Cyclic ADP-ribose-gated Ca\(^{2+}\) Release in Sea Urchin Eggs Requires an Elevated [Ca\(^{2+}\)]\(^*\)

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Cyclic ADP-ribose (cADPr) has been shown to release intracellular Ca\(^{2+}\) from sea urchin eggs and a variety of vertebrate cell types, although its mechanism of action remains elusive. We employed the caged version of cADPr to study the [Ca\(^{2+}\)] transient kinetics in intact sea urchin eggs for insights into how cADPr gates Ca\(^{2+}\) release. Ca\(^{2+}\) release triggered by photolytic production of cADPr was initially slow, with an effective delay of several hundred milliseconds before the onset of a rapid Ca\(^{2+}\) release phase. In contrast, Ca\(^{2+}\) release induced by photolysis of caged inositol 1,4,5-trisphosphate was immediate in onset and roughly an order of magnitude faster. The delay before cADPr-induced Ca\(^{2+}\) release was eliminated when the [Ca\(^{2+}\)] was step-elevated coincident with the photoliberation of cADPr and greatly prolonged in the presence of exogenous Ca\(^{2+}\) buffers. Thus, the slow onset of Ca\(^{2+}\) release does not reflect an intrinsically slow rate by which cADPr gates release channels. Rather, a [Ca\(^{2+}\)] rise from resting levels is needed to achieve more than minimal cADPr activity. Full release of Ca\(^{2+}\) by cADPr in intact sea urchin eggs requires a positive Ca\(^{2+}\) feedback.

Cyclic ADP-ribose (cADPr) has been found to be a potent mobilizer of intracellular Ca\(^{2+}\) in sea urchin eggs and in numerous vertebrate cell types (for review see Ref. 1). Over the last few years, the accumulating evidence has strengthened the proposal that cADPr is a true calcium-mobilizing second messenger. For example, both cADPr and its metabolic enzymes have been found to be widely distributed in mammalian tissue (2–4). Further, cADPr production and Ca\(^{2+}\) have been found to be widely distributed in mammalian tissue. For example, both cADPr and its metabolic enzymes (RyRCs) (8–11). Further, Clementi et al. (6) noted that cADPr-mediated Ca\(^{2+}\) release was only observed in a subclone of cultured PC12 cells that expressed type II RyRCs but was absence from a subclone not expressing RyRCs. These observations support the hypothesis that RyRCs are the ultimate target of this second messenger. Despite this rather strong circumstantial evidence, studies of the ability of cADPr to directly modulate skeletal and cardiac isofoms of the RyRC reconstituted in planar lipid bilayers have so far yielded equivocal results (12–16). However, photoaffinity labeling studies with cADPr analogues (17) have identified two specific binding proteins having molecular weights significantly smaller than that of known RyRCs. Moreover, at least in sea urchin eggs, cADPr-mediated Ca\(^{2+}\) release is obligately dependent on the presence of calmodulin (18–20). Thus, the activation of Ca\(^{2+}\) release channels by cADPr may involve one or more mediator proteins and may entail multiple activation steps.

We have previously noted (21) that Ca\(^{2+}\) release induced by flash photolysis of caged cADPr in intact sea urchin eggs occurred with a relatively slow onset (or delay), implying one or more slow steps in the activation pathway. To investigate the underlying basis for this slow onset in cADPr-induced Ca\(^{2+}\) release, we have examined the kinetic characteristics of the [Ca\(^{2+}\)] transients induced by photolysis of caged cADPr under conditions where we could simultaneously manipulate the cytosolic [Ca\(^{2+}\)]. We found that the delay before cADPr-induced Ca\(^{2+}\) release was eliminated when the [Ca\(^{2+}\)] was step-elevated coincident with the photoliberation of cADPr and greatly prolonged in the presence of exogenous Ca\(^{2+}\) buffers. Thus, the slow onset of Ca\(^{2+}\) release was not due to an intrinsically slow rate by which cADPr (or Ca\(^{2+}\)) gated release channels. Rather, our results show that cADPr is relatively ineffective at releasing Ca\(^{2+}\) at normal resting [Ca\(^{2+}\)] levels and that a positive Ca\(^{2+}\) feedback is required to achieve full Ca\(^{2+}\) release. Further, these findings underscore the importance of controlling the [Ca\(^{2+}\)] in investigations of cADPr-mediated Ca\(^{2+}\) release.

