Depletion of endoplasmic reticulum Ca\textsuperscript{2+} stores induces Ca\textsuperscript{2+} entry from the extracellular space by a process termed “store-operated Ca\textsuperscript{2+} entry” (SOCE). It has been suggested that the novel fungal metabolite adenophostin-A may be able to stimulate Ca\textsuperscript{2+} entry without stimulating Ca\textsuperscript{2+} release from stores. To test this idea further, we compared Ca\textsuperscript{2+} release, SOCE, and the stimulation of Ca\textsuperscript{2+}-activated Cl\textsuperscript{−} currents in response to inositol 1,4,5-trisphosphate (IP\textsubscript{3}) and adenophostin-A injection. IP\textsubscript{3} stimulated an outward Cl\textsuperscript{−} current, \(I_{\text{Cl}1-S}\), in response to Ca\textsuperscript{2+} release from stores followed by an inward current, \(I_{\text{Cl}2}\), in response to SOCE. In contrast, low concentrations of adenophostins (AdAs) activated \(I_{\text{Cl}2}\) without activating \(I_{\text{Cl}1-S}\) consistent with the suggestion that AdA can activate Ca\textsuperscript{2+} entry without stimulating Ca\textsuperscript{2+} release. However, when Ca\textsuperscript{2+} entry has been stimulated by AdA, Ca\textsuperscript{2+} stores are largely depleted of Ca\textsuperscript{2+}, as assessed by the inability of ionomycin to release additional Ca\textsuperscript{2+}. The Ca\textsuperscript{2+} release stimulated by AdA, however, was 7 times slower than the release stimulated by IP\textsubscript{3}, which could explain the minimal activation of \(I_{\text{Cl}1-S}\) when Ca\textsuperscript{2+} is released slowly, the threshold level required for \(I_{\text{Cl}1-S}\) activation is not attained.

\(Ca^{2+}\) signals regulate many cellular processes including cell growth, fertilization, gene transcription, and apoptosis (1). Increases in cytosolic Ca\textsuperscript{2+} levels are produced both by Ca\textsuperscript{2+} released from internal stores and Ca\textsuperscript{2+} influxed from the extracellular space. A major pathway for Ca\textsuperscript{2+} mobilization from internal stores is through inositol 1,4,5-trisphosphate receptors (IP\textsubscript{3}R)\textsuperscript{a} after stimulation of G-protein- or tyrosine kinase-coupled plasma membrane receptors linked to phospholipase C (2–4). The decrease in the Ca\textsuperscript{2+} content of the internal store then stimulates Ca\textsuperscript{2+} entry through plasma membrane store-operated Ca\textsuperscript{2+} channels (SOCs) by a process called store-operated Ca\textsuperscript{2+} entry (SOCE) (5, 6). The mechanism by which a reduction in the content of store Ca\textsuperscript{2+} results in opening of SOCs remains unknown, but there are two major hypotheses. The conformational coupling hypothesis suggests that there is direct physical contact between IP\textsubscript{3}R and SOCs such that conformational changes in the IP\textsubscript{3}R occurring upon Ca\textsuperscript{2+} depletion of the internal store can affect the opening of SOCs (7, 8). The diffusible messenger hypothesis suggests that the Ca\textsuperscript{2+} store (endoplasmic reticulum) produces a diffusible messenger that opens SOCs (9, 10).

Recently, a novel family of compounds called adenophostins (AdAs), which are structurally distinct from IP\textsubscript{3}\textsuperscript{a}, have been isolated from cultures of the fungus Penicillium brevicompactum (11, 12). The AdAs are 10–100-fold more potent than IP\textsubscript{3} in opening IP\textsubscript{3}R\textsubscript{b} and are capable of activating all three IP\textsubscript{3}R subtypes (13–15). Recently, Hartzell et al. (16) and DeLisle et al. (17) have shown that in Xenopus oocytes, low concentrations of AdA stimulate Ca\textsuperscript{2+}-activated Cl\textsuperscript{−} currents that are activated by Ca\textsuperscript{2+} influx more than Cl\textsuperscript{−} currents that are activated by Ca\textsuperscript{2+} released from stores. Based on these observations, DeLisle et al. (17) suggested that AdA may be capable of activating store-operated Ca\textsuperscript{2+} entry without first stimulating Ca\textsuperscript{2+} release from stores. This is significant because it suggests that AdA may share structural features with the putative diffusible Ca\textsuperscript{2+} entry signal released by Ca\textsuperscript{2+}-depleted endoplasmic reticulum.

