Activation of Frog (Xenopus laevis) Eggs by Inositol Trisphosphate.

I. Characterization of Ca\(^{2+}\) Release from Intracellular Stores

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ABSTRACT Iontophoresis of inositol 1, 4, 5-trisphosphate into frog (Xenopus laevis) eggs activated early developmental events such as membrane depolarization, cortical contraction, cortical granule exocytosis, and abortive cleavage furrow formation (pseudocleavage). Inositol 1,4-bisphosphate also triggered these events, but only at doses ~100-fold higher, whereas no level of fructose-1, 6-bisphosphate tested activated eggs. Using Ca\(^{2+}\)-selective microelectrodes, we observed that activating doses of inositol 1, 4, 5-trisphosphate triggered a Ca\(^{2+}\) release from intracellular stores that was indistinguishable from that previously observed at fertilization (Busa, W. B., and R. Nuccitelli, 1985, J. Cell Biol., 100:1325-1329), whereas subthreshold doses triggered only a localized Ca\(^{2+}\) release at the site of injection. The subthreshold IP\(_3\) response could be distinguished from the major Ca\(^{2+}\) release at activation with respect to their dose-response characteristics, relative timing, sensitivity to external Ca\(^{2+}\) levels, additivity, and behavior in the activated egg, suggesting that the Xenopus egg may possess two functionally distinct Ca\(^{2+}\) pools mobilized by different effectors. In light of these differences, we suggest a model for intracellular Ca\(^{2+}\) mobilization by sperm–egg interaction.

A transient increase in intracellular free Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) accompanies fertilization in both invertebrate (8, 9, 23) and vertebrate eggs (4, 6, 16, 21), and considerable evidence suggests that such Ca\(^{2+}\) pulses provide the primary regulatory signal that triggers the initiation of development in the zygote (13, 18). The means by which sperm–egg interaction triggers intracellular Ca\(^{2+}\) release in the egg is unknown, but Ca\(^{2+}\)-induced Ca\(^{2+}\) release has been suggested to be involved (18, 22).

Recently, considerable evidence has accumulated that implicates the turnover of inositol lipids in the mobilization of intracellular Ca\(^{2+}\) seen in a variety of cell types during hormonal stimulation. In response to hormones such as acetylcholine, vasopressin, or platelet-derived growth factor, responsive cells such as pancreatic acinar cells, hepatocytes, or cultured fibroblasts, respectively, undergo a pronounced increase in the hydrolysis of the plasma membrane lipid, phosphatidylinositol-4, 5-bisphosphate (PIP\(_2\)), releasing into the cytosol the water-soluble product, inositol-1, 4, 5-trisphosphate (IP\(_3\)), which, in turn, can trigger Ca\(^{2+}\) release from intracellular stores in these and other cells (see references 1 and 2 for review). That hydrolysis of PIP\(_2\), yielding IP\(_3\), may also be the trigger for Ca\(^{2+}\) release in eggs at fertilization is suggested by recent observations on sea urchin eggs. Fertilization of these eggs triggers a rapid turnover of PIP\(_2\) (24), and microinjection of IP\(_3\) into the unfertilized egg elicits fertilization envelope elevation, presumably due to cortical granule exocytosis triggered by an increase in [Ca\(^{2+}\)]\(_i\) (25).

In the present study, we demonstrate that iontophoresis of IP\(_3\) activates several aspects of early development in the eggs of a vertebrate—the frog, Xenopus laevis—and triggers a transient Ca\(^{2+}\) release essentially indistinguishable from that which we have previously shown to accompany fertilization (4). Finally, we report initial studies using a novel double-barreled microelectrode to observe the localized response of [Ca\(^{2+}\)]\(_i\) to subthreshold doses of IP\(_3\), and suggest, based on these data, that the egg may contain two functionally distinct pools of mobilizable Ca\(^{2+}\).