EXPERIMENTAL PROCEDURES

Egg Preparation—Eggs were released from female Lytechinus pictus sea urchins by intracoelomic injection of 0.5 M KCl and washed twice in artificial sea water containing (in mM): 460 NaCl, 27 MgCl\(_2\), 28 MgSO\(_4\), 10 CaCl\(_2\), 10 KC\(_1\), 2.5 NaHCO\(_3\), pH adjusted to 8.0 with NaOH. The jelly was removed by multiple filtrations through a 100-μm pore nylon filter. The eggs were transferred onto a poly-L-lysine-treated quartz coverslip that formed the bottom of a cell chamber filled with artificial sea water for microinjection and study. The microinjection solution contained a Ca\(^{2+}\) indicator dye (either 250 μM fluo-3 or 1–4 mM spectrally similar Calcium Green 5N (CG-5N)) and various concentrations of either caged cADPR, caged IP\(_3\), caged calcium, and/or heparin dissolved in a microinjection buffer (0.5 M KCl, 50 μM EGTA, 10 mM MOPS, pH 6.7, with KOH). The injected volume was estimated from the Ca\(^{2+}\) dye fluorescence calibrated against fluorescence measurements made of droplets of dye solution created in oil as described previously (21) and was typically 2–5% of the egg volume. Unless otherwise noted, caged IP\(_3\), and caged Ca\(^{2+}\) were loaded to intracellular concentrations greater than 10 μM. All experiments were performed at room temperature. All values reported in the text are the means ± S.E.

Detection of Ca\(^{2+}\) Dye Fluorescence—Whole egg Ca\(^{2+}\) dye fluorescence was monitored with a high time resolution microfluorimeter.
Ca\textsuperscript{2+} Dependence of cADPr-gated Ca\textsuperscript{2+} Release

previously described (21). Briefly, cells were illuminated with 470 nm excitation light for a 2.5-ms interval every 7.5 ms, and fluorescence emission light (500–550 nm) was detected with a photomultiplier tube/photocounter circuit. The longer wavelength, nonratiometric dyes fluo-3 and CG-SN were used to avoid photolysis of the caged compounds by the fluorescence excitation light.

**Flash Photolysis**—To photolyze caged compounds, the output of a xenon flashlamp (Hi-tech, UK) was passed through a UG-5 filter to select for ultraviolet light and merged into the excitation light path of the microfluorimeter with a dichroic beam splitter. The nominal flash lamp energy was set to 350 J, producing a light burst with a duration of ~2 ms. Based on the [Ca\textsuperscript{2+}] response to repeated flashes, we estimate that a single flash photolyses approximately 15% of intracellular NP-EGTA. The fraction of caged cADPr and caged IP\textsubscript{3} photolyzed by a single flash is unknown.

