Inositol trisphosphate analogues induce different oscillatory patterns in *Xenopus* oocytes

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Agonists that utilize the calcium-mobilizing second messenger inositol(1,4,5)trisphosphate $\text{Ins}(1,4,5)\text{P}_3$ usually generate oscillations in intracellular calcium. Such oscillations, based on the periodic release of calcium from the endoplasmic reticulum, can also be induced by injecting cells with $\text{Ins}(1,4,5)\text{P}_3$. The mechanism responsible for oscillatory activity was studied in *Xenopus* oocytes by injecting them with different inositol trisphosphates. The plasma membrane of *Xenopus* oocytes has calcium-dependent chloride channels that open in response to calcium, leading to membrane depolarization. Oscillations in calcium were thus monitored by recording membrane potential. The naturally occurring $\text{Ins}(1,4,5)\text{P}_3$ produced a large initial transient followed by a single transient or a burst of oscillations. By contrast, two analogues ($\text{Ins}(2,4,5)\text{P}_3$ and $\text{Ins}(1,4,5)\text{P}(\text{S})_3$) produced a different oscillatory pattern made up of a short burst of sharp transients. $\text{Ins}(1,3,4,5)\text{P}_4$ had no effect when injected by itself, and it also failed to modify the oscillatory responses to either $\text{Ins}(2,4,5)\text{P}_3$ or $\text{Ins}(1,4,5)\text{P}(\text{S})_3$. Both analogues failed to induce a response when injected immediately after the initial $\text{Ins}(1,4,5)\text{P}_3$ -induced response, indicating that they act on the same intracellular pool of calcium. The existence of different oscillatory patterns suggests that there may be different mechanisms for setting up calcium oscillations. The $\text{Ins}(2,4,5)\text{P}_3$ and $\text{Ins}(1,4,5)\text{P}(\text{S})_3$ analogues may initiate oscillations through a negative feedback mechanism whereby calcium inhibits its own release. The two-pool model is the most likely mechanism to describe the $\text{Ins}(1,4,5)\text{P}_3$ -induced oscillations.

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Introduction

Cell stimulation is frequently associated with oscillations in the intracellular levels of calcium (Berridge *et al.*, 1988; Berridge and Galione, 1988). Such oscillations are particularly evident when cells are stimulated with agonists that act through the phosphoinositide system to generate the second messengers diacylglycerol (DG) and inositol(1,4,5)-trisphosphate ($\text{Ins}(1,4,5)\text{P}_3$). Most of the models that have been put forward to explain such oscillatory activity fall into two categories, both of which are based on the calcium-mobilizing action of $\text{Ins}(1,4,5)\text{P}_3$ (Berridge and Galione, 1988). Receptor-controlled models suggest that various feedback interactions result in oscillations in the intracellular level of $\text{Ins}(1,4,5)\text{P}_3$ (Cobbold *et al.*, 1988; Meyer and Stryer, 1988). It is difficult to test such models because it is impossible to determine, using current techniques, whether or not the level of $\text{Ins}(1,4,5)\text{P}_3$ is oscillating. Some evidence arguing against such models has come from studies showing that oscillatory activity, similar to that induced by the natural agonist, can be duplicated by injecting or perfusing $\text{Ins}(1,4,5)\text{P}_3$ into cells such as *Xenopus laevis* oocytes (Oron *et al.*, 1985; Berridge, 1988), hamster eggs (Swann *et al.*, 1989), smooth muscle cells (Ohya *et al.*, 1988), and pancreatic acinar cells (Wakui *et al.*, 1989). Such observations have spawned other models that attempt to explain how a constant level of $\text{Ins}(1,4,5)\text{P}_3$ can generate oscillations in intracellular calcium. One mechanism is based on a negative feedback loop whereby calcium inhibits the action of $\text{Ins}(1,4,5)\text{P}_3$ (Payne *et al.*, 1988; Prentki *et al.*, 1988). A more complicated mechanism proposes that the calcium released from the $\text{Ins}(1,4,5)\text{P}_3$ -sensitive pool sets up oscillations in an $\text{Ins}(1,4,5)\text{P}_3$ -insensitive pool by a process of calcium-induced calcium release (Berridge, 1988; Berridge and Galione, 1988). The latter model emerged from work carried out on immature oocytes of *Xenopus*, which produce membrane potential oscillations in response to injections of $\text{Ins}(1,4,5)\text{P}_3$ (Oron *et al.*, 1985; Berridge, 1988, 1989). The sole function