MATERIALS AND METHODS

Procurement and Handling of Gametes: Xenopus laevis eggs were obtained and treated as previously described (4). The nominally Ca\(^{2+}\)-free medium used in certain experiments, as specified below, was standard F1 solution (17), except that no Ca\(^{2+}\) was added, and Ca\(^{2+}\) contamination was controlled by adding 1 mM EGTA. The resulting free Ca\(^{2+}\) concentration, as determined with the Ca\(^{2+}\)-selective microelectrodes described below, was <0.1 µM. All other experiments were performed in standard F1.

Abbreviations used in this paper. [Ca\(^{2+}\)]\(_i\), intracellular free Ca\(^{2+}\) concentration; IP\(_3\), inositol-1, 4-bisphosphate; IP\(_2\), inositol-1, 4, 5-trisphosphate; pCa, negative log of free Ca\(^{2+}\) concentration; PIP\(_2\), phosphatidylinositol-4, 5-bisphosphate.
Electrophysiological Recording and Iontophoresis: Measurements of \([Ca^{2+}]_i\) employing conventional single-barreled \(Ca^{2+}\)-selective microelectrodes were performed as previously described (4), except that \(Ca^{2+}\)-microelectrode tips were bevelled (instead of broken) before filling but after dipping in the polyvinyl-chloride-gelled sensor, using a jet-stream microevolver (19).

For studies of the local response of \([Ca^{2+}]_i\) to iontophoresis of IP3, double-barreled microelectrodes were constructed. Commercial double-barreled capillary tubing (A-M Manufacturing, Spokane, WA) was pulled in a single stage on a vertical puller to yield typical double-barreled micropipets. The two barrels were then separated by gently forcing a razor blade's edge between them, beginning at the back end and advancing toward the tip. One barrel was then silanized as previously described (4) and used to construct a \(Ca^{2+}\)-selective microelectrode as described above. The two barrels were then rejoined with cyanocrylate adhesive, using two micromanipulators and observing the tips at a magnification of 250. These electrodes functioned optimally when the two tips were left separated by 10–20 \(\mu\)m, and the results reported here are from such electrodes. After reassembly, the untreated barrel was loaded with the specified iontophoresis solution as described below.

All iontophoresis electrodes were backfilled with 1-mM solutions of either IP1, inositol-1, 4-bisphosphate (IP3), or fructose-1, 6-bisphosphate, as specified, in every case in 0.1 mM HEPES, pH 7.8. To conserve IP3, no more than 2 \(\mu\)l of solution was used in an iontophoresis electrode. Electrical connection with this filling solution was made via an Ag/AgCl wire glued into the iontophoresis electrode. Negative current was injected between the iontophoresis electrode and bath ground using the constant current injection circuit of a Biodyne amplifier (model 5DI0, Tektronix, Inc., Beaverton, OR), and both this oscilloscope and an Anapulse stimulator (model 301, W-P Instruments, Inc., New Haven, CT) were used to deliver the constant current injection circuit of a Biodyne amplifier using a Tektronix digital waveform analyzer (model 5D10, Tektronix, Inc., Beaverton, OR), and both this oscilloscope and a chart recorder were used to record the \(Ca^{2+}\) electrode output.

Cell impalement with single-barreled iontophoresis electrodes was achieved with the aid of a custom-built piezoelectric advance that rapidly advanced the electrode \(\sim 20 \mu\m\) to achieve shallow impalement. Deep impalements were achieved by manually advancing the iontophoresis electrode \(\sim 100–200 \mu\m\) beyond the initial shallow impalement using a micro-manipulator. During withdrawal from the egg, shallowly implanted electrodes (viewed under a dissecting microscope at 120\(\times\)) emerged from the cell readily, whereas with deeply implanted electrodes, a substantial (\(\sim 100 \mu\m\)) length of the electrode shaft could be seen to emerge slowly from the adhering plasma membrane of this 1.300-\(\mu\m\)-diam egg. Judged by this latter criterion, shallow impalements were determined using the Student's t test for unpaired data. The criterion for significance was \(P < 0.05\).