Ca\textsuperscript{2+}-activated Cl\textsuperscript{−} currents have been used for many years as real time indicators of sub-plasmalemmal Ca\textsuperscript{2+} in Xenopus oocytes (18–24), but clearly Cl\textsuperscript{−} currents are only indirect indicators of Ca\textsuperscript{2+} concentration. Consequently, conclusions about cytosolic Ca\textsuperscript{2+} concentration derived from these measurements are subject to different interpretations. We have recently found that there are two Ca\textsuperscript{2+}-activated Cl\textsuperscript{−} currents in the oocyte that are selectively activated by Ca\textsuperscript{2+} released from stores and by Ca\textsuperscript{2+} influx (24). The Ca\textsuperscript{2+} release-activated Cl\textsuperscript{−} current (\(I_{\text{Cl}1-S}\)) has an outwardly rectifying steady-state current-voltage relationship, whereas the Ca\textsuperscript{2+} influx-activated Cl\textsuperscript{−} current (\(I_{\text{Cl}2}\)) has an inwardly rectifying steady-state current-voltage relationship (24). This means that Ca\textsuperscript{2+}-activated Cl\textsuperscript{−} currents measured at constant negative membrane potentials, as was done in the experiments of DeLisle et al. (17), are relatively insensitive indicators of Ca\textsuperscript{2+} released from stores. In our experiments (16), we measured \(I_{\text{Cl}1-S}\) as an outward current at positive membrane potentials and \(I_{\text{Cl}2}\) as an inward current at negative membrane potentials to more clearly differentiate between Ca\textsuperscript{2+} influx and Ca\textsuperscript{2+} release and to increase the sensitivity of detection of Ca\textsuperscript{2+} release. Using this protocol, IP\textsubscript{3} activated both \(I_{\text{Cl}1-S}\) ("Ca\textsuperscript{2+} release") and \(I_{\text{Cl}2}\) ("Ca\textsuperscript{2+} influx"), but low concentrations of AdA often activated only a tiny amount of \(I_{\text{Cl}1-S}\), even though \(I_{\text{Cl}2}\) was robustly activated. But, because we could not find a concentration of AdA which could activate \(I_{\text{Cl}2}\) without activating some small amount of \(I_{\text{Cl}1-S}\), we concluded that AdA did not activate SOCE independently of Ca\textsuperscript{2+} release from stores. We hypothesized that AdA activated relatively little \(I_{\text{Cl}1-S}\) either because AdA released Ca\textsuperscript{2+} from stores very slowly or that AdA released Ca\textsuperscript{2+} from a subpopulation of stores which was tightly coupled to SOCs.


**Ca\(^{2+}\) Release by Adenophostin A and IP\(_3\) in Xenopus Oocytes**

The purpose of this paper was to examine further the mechanisms of AdA regulation of Ca\(^{2+}\)-activated Cl\(^{-}\) currents using confocal scanning microscopy of oocytes loaded with fluorescent Ca\(^{2+}\) indicators and two-microelectrode voltage clamp. Here we show that activation of SOCE following injection of low concentrations of AdA depends upon depletion of intracellular Ca\(^{2+}\) stores. However, at low AdA concentrations the kinetics of Ca\(^{2+}\) release from stores was greater than that observed with IP\(_3\). This slower mode of Ca\(^{2+}\) release is apparently not effective in activating \(I_{\text{Cl,L-S}}\). Therefore, different kinetics of Ca\(^{2+}\) release can differentially affect Cl\(^{-}\) current activation.

### EXPERIMENTAL PROCEDURES

**Isolation of Xenopus Oocytes**—Stage V-VI oocytes were harvested from adult albino or normal Xenopus laevis females (Xenopus 1) as described by Dascal (18). *Xenopus* were anesthetized by immersion in Tricaine (1.5 g/liter). Ovarian follicles were removed and digested in normal Ringer containing 123 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl\(_2\), 1.8 mM MgCl\(_2\), 5 mM HEPES, pH 7.4; Ca\(^{2+}\)-free Ringer solution was the same except that CaCl\(_2\) was omitted and MgCl\(_2\) was increased to 5 mM.