Figure 1. Membrane potential responses of a *Xenopus* oocyte after injection of either $\text{Ins}(1,4,5)\text{P}_3$ (at A) or $\text{Ins}(2,4,5)\text{P}_3$ (at B) from separate barrels of a double-barreled electrode. The amount of each inositol phosphate injected was varied by altering both the duration and the amplitude of the iontophoretic current: (A) 1 s/5 V; (B₁) 1 s/5 V; (B₂) 1 s/10 V.

of $\text{Ins}(1,4,5)\text{P}_3$ is to provide the calcium that drives the periodic uptake and release of calcium by the $\text{Ins}(1,4,5)\text{P}_3$ -insensitive pool. Analogues of $\text{Ins}(1,4,5)\text{P}_3$, which also mobilize calcium, should also give rise to oscillatory activity. This turns out to be the case, but the oscillations induced by $\text{Ins}(2,4,5)\text{P}_3$ and the nonhydrolyzable analogue of $\text{Ins}(1,4,5)\text{P}_3$, inositol(1,4,5)-trisphosphorothioate ($\text{Ins}(1,4,5)\text{P}(\text{S})_3$; for review see Nahorski and Potter, 1989), gave oscillatory patterns very different from those induced by $\text{Ins}(1,4,5)\text{P}_3$, indicating that there may be different mechanisms for setting up calcium oscillations.

Results

InsP₃-specific oscillatory patterns

Xenopus oocytes injected with $\text{Ins}(1,4,5)\text{P}_3$ display a characteristic membrane potential response that begins with a large depolarizing transient followed by a spontaneous series of oscillations (Figures 1 and 2). As noted previously, the initial transient declined when increasing amounts of $\text{Ins}(1,4,5)\text{P}_3$ were injected (Berridge, 1988). Using such high injection currents, there was little difference in the oscillatory response to $\text{Ins}(1,4,5)\text{P}_3$ and $\text{Ins}(1,4,5)\text{P}(\text{S})_3$ (Taylor *et al.*, 1988). However, when smaller amounts of these inositol phosphates were injected, there was a clear difference. Also, responses to $\text{Ins}(2,4,5)\text{P}_3$ were found to resemble closely those to $\text{Ins}(1,4,5)\text{P}(\text{S})_3$ in that the large initial transient was replaced by a brief series of rapid transients (Figures 1 and 2). The precise

Figure 2. Membrane potential responses of a *Xenopus* oocyte after injection of either (A) $\text{Ins}(1,4,5)\text{P}_3$ or (B) $\text{Ins}(1,4,5)\text{P}(\text{S})_3$ from separate barrels of a double-barreled electrode. The duration and amplitude of the iontophoretic injection were (A) 0.5 s/5 V and (B) 1 s/5 V.

pattern varied somewhat between oocytes; sometimes the initial transient was large, as for the $\text{Ins}(2,4,5)\text{P}_3$ responses in Figure 1, whereas in other cases the first transient after injection was small but then increased over the next few transients, as shown for the $\text{Ins}(1,4,5)\text{P}(\text{S})_3$ responses in Figure 2. To determine whether there was any consistent difference between these two analogues, we loaded them into the separate halves of a double-barreled electrode so that they could be injected alternatively into the same location of a single oocyte (Figure 3). The use of this approach resulted in no evidence for any difference in the oscillatory patterns induced by $\text{Ins}(2,4,5)\text{P}_3$ and $\text{Ins}(1,4,5)\text{P}(\text{S})_3$.