RESULTS

**IP3 Specifically Activates Xenopus Eggs**

To determine the minimum doses of IP3 or IP3 necessary to activate eggs, a sequence of increasingly larger injections (as specified below), separated by 4-min intervals, was performed on each egg. This interval was chosen because repeated subthreshold IP3 injections at 4-min intervals never activated eggs and, as we shall show below, the localized \([Ca^{2+}]_i\) increase elicited by a subthreshold IP3 injection required \(\sim 4 \text{ min}\) to return to the initial resting \([Ca^{2+}]_i\) level. The primary criteria used to score egg activation were a rapid, transient plasma membrane depolarization (the so-called activation potential), followed \(\sim 5 \text{ min}\) later by contraction of the pigmented animal hemisphere toward the animal pole (cortical contraction). After removal of the electrodes, a subset of eggs was further observed for rotation within the vitelline envelope (reflecting cortical granule exocytosis) and abortive cleavage furrow formation (so-called pseudocleavage, typical of artificially activated eggs). In every instance, eggs which displayed an activation potential and cortical contraction also rotated and pseudocleaved. As reviewed in reference 10, all these events are typical of activated frog eggs.

For eggs shallowly impaled (see Materials and Methods) in the animal hemisphere with a single-barreled iontophoresis electrode, injection of as little as 0.32 nC from an IP3-filled electrode \((3.2 \text{ nA for } 100 \text{ ms})\) triggered a rapid depolarization of the plasma membrane (Fig. 1) and cortical contraction, signaling egg activation. Using a sequence of 100-ms injections beginning at 3.2 nA and increasing by about this same value at each step, the minimum activating charge ranged from 0.32 to 1.6 nC, and averaged 0.84 \(\pm\) 0.04 nC for nine eggs from three females. Assuming, as a first approximation, that all of the outward component of this charge was carried by IP3 with a net molecular charge of \(-6\), this would represent a mean activating dose of 0.73 fmol IP3 per egg. This value is an upper limit only, however. The actual dosage of IP3 delivered may have been as little as a fifth of this value, since transport numbers for iontophoresis of anions can be \(<0.1\) (20), rather than the ideal value of 0.5 we have assumed here.

Eggs deeply impaled with the iontophoresis electrode required significantly more IP3 to trigger their activation. Using a sequence of 33-nA injections of 0.1, 0.2, . . ., 1.0-s durations, the minimum activating charge ranged from 6.6 nC to 32.8 nC for ten eggs from three females, and averaged 18.0 \(\pm\) 0.8 nC (significantly greater than the average activating charge of 0.84 nC for shallow injections; \(P < 0.01\)). In contrast with shallow IP3 injections, which invariably triggered activation potentials either during or immediately after the 100-ms injection interval (Fig. 1), activation potentials after deep injections of IP3 commenced an average of 9 \(\pm\) 2 s \((n = 10)\) after onset of the iontophoresis current.

To ensure that the activation elicited by IP3 iontophoresis was specific for this compound and not due, for instance, to
some indirect effect of current injection, we tested the effect of iontophoresing fructose-1, 6-bisphosphate into shallowly impaled eggs. For seven eggs from two females, fructose-1, 6-bisphosphate injections of 131 nC (32.8 nA for 4 s) never elicited any sign of activation. In contrast, shallow injections of IP$_2$ did activate eggs, but only at much higher doses than those required with IP$_3$. Using sequential 33-nA injections of 0.5, 1.0, ... 3.0-s durations, the minimum activating charge ranged from 33 to 99 nC and averaged 60.9 ± 3.2 nC for seven eggs from three females. Based on a net molecular charge of ~4 and transport number of 0.5, this represents an upper limit of 79 fmol IP$_2$ per egg, more than 100 times the average calculated dose of IP$_3$ required to trigger activation. We cannot rule out the likely possibility that this apparent activating effect of IP$_2$ was due to the minor contaminant detected in this preparation (see Materials and Methods).