Oocytes were injected with IP\(_3\) using a Nanoject automatic oocyte injector (Drummond Scientific Co., Broomall, PA). Solutions of IP\(_3\) or AdA were prepared in normal Ringer with no added calcium, containing 2 mg/ml collagenase type IA (Sigma Chemical Co., St. Louis, MO), for 2 h at room temperature. The oocytes were extensively rinsed with normal Ringer, placed in L-15 medium (Life Technologies, Inc., Gaithersburg, MD) and stored at 18 °C. Oocytes were usually used within 1–5 days after isolation.

**Imaging and Electrophysiological Methods**—Xenopus oocytes were injected with 9 nl Ca-green-1 coupled to 70-kDa dextran (333 µM) for a final calculated oocyte concentration of ~3 µM, and voltage-clamped with two-microelectrodes using a GeneClamp 500 (Axon Instruments, Foster City, CA). Electrodes were filled with 3 M KCl and had resistances of 1–4 MΩ. Oocyte resting potentials were between −20 mV and −50 mV. Typically, the membrane was held at 0 mV and stepped to +40 mV for 1.5 s every 15 s to monitor \(I_{\text{Cl,T}}\). Every 2.25 min, a 1.5-s duration pulse to −140 mV followed by a 1.5-s duration pulse to +40 mV was given to monitor \(I_{\text{Cl,S}}\) and \(I_{\text{Cl,T}}\), respectively. Images (256 × 256 pixels) were acquired 500 ms after the onset of each voltage pulse using a Zeiss LSM 410 confocal box fitted to a Zeiss Axiovert 100 TV inverted microscope using a Zeiss 63× objective (0.5 numerical aperture). The confocal aperture was set at the maximal opening, resulting in a focal section 1267 × 1267 × 35 μm. Image data was analyzed using the LSM 410 software or NIH image 1.60 on a Mac IIfx. Current data was analyzed on a Pentium PC using Origin 5.0 (Microcal Software, Northampton, MA). For plots of Ca\(^{2+}\) fluorescence, the fluorescence intensity of the entire focal section was averaged and expressed as a ratio of the background fluoroscent taken before IP\(_3\) injection. Experiments were performed at room temperature (22–28 °C). Normal Ringer solution contained 123 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl\(_2\), 1.8 mM MgCl\(_2\), 10 mM HEPES, pH 7.4; Ca\(^{2+}\)-free Ringer solution was the same except that CaCl\(_2\) was omitted and MgCl\(_2\) was increased to 5 mM.

Oocytes were injected with IP\(_3\) using a Nanoject automatic oocyte injector (Drummond Scientific Co., Broomall, PA). The injection pipette was pulled from glass capillary tubing in a manner similar to the recording electrodes and then broken so that it had a beveled tip with an inside diameter ~20 μm. Solutions of IP\(_3\) or AdA were prepared in Chelex resin-treated H\(_2\)O. The Ca\(^{2+}\) concentration in this solution was not buffered, but injection of H\(_2\)O produced no change in Ca-green fluorescence. The voltage protocol used is shown at the top. a. Summary of Cl\(^{-}\) currents and Ca\(^{2+}\) fluorescence levels measured from the entire focal section and normalized to Ca\(^{2+}\)-dependent fluorescence before IP\(_3\) injection. At the end of the experiment, the oocyte was exposed to 140 mV pulses labeled \(I_{\text{Cl,S}}\) and at −140 mV (\(I_{\text{Cl,T}}\)) to release any residual Ca\(^{2+}\) from stores (see Fig. 6). This cell is representative of 13 cells.
fluorescence or membrane current. Levels of the IP$_3$R were lowered by injection of 60 ng of the IP$_3$R antisense primer (AACTAGACATCTTGTCAGACATTGCTGCA) one day before the experiment as described by Kume et al. (25). The reverse sense primer (CTGCAGCAATGTCAACACTAGACATCTTGTCAGAGACAAGATGTCTAGTT) was injected at the same level as a control.