Dose-response relationships

The different InsP_3 isomers described above vary in their ability to mobilize calcium when ap-

Figure 3. Comparison of the effects of injecting either (electrode A) $\text{Ins}(2,4,5)\text{P}_3$ or (electrode B) $\text{Ins}(1,4,5)\text{P}(\text{S})_3$ on the membrane potential of a single *Xenopus* oocyte. The inositol phosphate or phosphorothioate, placed in separate barrels of a double-barreled electrode, was injected at the arrows (large arrows, 2 s/5 V; small arrows, 1 s/5 V).

plied to permeabilized cells, with their order of effectiveness being Ins(1,4,5)P₃ ($EC_{50} = 2.1 \times 10^{-7}$ M) > Ins(1,4,5)P(S)₃ ($EC_{50} = 9.6 \times 10^{-7}$ M) > Ins(2,4,5)P₃ ($EC_{50} = 2.8 \times 10^{-6}$ M) (Strupish *et al.*, 1988; Willcocks *et al.*, 1989). To determine whether the different oscillatory patterns described above (Figures 1 and 2) were concentration dependent, we studied the effect of varying the amounts of Ins(1,4,5)P₃ and Ins(1,4,5)P(S)₃ injected (Figure 4). As the amount of Ins(1,4,5)P₃ injected was increased, the initial effect was to increase the magnitude of the initial transient. At the higher doses, the recovery phase became longer and was interrupted by spontaneous transients that began ~30 s after the injection of Ins(1,4,5)P₃ (Figure 4, top trace). The first evidence of spontaneous activity was a single second transient (Figure 4, injection d, top trace). After a larger injection of Ins(1,4,5)P₃, the spontaneous transients ran into each other to give a prolonged transient (Figure 4, injection and upper trace). A qualitatively different pattern of responses was recorded after injection of increasing amounts of Ins(1,4,5)P(S)₃. The initial small depolarizing responses were similar to those produced by Ins(1,4,5)P₃, but, as the amount of Ins(1,4,5)P(S)₃ injected was increased, there was no evidence of a large early transient—the response consisted of a series of sharp transients that ceased within 20 s after the injection (Figure 4, bottom trace), i.e., at a time before the transient activity induced by Ins(1,4,5)P₃ had begun (Figure 4, top trace). A series of responses similar to those just described for Ins(1,4,5)P(S)₃ was observed after injection of increasing amounts of Ins(2,4,5)P₃ (data not shown). At no stage was it possible to duplicate the Ins(1,4,5)P₃ response pattern by increasing the amount of injected Ins(1,4,5)P(S)₃ or Ins(2,4,5)P₃, indicating that these last two compounds induce a physiological response that is qualitatively different from that of Ins(1,4,5)P₃.

Heterologous desensitization

Previous studies have indicated that the Ins(1,4,5)P₃-induced membrane depolarization displays homologous desensitization (Berridge, 1988, 1989). Injection of sufficient Ins(1,4,5)P₃ to induce oscillatory activity renders the oocyte insensitive to subsequent injections. Oocytes desensitized to Ins(1,4,5)P₃ were also found to be insensitive to Ins(1,4,5)P(S)₃ (Figure 5a). The typical rapid transients produced by injecting Ins(1,4,5)P(S)₃ are clearly evident at the beginning and end of the trace but were totally absent

Figure 4. Dose-response relationships for the effect of injecting (top trace) Ins(1,4,5)P₃ and (bottom trace) Ins(1,4,5)P(S)₃ on membrane potential of *Xenopus* oocytes. The inositol phosphate or phosphorothioate was loaded into the separate barrels of a double-barreled electrode, and the two traces shown here were taken from a continuous recording from the same oocyte. The amount of each inositol phosphate or phosphorothioate injected (arrows) was varied by altering both the duration and the amplitude of the iontophoretic current: (a) 0.5 s/0.5 V, (b) 0.5 s/1 V, (c) 0.5 s/2 V, (d) 0.5 s/5 V, (e) 1 s/5 V.

when injected 15 s after an injection of Ins(1,4,5)P₃ (Figure 5a). Once the oocyte had undergone its series of spontaneous transients, it once again became sensitive to Ins(1,4,5)P(S)₃. Such examples of heterologous desensitization were also observed when Ins(2,4,5)P₃ was injected after Ins(1,4,5)P₃ (Figure 5b). However, when the order of injecting these inositol phosphates was reversed, there was no evidence for Ins(2,4,5)P₃ being able to desensitize the oocyte against Ins(1,4,5)P₃ (Figure 5b). Even when the oocyte was injected with a larger amount of Ins(2,4,5)P₃, injection of Ins(1,4,5)P₃ given 15 s later still gave the characteristic initial depolarizing spike followed by a burst of oscillations (data not shown).