**Calibration of Ca\textsuperscript{2+} Dye Fluorescence**—Fluorescence emission intensity (FI) was used as an index of the intracellular [Ca\textsuperscript{2+}], but no attempt was made to calibrate the fluorescence intensity to an actual concentration scale. In eggs injected with only minimal amounts of Ca\textsuperscript{2+}-chelators, we presume that the normal resting [Ca\textsuperscript{2+}] was ~150 nM based on estimates made by others in eggs studied under similar conditions (10, 22, 23). In eggs loaded with millimolar levels of the caged calcium NP-EGTA, the preflash [Ca\textsuperscript{2+}] presumably was somewhere between the normal resting [Ca\textsuperscript{2+}] and the free [Ca\textsuperscript{2+}] of the injected Ca\textsuperscript{2+}-NP-EGTA has a $K_D$ of 80 nM for Ca\textsuperscript{2+} in a solution with an ionic strength of 0.10–0.15 M at pH 7.2 (24). Using this value, the injectate-free [Ca\textsuperscript{2+}] would be estimated to have been 120 and 53 nM for Ca-NP-EGTA molar ratios of 0.6 and 0.4, respectively. However, most Ca\textsuperscript{2+}-chelators, including EGTA, BAPTA, fluo-3, and CG-SN, have much lower affinities at higher ionic strengths and lower pH levels (25–27). For example, the affinity of EGTA for Ca\textsuperscript{2+} decreases 11-fold going from a 0.15 M ionic strength solution at pH 7.2 to one of 0.6 M ionic strength at pH 6.7 (the approximate cytosolic environment in sea urchin eggs (28)). If the affinity of the structurally similar NP-EGTA also decreases 11-fold under these conditions, the injectate-free [Ca\textsuperscript{2+}] would be estimated to have been 1320 nM and 587 nM for the 0.6 and 0.4 Ca-NP-EGTA ratios, respectively.

**Materials**—Sea urchins were purchased from Marinus, Inc. (Long Beach, CA). Caged cADPr (29) and the Ca\textsuperscript{2+}-sensitive fluorescent dye fluo-3 to approximately 10 and 5 μM, respectively, and subjected to a single UV flash. Whole cell fluo-3 FI was normalized to the preflash fluorescence (F\textsubscript{0}) in A and B. The right-hand panels show the rising phase of the ([Ca\textsuperscript{2+}]) transients at higher time resolution. The delay was estimated by extrapolating a line tangent to the instant of maximum dF/dt back to the intersection with the preflash FI as illustrated in A. Similar recordings were obtained in at least 10 eggs each. For C, eggs were loaded with ~100 nM fluo-3 and ~10 μM of caged cADPr and then subjected to a single UV flash. The higher noise level reflects the lower fluo-3 concentration. The preflash FI was just barely greater (by 20 photons/ms) than that of the unloaded egg. Nonetheless, the amplitude and overall kinetics of the ([Ca\textsuperscript{2+}]) transients were similar to those observed in the presence of 5 μM fluo-3. Response is representative of three experiments.

**RESULTS**

The kinetics of the Ca\textsuperscript{2+} release activated by flash photolytic production of cADPr were studied in intact eggs from the sea urchin *L. pictus*. Eggs were microinjected with caged cADPr (29) and the Ca\textsuperscript{2+}-sensitive fluorescent dye fluo-3 to approximately 10 and 5 μM, respectively, and subjected to a single high intensity flashlamp burst. As we have previously noted (21), the onset of rapid Ca\textsuperscript{2+} release after a single flash occurred only after a several hundred milliseconds delay (Fig. 1A and Table I). Peak [Ca\textsuperscript{2+}] generally was achieved in ~4 s (Table I), and the [Ca\textsuperscript{2+}] then gradually fell back to preflash levels over the next 60 s or so. The rise in [Ca\textsuperscript{2+}] was initially quite slow but accelerated during the first 1–2 s after the flash. In many cases there was no detectable change in the fluo-3 fluorescence over the first 200 ms, but even when the fluorescence did increase during this period, its rate of rise eventually increased more than 100-fold. Thus, the rate of Ca\textsuperscript{2+} release appears to be less than 1% of its maximum for a significant period of time after the photoillumination of cADPr.

In contrast, ([Ca\textsuperscript{2+}]) transients induced by photolysis of caged IP\textsubscript{3} had a similar peak amplitude to those induced by cADPr (Fig. 1B) but strikingly different kinetics. By a number of criteria, the overall response was roughly an order of magnitude faster (Table I). In particular, the onset of Ca\textsuperscript{2+} release occurred within 30 ms of the flash. Thus, the slow onset of Ca\textsuperscript{2+} release appears to be a characteristic specific to the cADPr-activated release mechanism, not of Ca\textsuperscript{2+} release in general.