**RESULTS**

**Ca$^{2+}$ Transient and Cl$^{-}$ Currents Activated by High Concentrations of IP$_3$—**The protocol used to measure Ca$^{2+}$-activated Cl$^{-}$ currents in Xenopus oocytes in response to IP$_3$ or AdA injection while simultaneously measuring cytosolic Ca$^{2+}$ with confocal microscopy and Ca-green dextran is shown in Fig. 1. About 30 min after injection of Ca-green dextran, the oocytes were voltage-clamped at 0 mV and stepped to +40 mV every 15 s to monitor $I_{\text{Cl1-S}}$ (current at the end of the +40 mV pulse, Fig. 1b) as an outward current at depolarizing potentials that is activated quickly (~10 s) after IP$_3$ injection by Ca$^{2+}$ released from intracellular stores (16, 24, 26). In addition, once every 2.25 min, the oocyte was also stepped to -140 mV to monitor $I_{\text{Cl2}}$ and then to +40 mV to monitor $I_{\text{Cl1-T}}$ (current at the end of the -140 mV pulse, Fig. 1c) as an inward current that is activated by Ca$^{2+}$ entry through SOCs driven by the negative membrane potential. $I_{\text{Cl1-T}}$ is a transient outward current (peak outward current during the +40 mV pulse, Fig. 1c) that was activated by a depolarizing pulse preceded by a hyperpolarizing pulse to stimulate Ca$^{2+}$ influx. The -140 mV pulse was given only every 2.25 min to minimize Ca$^{2+}$ influx (and store refilling) during the experiment. For a more detailed discussion of the Cl$^{-}$ currents see Hartzell and co-workers (24, 27, 28).

**Ca$^{2+}$ Waves Stimulated by AdA Are Very Slow—**Injection of large amounts of AdA (estimated intra-oocyte concentration ~20 μM). When saturating levels of IP$_3$ were injected, $I_{\text{Cl1-S}}$ (filled squares) was activated immediately. As the stores became depleted of Ca$^{2+}$ and SOCE developed, $I_{\text{Cl1-T}}$ (open triangles) and $I_{\text{Cl2}}$ (open circles) were activated. Injection of IP$_3$ caused a large increase in Ca$^{2+}$ fluorescence at all potentials (Fig. 1d) because of Ca$^{2+}$ release from stores. Before the peak fluorescence was reached, the fluorescence was the same at all potentials, but afterward the fluorescence during the -140 mV pulse became greater than the fluorescence during the +40 mV pulse. The difference between the fluorescence at -140 mV and +40 mV is the voltage-dependent Ca$^{2+}$ fluorescence, which we have shown is related to Ca$^{2+}$ entry through SOCs (28).
which are measured from the entire surface of the oocyte, increase as soon as Ca$^{2+}$ is released from stores near the injection site. However, the slow increase in Ca$^{2+}$ fluorescence partly reflects the very slow transit time of the Ca$^{2+}$ wave from the injection site to the confocal image plane ~1 mm away. There is some variability in the lag period between AdA injection and the increase in Ca$^{2+}$ fluorescence. This variability is most likely related to the depth and position of the injection pipette in the oocyte.

The Ca$^{2+}$ waves induced by injection of smaller amounts of AdA moved even more slowly. In Fig. 3a, typical traces of Ca$^{2+}$ fluorescence at +40 mV in response to injection of large amounts of IP$_3$ (~20 μM, filled squares), large amounts of AdA (~2 μM, open circles), and small amounts of AdA (~5 nM, open triangles) are superimposed. In the case of low concentrations of adenosphin, the time-to-peak of the Ca$^{2+}$ fluorescence was ~20 min. Fig. 3b shows averages of the time-to-peak of the Ca$^{2+}$ fluorescence to these injections. The time-to-peak for large concentrations of AdA was >2 times slower than for large concentrations of IP$_3$, and the time-to-peak for small concentrations of AdA was >7 times slower than for large concentrations of IP$_3$. It was not possible to measure the time-to-peak for small IP$_3$ concentrations because small IP$_3$ concentrations produced oscillating Ca$^{2+}$ waves that exhibited no clear peak. The slowness of the Ca$^{2+}$ wave is illustrated in a different way in the images in Fig. 3c. After injection of AdA, the spread of the Ca$^{2+}$ fluorescence is very slow relative to the spread of the IP$_3$-induced wave of Ca$^{2+}$ release.

These data confirm our earlier suggestion that AdA causes release of Ca$^{2+}$ from stores much more slowly than IP$_3$ does. These findings support the idea that small concentrations of AdA do not stimulate $I_{Cl-S}$ because slow release of Ca$^{2+}$ from stores does not elevate Ca$^{2+}$ in the vicinity of the Cl$^-$ channels to an activating level. This could occur if efflux and/or local Ca$^{2+}$ buffering removes free Ca$^{2+}$ as rapidly as it is released, so that an effective Ca$^{2+}$ concentration is not attained.

