RESEARCH COMMUNICATION

Kinetics of elementary Ca^{2+} puffs evoked in *Xenopus* oocytes by different Ins(1,4,5)P_3 receptor agonists

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Elementary Ca^{2+} puffs form the basic building blocks of global Ins(1,4,5)P_3-evoked Ca^{2+} signals. In *Xenopus* oocytes, Ca^{2+} puffs evoked by the high-affinity agonist adenophostin were shorter and smaller than puffs evoked by Ins(1,4,5)P_3 and the lower affinity analogue Ins(2,4,5)P_3. Agonist-specific mechanisms, therefore, play a role in shaping local Ca^{2+} release events, but termination of Ca^{2+} flux is not delimited simply by agonist dissociation.

INTRODUCTION

The coordinated activation of Ins(1,4,5)P_3 receptors in many cell types generates repetitive oscillations in cytoplasmic Ca^{2+} concentration that serve to control the activity of numerous proteins [1,2]. The basic building blocks of these global Ca^{2+} signals are 'Ca^{2+} puffs', spatially restricted, subcellular Ca^{2+} release events, persisting for a few hundred milliseconds, that become progressively recruited by Ca^{2+}-induced Ca^{2+} release at increasing Ins(1,4,5)P_3 concentrations to generate propagating Ca^{2+} waves [3–7]. The time-course of individual Ca^{2+} puffs recorded at a given release site varies considerably, such that varying amounts of Ca^{2+} are liberated [3,8,9]. This variable capacity for local Ca^{2+} release is an important factor that controls the initiation of Ca^{2+} waves [3]. Consequently, it is important to understand the regulatory mechanisms that act upon Ins(1,4,5)P_3 receptors to determine the time-course and magnitude of Ca^{2+} puffs and thereby the capacity for initiation of Ca^{2+}-induced Ca^{2+} release [3,10]. One possibility is that Ins(1,4,5)P_3 dissociation from its receptor terminates Ca^{2+} liberation during a puff, and hence delimits the total Ca^{2+} efflux. In that case, the affinity of the challenging agonist (which is partly determined by its dissociation rate) is expected to influence the duration of these events, with higher affinity agonists producing longer durations of Ca^{2+} release [11]. To test this prediction, we employed high-resolution laser scanning confocal microscopy [12] to compare the kinetics and magnitudes of Ca^{2+} puffs evoked in *Xenopus* oocytes by Ins(1,4,5)P_3 with those evoked by other agonists of differing affinities.

MATERIALS AND METHODS

Materials

[^{3}H]Ins(1,4,5)P_3 (58 Ci/mmol) was obtained from Amersham (Little Chalfont, U.K.). Caged Ins(1,4,5)P_3 [Ins(1,4,5)P_3, $P^{4(5)}$-1-(2-nitrophenyl) ethyl ester] and Oregon Green 488 BAPTA-1 were from Molecular Probes Inc. (Eugene, OR, U.S.A.). Ins(1,4,5)P_3 was from American Radiolabelled Chemicals (St. Louis, MO, U.S.A.), D-Ins(2,4,5)P_3 was purified from bovine brain [13] and adenophostin A was a generous gift from Dr. M. Takahashi (Sankyo Co., Tokyo, Japan). All other reagents were from suppliers reported previously [8,14].