Effect of repetitive injections on oscillatory activity

Membrane potential oscillations can be induced by Ins(1,4,5)P₃ given either as a single large injection (Figures 1, 2, and 4) or by much smaller injections given repetitively (Figure 6). The first injection gave a large transient, but the next few injections gave very small responses, indicating the onset of desensitization. As injections continue, however, spontaneous oscillations sud-

Figure 5. Heterologous desensitization. (a) At the beginning of the trace are shown typical responses to injecting Ins(1,4,5)P₃ (0.5 s/5 V at A) or Ins(1,4,5)P(S)₃ (0.5 s/5 V at B). There was no response to the latter when injected 15 s after an equivalent injection of Ins(1,4,5)P₃. The asterisks indicate the spontaneous transients induced by injecting Ins(1,4,5)P₃. (b) Injection of Ins(1,4,5)P₃ (100 ms/5 V given at A) reduced the effect of Ins(2,4,5)P₃ (0.5 s/5 V) injected at B, but there was no effect when the order was reversed (i.e., when A followed B).

denly begin and continue for a considerable period after the injections have ceased (Figure 6). After a brief rest period, the same oocyte was injected repetitively with Ins(1,4,5)P(S)₃. There was no evidence of desensitization; a series of large brief transients appeared that ceased immediately when the injections of Ins(1,4,5)P(S)₃ ceased (Figure 6). There was no evidence of the broader transients characteristic of the spontaneous oscillatory activity initiated by the repetitive injections of Ins(1,4,5)P₃.

Effect of Ins(1,3,4,5)P₄

Ionophoretic injection of Ins(1,3,4,5)P₄ into *Xenopus* oocytes failed to induce membrane depolarization or oscillations (data not shown). The response to Ins(2,4,5)P₃ was unaltered by co-injection with Ins(1,3,4,5)P₄. The Ins(1,3,4,5)P₄ was either injected together with the Ins(2,4,5)P₃ or was placed in the second barrel of a double-barreled pipette. In the latter case, the typical brief oscillatory burst of Ins(2,4,5)P₃ was unaltered by injecting

Ins(1,3,4,5)P₄ 2 s before or after the injection of the trisphosphate.

Discussion

The unexpected observation to emerge from this study is that analogues of Ins(1,4,5)P₃ induce very different oscillatory patterns when injected into *Xenopus* oocytes. Previous studies on oocytes have established that Ins(1,4,5)P₃ acts to mobilize calcium, which then opens chloride channels leading to membrane depolarization. In this study, therefore, the calcium-activated chloride channels are used as an endogenous detector of calcium, which can thus be monitored continuously simply by recording membrane potential. Previous studies revealed that injection of Ins(1,4,5)P₃ gave an initial transient depolarization that displayed a series of oscillations during the recovery phase (Berridge, 1988, 1989). Because these oscillations developed at a time when the oocyte was insensitive to further injections of Ins(1,4,5)P₃, it was proposed that they may be produced by the periodic release of calcium by an Ins(1,4,5)P₃-insensitive pool. The idea is that Ins(1,4,5)P₃ mobilizes calcium, which is then taken up and released in a repetitive manner by the Ins(1,4,5)P₃-insensitive pool by a process of calcium-induced calcium release (Goldbeter *et al.*, 1990). Although this model can adequately explain the Ins(1,4,5)P₃-induced oscillations, it cannot explain the oscillations produced by Ins(2,4,5)P₃ or Ins(1,4,5)P(S)₃.

