Inositol 1,4,5-trisphosphate receptor function in human oocytes: calcium responses and oocyte activation-related phenomena induced by photolytic release of InsP₃ are blocked by a specific antibody to the type I receptor*

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Type I inositol 1,4,5-trisphosphate-sensitive receptors (InsP₃R) are expressed in human oocytes and may be involved in operating the Ca²⁺ release triggered by the fertilizing sperm. This study examines the contribution of type I InsP₃R in operating Ca²⁺ release in human oocytes secondary to InsP₃ itself, using a specific function-blocking antibody in conjunction with photolytic release of microinjected InsP₃. Intracellular Ca²⁺ responses were assessed in oocytes microinjected with only caged InsP₃ in experiment set A, while in experiment sets B and C, sibling oocytes were injected with caged InsP₃ and the blocking antibody or a corresponding volume of medium, prior to flash photolysis. In experiment set C, certain fertilization-related phenomena (cortical granule exocytosis and chromatin configurations) were assessed using optical sections and three-dimensional image reconstructions obtained from a confocal laser scanning microscope. In experiment set A, photolytic release of InsP₃ triggered a Ca²⁺ response (increase from ~100 to 220 nmol/l followed by an exponential recovery, n = 8) and a wave in the oocytes that spread from the stimulation point to the opposite pole. In set B, photolytic InsP₃ release generated Ca²⁺ responses in control oocytes (n = 9), but not in the antibody-injected oocytes (n = 7). In set C, cortical granule exocytosis and anaphase chromosome configurations were noted in the control oocytes after flash photolysis (n = 6). These changes were completely absent in antibody injected oocytes as their cortical granules were intact and the chromosomes were in metaphase. These oocytes had also lacked Ca²⁺ responses as in set B (n = 5). This study demonstrates the functional presence of type I InsP₃R-operated Ca²⁺ channels in human oocytes and further suggests an active role of InsP₃ in triggering the Ca²⁺ rise and secondary activation phenomena at fertilization.

Key words: Ca²⁺ transients/caged IP₃/fertilization/flash photolysis/inositol 1,4,5-trisphosphate receptors

Introduction

A wavelike increase of intracellular Ca²⁺ followed by oscillations is the hallmark of mammalian fertilization (Miyazaki et al., 1993). This event initiates the process of oocyte activation and related phenomena, which mark the onset of embryo development. Furthermore, the spatiotemporal characteristics of the Ca²⁺ release at fertilization are particularly vital for embryo development, possibly because they influence cellular processes and gene expression (Ozil, 1990; Bos-Mikich et al., 1997; Rout et al., 1997; Dolmetsch et al., 1998). Understanding the mechanisms determining the occurrence and spatiotemporal dynamics of Ca²⁺ response in the oocyte is therefore crucial.

Two types of receptor-operated channels are thought to mediate Ca²⁺ release in the oocytes, namely receptors that are sensitive to ryanodine (RyR) and those that are sensitive to the second messenger inositol 1,4,5-trisphosphate (InsP₃R) (Berridge, 1993; Coronado et al., 1994). Of the two, the type I InsP₃R are most prominently expressed in oocytes and hence, are likely to be the main Ca²⁺ channel-regulating receptors releasing Ca²⁺ from intracellular stores at fertilization (Miyazaki et al., 1992b; Fujikura et al., 1993; Yue et al., 1995; Mehlmann et al., 1996; He et al., 1997). The functional role of type I InsP₃R in operating Ca²⁺ release in oocytes at fertilization has been demonstrated in the golden hamster and mouse using a specific blocking antibody, and in frogs using a polyclonal...
antibody against a C-19 peptide of rat type I InsP₃R (Miyazaki et al., 1992b; Xu et al., 1994; Runft et al., 1999). In human oocytes, the type I InsP₃R are prominently expressed in oocytes, zygotes and embryos and are dynamically redistributed through maturation, fertilization and early embryogenesis (Goud et al., 1999). Nonetheless, there is no direct proof as to the functional role of the InsP₃R in human oocytes and the information available in the literature is indirectly derived from studies on Ca²⁺ responses after exposure to various agonists/antagonists (Herbert et al., 1995, 1997; Sousa et al., 1996a,b).