Confocal line-scanning microscopy in *Xenopus* oocytes

Experiments were performed using defolliculated immature oocytes from *Xenopus laevis*, prepared as detailed previously [8]. Stage V and VI oocytes, bathed in Ringer's solution (120 mM NaCl/2 mM KCl/1.8 mM $CaCl_2$/5 mM Hepes, pH 7.2, at room temperature) were microinjected with the fluorescent calcium indicator Oregon Green 488 BAPTA-1 (K_d for Ca^{2+} ~ 170 nM) and caged Ins(1,4,5)P_3 (final intracellular concentrations ~ 40 μM and ~ 5 μM respectively) 1 h before recording images at room temperature. Confocal line-scan imaging and flash photolysis of caged Ins(1,4,5)P_3 were achieved using the 'home-brew' confocal microscope and UV illumination system described previously [12]. Recordings were obtained using an Olympus IX70 inverted microscope, with the scan line focused on the underside of the oocyte at the depth of the pigment granules (~ 6 μm inward from the surface membrane), where Ins(1,4,5)P_3-sensitive release sites are localized [15]. Line-scan images were collected at a scan rate of 8 ms per 100 μm line, with a nominal pixel size of 0.13 μm and an integration time of 10 μs per pixel. Ins(1,4,5)P_3 receptor agonists were injected into the oocyte by ionophoresis (pipette-negative pulses of about 500 nA and 100–200 ms at 1 Hz, with a steady backing current of about 50 nA to minimize unwanted leakage) from pipettes positioned relative to the scan line using a Narishige hydraulic micromanipulator. Line-scan Ca^{2+}-fluorescence images were constructed and processed using routines written in the IDL programming language (Research Systems Inc., Boulder, CO, U.S.A.). Fluorescence is expressed as a pseudo-ratio (F/F_0 or $\Delta F/F_0$) of fluorescence at a given pixel during a response (F) relative to the mean value before stimulation (F_0). Fluorescence signal mass [8] provides an estimate of the amount of Ca^{2+} released during an elementary event, and was obtained by integration of the fluorescence profile from sharply defined, discrete events through three dimensions. The resulting quantity has units of $\Delta F/F_o \times 1 \times 10^{-15}$ litres; where 1 unit corresponds to

Abbreviations used: caged Ins(1,4,5)P_3, Ins(1,4,5)P_3, $P^{4(5)}$-1-(2-nitrophenyl) ethyl ester; $[Ca^{2+}]_{cyt}$, cytosolic free Ca^{2+} concentration; FKBP, FK506-binding protein molecule.

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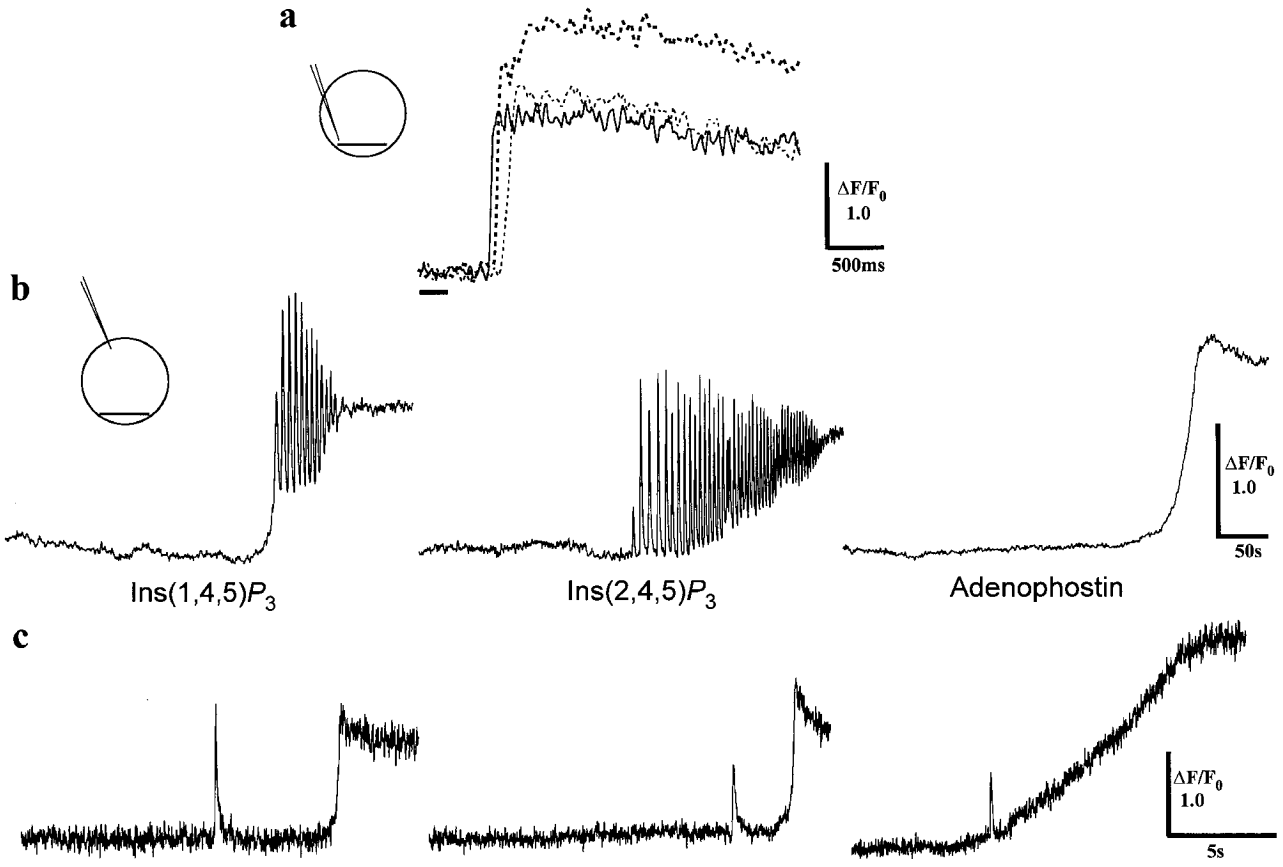