IP$_3$-Induced Activation Involves a Transient [Ca$^{2+}$], Increase Identical to That Accompanying Fertilization

The ability of microinjected IP$_3$ to trigger fertilization envelope elevation in sea urchin eggs, as recently reported by Whitaker and Irvine (25), was presumed by them to be due to an IP$_3$-induced transient increase in [Ca$^{2+}$]. To determine whether a [Ca$^{2+}$] increase accompanied IP$_3$-induced activation in Xenopus eggs, we used Ca$^{2+}$-selective microelectrodes to measure [Ca$^{2+}$], directly during IP$_3$ iontophoresis. Fertilization of Xenopus eggs triggers an increase in [Ca$^{2+}$], beginning (as detected by deep impaling electrodes) ~1 min after the activation potential, rising over 2 min from an average resting level of 0.4 µM (pCa 6.4 [negative log of free Ca$^{2+}$ concentration]) to a peak of 1.2 µM (pCa 5.9), and returning over 10 min to its initial value (4). As shown in Fig. 2, iontophoresis of a nonactivating dose of IP$_3$ (henceforth referred to as a sub-threshold dose) had no detectable effect on [Ca$^{2+}$], measured ~275 µm from the site of iontophoresis (point 1 in Fig. 2), while an activating dose of IP$_3$ (here, 16 nC; point 2) triggered a transient [Ca$^{2+}$], increase (as detected by deeply impaling electrodes) ~1 min after the activation potential, rising over 2 min from an average resting level of 0.25 µM (pCa 6.6 ± 0.1) to 1.58 µM (pCa 5.8 ± 0.2) as observed at a Ca$^{2+}$-selective microelectrode located >250 µm (along the egg surface) from the iontophoresis electrode.

In three experiments with eggs from two females, activating injections of IP$_3$ triggered transient [Ca$^{2+}$], increases, from an average resting level of 0.25 µM (pCa 6.6 ± 0.1) to 1.58 µM (pCa 5.8 ± 0.2) as observed at a Ca$^{2+}$-selective microelectrode located >250 µm (along the egg surface) from the iontophoresis electrode. As we shall show below, the local [Ca$^{2+}$] response to IP$_3$ injection begins almost immediately at the injection site; hence, the 31-s delay between iontophoresis and detection of the Ca$^{2+}$ pulse in Fig. 2 reflects a propagation rate of 8.9 µm/s (here, at 22.5°C), in good agreement with the propagation rate of 9.7 ± 1.5 µm/s previously determined for the Ca$^{2+}$ wave accompanying fertilization in these eggs (4).

Comparison of IP$_3$- and Iontophore-induced Ca$^{2+}$ Release Using Double-Barreled Microelectrodes

As discussed above, the transient [Ca$^{2+}$], increase seen in Fig. 2 and similar experiments is apparently identical to the propagated wave of [Ca$^{2+}$], increase that we have previously shown to traverse the egg as a band of elevated free Ca$^{2+}$ concentration at fertilization (4). To study separately the characteristics of the local response of [Ca$^{2+}$], to injection of IP$_3$ and the (possibly distinct) global Ca$^{2+}$ release which constitutes the propagated Ca$^{2+}$ wave in Xenopus eggs, we have employed a novel double-barreled microelectrode incorporating both a Ca$^{2+}$-selective microelectrode and an IP$_3$ iontophoresis electrode whose tips are separated by no more than 20 µm. As shown in Fig. 3A, passage of current through the iontophoresis barrel (here, filled with IP$_3$ solution) elicited a transient spike artifact in the Ca$^{2+}$ electrode output due, presumably, to charging of the latter electrode's capacitance during current ejection from the nearby iontophoresis electrode. Similar artifacts, requiring ~4 s to recover, were consistently observed whether the electrode tip was in a cell (Fig. 3A, points 1-5) or simply immersed in buffer (Fig. 3A, point 6) when the iontophoresis electrode was filled with IP$_3$ (which, as discussed above, did not trigger activation at the doses used here). No longer-lasting response to IP$_3$ iontophoresis was observed intracellularly, indicating that IP$_3$ at the doses used here did not trigger detectable Ca$^{2+}$ release in the egg. The typical Ca$^{2+}$ pulse observed here during activation with the Ca$^{2+}$ ionophore, A23187 (2 µM), demonstrated that the Ca$^{2+}$ electrode could detect [Ca$^{2+}$] changes.