Several experiments were performed to determine whether delayed Ca\textsuperscript{2+} release was a consequence of our activation or detection methodology. The slow onset of Ca\textsuperscript{2+} release was still observed when the amount of fluo-3 loaded in eggs was decreased 50-fold to approximately 100 nM (Fig. 1C), making it unlikely that this feature of the ([Ca\textsuperscript{2+}]) transient was due to the presence of the Ca\textsuperscript{2+} dye. The characteristics of these cADPr-induced ([Ca\textsuperscript{2+}]) transients were not affected by coloading eggs with heparin (200 μg/ml egg volume; data not shown), ruling out the possibility that the more rapid IP\textsubscript{3}-gated process was involved. Further, using an egg extract assay (30), we found that caged cADPr (5 μM) did not increase the ED\textsubscript{50} of cADPr to release Ca\textsuperscript{2+} (data not shown), indicating that the caged compound does not interfere with cADPr binding.

To assess whether the kinetics of the ([Ca\textsuperscript{2+}]) transients were a consequence of submaximal or slow photoliberation of cADPr, we examined the dependence of the ([Ca\textsuperscript{2+}]) transient amplitude and kinetics on the amount of caged-cADPr loaded into eggs. Fig. 2 shows the peak amplitude and several kinetic descriptors of the ([Ca\textsuperscript{2+}]) transients induced by cADPr and IP\textsubscript{3}, each plotted as a function of the estimated caged compound concentration. In eggs loaded with less than 5 μM of caged cADPr or less than 3 μM of caged IP\textsubscript{3}, the ([Ca\textsuperscript{2+}]) transient kinetics and amplitude


**Ca\(^{2+}\) Dependence of cADPr-gated Ca\(^{2+}\) Release**

TABLE I

| Release | Peak \(F/F_0\) | Delay | Maximum \(dF/dt\) | Time to half peak | Time to peak |
|---------|----------------|-------|-------------------|------------------|-------------|
| Caged cADPr (n = 14) | 4.3 ± 0.2 | ms | 2.7 ± 0.3 | 1.17 ± 0.11 | 1.46 ± 0.40 |
| Caged IP\(_3\) (n = 11) | 5.7 ± 0.4* | 35 ± 3* | 27.0 ± 2.4* | 0.13 ± 0.01* | 0.59 ± 0.03* |

*Significantly different (p < 0.005) from caged cADPr.

Sea urchin eggs were loaded with >5 \(\mu M\) caged cADPr or >3 \(\mu M\) caged IP\(_3\).

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were dependent on the degree of caged compound loading. Thus, slower overall kinetics and a more pronounced delay in the onset of Ca\(^{2+}\) release can be observed, even in response to IP\(_3\), if the production of second messenger is limited. However, when loaded above these levels, the characteristics of the [Ca\(^{2+}\)] transients were independent of the caged compound concentration, indicating that they were not a function of sub-maximal or slow photoliberation of cADPr. Therefore, based on all these observations, we conclude that these characteristics of the [Ca\(^{2+}\)] transients reflect the intrinsic kinetic properties of the Ca\(^{2+}\) release process responding to the rapid appearance of a maximal cADPr concentration.