Therefore, as an initial step towards exploring the role of the InsP₃R in human oocytes, we investigated the Ca²⁺ responses to InsP₃ in oocytes injected with or without a specific function-blocking antibody to the type I InsP₃R (18A10 mAb) (Miyazaki et al., 1992a,b). A novel method of photolytic release of injected InsP₃ was employed and the responses were studied by assessing the calcium release as well as early phenomena related to oocyte activation such as cortical granule release and exit from the meiotic metaphase. Photolytic release of the injected caged InsP₃ resulted in Ca²⁺ release, which spread globally in the oocyte in a wavelike manner. This phenomenon was followed by cortical granule release as well as metaphase–anaphase transition. On the other hand, blockade of the type I receptor in the antibody-injected oocytes showed both complete absence of the calcium responses as well as maintenance of the chromosomes in the meiotic metaphase without any cortical granule release.

Materials and methods

Study design

In experiment set A, oocytes were loaded with a Ca²⁺-sensitive dye and injected with caged InsP₃ at the desired concentration and Ca²⁺ responses were recorded during and after flash photolysis. In experiment set B, Ca²⁺ measurements were performed in a similar way in sibling oocytes that were either sham-injected (controls) or injected with a blocking antibody (18A10 mAb), then injected with caged InsP₃ and subjected to flash photolysis. In experiment set C, sibling oocytes were treated as in set B and were subsequently subjected to fluorescent staining for cortical granules and chromatin after flash photolysis of injected caged InsP₃. Assessment for cortical granules and chromatin was performed under a confocal laser scanning microscope (CLSM).

Source of oocytes

The project was approved by the Institutional Ethical Review Board of Ghent University. Accordingly, donated spare germinal vesicle (GV) and metaphase I (MI) stage oocytes from patients undergoing ICSI were subjected to in-vitro maturation (Goud et al., 1998). The oocytes that matured to the metaphase (MII) stage at the end of 30–32 h of culture without gross size, shape and cytoplasmic abnormalities were selected for the study. As the experiment sets B and C involved a comparison of two groups, care was taken to assign sibling oocytes with similar features into groups that would be compared subsequently.

Fluo-3 loading and microinjection of caged InsP₃ and antibody

The mean diameter of each oocyte was measured using an ocular grid in three perpendicular axes at ×400 magnification, and the approximate volume of each oocyte was deduced from this, assuming a spherical shape. This helped to estimate the amount of caged InsP₃ and/or antibody to be injected into each individual oocyte in order to attain a desired concentration. The oocytes in either group were then incubated for 1 h in human tubal fluid medium (HTF; Irvine Scientific, Irvine, CA, USA) containing 10 µmol/l of the Ca²⁺-sensitive cell permeable dye fluo-3-AM (Molecular Probes, Eugene, OR, USA). The dye loading was continued after further steps involving the microinjection of caged InsP₃ and/or 18A10 mAb.

The caged InsP₃ (Calbiochem, San Diego, CA, USA) was dissolved at 5 mMol/l in an intracellular buffer containing 134 mMol/l KC1, 7.8 mMol/l NaCl, 7.8 mMol/l Na₂HPO₄, and 1.4 mMol/l KH₂PO₄, pH 7.2. About 10–15 pl (11.8 ± 0.1 pl) of caged InsP₃ was microinjected into each oocyte to be studied, using a glass ICSI micropipette with a shaft of 10 µm internal diameter that was constant for >500 µm length. The injection technique was similar to ICSI (Goud et al., 1997) and a predetermined amount of caged InsP₃ was injected by calculating the length of the column and consequently the volume within the micropipette segment. Leakage of caged InsP₃ from the micropipette during the procedure was avoided by protecting the column with 10 µm columns (~0.5–1.0 pl) of mineral oil on either side transiently before microinjection. The first mineral oil column was expelled just prior to the penetration of the oolemma, and microinjection was stopped and the micropipette withdrawn as the second column reached the tip of the injection pipette. This allowed a fair amount of accuracy in microinjecting the desired quantity of caged InsP₃ in the oocytes. In both the experimental sets, the approximate intracellular concentration of caged InsP₃ was ~50 µmol/l (Table I).