Figure 1 Patterns of Ca^{2+} liberation evoked by Ins(1,4,5)P_3, Ins(2,4,5)P_3 and adenophostin in *Xenopus* oocytes

(**a**) Superimposed records showing the time-course of elevation of Ca^{2+} at the centre of a 100 μm scan line following the ionophoretic release (200 ms pulse, black bar) of Ins(1,4,5)P_3 (black line), Ins(2,4,5)P_3 (heavy dotted line) and adenophostin (light dotted line) from a pipette positioned near the end of the laser line. The concentration of all analogues in the ionophoretic pipette was 500 μM. Traces show fluorescence ratios (F/F_0) measured from a 3 pixel region. (**b**) Traces monitor the cytosolic Ca^{2+} concentration within a 2 μm region along the laser scan line following the ionophoretic injection of Ins(1,4,5)P_3, Ins(2,4,5)P_3 and adenophostin from pipettes positioned at the opposite side of the oocyte to the laser line (about 1 mm distant). In each case, ionophoretic pulses (100 ms duration, 1 Hz) began at the start of the trace and continued for the duration of the record. Inset diagrams in (**a**) and (**b**) show side views of the oocyte, illustrating the positioning of the ionophoresis pipette relative to the confocal scan line. (**c**) Fluorescence profiles during continued ionophoresis of low concentrations of (from left to right) Ins(1,4,5)P_3, Ins(2,4,5)P_3 and adenophostin, with the pipettes positioned as in (**b**), showing discrete elementary events before a Ca^{2+} wave. Measurements were made from 0.4 μm regions, positioned over active puff sites.

a doubling of fluorescence in a volume of 1 fl, and results from release of roughly 2×10^{-20} moles of Ca^{2+} [8].

Membrane binding assay

Binding assays were performed on rat cerebellar membranes, as *Xenopus* membrane preparations showed unacceptably low levels of specific binding ($22.1 \pm 3.0\%$ of total, $n = 7$ independent preparations). *Xenopus* Ins(1,4,5)P_3 receptors share 90% structural identity with cerebellar Ins(1,4,5)P_3 receptors and exhibit a similar rank order of analogue potency when binding is assessed in either purified or transfected preparations [16,17]. Membranes prepared from rat cerebellum [14] were stored in liquid nitrogen for up to 2 weeks (~ 0.5 mg of protein/ml in binding buffer) and equilibrium binding of [^{3}H]Ins(1,4,5)P_3 was subsequently measured using a centrifugation assay [14]. Briefly, membranes (0.04 mg of protein/tube) were added to binding buffer (50 mM Tris/HCl/1 mM EDTA, pH 8.3, at 4 °C), containing [^{3}H]-Ins(1,4,5)P_3 (30 nCi, final concentration = 1 nM) and the appropriate concentration of competing ligand. After incubation for 5 min on ice, bound and free [^{3}H]Ins(1,4,5)P_3 were separated by centrifugation (200000 ***g***, 5 min, 0 °C). Competition binding data were fitted using non-linear least-squares regression analysis to determine IC_{50}, the concentration of competing ligand causing displacement of half the specifically bound radiolabel. All results are presented as means $\pm$ S.E.M.

