$InsP_3$- and Ca^{2+}-induced Ca^{2+} release in single mouse oocytes

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To better understand the mechanism of intracellular Ca^{2+} mobilization, mouse oocytes were micro-injected with 'caged'-inositol-1,4,5 trisphosphate (caged-$InsP_3$) together with the Ca^{2+} indicator Fluo-3 to directly induce and monitor Ca^{2+} redistribution. Photo-released $InsP_3$ elicits $[Ca^{2+}]_i$ changes exhibiting several kinetic phases and threshold behaviour. Repetitive Ca^{2+} oscillations were induced after a single $InsP_3$ pulse. Autoregenerative Ca^{2+} transients could also be induced by injections of Ca^{2+} itself, demonstrating unequivocally the presence of a Ca^{2+}-induced Ca^{2+}-release mechanism in these cells.

Inositol 1,4,5 trisphosphate; Flash photolysis; Calcium ion, cytosolic; Oscillation; Oocyte (mouse)

1. INTRODUCTION

An increase in $[Ca^{2+}]_i$, often taking the form of periodic oscillations, is commonly observed in a variety of cells under different kinds of stimulation [3-5]. Neither their originating mechanism nor their physiological role have yet been conclusively clarified, although several models [6-8] have been proposed. Mammalian oocytes represent a convenient cellular model known to generate repetitive Ca^{2+} spikes during activation [9-11], probably mediated by $InsP_3$ [12].

The way in which oscillatory Ca^{2+} changes are generated in response to extracellular stimulation appears to involve two types of intracellular Ca^{2+} sources functionally defined as $InsP_3$-sensitive stores, sites of $InsP_3$-induced Ca^{2+} release (IICR), and $InsP_3$-insensitive stores, probably sites of Ca^{2+}-induced Ca^{2+} release (CICR), although the existence of this latter process in non-excitable cells needs to be more generally assessed [13]. Appropriate feedback connections between these two kinds of store may originate the spiking behaviour [6,14,15].

Coupling of electrophysiological and microfluorimetric techniques allows the testing of the presence of both kinds of processes in the same single cell: the cytosolic Ca^{2+} concentration can be directly monitored by Fluo-3 [16], while $InsP_3$ is photolytically generated and graded Ca^{2+} increases can be produced by voltage-driven Ca^{2+} influx.

2. MATERIALS AND METHODS

A single mouse oocyte with an intact germinal vesicle (GV stage) was internally perfused by means of a patch-clamp pipette connected to a conventional patch-clamp amplifier [17]. The pipettes (1-2 MΩ resistance) contained (in mM): 140 KCl, 4 $MgCl_2$, 10 Hepes-KOH, pH 7.2, to which 100 μM Fluo-3 as Ca^{2+} indicator [16,18] and 20 μM 'caged'-$InsP_3$ (Calbiochem) were added. The external solution contained (in mM): 125 NaCl, 5 KCl, 1.2 $MgCl_2$, 2 $CaCl_2$, 25 Hepes-NaOH, 6 glucose, pH 7.3, temperature 28-30°C. The light from the 150 W Xenon arc lamp (Muller) was passed through a narrow-band interference filter centred at 490 nm to excite the fluo-3 fluorescence. Photolysis of caged $InsP_3$ was induced by electronically changing the filter for a controlled time with a broad-band UV-A filter. A light guide connected the filter changer to an inverted microscope equipped for epifluorescence. The emitted light was collected by a high N.A. objective (Nikon Fluor 40×) and measured by a photomultiplier (Cairn Research Ltd Sittingbourne, Kent, UK). The amount of $InsP_3$ produced, as estimated by a competitive binding assay (Amersham), was only a small percent of the pipette concentration (between 10 and 100 nM) and approximately proportional to the flash duration.

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3. RESULTS

Generation of small quantities of $InsP_3$ (10-100 nM) revealed a 'threshold' behaviour of the Ca^{2+} transients and also the existence of characteristic kinetic phases. This is illustrated in Fig. 1 (left column): a 50 msec flash caused only an almost undetectable Ca^{2+} increase (trace A); doubling the amount of photo-released $InsP_3$ with a 100 msec flash, induced initially a slow and steady Ca^{2+} rise which eventually triggered a larger transient lasting for a few seconds (trace B); a 200 msec flash (trace C) caused a more steep initial rise which accelerated the generation of a larger and longer transient; the last trace (D) shows the response to a 400 msec pulse consisting of an immediate and larger rise, fast partial decline followed by a plateau phase, ending with a steep decline phase.