The rapid activation observed with IP\(_3\) is consistent with the known ability of this second messenger to directly gate Ca\(^{2+}\) release channels (31). In contrast, the delayed release triggered by cADPr suggests a more complex activation scheme. Ca\(^{2+}\) has been shown to facilitate Ca\(^{2+}\) release triggered by submaximal cADPr concentrations (11, 20). Thus, the initially slow but progressively increasing rate of Ca\(^{2+}\) release could reflect, at least in part, the progressive enhancement of cADPr activity as the [Ca\(^{2+}\)] rises. Additionally, the slow onset of Ca\(^{2+}\) release might also reflect the intrinsic rate of the steps by which cADPr acts to open release channels, independent of Ca\(^{2+}\) facilitation. To determine the extent to which Ca\(^{2+}\) influenced the kinetics of cADPr-induced [Ca\(^{2+}\)] transients, we manipulated the [Ca\(^{2+}\)] in an attempt to alter Ca\(^{2+}\) feedback signals in situ. We first employed caged calcium to elevate the [Ca\(^{2+}\)] coincident with cADPr liberation. NP-EGTA and Ca\(^{2+}\) were added to the injectate at a Ca:NP-EGTA molar ratio of 0.6 and coinjected into eggs along with caged cADPr. Total NP-EGTA loading was estimated to be approximately 1 mM. Because initial experiments employing fluo-3 showed near-saturation of this dye after NP-EGTA photolysis, we switched to the lower affinity dye CG-5N, which was loaded into eggs to a concentration of ~20 \(\mu M\). Under these conditions, a single flash produced a step [Ca\(^{2+}\)] jump followed by a kinetically distinct secondary rise that occurred without appreciable delay or acceleration (Fig. 3A). This secondary rise in [Ca\(^{2+}\)] was not observed in the absence of caged cADPr (Fig. 3B), indicating that it was not a result of direct Ca\(^{2+}\)-induced Ca\(^{2+}\) release. Thus, in the absence of a permissive [Ca\(^{2+}\)], cADPr can rapidly activate Ca\(^{2+}\) release without delay. Therefore, it is unlikely that delayed Ca\(^{2+}\) release under more physiological conditions is due to an intrinsically slow rate at which cADPr binds to and gates release channels.

Due to uncertainty regarding the preflash resting [Ca\(^{2+}\)] in the above experiments (see “Experimental Procedures”), we cannot rule out the possibility that a permissive [Ca\(^{2+}\)] existed prior to the flash. Thus, there remained the question of how rapidly Ca\(^{2+}\) could act to facilitate cADPr-activated release. To address this, additional eggs were injected with a similar solution having a lower Ca:NP-EGTA molar ratio of 0.4. We presume that under these conditions the preflash resting [Ca\(^{2+}\)] would be lower, as would the magnitude of the step change in the [Ca\(^{2+}\)] resulting from a single flash. As expected, a single flash produced a smaller [Ca\(^{2+}\)] jump and only a slow cADPr-dependent secondary [Ca\(^{2+}\)] rise (Fig. 3C). However, a second flash ~2.5 s later jumped the [Ca\(^{2+}\)] further, and the Ca\(^{2+}\) release rate immediately increased. The cADPr produced by the first flash was unlikely to be significantly degraded during this 2.5-s interval, particularly because, as will be described below, the onset of rapid calcium release could occur more than 10 s after a single flash in the presence of excess calcium buffers. Thus, the rapid increase in the Ca\(^{2+}\) release rate after the second flash likely can be ascribed to the elevated [Ca\(^{2+}\)], not to a change in the cADPr concentration. We conclude that Ca\(^{2+}\) can also rapidly activate release if cADPr is present at a permissive concentration.

If the acceleration of Ca\(^{2+}\) release was Ca\(^{2+}\)-dependent, one would predict that the onset of rapid Ca\(^{2+}\) release could be prevented or postponed by attenuating the [Ca\(^{2+}\)] rise with exogenous Ca\(^{2+}\) buffers. In fact, this outcome was suggested by the slow, nonaccelerating Ca\(^{2+}\) release observed following the first flash in the set of experiments illustrated by Fig. 3C on eggs loaded with ~1 mM of the Ca\(^{2+}\) chelator NP-EGTA. No
FIG. 3. A [Ca\textsuperscript{2+}] rise coincident with the photolysis of caged cADPr eliminates the delay in the onset of rapid Ca\textsuperscript{2+} release. For A and B, eggs were loaded with approximately 20 \(\mu\)M CG-SN, 1 mM NP-EGTA, and 600 \(\mu\)M Ca\textsuperscript{2+} with (A) or without (B) 10 \(\mu\)M caged cADPr and then subjected to a single flashlamp burst (UV). In C, the egg was loaded with caged cADPr and caged calcium as in A, but with only 400 \(\mu\)M Ca\textsuperscript{2+}, and subjected to two flashlamp bursts (UV1 and UV2). The rate of fluorescence rise (dF/dt) abruptly increased 9-fold after the second flash. Because we estimate the fractional photolysis of caged NP-EGTA to be approximately 15% for a single flash, the change in total buffer power could account for only a very small fraction of the change in dF/dt. The insets of A and C show the initial [Ca\textsuperscript{2+}] change at higher time resolution immediately after the flash. Responses are representative of at least four experiments each.