RESULTS

To compare Ca^{2+} release events elicited by Ins(1,4,5)P_3 with those evoked by agonists of differing affinities, we examined responses to adenophostin A, a metabolically stable glyconucleotide isolated from the fungus *Penicillium brevicompactum*, which is the most potent agonist of Ins(1,4,5)P_3 receptors yet identified [18,19], and the lower affinity agonist Ins(2,4,5)P_3 [20,21]. The binding affinities for Ins(1,4,5)P_3 receptors of these analogues were determined in an equilibrium binding assay using cerebellar membranes, as attempts to measure binding to oocyte membranes were confounded by contamination with endogenous lipids, leading to low levels of specific binding [16,17]. Equilibrium dissociation constants (K_d) were: 1.85 ± 0.23 nM, adenophostin A; 6.55 ± 0.67 nM, Ins(1,4,5)P_3; and 164.4 ± 17.5 nM, Ins(2,4,5)P_3 ($n \geqslant 3$).

The effects of Ins(1,4,5)P_3, Ins(2,4,5)P_3 and adenophostin on

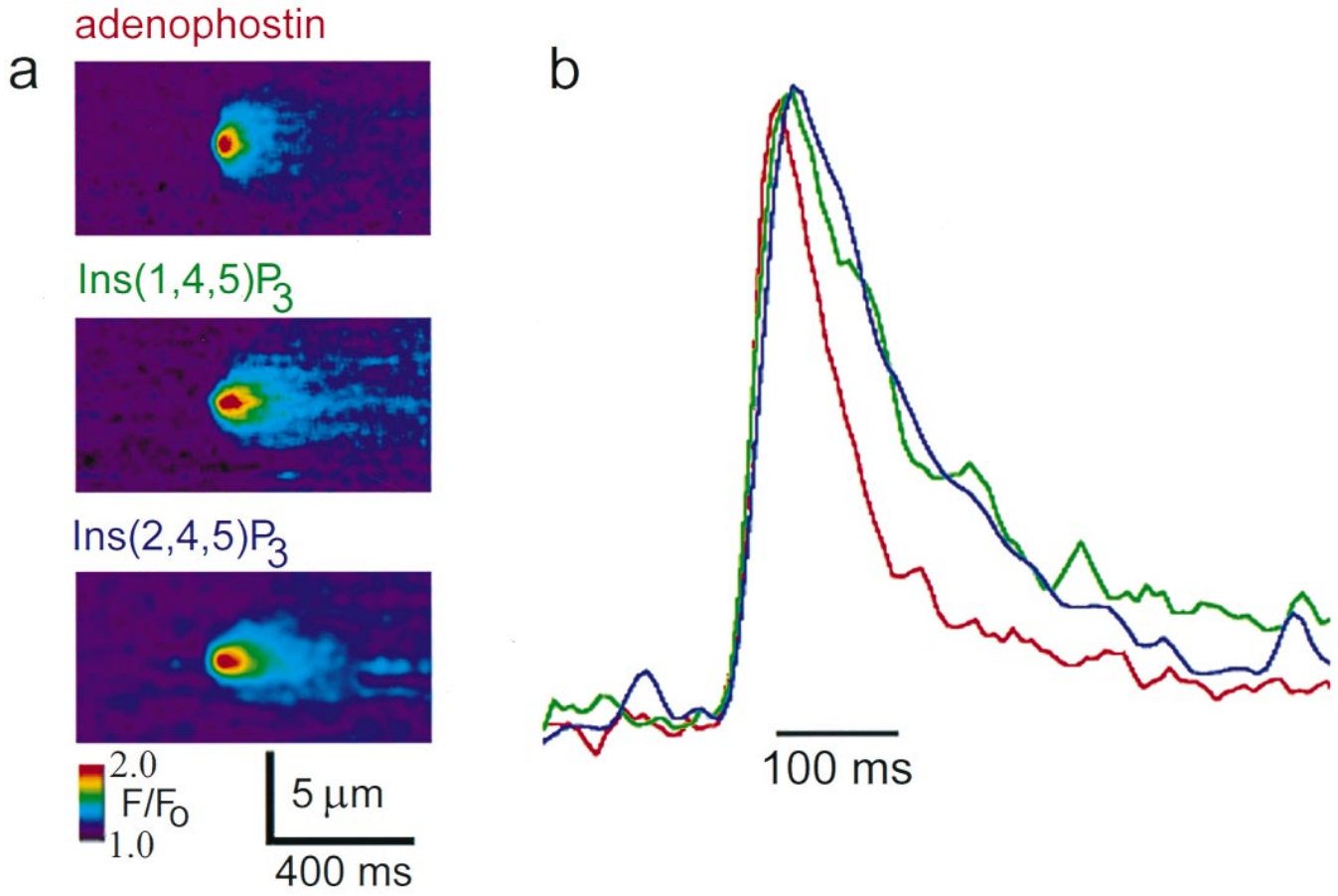

Figure 2 Ca^{2+} puffs evoked by different agonists

(**a**) Line-scan images show averages of puffs ($n > 10$ events each, from $\geqslant 3$ oocytes) evoked by Ins(1,4,5)P_3, Ins(2,4,5)P_3 and adenophostin. In each image, distance along the scan line is depicted vertically, and time runs from left to right. Increasingly 'warm' colours correspond to increasing fluorescence ratio (F/F_0). Averages were formed by aligning in space and time the peak fluorescence during individual Ca^{2+} puffs. (**b**) Traces show fluorescence ratios (F/F_0) measured from a 3 pixel region (~ 0.4 μm) through the point of peak fluorescence on each image: Ins(2,4,5)P_3, blue line; adenophostin, red line; Ins(1,4,5)P_3, green line.

cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) were monitored by iontophoresing each analogue into *Xenopus* oocytes previously microinjected with the fluorescent Ca^{2+} indicator Oregon Green 488 BAPTA-1 and caged Ins(1,4,5)P_3. Ca^{2+}-dependent fluorescence was visualized by line-scan confocal microscopy, imaging along a 100 μm line focused by an inverted microscope in the vegetal hemisphere of the oocytes. With the ionophoretic pipette inserted through the oocyte so that its tip lay adjacent to one end of the laser scan line, iontophoresis of amounts of analogues sufficient to evoke maximal responses triggered rapid