A single $InsP_3$ pulse could also lead to the establishment of sustained Ca^{2+} oscillations. This is shown in Fig. 1E and F which illustrates two oocytes in which a 400 msec light pulse triggered Ca^{2+} transients similar to that shown in Fig. 1D. In these two cells however, the

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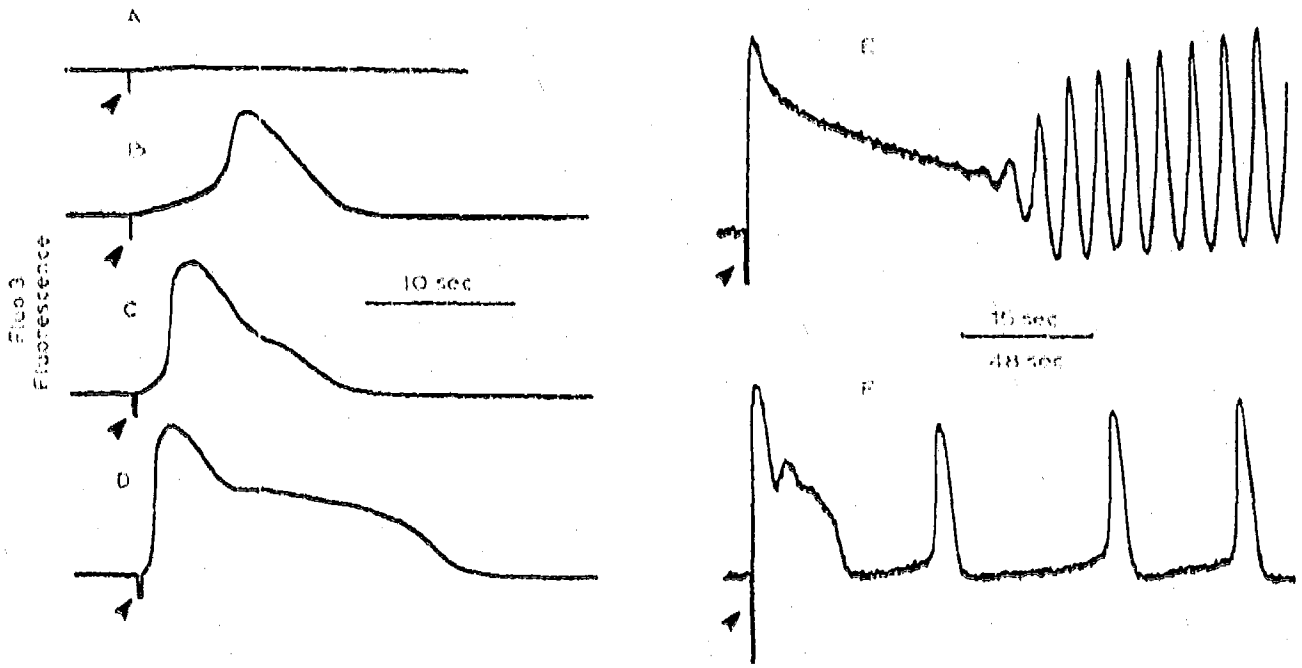

Fig. 1. Ca^{2+} responses induced by variable amounts of photo-released $InsP_3$: a barely detectable Ca^{2+} increase is caused by a 50 msec flash in (A) ($InsP_3$-releasing flashes appear as interruptions in the fluorescence trace and are indicated by arrowheads); doubling the flash duration makes the Ca^{2+} level slowly increase towards the generation of a Ca^{2+} spike (B); successive doubling of the flash duration elicits Ca^{2+} transients having a larger peak and a shorter lag (C,D); in the bottom trace a slowly declining plateau also appears, which is terminated by a fast descent to the basal level. (E) a 500 msec flash in another oocyte caused the characteristic kinetic phases already seen in (D) followed by the occurrence of fast sustained Ca^{2+} oscillations. (F) in another oocyte stimulated with a 400 msec flash the usual transient was followed by slow frequency oscillations.