In experiment sets B and C, oocytes were pre-injected with the 18A10 antibody (estimated final concentration ~70 µg/ml, Table I) using the same technique described above. The control oocytes in both these experiment sets received injection of the corresponding volume of the vehicle (phosphate-buffered saline, 0.4% bovine serum albumin). The oocytes were allowed to recover over the next 30 min before injection of the caged InsP₃. Fluo-3 loading then continued after caged InsP₃ microinjection until the time when the oocytes were subjected to flash photolysis.

Flash photolysis

The oocytes were transferred to the warm stage (37°C) of a fluorescence microscope equipped to perform Ca²⁺ imaging and flash photolysis. The flash photolysis light was generated with a band-pass filtered mercury-arc bulb focused to a small spot. The UV light exposure was controlled with a diaphragm and an exposure time of 250–500 ms was used. The Ca²⁺ responses were recorded on a PC or VCR and were subsequently analysed.

Staining for cortical granules and chromatin

In experiment set C, oocytes and their sibling controls were subjected to flash photolysis of caged InsP₃ using the same protocol used for experiment set B,
but were allowed to recover for 30–45 min in HTF at 37°C. They were then subjected to zona removal with acid Tyrode’s solution (Sigma-Aldrich NV/SA, Bornem, Belgium), attached to poly-L-lysine-coated coverslips, fixed for 1 h in freshly prepared 4% paraformaldehyde, blocked overnight in a 3% blocking solution (Sigma), stained with rhodamine-conjugated lens culinaris agglutinin (LCA; Vector Laboratories, Burlingame, CA, USA) (Ghetler et al., 1998) and mounted in Vectashield containing 4,6-diamidino-2-phenylindole (DAPI; Vector). The oocytes along with the suitable negative and positive controls were subjected to confocal microscopy (Bio-Rad 1024 UV). Image processing and three-dimensional (3-D) image reconstruction were done with Imaris and Huygens system-2 (SVI, Hilversum, The Netherlands).

**Results**

**Oocyte Ca\(^{2+}\) responses following flash photolysis of caged InsP\(_3\) (experiment set A)**

In experiment set A, eight MII stage oocytes were obtained from three patients and were successfully injected with caged InsP\(_3\). All oocytes survived the injection procedure. The estimated volume of caged InsP\(_3\) within the oocytes was 11.8 ± 0.1 pl (mean ± SE). Estimates of oocyte volume, concentrations of caged InsP\(_3\) and 18A10 mAb, as well as the fluorescence changes (∆F/F₀) are presented and illustrated in Table I and Figure 1 respectively. Overall, flash photolysis of caged InsP\(_3\) (250 ms duration) triggered a Ca\(^{2+}\) response in all the oocytes, and the relative fluorescence change corresponded roughly to a Ca\(^{2+}\) rise from ~100 to 220 nmol/l. The Ca\(^{2+}\) response was followed by an exponential recovery characterized by a time constant of 34 ± 9 s (Figure 1A,B). In seven oocytes, application of the flash at the oocyte periphery demonstrated a Ca\(^{2+}\) wave spreading from the stimulation point to the opposite side of the cell at a speed of 19.3 ± 1.5 µm/s (Figure 1B–D). Oocyte Ca\(^{2+}\) changes did not propagate to the polar bodies.

**Flash photolysis of caged InsP\(_3\) in antibody-injected and control oocytes (experiment sets B and C)**

In experiment set B, 22 sibling oocytes from nine patients were assigned for injection with 18A10 mAb (test subgroup, \(n = 11\)) or sham injection (control subgroup, \(n = 11\)) in addition to caged InsP\(_3\) injection. In the control group, nine of the 11 oocytes survived microinjection of caged InsP\(_3\) and medium. The estimated volume of caged InsP\(_3\) in control oocytes was 12.9 ± 0.2 pl, similar to that for the test subgroup (12.9 ± 0.3 pl). Also, the estimated oocyte volumes and concentrations of InsP\(_3\) were similar in the test and control groups (Table I). The volumes of the injected 18A10 antibody and medium (sham injection) were also similar (data not shown). Flash photolysis of caged InsP\(_3\) (500 ms duration) resulted in relative fluorescence increase in all nine control oocytes indicating a Ca\(^{2+}\) response (Table I, Figure 1A–D), corresponding to a Ca\(^{2+}\) increase from a baseline value of ~100 to ~300 nmol/l.