Ca^{2+} release from the intracellular stores, after a latency that was similarly brief for each analogue (Figure 1a). When the ionophoretic pipette was inserted with its tip positioned near the top of the oocyte (~ 1 mm from the scan line), the latency to Ca^{2+} release for each analogue was much longer and, with Ins(1,4,5)P_3 and Ins(2,4,5)P_3, repetitive Ca^{2+} waves were observed before a sustained elevation in $[Ca^{2+}]_{cyt}$ (Figure 1b). In contrast, repetitive Ca^{2+} waves were never observed ($n = 18$ cells) with a range of adenophostin concentrations (Figure 1b). Furthermore, the rate of rise of $[Ca^{2+}]_{cyt}$ with adenophostin was less abrupt than with the other agonists (Figure 1c) and, even after $[Ca^{2+}]_{cyt}$ had declined almost to the resting level following its peak, responses could no longer be evoked by photolytic release of concentrations of Ins(1,4,5)P_3 up to 100-fold higher than required previously to mobilize Ca^{2+} at the same site.

When the amount of Ins(1,4,5)P_3 injected through the ionophoretic pipette was reduced, discrete elementary events were resolved preceding the initiation of a Ca^{2+} wave (Figure 1c). At lower doses still, Ca^{2+} puffs were observed as discrete independent events that failed to trigger a global Ca^{2+} rise. Because of their resistance to metabolic inactivation [18,22], continued iontophoresis of even low concentrations of Ins(2,4,5)P_3 and adenophostin eventually resulted in the initiation of a widespread Ca^{2+} elevation. Nonetheless, discrete Ca^{2+} puffs were resolved during the early stages of the response to low doses of all agonists (Figure 1c). Quantitative comparisons of the properties of Ca^{2+} puffs evoked by these different agonists were made using four parameters determined from the fluorescence profile of individual events (Figure 2b): peak fluorescence, duration, rise time and 'signal mass' (a measure of the total amount of Ca^{2+} liberation). Paired measurements of puffs evoked by iontophoresis of adenophostin or Ins(2,4,5)P_3 were compared with Ca^{2+} puffs evoked previously in the same oocytes by photoreleasing low concentrations of Ins(1,4,5)P_3. Despite the different means of delivery (iontophoresis versus photorelease), this comparison is valid, since iontophoresed or photoreleased Ins(1,4,5)P_3 evoked puffs