Small Concentrations of AdA Completely Deplete Ca$^{2+}$ Stores—Although Fig. 3 shows that low concentrations of AdA release Ca$^{2+}$ from stores, the question remains whether the stimulation of Ca$^{2+}$ entry by low concentrations of AdA is because of depletion of stores. For example, the AdA-stimulated Ca$^{2+}$ release might be so slow that the stores refill. To examine this question, we measured the effects of low concentrations of AdA (~5 nM) that did not activate $I_{Cl-S}$ on Ca$^{2+}$ store depletion. Fig. 4 shows the results of a typical experiment. Injection of 10 nl of 0.5 μM AdA did not detectably stimulate $I_{Cl-S}$ (Fig. 4, a–c), but both $I_{Cl-T}$ and $I_{Cl-2}$ developed robustly. $I_{Cl-T}$ and $I_{Cl-2}$ were dependent on extracellular Ca$^{2+}$, and their activation corresponded to the activation of SOCE (16). Ca$^{2+}$ fluorescence began to increase about 5 min after AdA injection and continued to increase for 20 min (Fig. 4d).

Voltage-dependent Ca$^{2+}$ fluorescence (open circles), which reflects SOCE, developed shortly after Ca$^{2+}$ release and remained at a high level for the duration of the experiment. To test whether stores were depleted of Ca$^{2+}$, ionomycin in Ca$^{2+}$-free Ringer was applied to release Ca$^{2+}$ from any remaining stores. Ionomycin had only a very small effect on $I_{Cl-L}$, and had no effect on the Ca$^{2+}$ fluorescence at +40 mV. This showed that the stores had been virtually completely depleted of Ca$^{2+}$ by AdA.

This result contrasts to that observed when small amounts of IP$_3$ were injected (Fig. 5). Concentrations of IP$_3$ that stimulated Ca$^{2+}$ influx, as determined by the presence of voltage-dependent Ca$^{2+}$ fluorescence and activation of $I_{Cl-T}$ and $I_{Cl-2}$, inevitably stimulated $I_{Cl-S}$. In some cells, as in Fig. 5, the increase in $I_{Cl-S}$ was not accompanied by a significant increase in Ca$^{2+}$ fluorescence, because the IP$_3$ effect was local and did not propagate into the region of the oocyte that was imaged. Both voltage-dependent Ca$^{2+}$ fluorescence and $I_{Cl-T}$ and $I_{Cl-2}$ eventually declined to baseline. Application of ionomycin at the end of the experiment evoked a large increase in Ca$^{2+}$ fluorescence and in $I_{Cl-S}$, showing that the stores were not completely depleted of Ca$^{2+}$.

To obtain a more quantitative measure of the extent of store depletion of Ca$^{2+}$, because the IP$_3$ effect was local and did not propagate into the region of the oocyte that was imaged. Both voltage-dependent Ca$^{2+}$ fluorescence and $I_{Cl-T}$ and $I_{Cl-2}$ eventually declined to baseline. Application of ionomycin at the end of the experiment evoked a large increase in Ca$^{2+}$ fluorescence and in $I_{Cl-S}$, showing that the stores were not completely depleted of Ca$^{2+}$.
depletion after injection of IP$_3$ or AdA, we calculated the ratio of ionomycin-induced Ca$^{2+}$ release to IP$_3$- or AdA-induced Ca$^{2+}$ release. This ratio gives a measure of the level of residual Ca$^{2+}$ in intracellular stores after IP$_3$R agonist injection. The results from these experiments are shown in Fig. 6. Injections of high IP$_3$, high AdA, or low AdA all left the stores largely depleted of Ca$^{2+}$. In contrast, low IP$_3$ concentrations were less effective in depleting the stores. These data show that concentrations of AdA that did not noticeably activate $I_{Cl1-S}$ were capable of depleting intracellular Ca$^{2+}$ stores to similar levels as high concentrations of IP$_3$.

Thus, we conclude that AdA stimulates SOCE as a consequence of depletion of internal Ca$^{2+}$ stores and not by some direct effect on SOCs. Furthermore, previous conclusions, based on Ca$^{2+}$-activated Cl$^-$ current activation, which suggested that low concentrations of AdA stimulate SOCE without releasing Ca$^{2+}$ from stores (17), can be explained by the observation that slow release of Ca$^{2+}$ from stores is often insufficient to activate $I_{Cl1-S}$.