acceleration of Ca\textsuperscript{2+} release was observed if a second flash was not imposed under these conditions, and the [Ca\textsuperscript{2+}] \(\text{O} [\text{Ca}^{2+}]\) returned to resting levels within 30 s. To determine if a less potent Ca\textsuperscript{2+} buffer load would merely prolong the delay, an additional eggs were loaded with approximately 120 \(\mu\)M CG-SN as the only Ca\textsuperscript{2+} buffer. Under these conditions, the postflash delay before rapid cADPr-induced Ca\textsuperscript{2+} release was greatly prolonged (Fig. 4A) to a mean of 12.5 \(\pm\) 3.5 s \((n = 6)\). In contrast, these buffer conditions did not significantly alter the onset of IP\textsubscript{3}-induced Ca\textsuperscript{2+} release (Fig. 4B). These observations further confirm that the onset of rapid Ca\textsuperscript{2+} release is determined by the [Ca\textsuperscript{2+}] and not the inherent speed by which photoliberated cADPr can gate open release channels.

**DISCUSSION**

Flash photolysis of caged second messengers permits one to impose rapid, homogeneous increases in the second messenger concentration, eliminating intracellular diffusion and native production rates as determinants of the activation rate of target processes. As expected, the [Ca\textsuperscript{2+}] transients induced by photolysis of caged cADPr in sea urchin eggs reported here were significantly faster than those induced by fertilization or by direct microinjection of cADPr reported by others (22, 30). Control experiments indicate that the kinetics of the [Ca\textsuperscript{2+}] transients were not limited by our activation or detection methodology nor by the amount of cADPr liberated. Thus, the characteristics of the [Ca\textsuperscript{2+}] transients appear to reflect the intrinsic kinetic properties of the Ca\textsuperscript{2+} release system responding to the rapid appearance of a maximally effective concentration of cADPr.

In eggs loaded only with caged cADPr and fluo-3, the postflash rate of Ca\textsuperscript{2+} release was very low during the first 100–200 ms but then accelerated more than 100-fold over the next 1–2 s. Given that Ca\textsuperscript{2+} is known to facilitate cADPr-induced release (11, 20), it is reasonable to expect that positive Ca\textsuperscript{2+} feedback will contribute to this increase in release rate. The relatively slow initial rate of Ca\textsuperscript{2+} release could also be a consequence of the intrinsic rate of the cADPr and Ca\textsuperscript{2+} binding steps and/or of the gating process itself. However, we found that a step change in either the Ca\textsuperscript{2+} or cADPr concentration could lead to rapid and immediate activation of Ca\textsuperscript{2+} release when the other was present at a permissive concentration, indicating that the steps linking these messengers to release channel opening are not intrinsically slow. Thus, [Ca\textsuperscript{2+}] appears to be the sole factor limiting Ca\textsuperscript{2+} release during the early postflash period, with acceleration of release reflecting positive feedback enhancement of cADPr activity by released Ca\textsuperscript{2+}. This conclusion is reinforced by the finding that the onset of rapid Ca\textsuperscript{2+} release could be prevented or significantly delayed by the presence of excess exogenous Ca\textsuperscript{2+} buffers. Presumably calcium buffers are effective because they slow the (Ca\textsuperscript{2+}) \text{rise resulting from the initial release and perhaps limit the spatial range over which that Ca\textsuperscript{2+} can signal neighboring channels.}