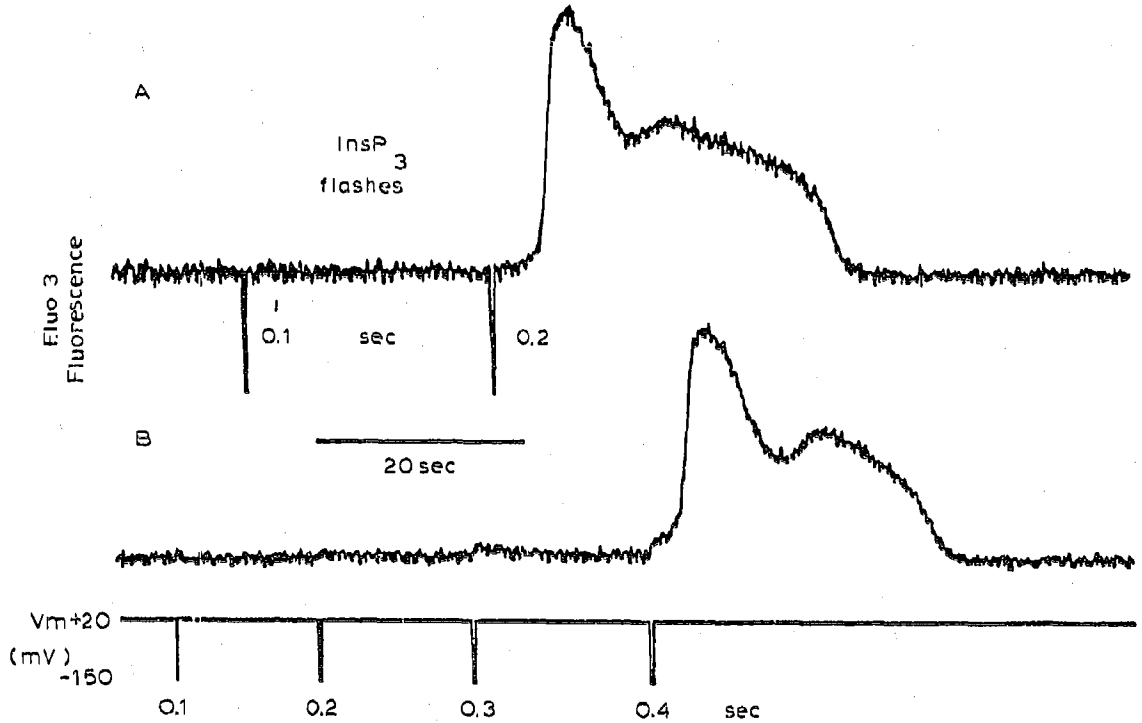

Fig. 2. The process of Ca^{2+}-induced Ca^{2+} release may be activated in mouse oocytes by hyperpolarizing the cytoplasm for various times from V_h = +20 mV to −150 mV (B bottom trace): Ca^{2+} entering through unspecific membrane leaks increases the Ca^{2+} level almost undetectably for the 0.1 and 0.2 sec pulses; the 0.3 sec pulse is just subthreshold, while the initial Ca^{2+} rise due to the 0.4 sec pulse triggers an autocatalitic Ca^{2+} spike with a second slowly declining phase. A very similar Ca^{2+} transient could be generated in the same oocyte by photo releasing a super-threshold amount of $InsP_3$ (upper trace).