**Effect of AdA on SOCE Requires Active IP$_3$R—**If this conclusion is correct, the effects of AdA on SOCE should depend on the ability of the IP$_3$R to release Ca$^{2+}$. Thus, treatments that suppress IP$_3$R function should inhibit the effects of AdA injections. We suppressed IP$_3$R function either by injecting the competitive inhibitor heparin (Fig. 7) or by reducing IP$_3$R expression by injection of antisense oligonucleotides to the Xenopus IP$_3$R (Fig. 8).

Injection of heparin to block the IP$_3$R significantly reduced $I_{Cl12}$ and $I_{Cl1-T}$ currents induced by small AdA injections (Fig. 7, a–b). In a similar fashion, heparin blocked the Cl$^-$ current response induced by IP$_3$ (Fig. 7, c–d). Reducing IP$_3$R levels by antisense oligonucleotides as described previously by others (25, 29) also reduced the effects of IP$_3$ and AdA treatments on $I_{Cl1-S}$ and $I_{Cl12}$ (Fig. 8). The effects of antisense treatment were less pronounced than the effects of heparin, but it was clear that antisense had a significant effect. Note that although antisense treatment inhibited $I_{Cl12}$ and $I_{Cl1-T}$ in response to IP$_3$ injection, there was no decrease in levels of $I_{Cl1-S}$ (Fig. 8d). Actually, $I_{Cl1-S}$ was slightly potentiated as compared with sense-injected cells (Fig. 8c). This observation could be explained if one assumes there are two distinct subpopulations of IP$_3$ receptors with differential turnover rates of the IP$_3$R. If we postulate the existence of subsets of stores, one close to the $I_{Cl1-S}$ Cl$^-$ channels containing IP$_3$Rs with a very slow turnover rate and a second located further from the Cl$^-$ channels containing an IP$_3$R population that turns over rapidly, then injection of antisense IP$_3$ oligonucleotides will reduce the levels of IP$_3$Rs in the latter subset faster, resulting in sufficient Ca$^{2+}$ release after IP$_3$ injection to activate $I_{Cl1-S}$ but insufficient release from most of the stores to induce significant SOCE.

**DISCUSSION**

In many cell types, release of Ca$^{2+}$ from endoplasmic reticulum stores stimulates Ca$^{2+}$ influx into the cytosol from the extracellular space through SOCs by a process termed SOCE. The mechanisms by which release of Ca$^{2+}$ from stores stimu-
lates SOCE is unknown, but one hypothesis states that the endoplasmic reticulum releases a diffusible chemical messenger that opens SOCs. The search for such a calcium influx factor has so far not been very fruitful, and the putative calcium influx factors that have been discovered have not found universal acceptance (9, 10). When it was suggested that AdA could stimulate Ca\(^{2+}\) influx without stimulating Ca\(^{2+}\) release from stores (17), some hope was raised that clues to the structure of calcium influx factors would be learned from AdA. The suggestion that AdA could stimulate Ca\(^{2+}\) entry without depleting Ca\(^{2+}\) from stores was based on the observation that low concentrations of AdA did not stimulate Cl\(^{-}\) currents in the absence of extracellular Ca\(^{2+}\) and therefore did not release Ca\(^{2+}\) from stores but did stimulate Ca\(^{2+}\)-activated Cl\(^{-}\) currents in the presence of Ca\(^{2+}\) influx. The present studies using Ca\(^{2+}\) imaging demonstrate, however, that even very low amounts of AdA (calculated oocyte concentration; 5 nM) released Ca\(^{2+}\) from stores. Under these conditions, even though \(I_{Cl1-S}\) was not activated, the stores were completely depleted of Ca\(^{2+}\) as demonstrated by the inability of ionomycin to increase Ca\(^{2+}\) fluorescence. We believe that the Ca\(^{2+}\) released from stores by low concentrations of AdA is unable to activate \(I_{Cl1-S}\) because of its significantly slower rate of Ca\(^{2+}\) release (7 times slower than IP\(_3\)).