The oscillations produced by these two analogues are characterized by sequences of very narrow transients that develop immediately after injection and terminate equally abruptly. When paired with Ins(1,4,5)P₃, they display heterologous desensitization (Figure 5), indicating that they release calcium from the same Ins(1,4,5)P₃-sensitive pool. A possible reason for

Figure 6. Effect of repetitive injections of either Ins(1,4,5)P₃ (0.5 s/1 V at arrowheads) or Ins(1,4,5)P(S)₃ (0.5 s/2 V at arrowheads).

their failure to produce the delayed oscillatory burst may be that they cannot displace sufficient calcium from the Ins(1,4,5)P₃-sensitive pool to prime the Ins(1,4,5)P₃-insensitive pool. Such an explanation is consistent with the observation that Ins(1,4,5)P(S)₃ was incapable of setting up oscillations when injected repetitively (Figure 6). The oscillations induced by repetitive injections of Ins(1,4,5)P₃ develop slowly after the calcium-mobilizing response has desensitized (Figure 6; Berridge, 1989). No such desensitization was observed after the repetitive injections of Ins(1,4,5)P(S)₃. There was also no evidence for desensitization of the calcium-mobilizing response when the amount of Ins(1,4,5)P(S)₃ injected was increased (Figure 4), and this may provide the clue to the action of these analogues. It seems that these analogues can release calcium in short bursts to give very rapid transients but cannot release calcium over a prolonged period to produce the long-lasting initial transient depolarization characteristic of the action of Ins(1,4,5)P₃. The fluttering action of Ins(1,4,5)P(S)₃ and Ins(2,4,5)P₃ might be explained if their binding to the receptor were rapidly reversible.

Calcium itself has a marked effect on the ability of Ins(1,4,5)P₃ to release calcium from the endoplasmic reticulum. The operation of such a feedback loop might be capable of setting up calcium oscillations (Gray, 1988; Payne *et al.*, 1988; Joseph *et al.*, 1989; Parker and Ivorra, 1990). In *taenia coli*, the initial rate of Ins(1,4,5)P₃-induced release of calcium is maximal at an ambient calcium concentration of 0.1 μ M (Iino 1987) but is reduced as the level rises with an IC₅₀ of 1 μ M (Jean and Klee, 1986). Injection of calcium into *Xenopus* oocytes inhibited the calcium signal induced by Ins(1,4,5)P₃ (Parker and Ivorra, 1990). Because the inhibitory effect of calcium was much more pronounced at submaximal levels of Ins(1,4,5)P₃ (Joseph *et al.*, 1989), analogues such as Ins(2,4,5)P₃ and Ins(1,4,5)P(S)₃, which have a lower affinity for the receptor, conceivably may be much more susceptible to this negative feedback loop exerted by a rise in cytoplasmic calcium. The existence of such a feedback loop may account for the rapid transients induced by either Ins(2,4,5)P₃ or Ins(1,4,5)P(S)₃.

Finally, it should be mentioned that both Ins(2,4,5)P₃ and Ins(1,4,5)P(S)₃ are poorly metabolized by both the 5-phosphatase and the 3-kinase. Despite not being metabolized, these two inositol phosphates give very brief responses. This apparent paradox can probably be explained by the large volume of the oocyte

acting as a sink into which these inositol phosphates can disperse after injection. In a previous study it was argued that the oscillations induced by Ins(1,4,5)P₃ might be highly localized to the site of injection (Berridge, 1989). It seems likely, therefore, that the brief oscillatory bursts produced by Ins(2,4,5)P₃ and Ins(1,4,5)P(S)₃ were terminated as the local high concentration immediately after an injection was reduced by diffusion into the bulk of the cytoplasm. Because these two analogues are poor substrates for the inositol trisphosphate 3-kinase, they will not be converted to the corresponding tetrakis compound. The unusual oscillatory pattern displayed by these two analogues may be explained by the absence of some effect normally provided by Ins(1,3,4,5)P₄. Attempts to modify the Ins(2,4,5)P₃-induced oscillatory pattern by co-injecting Ins(1,3,4,5)P₄ were unsuccessful. Previous studies have shown that *Xenopus* oocytes pressure-injected with Ins(1,3,4,5)P₄ displayed an oscillatory response similar to that induced by Ins(1,4,5)P₃ but lacking the large initial depolarization (Parker and Miledi, 1987; Snyder *et al.*, 1988). No such oscillations were observed after the ionophoretic injection of Ins(1,3,4,5)P₄ (this paper), and this discrepancy may have arisen from differences in the amounts injected. To record a response resembling that of Ins(1,4,5)P₃, Parker and Miledi (1987) injected 20 times more Ins(1,3,4,5)P₄. Because there is evidence that cells possess an inositol 1,3,4,5-tetrakisphosphate 3-phosphomonoesterase (Cunha-Melo *et al.*, 1988; Höer *et al.*, 1988; Oberdisse *et al.*, 1990), the oscillations derived from injecting large amounts of Ins(1,3,4,5)P₄ conceivably may have arisen from the production of Ins(1,4,5)P₃. The difficulty of injecting large amounts of Ins(1,3,4,5)P₄ by ionophoresis may explain the absence of oscillations in the present studies. A similar explanation may account for the failure of Ins(1,3,4,5)P₄ to modify the oscillatory activity of Ins(2,4,5)P₃. The possibility that the conversion of Ins(1,4,5)P₃ to Ins(1,3,4,5)P₄ might play some role in setting up calcium oscillations (Irvine *et al.*, 1988; Yule and Gallacher, 1988) remains an open question.