In contrast with the absence of an observable local [Ca$^{2+}$], response to IP$_3$ iontophoresis, iontophoresis of subthreshold doses of IP$_3$ into the unactivated egg elicited both the initial current-flow artifact discussed above as well as a further, transient increase in [Ca$^{2+}$], requiring ~4 min to recover to the initial resting value of [Ca$^{2+}$], (Fig. 3B, points 2-5). This latter response was only observed intracellularly and never when the double-barreled electrode was simply immersed in buffer (Fig. 3B, points 1 and 10), indicating that it accurately reflects a localized increase of [Ca$^{2+}$], in response to IP$_3$ injection. Injection of increasing amounts of IP$_3$ demonstrated a typical dose-response relationship by [Ca$^{2+}$], in both the unactivated (Fig. 3B, points 2-5) and activated egg (points 7-9) with larger [Ca$^{2+}$], increases in response to larger doses of IP$_3$. As illustrated in Fig. 2, transient [Ca$^{2+}$], increases were
that the IP3-sensitive Ca\(^{2+}\) pool in the Xenopus egg were widely separated along the surface of this 1.3-mm-diam egg. After withdrawal from egg, indicating the local and nonpropagated nature of the [Ca\(^{2+}\)] response to subthreshold injections of IP\(_3\) (illustrated in Fig. 4B), whereas six showed little or no difference between the response in Ca\(^{2+}\)-free and Ca\(^{2+}\)-containing media (Fig. 4A). Additionally, even eggs that displayed a decreased IP\(_3\) response in Ca\(^{2+}\)-free medium for a minimum of 5 min before initiating iontophoresis. Thus, these studies suggest that both the subthreshold IP\(_3\) and the major Ca\(^{2+}\) pulse at activation are due to a release of sequestered Ca\(^{2+}\) from some intracellular store(s). It appears, however, that under conditions in which cytosolic Ca\(^{2+}\) might leak out of the cell (due to impalement damage in Ca\(^{2+}\)-free medium), the IP\(_3\)-sensitive intracellular Ca\(^{2+}\) pool can be partially depleted, even under conditions that apparently do not decrease the magnitude of the major Ca\(^{2+}\) pulse at activation.

**DISCUSSION**

**New Findings**

Using Ca\(^{2+}\)-selective microelectrodes, we have directly demonstrated for the first time with germ cells what has previously been demonstrated with somatic cells from a variety of vertebrates, i.e., that intracellular Ca\(^{2+}\) stores can be specifically mobilized, leading to transient increases in [Ca\(^{2+}\)], by the intracellular application of very small doses of IP\(_3\). This is also the first demonstration that IP\(_3\) can mobilize intracellular Ca\(^{2+}\) stores in intact cells, since all previous studies in which Ca\(^{2+}\) release was directly measured have utilized permeabilized cell models to facilitate delivery of exogenously applied Ca\(^{2+}\) ions.

