized release of Ca^{2+}, and a Ca^{2+} wave which is propagated across the cell (23). The inability to trigger a propagated Ca^{2+} wave in the immature oocyte with low amounts of Ins(1,4,5)P_3 may be due to the fact that the immature oocyte has a different endoplasmic reticulum network than the mature egg (17, 18, 19). Our results also suggest that small distinct areas of the cell each may have different Cl^- current patterns and all of these areas combine to give an overall current pattern.

In experiments with permeabilized cells, the Ins(1,4,5)P_3[S]$_3$ is not able to diffuse away from its site of action. Ins(1,4,5)P_3[S]$_3$ causes a sustained elevation of intracellular Ca^{2+} in permeabilized human SY5Y neuroblastoma cells (8). We found that Ins(1,4,5)P_3[S]$_3$ does not cause a sustained elevation in Ca^{2+} in the intact oocyte. Since Ins(1,4,5)P_3 diffuses at a rate of 50 μm per 3 seconds in *Limulus* photoreceptors, our result may be due to the rapid diffusion of Ins(1,4,5)P_3[S]$_3$ from the site of injection (24).

With two different concentrations we found that Ins(1,4,5)P_3[S]$_3$ was approximately 10-fold less potent than Ins(1,4,5)P_3 at releasing intracellular Ca^{2+}. In permeabilized cells Ins(1,4,5)P_3[S]$_3$ has been reported to be approximately 3 to 4 times less potent than Ins(1,4,5)P_3 (10, 11, 13). This small difference between the permeabilized cell data and our results may be due to either the different techniques of Ca^{2+} measurement or to our assumption that both Ins(1,4,5)P_3 and Ins(1,4,5)P_3[S]$_3$ have the same overall charge. We have previously reported that 320 nC of Ins(1,3,4,5)P_4 is approximate 5-fold less potent than Ins(1,4,5)P_3 in releasing intracellular Ca^{2+} (7). Therefore both Ins(1,3,4,5)P_4 and Ins(1,4,5)P_3[S]$_3$ have similar Ca^{2+} releasing characteristics and effects on Cl^- conductance. But, as explained previously, the Ca^{2+}-releasing characteristics alone do not explain why Ins(1,4,5)P_3[S]$_3$ and Ins(1,3,4,5)P_4 cannot elicit the fast, immediate Cl^- current pulse.

In conclusion, we have shown in the *Xenopus* oocyte that Ins(1,4,5)P_3[S]$_3$ causes a Cl^- current pattern similar to the Ins(1,3,4,5)P_4-induced Cl^- current pattern, not the Ins(1,4,5)P_3 pattern. Ins(1,4,5)P_3[S]$_3$ causes a transient release of internal Ca^{2+} from the intact *Xenopus* oocyte.

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References

1. Berridge, M. J. (1987) Ann. Rev. Biochem. **56**, 159-193
2. Oron, Y., Dascal, N., Nadler, E., and Lupu, M. (1985) Nature **313**, 141-143.
3. Gillo, B., Lass, Y., Nadler, E. and Oron, Y. (1987) J. Physiol. **392**, 349-361.
4. Parker, I., and Miledi, R. (1987) Pro. R. Soc. Lon. B. **235**, 59-70.
5. Nuccitell, R. Ferguson, J. and Han, J-K. (1989) in Mechanisms of Egg Activation (Nuccitelli, R., Cherr,G.N. and Clark, W. H. eds) Plenum Press, New York pp. 215-230.
6. Synder, P. M., Krasue, K-H, and Welsh, M. J. (1988) J. Biol. Chem. **262**, 11048-11051.
7. Ferguson, J. E. Han, J-K., Kao, J. P. Y. and Nuccitelli, R. (1990) Exp. Cell Res. (submitted)
8. Nahorski, S. R. and Potter, B. V. L. (1989) Trends Pharmacol. Sci **10**, 139-144.
9. Willcocks, A. L. Potter, B. V. L., Cooke, A. M. and Nahorski, S. R. (1988) Eur. J. Pharmacol. **155**, 181-183

10. Taylor, C. W., Berridge, M. J., Cooke, A. M., and Potter, B. V. L. (1989) Biochem. J. **259**, 645-650.
11. Taylor, C. W., Berridge, M. J., Brown, K. D., Cooke, A. M. and Potter, B. V. L. (1988) Biochem. Biophys. Res. **150**, 626-632.
12. Thevenod, F., Dehlinger-Kremer, M. Kemmer, T. P., Christian, A-L., Potter, B. V. L. and Schulz, I. (1989). J. Membrane Biol. **109**, 173-186.
13. Strupish, J. Cooke, A. M., Potter, B. V. L., and Gigg, R. and Nahorski, S. R. (1988) Biochem. J. **253**, 901-905.
14. Ferguson, J. E., Potter, B. V. L., and Nuccitelli, R. (1989). J. Cell Biol **109**, 1150a.
15. Robinson, K. R. (1979) Proc. Natl. Acad. Sci. U.S.A. **76**, 837-841.
16. Miledi, R. and Parker, I. (1984) J. Physiol. **357**, 173-183.
17. Gardiner, D. M. and Grey, R. (1983) J. Cell Biol **96**, 1159-1163.
18. Campenella, C. Andreuceetti, P., Taddei, C., and Televi, R. (1984) J. Exp. Zool. **229**, 283-293.
19. Charbonneau, M. and Grey, R. D. (1984) Dev. Biol. **102**, 90-97.
20. Cooke, A. M. Gigg, R., and Potter, B. V. L. (1987) J. Chem. Soc. Chem. Commun. **698**, 1525-1526.
21. Barish, M. E. (1983). J. Physiol. **343**, 309-325.
22. Irvine, R. F. and Moor, R. M. (1986) Biochem. J. **240**, 917-920.
23. Busa, W. B. and Nuccitelli, R. (1985) J. Cell Biol. **100**, 1329-1329.
24. Payne, R. and Fein, A. (1987) J. Cell Biol. **104**, 933-937.