How do the kinetics of Ca\(^{2+}\) release from stores determine the response of the Cl\(^{-}\) channels? It has been suggested that \(I_{Cl1-S}\) responds to the rate-of-change of cytosolic Ca\(^{2+}\) (20) because the peak activation of \(I_{Cl1-S}\) corresponds to the maximum rate of change of cytosolic Ca\(^{2+}\) and because the amplitude of \(I_{Cl1-S}\) does not correlate with the steady-state levels of cytosolic Ca\(^{2+}\). However, we have shown (27) that the turn-off of \(I_{Cl1-S}\) is not explained by inactivation of the current as previously sug-
Furthermore, we have found that the Ca\(^{2+}\) concentration measured by cytosolic Ca\(^{2+}\) dyes (such as Ca\(^{2+}\)-green dextran) does not reflect the concentration of Ca\(^{2+}\) just below the plasma membrane (measured by lipophilic Ca\(^{2+}\) dyes such as Ca-green C18) (28). We have presented evidence that the subplasmalemmal Ca\(^{2+}\) concentration changes much more...
quickly than does the Ca\textsuperscript{2+} concentration deeper in the cytosol because plasma membrane Ca\textsuperscript{2+} efflux systems can rapidly clear Ca\textsuperscript{2+} from the subplasmalemmal space. Consequently, we would predict that the subplasmalemmal Ca\textsuperscript{2+} concentration would depend on the relative rates of Ca\textsuperscript{2+} release from stores and cytosolic Ca\textsuperscript{2+} buffering and Ca\textsuperscript{2+} efflux from the oocyte. If Ca\textsuperscript{2+} release is slow, the concentration of Ca\textsuperscript{2+} in the subplasmalemmal space may not rise sufficiently to activate Ca\textsuperscript{2+}-activated Cl\textsuperscript{−} channels.

The different kinetics of Ca\textsuperscript{2+} release produced by AdA and IP\textsubscript{3} are probably related to differences between AdA and IP\textsubscript{3} activation of IP\textsubscript{3}Rs. First, the apparent diffusion coefficient of AdA or IP\textsubscript{3} in the cytosol will depend on the fraction of molecules (k) that are bound to the IP\textsubscript{3}R at any one time (D\textsubscript{app} = D/k). Because AdA has a 100-fold higher affinity for the IP\textsubscript{3}R than IP\textsubscript{3} does, AdA diffusion will be slower because a larger fraction of the total AdA (compared with IP\textsubscript{3}) will be bound to IP\textsubscript{3}Rs. Second, AdA exhibits a higher cooperativity in activating IP\textsubscript{3}Rs than IP\textsubscript{3} does. Hirota et al. (1995) (13) have shown that IP\textsubscript{3} has a Hill coefficient of 1.8 for Ca\textsuperscript{2+} release by the type 1 IP\textsubscript{3}R, whereas the Hill coefficient for AdA was 3.9. This implies that at least 2 molecules of IP\textsubscript{3} and 4 molecules of AdA are needed to open an IP\textsubscript{3}R. This factor will also contribute to the slow movement of the Ca\textsuperscript{2+} release wave in response to small amounts of AdA. Accordingly, the elementary Ca\textsuperscript{2+} release events (“Ca\textsuperscript{2+} puffs”) induced by AdA have been shown by Marchant and Parker (30) to be smaller and faster than those induced by IP\textsubscript{3}. Because Ca\textsuperscript{2+} waves are initiated by the summation of Ca\textsuperscript{2+} puffs, the smaller and faster puffs induced by AdA may contribute to the slower propagation of the AdA wave. However, the mechanisms by which AdA releases Ca\textsuperscript{2+} from stores remains to be fully elucidated.

Although low concentrations of AdA evoke a slow release of Ca\textsuperscript{2+} from stores and little or no I\textsubscript{C11,S} responses, high concentrations of AdA can evoke 2-fold more slowly than IP\textsubscript{3} and also evoke significant I\textsubscript{C11,S} responses. This finding that AdA activates different I\textsubscript{C11,S} responses depending upon the kinetics of Ca\textsuperscript{2+} release from intracellular stores is interesting because it provides another example of how the temporal features of a Ca\textsuperscript{2+} signal contribute to its physiological consequences. Different receptors can induce different Ca\textsuperscript{2+} release kinetics depending on factors including spatial localization of the receptor and/or IP\textsubscript{3}-sensitive stores or the activation of different PLC isoforms (31–36). These different release kinetics can then play an important role in determining which effectors are activated.

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