In the test subgroup, seven of 11 oocytes survived microinjection of caged InsP\(_3\) and 18A10 antibody. Flash photolysis of the caged InsP\(_3\) resulted in no fluorescence increase in any of the oocytes injected with the 18A10 antibody, indicating a complete lack of a Ca\(^{2+}\) response in all of the seven surviving oocytes.

**Spatiotemporal characteristics of the Ca\(^{2+}\) response**

In experiment set B, the control and antibody-injected oocytes were monitored for fluorescence increase for 10 min subsequent to photolytic release of caged InsP\(_3\). In the control group, a single Ca\(^{2+}\) rise was noted in six out of nine oocytes and no subsequent Ca\(^{2+}\) rises were noted. However, in the other three control oocytes, we observed at least three spontaneous Ca\(^{2+}\) rises during the 10 min after flash photolysis of caged InsP\(_3\) (Figure 1E). Such secondary spontaneous Ca\(^{2+}\) rises were never observed in the oocytes injected with the 18A10 antibody.

**Cortical granule and chromatin status in oocytes after flash photolysis (experiment set C)**

In experiment set C, test and control oocytes were injected with 18A10 antibody (\(n = 6\)) or were sham-injected with medium (\(n = 6\)), before injection of caged InsP\(_3\) as in experiment set B. They were subjected to flash photolysis and subsequently processed for cortical granule and chromatin staining. In the control group, all six oocytes survived the injection procedure while in the test group five out of six oocytes survived the microinjection procedure. Flash photolysis resulted in a Ca\(^{2+}\) response in the control group, similar to that in experiment set B (Table I). Also, the antibody-injected oocytes exhibited a complete lack of Ca\(^{2+}\) response as in experiment set B.

Analysis of the individual 2–5 µm optical sections of the oocytes as well as the 3-D reconstructions revealed cortical granule exocytosis. This was evident from the extrusion of cortical granule contents in the perivitelline space (Figure 2A,B). In some control oocytes, the cortical granule contents formed aggregates just outside the oolemma and these were clearly noted in 3-D reconstructed images (Figure 2D). On the other hand, the cortical granules in the 18A10 mAb-injected oocytes were found to be intact (Figure 2C,E). The chromosomes in all the control oocytes were found to be in anaphase (\(n = 6\), Figure 2A,B inset). However, the chromosomes in the antibody-injected oocytes were found to be in metaphase configuration (Figure 2C). Thus, the control oocytes showed both cortical granule exocytosis as well as metaphase–anaphase transition, whereas the antibody-injected oocytes lacked both of these phenomena.

**Discussion**

A rise of intracellular calcium in the oocytes is known to play a key role during fertilization and possibly also during oocyte maturation (Miyazaki et al., 1993; Homa, 1995; Mattioli et al., 1998; Pesty et al., 1998). The Ca\(^{2+}\) release during fertilization in mammals is peculiar as it takes the form of repetitive oscillations. This is in contrast to the single rise in Ca\(^{2+}\) that occurs in other species such as the amphibians (Swann and Parrington, 1999). Furthermore, spatiotemporal characteristics of the Ca\(^{2+}\) oscillations are thought to directly influence embryonic development (Ozil, 1990; Bos-Mikich et al., 1997). Therefore, elucidation of the intracellular Ca\(^{2+}\) release mechanisms is vital in mammalian oocytes.

Oscillatory release of Ca\(^{2+}\) has also been noted during human fertilization (Taylor et al., 1993; Tesarik et al., 1994; Tesarik and Sousa, 1995). However, the mechanisms involved in this process are as yet unclear. A sperm-specific factor has been suggested to initiate Ca\(^{2+}\) oscillations in oocytes, but the exact nature of this factor in not known (Parrington et al., 1996; Sette et al., 1997; Dale et al., 1999).