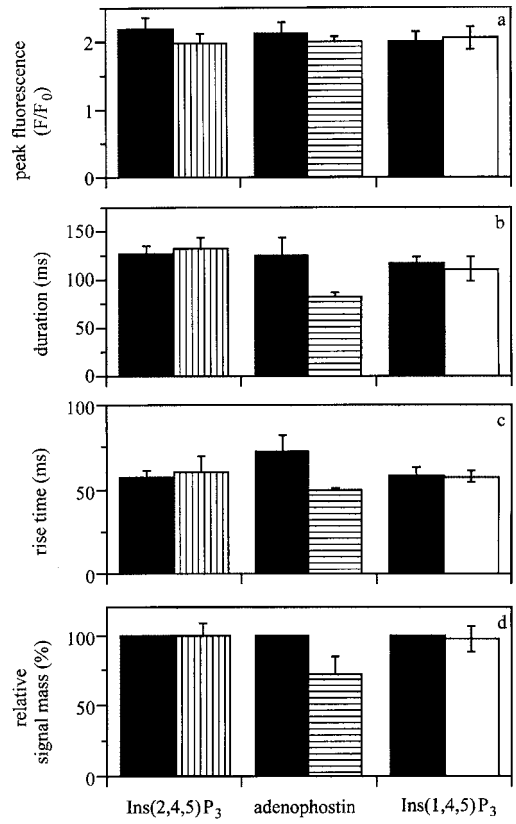

Figure 3 Magnitudes and kinetics of Ca^{2+} puffs evoked by Ins(2,4,5)P_3, adenophostin and Ins(1,4,5)P_3

The characteristics of Ca^{2+} puffs evoked following ionophoresis of Ins(2,4,5)P_3 (vertical striped bars) adenophostin (horizontal striped bars) and Ins(1,4,5)P_3 (clear bars) were compared within the same oocyte with Ca^{2+} puffs evoked by photorelease of caged Ins(1,4,5)P_3 (black bars). Bars show means $\pm$ S.E.M. of $>$ 50 individual events in at least three oocytes. (**a**) Peak fluorescence of Ca^{2+} puffs expressed as F/F_0. (**b**) Durations of puffs, defined as the time for which the fluorescence signal exceeded one-half of the peak amplitude during a puff. (**c**) Rise times of puffs, measured from baseline to peak fluorescence. (**d**) Relative signal mass of puffs, expressed as a percentage of that evoked by photoreleased Ins(1,4,5)P_3 in the same cells. Signal mass values (see Materials and methods section) are expressed relative to the amount of Ca^{2+} released during Ins(1,4,5)P_3-evoked Ca^{2+} puffs in the same oocyte.

with identical characteristics (black and clear bars, respectively, in Figure 3).

Similar to the variation in magnitude and kinetics of individual puffs evoked by Ins(1,4,5)P_3 [8], puffs evoked by adenophostin and Ins(2,4,5)P_3 were not stereotyped events. To illustrate the mean characteristics of puffs evoked by each agonist, Figure 2(a) shows average images formed from more than ten individual events, and the corresponding kinetics of the average fluorescence signals are plotted in Figure 2(b). The peak fluorescence changes during puffs were almost identical with all three agonists (Figure 3a), suggesting that the peak rates of Ca^{2+} liberation were similar [8]. Furthermore, no differences were apparent between Ins(1,4,5)P_3 and Ins(2,4,5)P_3 in terms of half-maximal duration (Figure 3b) or rise time (Figure 3c) of puffs, nor in the amounts of Ca^{2+} liberated during puffs (Figure 3d). Estimates of Ca^{2+} liberation based on the fluorescence 'signal mass' [8] yielded values of $(3.6 \pm 0.2) \times 10^{-19}$ moles of Ca^{2+} for puffs evoked by Ins(1,4,5)P_3 and Ins(2,4,5)P_3. On the other hand, puffs evoked by adenophostin showed appreciably faster kinetics (Figure 2; and horizontal striped bars in Figure 3), with shorter duration and more rapid rise, resulting in a smaller relative signal mass, corresponding to liberation of, on average, $(2.6 \pm 0.3) \times 10^{-19}$ moles of Ca^{2+}.