The lack of response to subthreshold IP\(_3\) injection in Ca\(^{2+}\)-free medium illustrated here has only been observed in two eggs. Unfortunately, we did not perform prior controls using Ca\(^{2+}\)-containing media with these two cells (as was done in the experiment of Fig. 4A and all other experiments).

\[\text{[Ca}^{2+}\text{]}_i \text{ Increases during IP}_3 \text{ Injection and Activation Reflect Release of Ca}^{2+} \text{ from Intracellular Stores}\]

To determine the source (i.e., intracellular store or extracellular medium) of the Ca\(^{2+}\) released into the cytosol either in response to a subthreshold IP\(_3\) injection or during the major Ca\(^{2+}\) pulse accompanying activation, we studied the response of [Ca\(^{2+}\)] in eggs bathed in nominally Ca\(^{2+}\)-free medium (Fig. 4). These experiments were complicated, however, by the destabilizing effect of Ca\(^{2+}\)-free media on the egg plasma membrane, as indicated by both a decrease in membrane potential under Ca\(^{2+}\)-free conditions (Fig. 4A) and, occasionally, by a clearing of pigment from around the electrode impalement sites (this was observed in the egg used in Fig. 4B, but not in that of Fig. 4A). For the eight eggs (from five females) studied, two failed to display a typical local [Ca\(^{2+}\)] response to subthreshold injections of IP\(_3\); (illustrated in Fig. 4B), whereas six showed little or no difference between the response in Ca\(^{2+}\)-free and Ca\(^{2+}\)-containing media (Fig. 4A). Additionally, even eggs that displayed a decreased IP\(_3\) response in Ca\(^{2+}\)-free medium for a minimum of 5 min before initiating iontophoresis. Thus, these studies suggest that both the subthreshold IP\(_3\) response and the major Ca\(^{2+}\) pulse at activation are due to a release of sequestered Ca\(^{2+}\) from some intracellular store(s). It appears, however, that under conditions in which cytosolic Ca\(^{2+}\) might leak out of the cell (due to impalement damage in Ca\(^{2+}\)-free medium), the IP\(_3\)-sensitive intracellular Ca\(^{2+}\) pool can be partially depleted, even under conditions that apparently do not decrease the magnitude of the major Ca\(^{2+}\) pulse at activation.
The following intervals: (I) 100 ms, injected into calibration buffer displayed by six of eight eggs, illustrating lack of dependence on Ca$^{2+}$-free FI with 1 mM EGTA (indicated by bar). Traces at bottom free Ca$^{2+}$ (pCa$^{-}$) recordings during iontophoresis with IP3 of eggs bathed in complete F1, containing 0.25 mM Ca$^{2+}$, or in nominally Ca$^{2+}$-free F1 with 1 mM EGTA (indicated by bar). Traces at bottom left and right in each figure show Ca$^{2+}$ electrode calibrations (pCa 7, 6.5, and 6) before and after impalement. (A) Typical result displayed by six of eight eggs, illustrating lack of dependence on external Ca$^{2+}$. All injections used a 30-nA iontophoresis current for the following intervals: (1) 100 ms, injected into calibration buffer before impalement. (2) 10 ms. (3) 20 ms. (4) 35 ms. (5-7) Same as 2-4, respectively, but after changing bathing medium to Ca$^{2+}$-free F1. (B) Response displayed by two of eight eggs, illustrating partial dependence on external Ca$^{2+}$. All injections were 30 nA for the following intervals: (1) 100 ms, injected into calibration buffer before impalement. (2) 10 ms. (3) 20 ms. (4) 50 ms. (5) 100 ms. (6) 300 ms. Note resolution of the local IP3 response from the major Ca$^{2+}$ pulse accompanying activation. (7) 20 ms. (8) 75 ms. (9) 100 ms. (10) 300 ms.

IP3 to its intracellular target(s) (see references 1 and 2 for review). Since cell permeabilization inevitably leads to uncontrollable and nonphysiological changes in the internal milieu, with potentially confounding effects, the present demonstration of IP3-induced Ca$^{2+}$ release in intact cells is an important addition to the study of IP3-mediated regulation of [Ca$^{2+}$].