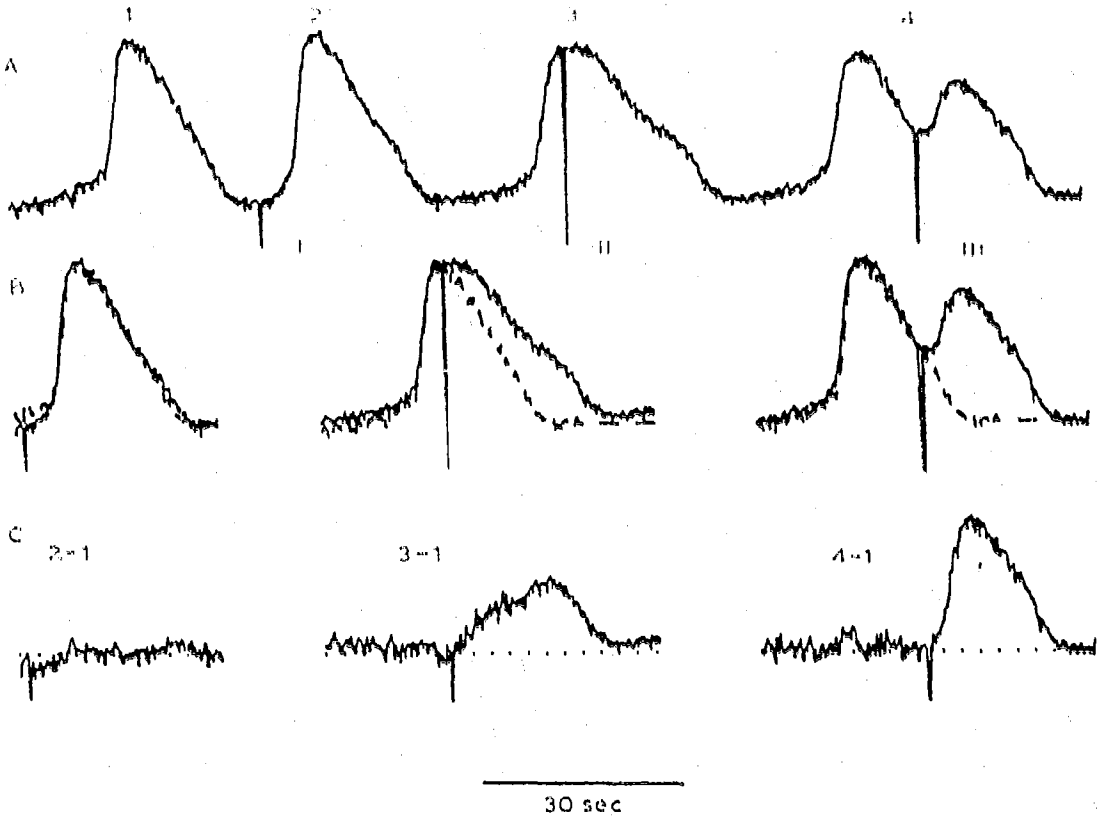

Fig. 3. Interference of $InsP_3$-induced with spontaneous Ca^{2+} transients. (A) original recording showing a spontaneous transient (1), an $InsP_3$-induced transient (2); transient 3 was spontaneously triggered but at its peak a 100 msec flash was applied; transient 4 was again spontaneous and the flash was given halfway during the decline phase. In (B) transients 2, 3 and 4 have been superimposed to the control spontaneous transient 1 (plotted as a dashed line; transient 4 was also multiplied by 1.13 to match the control amplitude). (C) shows the indicated difference traces. Holding potential −50 mV.

Ca^{2+} concentration, instead of stabilizing to the initial level, began to oscillate at fast (E) or slow (F) frequency.

The threshold behaviour of the $InsP_3$-induced cytosolic Ca^{2+} rises, illustrated in Fig. 1, suggests the presence of a regenerative mechanism for Ca^{2+} release [19]. The presence of a Ca^{2+}-induced Ca^{2+}-release process would provide such a regenerative mechanism in oocytes. Fig. 2 shows that this is indeed the case. Here, Ca^{2+} transients could be induced in the same cell in two ways: by photo release of $InsP_3$, or by direct Ca^{2+} injections. These were done by applying strong hyperpolarizing pulses of brief duration to allow Ca^{2+} entry through unspecific membrane leaks. In the experiment of Fig. 2, the oocyte was voltage-clamped at +20 mV and Ca^{2+} transients could be normally induced by photo releasing $InsP_3$ (Fig. 2A). The bottom trace in Fig. 2B shows brief hyperpolarizations (to −150 mV) of increasing duration causing progressively larger Ca^{2+} elevations. These eventually triggered a full-sized Ca^{2+} transient with a time course very similar to the one generated by $InsP_3$.

The $InsP_3$ photo-release technique allows one to repeat the stimulation after short delays to investigate the ability of the cell to produce successive calcium transients. In the cell illustrated in Fig. 3 periodic Ca^{2+} oscillations were induced by a priming $InsP_3$ pulse several minutes before. The top trace (A) shows spontaneous Ca^{2+} transients intermixed with $InsP_3$-induced Ca^{2+} transients. Three 100 msec $InsP_3$ pulses were given at different times during the spontaneous transients: just at the end (I), at the peak (II) and during the decline (III) of the spontaneous transients.

This recording was analysed in the following way: the spontaneous transient no. 1 was considered the control transient and was subtracted from the test transients 2, 3 and 4 after alignment of the traces to make the peaks correspond in time (Fig. 3B). Traces in C are the results of the subtraction and show that (i) the $InsP_3$-induced transient is practically identical to the spontaneous one (2–1), (ii) an $InsP_3$ pulse at the peak of a spontaneous transient is able to mobilize some more Ca^{2+}, although at a strongly reduced rate (3–1) and, (iii) an $InsP_3$ pulse during the decline phase of a spontaneous transient is able to generate an almost full-sized Ca^{2+} transient (4–1).