The qualitative differences in the way *Xenopus* oocytes respond to various inositol trisphosphates suggest that separate oscillatory mechanisms may coexist in the same cell. The immediate but short-lived transients induced by Ins(2,4,5)P₃ or Ins(1,4,5)P(S)₃ may arise through calcium inhibition of the release mechanism located on the Ins(1,4,5)P₃-sensitive store of calcium. It is argued that Ins(1,4,5)P₃ is less affected by such calcium inhibition and is thus

able to induce a much larger initial calcium transient, which may then trigger a secondary phase of calcium oscillations by a process of calcium-induced calcium release from an $\text{Ins}(1,4,5)\text{P}_3$ -insensitive pool of calcium.

Methods

Electrophysiological measurements were carried out on oocytes obtained from *X. laevis* as described previously (Berridge, 1988, 1989). The oocytes were maintained in modified Barths solution (MBS) containing 88 mM NaCl, 2 mM KCl, 1 mM CaCl_2 , 1 mM MgCl_2 , 0.5 mM Na_2HPO_4 , 2 mM NaHCO_3 , and 15 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) (pH 7.6). Oocytes were placed in a small depression in the base of a perspex chamber, which was perfused at 1 ml/min with a physiological saline containing 115 mM NaCl, 2 mM KCl, 1.8 mM CaCl_2 , 1 mM MgCl_2 , and 7.5 mM tris(hydroxymethyl)amino-methane-HCl (Tris-HCl) (pH 7.6).

Inositol trisphosphates were injected through the same glass microelectrode used to record membrane potential. Double-barreled electrodes were prepared from theta-glass (TGC200-10, Clark Electromedical Instruments). When two separate electrodes were used, one was inserted in the middle of the animal pole and the other at an angle of 45° , giving a separation of $\sim 200 \mu\text{M}$. Electrodes were backfilled with small volumes ($1\text{--}2 \mu\text{l}$) of 200 mM KCl containing 1 mM of the various inositol trisphosphates. Microelectrodes were connected to the headstages of a conventional dual-channel high-impedance amplifier through an Ag-AgCl silver wire, and the signal was recorded continuously on a dual-channel potentiometric chart recorder. Electrodes had resistances of 50–70 M Ω ; different amounts of the inositol trisphosphates were injected by varying the period and the magnitude of the ionophoretic current by means of a Digitimer Stimulator DS9A.

$\text{Ins}(1,4,5)\text{P}_3$ and $\text{Ins}(2,4,5)\text{P}_3$ were obtained from Dr. R.F. Irvine of the AFRC Institute of Animal Physiology, Babraham, United Kingdom. $\text{DL-Ins}(1,4,5)\text{P}(\text{S})_3$ was synthesized according to Cooke *et al.* (1987) and was purified by ion-exchange chromatography. $\text{L-Ins}(1,4,5)\text{P}_3$ was inactive in causing oscillations when injected into *Xenopus* oocytes (Taylor *et al.*, 1988).

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