Based on the observed initial rate of Ca\textsuperscript{2+} release, we estimate that the ability of cADPr to gate release channels in the presence of normal resting [Ca\textsuperscript{2+}] to be less than 1% of its maximum at higher [Ca\textsuperscript{2+}]. The precise concentration range over which Ca\textsuperscript{2+} modulates cADPr activity remains uncertain, due largely to uncertainty about the affinity of the Ca\textsuperscript{2+} dyes and chelators we employed in intact eggs (see “Experimental Procedures”). Furthermore, a Ca\textsuperscript{2+} feedback site might be capable of sensing the locally higher [Ca\textsuperscript{2+}] near open release channels and/or (in experiments employing NP-EGTA) the submillisecond transient [Ca\textsuperscript{2+}] overshoots that immediately follow photolysis of caged calcium (32). Hence, even accurate calibration of the whole egg fluorescence signal might not allow the relevant [Ca\textsuperscript{2+}] values to be determined. A more detailed description of the Ca\textsuperscript{2+} dependence of cADPr action will require a more appropriate model preparation.

Calmodulin, a required cofactor for cADPr-induced Ca\textsuperscript{2+} release in sea urchin eggs (18–20), is a possible candidate for mediating Ca\textsuperscript{2+}-enhancement of cADPr activity. The very low activity of cADPr at normal resting [Ca\textsuperscript{2+}], which suggests the involvement of a cooperative Ca\textsuperscript{2+} binding site, is a common feature of other processes regulated by this protein (33). How-
ever, there is evidence that at least one other divergent cation binding site is capable of enhancing cADPr activity in the absence of calmodulin (20). Thus, further investigation will be needed to establish the identity of the Ca\(^{2+}\) feedback site(s).

Recently, Chini and Doussa (34) compared Ip\(_2\) and cADPr-induced Ca\(^{2+}\) release from sea urchin egg extracts. They observed that both second messengers had a bell shaped [Ca\(^{2+}\)] dependence, with Ip\(_2\) having greater relative activity at lower [Ca\(^{2+}\)]. Although they examined only the steady state Ca\(^{2+}\) dependence and used a rather low resolution [Ca\(^{2+}\)] range (full log unit intervals), their data strongly suggest the existence of both sensitization and desensitization Ca\(^{2+}\) sites that regulate cADPr-mediated Ca\(^{2+}\) release. The actual Ca\(^{2+}\) dependence of cADPr activity reported by this group (no activity in the absence of Ca\(^{2+}\), maximum at 1 \(\mu M\) [Ca\(^{2+}\)], and about 60% of maximum activity at 100 nM [Ca\(^{2+}\)]) in egg extracts appears inconsistent with our estimate that cADPr activity in the intact egg was less than 1% of maximum at normal resting [Ca\(^{2+}\)] (measured by others to be in the 150—200 nM range (22, 23)). Part of this discrepancy might reflect the experimental conditions Chini and Doussa employed in their study. Because cADPr action was determined in the steady state presence of [Ca\(^{2+}\)], the Ca\(^{2+}\)-dependent desensitization process likely would have attenuated the maximum release rate observed. In addition, the [Ca\(^{2+}\)] range was examined only at full log unit intervals and so may have missed the optimal [Ca\(^{2+}\)] having the greatest release rate. Underestimating the maximum Ca\(^{2+}\) release rate will tend to inflate estimates of the relative release rate at lower [Ca\(^{2+}\)]. Nonetheless, full reconciliation of these independent estimates of cADPr activity at lower [Ca\(^{2+}\)] will require further investigation.