Our present report also demonstrates for the first time that vertebrate eggs can be triggered to initiate the early events of development by intracellular application of low doses of IP3 (presumably due to the transient increase in [Ca$^{2+}$], just discussed), thus confirming and expanding upon the recent report by Whitaker and Irvine (25) that microinjection of IP3 activates sea urchin eggs (see Noted Added in Proof). These authors used as the criteria for egg activation the elevation of the fertilization envelope (reflecting cortical granule exocytosis) and the increase in intracellular fluorescein fluorescence which signals an increase in intracellular pH (another event accompanying egg activation; see reference 3 for review). In our studies, we have used an extensive set of criteria, including the activation potential (a transient depolarization of the plasma membrane), cortical contraction, cortical granule exocytosis (reflected by egg rotation within the vitelline envelope), and the abortive cleavage furrow formation (pseudocleavage) which commonly accompanies artificial activation of unfertilized frog eggs. Thus, the present study demonstrates that a wide variety of processes that normally comprise the early development of the zygote are all triggered by IP3. In light of the previous demonstration that fertilization triggers a dramatic activation of PIP2 hydrolysis in the sea urchin egg (24), these findings provide compellingly suggestive evidence that Ca$^{2+}$-mediated developmental activation of eggs may be triggered at fertilization by the generation of IP3. It remains to be demonstrated directly that fertilization triggers a sufficient increase in cytosolic IP3 concentration in any egg to elicit the responses seen with artificial applications of IP3.