DISCUSSION

The mechanisms which regulate the duration of Ins(1,4,5)P_3 receptor openings during a Ca^{2+} puff, and consequently the amount of Ca^{2+} released into the cytoplasm, are unresolved [3,8]. In *Xenopus* oocytes, the smallest Ins(1,4,5)P_3-evoked events liberate $\sim 2 \times 10^{-20}$ moles of Ca^{2+} [8], arising from a Ca^{2+} release flux (current of < 0.5 pA) that is consistent with estimates of calcium current through a single Ins(1,4,5)P_3 receptor under physiological conditions [23,24]. In contrast, larger Ca^{2+} release events liberate as much as 5×10^{-18} moles of Ca^{2+}, because several clustered receptors activate synchronously and remain open longer [8]. The time course of Ins(1,4,5)P_3 dissociation from its receptor under physiological conditions ($t_{\frac{1}{2}} = 125$ ms [25]) is compatible with the durations of Ca^{2+} liberation during puffs (~ 150 ms [8]), suggesting that the time course of elementary release events may be determined by the stochastic dissociation of agonist molecules from the Ins(1,4,5)P_3 receptor, in a manner analogous to the agonist-specific open life-time of other ligand-gated channels [11,26].

To assess this possibility, we compared the properties of Ca^{2+} puffs in *Xenopus* oocytes evoked by three agonists differing by ~ 90-fold in their affinity for the Ins(1,4,5)P_3 receptor. With similar diffusion-limited association rates [27], the mean open duration of Ins(1,4,5)P_3 receptors/channels is expected to shorten with decreasing agonist affinity, thereby resulting in smaller and briefer puffs. In contrast to this, experimental observations showed the average characteristics of puffs evoked by Ins(1,4,5)P_3 and Ins(2,4,5)P_3 to be almost identical, despite the 25-fold lower affinity of Ins(2,4,5)P_3, whereas puffs evoked by the high-affinity agonist adenophostin were shorter and involved a smaller liberation of Ca^{2+} (Figures 2 and 3). We conclude that the duration of a Ca^{2+} puff bears no simple relationship to the affinity of the agonist that evoked it, and, consequently, propose that regulatory mechanisms other than agonist dissociation must delimit the period of Ca^{2+} flux through Ins(1,4,5)P_3 receptors at the microscopic level. Possibilities include the processes of Ca^{2+}-mediated inhibition [28,29] and Ins(1,4,5)P_3-induced inactivation [30,31], both of which occur sufficiently rapidly to influence Ins(1,4,5)P_3 receptor behaviour on a timescale compatible with the durations of elementary events. Association of Ins(1,4,5)P_3 receptors with FK506-binding protein molecules (FKBP) [32,33] may be involved, as disruption of FKBP association with cardiac ryanodine receptors prolongs the duration of Ca^{2+} sparks in rat heart [34,35].

Adenophostin is the first compound reported that modulates the time-course of receptor activity during Ca^{2+} puffs. Furthermore, Ca^{2+} liberation induced by adenophostin shows characteristics that are qualitatively different from those induced by Ins(1,4,5)P_3 and Ins(2,4,5)P_3, including its consistent failure to evoke repetitive Ca^{2+} spikes (Figure 2b) and oscillatory Ca^{2+}-activated Cl^- currents [36], and the absence of abrupt, regenerative Ca^{2+} release in response to gradually increasing concentrations of adenophostin (Figure 2c). The mechanisms underlying the actions of adenophostin remain to be elucidated, but it appears that it may cause Ca^{2+} release through Ins(1,4,5)P_3 receptors as both discrete Ca^{2+} puffs, and in a more continuous fashion, without resolvable elementary events.