Recently, Genazzani et al. (35) examined the kinetics of cADPr-induced Ca\(^{2+}\) release in sea urchin egg homogenates as a function of the cADPr concentration using a stop flow apparatus with a 25-ms time resolution. They reported an apparent acceleration of release in response to low, submaximal levels of cADPr, which they attributed to a Ca\(^{2+}\)-dependent facilitation. However, in contrast to our observations in intact eggs, they observed no significant acceleration or delay of Ca\(^{2+}\) release in response to near maximal cADPr concentrations. The reason for this important difference in the behavior of Ca\(^{2+}\) release in these two models is not clear. A possible factor is that the homogenate extract is diluted 40-fold relative to its normal intracellular density, perhaps altering the normal spatial relations that permit efficient communication between neighboring channels. These relations might be critical if the [Ca\(^{2+}\)] range needed for maximal cADPr action corresponds to local [Ca\(^{2+}\)] levels achieved only near open release channels. We note that the rate of Ca\(^{2+}\) release from homogenates observed by these authors were quite slow (although typical of those reported by others in this model), and peak [Ca\(^{2+}\)] was achieved more than a minute after addition of cADPr. Thus, it is possible that Ca\(^{2+}\) release in this model occurs with little or no calcium facilitation. An understanding of the basis for these differences in the [Ca\(^{2+}\)] transient kinetics of these two models could reveal important insights into the mechanism by which cADPr gates release.

The sea urchin egg [Ca\(^{2+}\)] transients we observed following photolysis of caged IP\(_2\) were immediate in onset and were resistant to the levels of exogenous Ca\(^{2+}\) buffers that compromised cADPr-induced release. Based on these qualitative assessments, Ip\(_2\)-mediated release appears less critically dependent on Ca\(^{2+}\) feedback. As noted previously, Chini and Doussa (34) found Ip\(_2\) to be more effective than cADPr at releasing Ca\(^{2+}\) in egg extracts at lower [Ca\(^{2+}\)]. However, other factors could also contribute to this apparent difference. Despite re-

leasing similar amounts of Ca\(^{2+}\), the maximum rate of IP\(_2\)-induced Ca\(^{2+}\) release was about 10-fold higher than that induced by cADPr (Table I). A 10-fold greater release flux, whether due to more channels or a larger conductance, would accelerate Ca\(^{2+}\) feedback and help resist attenuation by exogenous Ca\(^{2+}\) buffers, even if the initial fractional activity was the same.

Functionally, a maximal effective concentration of cADPr appears merely to enable a Ca\(^{2+}\)-induced Ca\(^{2+}\) release pathway, with subsequent Ca\(^{2+}\) release critically dependent on a facilitating [Ca\(^{2+}\)] signal. Lee (1) has proposed that the cADPr-gated release pathway can operate along a continuum between two extreme modes: a "modulator" mode at submaximal cADPr levels, where Ca\(^{2+}\) release is a function of the ambient [Ca\(^{2+}\)] (thus permitting cADPr to amplify Ca\(^{2+}\) signals generated by other mechanisms), and a "messenger" mode at high cADPr levels, where full Ca\(^{2+}\) release occurs independent of the ambient [Ca\(^{2+}\)]. Our findings challenge a strict mechanistic description of this dual mode hypothesis. Full activation of release, even in the presence of maximal cADPr levels, required a facilitating [Ca\(^{2+}\)] rise, and conditions that altered this Ca\(^{2+}\) signal changed the characteristics of the [Ca\(^{2+}\)] transients. Nonetheless, from a functional perspective, our results are in accord with the dual mode hypothesis in sea urchin eggs. The initially slow postflash Ca\(^{2+}\) release was always sufficient to ignite a positive Ca\(^{2+}\) feedback that ensured eventual full activation of release.

However, the inevitability of full Ca\(^{2+}\) release to a supramaximal cADPr signal in other cell types is not a given. In cells that have more active basal Ca\(^{2+}\) uptake processes, a lower resting [Ca\(^{2+}\)], or a lower density of cADPr-gated channels, the initial release rate might be insufficient to initiate a positive Ca\(^{2+}\) feedback before the cADPr is degraded. Indeed, a cADPr-gated release pathway might play an essential role in shaping and amplifying Ca\(^{2+}\) signals induced by other mechanisms, yet go undetected under experimental conditions that interfere with or fail to provide a triggering Ca\(^{2+}\) signal.

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