**Characteristics of the Subthreshold IP3-induced Ca$^{2+}$ Release and the Ca$^{2+}$ Pulse Accompanying Activation**

Development of the double-barreled IP3-iontophoresis/Ca$^{2+}$-measurement electrode has enabled us to begin the in vivo characterization of the mobilizable Ca$^{2+}$ pool(s) responsible for the transient [Ca$^{2+}$], increase accompanying the activation of Xenopus eggs. As shown in Fig. 4 (A and B), both the localized Ca$^{2+}$ release triggered by IP3 iontophoresis, as well as the major [Ca$^{2+}$], increase at activation, are observable in eggs in Ca$^{2+}$-free medium, suggesting that both responses arise from the release into the cytosol of Ca$^{2+}$ sequestered in some intracellular store(s). Several lines of evidence suggest that the localized IP3-triggered [Ca$^{2+}$], response documented in the present study and the global Ca$^{2+}$ wave at activation (4) arise from the activities of functionally (and, perhaps, structurally) distinct Ca$^{2+}$ pools. First, local IP3-induced Ca$^{2+}$ release can be demonstrated in either the unactivated or activated egg; in contrast, the ionophore-triggered Ca$^{2+}$ pulse can only be elicited in the unactivated egg (Fig. 3 B). In this respect, ionophore- (but not IP3-) triggered Ca$^{2+}$ release coincides with the presence of cortical endoplasmic reticulum-plasma membrane junctions in the Xenopus egg, which have been implicated in [Ca$^{2+}$], regulation (4a, 5, 15), since these junctions are numerous in the unactivated egg but apparently disappear within 30–60 s of the completion of cortical granule exocytosis (15). Secondly, as seen in Fig. 4 B (point 6), the local IP3 response (even in the absence of external Ca$^{2+}$) and the major Ca$^{2+}$ pulse that it elicits can be temporally separated at a discrete site in the cytoplasm. Further, even when the IP3-sensitive store is partially depleted, a major Ca$^{2+}$ pulse of normal magnitude can still be observed to accompany activation. Interestingly, while the egg in Fig. 4 B failed to respond to a 3-nC injection of IP3 before activation, it responded normally to this same dose after the major Ca$^{2+}$ pulse accompanying activation (compare points 5 and 9), suggesting the...
possibility that the Ca\(^{2+}\) released by the major pulse may have partially recharged the IP\(_3\)-sensitive store. Finally, even at the peak of the [Ca\(^{2+}\)]\(_c\) transient elicited by A23187, a further incremental increase in [Ca\(^{2+}\)]\(_c\) can still be achieved by injection of IP\(_3\) (Fig. 3B). All of these observations are consistent with the possibility that the Ca\(^{2+}\) released by the major pulse may have sufficient IP\(_3\) into the cortical cytosol to elicit a local increase in [Ca\(^{2+}\)]\(_c\) via Ca\(^{2+}\) release from the IP\(_3\)-sensitive pool. This local response, in turn, may then trigger a more extensive Ca\(^{2+}\)-induced Ca\(^{2+}\) release from the second (presumably IP\(_3\)-insensitive) pool, which would propagate across the egg via further Ca\(^{2+}\)-induced Ca\(^{2+}\) release (as previously suggested by Gilkey et al. [16]), thus giving rise to the Ca\(^{2+}\) wave we have previously documented with this egg. The principal role of this latter release is presumably to effect this propagated Ca\(^{2+}\) wave (observed, to date, in the eggs of four species [4, 8, 9, 16], but not in any somatic cell type) to protect against polyspermy by triggering the propagated excocytosis of the cortical granules. It may thus be of no further utility (and is therefore inactivated) after the initiation of development, leaving only the IP\(_3\)-sensitive Ca\(^{2+}\) pool which appears to be a rather common feature of somatic cells. This model differs from that previously proposed for the activating sea urchin egg by Whitaker and Irvine (25), who envisioned "...a mechanism in which IP\(_3\)-stimulated calcium release and calcium-stimulated IP\(_3\) production cooperate to produce a wave of calcium release which propagates through the egg cytoplasm," in that it substitutes Ca\(^{2+}\)-induced Ca\(^{2+}\) release for Ca\(^{2+}\)-stimulated IP\(_3\) production as the mechanism responsible for propagation of the Ca\(^{2+}\) wave beyond the sperm entry site. Both models, however, propose IP\(_3\) production via IP\(_3\) hydrolysis as the proximate mechanism coupling sperm/egg interaction with the initial mobilization of intracellular Ca\(^{2+}\). While these two models are not mutually exclusive, and might even simply reflect species differences, it is important to attempt to distinguish between them, as we are presently doing.

The notion that a cortically located Ca\(^{2+}\) pool (e.g., the cortical endoplasmic reticulum forming junctions with the plasma membrane) which exhibits Ca\(^{2+}\)-induced Ca\(^{2+}\) release may be responsible for the propagated Ca\(^{2+}\) wave at activation is in keeping with our observation that 20-fold more IP\(_3\) is required to trigger activation when injected deeply (as opposed to shallowly) into the egg. If the local [Ca\(^{2+}\)] increase induced by IP\(_3\) triggers the major Ca\(^{2+}\) pulse via Ca\(^{2+}\)-induced Ca\(^{2+}\) release, as we have suggested, then the depth dependence of the IP\(_3\) dose required to trigger activation could reflect the necessity for the IP\(_3\)-triggered [Ca\(^{2+}\)]\(_c\) increase to reach these cortical targets. Larger doses of IP\(_3\) elicit larger local [Ca\(^{2+}\)]\(_c\) increases (Fig. 3), which, by diffusion, should involve larger volumes of cytosol. According to our model, then, deeply injected IP\(_3\) should only trigger a global Ca\(^{2+}\) wave when the local [Ca\(^{2+}\)] increase it elicits can reach the egg cortex.

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Note Added in Proof: While this paper was in press, it was reported that microinjection of IP\(_3\) triggers activation of mature Xenopus oocytes (Picard, A., F. Giraud, F. Le Boutant, F. Sladeczek, C. Le Peuch, and M. Dorée, 1985, FEBS [Fed. Eur. Biochem. Soc.] Lett., 182:446-450).

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