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REFERENCES

1 Berridge, M. J. (1993) Nature (London) **361**, 315–325
2 Berridge, M. J. and Dupont, G. (1994) Curr. Opin. Cell Biol. **6**, 267–274
3 Bootman, M. D., Berridge, M. J. and Lipp, P. (1997) Cell **91**, 367–373
4 Yao, Y., Choi, J. and Parker, I. (1995) J. Physiol. **482**, 533–553
5 Parker, I., Choi, J. and Yao, Y. (1996) Cell Calcium **20**, 105–121
6 Bootman, M. D. and Berridge, M. J. (1996) Curr. Biol. **6**, 855–865
7 Bootman, M. D., Niggli, E., Berridge, M. J. and Lipp, P. (1997) J. Physiol. **499**, 307–314
8 Sun, X.-P., Callamaras, N., Marchant, J. S. and Parker, I. (1998) J. Physiol. **509**, 67–80
9 Thomas, D., Lipp, P., Berridge, M. J. and Bootman, M. D. (1998) J. Physiol. Proc., **509P**, 186–187
10 Berridge, M. J. (1997) J. Physiol. **499**, 291–306
11 Lester, R. A. J. and Jahr, C. E. (1992) J. Neurosci. **12**, 635–643
12 Parker, I., Callamaras, N. and Wier, W. G. (1997) Cell Calcium **21**, 441–452
13 Irvine, R. F., Brown, K. D. and Berridge, M. J. (1984) Biochem. J. **222**, 269–272
14 Nunn, D. L. and Taylor, C. W. (1990) Biochem. J. **270**, 227–232
15 Parys, J. B. and Bezprozvanny, I. (1995) Cell Calcium **18**, 353–363
16 Parys, J. B., Sernett, S. W., DeLisle, S., Snyder, P. M., Welsh, M. J. and Campbell, K. P. (1992) J. Biol. Chem. **267**, 18776–18782
17 Kume, S., Muto, A., Aruga, J., Nakagawa, T., Michikawa, T., Furuichi, T., Nakade, S., Okano, H. and Mikoshiba, K. (1993) Cell **75**, 555–570
18 Takahashi, M., Tanzawa, K. and Takahashi, S. (1994) J. Biol. Chem. **269**, 369–372
19 Hirota, J., Michikawa, T., Miyawaki, A., Takahashi, M., Tanzawa, K., Okura, I., Furuichi, T. and Mikoshiba, K. (1995) FEBS Lett. **368**, 248–252
20 Loomis-Husselbee, J. W., Cullen, P. J., Dreikhausen, U. E., Irvine, R. F. and Dawson, A. P. (1996) Biochem. J. **314**, 811–816
21 Marchant, J. S., Chang, Y.-T., Chung, S.-K., Irvine, R. F. and Taylor, C. W. (1997) Biochem. J. **321**, 573–576
22 Hill, T. D., Dean, N. M. and Boynton, A. L. (1988) Science **242**, 1176–1178
23 Bezprozvanny, I. and Ehrlich, B. E. (1994) J. Gen. Physiol. **104**, 821–856
24 Mak, D. O. and Foskett, J. K. (1997) J. Gen. Physiol. **109**, 571–587
25 Hannaert-Merah, Z., Coquil, J.-F., Combettes, L., Claret, M., Mauger, J.-P. and Champeil, P. (1994) J. Biol. Chem. **269**, 29642–29649
26 Leibowitz, M. and Dionne, V. E. (1984) Biophys. J. **45**, 153–163
27 Meyer, T., Wensel, T. and Stryer, L. (1990) Biochemistry **29**, 32–37
28 Finch, E. A., Turner, T. J. and Goldin, S. M. (1991) Science **252**, 443–446
29 Parker, I. and Ivorra, I. (1990) Proc. Natl. Acad. Sci. U.S.A. **87**, 260–264
30 Hajnóczky, G. and Thomas, A. P. (1994) Nature (London) **370**, 474–477
31 Dufour, J.-F., Arias, I. M. and Turner, T. J. (1997) J. Biol. Chem. **262**, 2675–2681
32 Cameron, A. M., Steiner, J. P., Roskams, A. J., Ali, S. M., Ronnett, G. V. and Snyder, S. H. (1995) Cell **83**, 463–472
33 Cameron, A. M., Nucifora, F. C., Fung, E. T., Livingston, D. J., Aldape, R. A., Ross, C. A. and Snyder, S. H. (1997) J. Biol. Chem. **272**, 27582–27588
34 Xiao, R.-P., Valdivia, H. H., Bogdanov, K., Valdivia, C., Lakatta, E. G. and Cheng, H. (1997) J. Physiol. **500**, 343–354
35 Lukyanenko, V., Wiesner, T. F. and Györke, S. (1998) J. Physiol. **507**, 667–677
36 DeLisle, S., Marksberry, E. W., Bonnett, C., Jenkins, D. J., Potter, B. V. L., Takahashi, M. and Tanzawa, K. (1997) J. Biol. Chem. **272**, 9956–9961

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