The experiments reported here were normally performed under a voltage-clamp, different from the case

of human fibroblasts [17]. No effect of the membrane potential could be detected and the Ca^{2+} responses could be induced also in current-clamp conditions.

4. DISCUSSION

The results illustrated above demonstrate that mouse oocytes possess intracellular stores that are able to discharge Ca^{2+} ions in the cytoplasm in response to $InsP_3$ and to Ca^{2+} itself. The kinetics of the $InsP_3$-induced Ca^{2+} release show complex phases that can be matched by the Ca^{2+}-induced Ca^{2+} release (see Fig. 2). Moreover, the time course of the spontaneous oscillations is identical to that of the $IsnP_3$-induced Ca^{2+} transient (Fig. 3). Then the role of the $InsP_3$-induced release of Ca^{2+} appears to be that of gradually raising the Ca^{2+} level up to the point where Ca^{2+}-induced Ca^{2+} release is triggered.

These observations, therefore, produce evidence supporting the oscillatory model of [8,14] which is based on the interplay between $InsP_3$-sensitive and Ca^{2+}-sensitive stores. However in order to produce oscillations, this model requires a continuous generation of $InsP_3$, which does not appear essential in the experiments shown here (see Fig. 1). A possible explanation of this result is that another feedback loop involving $InsP_3$ exists, i.e., if Ca^{2+} could act by enhancing the activity of phospholipase C [19,20], then a cross activation of $InsP_3$ and Ca^{2+} could be established, leading to an oscillatory behaviour [7].

It is important to note that usually sustained Ca^{2+} oscillations arose at the moment of the rapid re-uptake phase ending in a plateau, as illustrated in Fig. 1. This may suggest that loading of a particular Ca^{2+} store is the specific triggering event for oscillations.

A last point which deserves to be commented upon is the ability of the cell to produce repetitive releases of Ca^{2+}, even at very short stimulation intervals. This indicates that the CICR-positive feedback is not terminated by depletion of available Ca^{2+} and therefore it appears necessary to hypothesize the existence of a spontaneous inactivation process of the CICR mechanism.

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REFERENCES

[1] Walker, J.W., Somlyo, A.V., Goldman, Y.E., Somlyo, A.P. and Trentham, D.R. (1987) Nature 327, 249–252.
[2] Gurney, A.M. and Lester, H.A. (1987) Physiol. Rev. 67, 583–617.
[3] Berridge, M.J. and Galione, A. (1988) FASEB J. 2, 3074–3082.
[4] Rink, T.J. and Jacob, R. (1989) Trends Neuro. Sci. 12, 43–46.
[5] Peres, A. and Giovannardi, S. (1990) FEBS Lett. 261, 35–38.
[6] Kuba, K. and Takeshita, S. (1981) J. Theor. Biol. 93, 1009–1031.
[7] Meyer, T. and Stryer, L. (1988) Proc. Natl. Acad. Sci. USA 85, 5051–5055.
[8] Goldbeter, A., Dupont, G. and Berridge, M.J. (1990) Proc. Natl. Acad. Sci. USA 87, 1461–1465.
[9] Cuthbertson, K.S.R., Whittingham, D.G. and Cobbold, P.H. (1981) Nature 294, 754–757.
[10] Cuthbertson, K.S.R. and Cobbold, P.H. (1985) Nature 316, 541–542.
[11] Miyazaki, S. (1988) Dev. Growth Differ. 30, 603–610.
[12] Miyazaki, S. (1988) J. Cell Biol. 106, 345–353.
[13] Osipchuk, Y.V., Wakui, M., Yule, D.I., Gallacher, D.V. and Petersen, O.H. (1990) EMBO J. 9, 697–704.
[14] Berridge, M.J.and Irvine, R.F. (1989) Nature 341, 197–205.
[15] Berridge, M.J. (1989) J. Am. Med. Assoc. 262, 1834–1841.
[16] Minta, A., Kao, J.P.Y. and Tsien, R.Y. (1989) J. Biol. Chem. 264, 8171–8178.
[17] Pallotta, T. and Peres, A. (1989) J. Physiol. 416, 589–599.
[18] Harootunian, A.T., Kao, J.P.Y. and Tsien, R.Y. (1988) Cold Spring Harbor Symposia on Quantitative Biology LIII, 935–943.
[19] Swann, K. and Whitaker, M. (1986) J. Cell Biol. 103, 2333–2342.
[20] Chandler, L.J. and Crews, F.T. (1989) Ann. N.Y. Acad. Sci. 568, 187–192.