According to an alternate theory proposed to explain the Ca\(^{2+}\) release at fertilization, an interaction between a sperm surface ligand and putative oocyte surface receptors activates Gq-protein mediated mechanisms. This generates the secondary messenger, InsP\(_3\), via the activation of phospholipase C (Schultz and Kopf, 1995). However, even this theory is not entirely accepted as a Gq protein antibody fails to block Ca\(^{2+}\) release at fertilization (Williams et al., 1998).

Recently, a combination of the above-mentioned two theories was proposed (Swann and Parrington, 1999). Accordingly, phospholipase C activity in the sperm factor is thought to be involved in generation of InsP\(_3\) in the oocytes (Jones et al., 1998). Therefore, involvement of InsP\(_3\) and its receptors may still be important in the generation of Ca\(^{2+}\) transients in oocytes.
InsP$_3$-sensitive receptors in human oocytes

Figure 1. Calcium responses of oocytes to photolytic release of InsP$_3$ in human oocytes. (A) Raw fluorescence image of an oocyte loaded with the calcium dye fluo-3. The scalebar measures 40 µm. (B) Calcium image sequence showing changes of fluo-3 fluorescence relative to the fluorescence before stimulation (ΔF/F). Application of the UV light to the right side of the oocyte triggered an increase of calcium that swept over the cell like an intracellular calcium wave. The times in the upper right corners indicate the time since the start of the flash photolysis. In oocytes that were injected with the InsP$_3$ receptor antibody (18A10), no calcium responses were observed (not shown). (C) Location of the analysis points from which the traces shown under D and E were drawn. (D) Time course of calcium changes (ΔF/F) at the different analysis points shown in C. Note that the first apparent peak Ca$^{2+}$/H$_{11001}$ observed at point 1 is due to the flash itself during first 250 ms, whereas the Ca$^{2+}$ changes observed at other time points have similar peaks. (E) Calcium trace showing the time course of calcium changes at analysis point 5 shown in (C). In this oocyte, the initial response to photolytic stimulation was followed by two calcium oscillations occurring with a period of ~1 min. Although derived from experiment set B, the Figure also represents experiment set A, barring some differences (see text for details).

InsP$_3$R are the major Ca$^{2+}$/H$_{11001}$ channel operating receptors in human as in other mammalian oocytes (Shiraishi et al., 1995; Mehlmann et al., 1996; He et al., 1997; Macháty et al., 1997; Goud et al., 1999). Moreover, between the ryanodine-sensitive receptors (RyR) and the InsP$_3$R, the latter seem to be the main contributors of Ca$^{2+}$/H$_{11001}$ release at fertilization. This is supported by the prominent expression of InsP$_3$R compared to the very low or absent expression of the RyR seen in various species (Ayabe et al., 1995; Yue et al., 1995; He et al., 1997). Furthermore, InsP$_3$ itself, and its agonists can induce an intracellular Ca$^{2+}$/H$_{11001}$ rise in human and other mammalian species (Fujitawa et al., 1993; Fissore et al., 1995).

Among the various subtypes of the InsP$_3$R identified so far, the type I receptor is most prominently expressed in oocytes (Parrington et al., 1998; Fissore et al., 1999). In the human oocytes, as in other species, the type I InsP$_3$R undergoes an increase and a dynamic redistribution through maturation (Shiraishi et al., 1995; Mehlmann et al., 1996; He et al., 1997; Goud et al., 1999). Finally, in the MII stage oocyte, the type I InsP$_3$R is expressed in close proximity with the oolemma, which encounters the sperm after zona penetration and also with the cortical granules, which are released at fertilization (Kline, 2000).

We have previously shown that human oocytes express the type I InsP$_3$R as seen from Western blots and immunocytochemistry (Goud et al., 1999). In continuation of this study, we aimed to investigate the functional presence of the type I InsP$_3$R in human oocytes. We preferred the use of InsP$_3$ itself and its specific antibody rather than...
Figure 2. Status of cortical granules and chromatin following photolytic release of InsP3 in oocytes. (A) An approximately equatorial optical section through an oocyte after photolytic release of InsP3. Cortical granules are in the process of exocytosis (two short arrows in left upper corner) immediately adjacent to the oolemma. The solid arrow points to the chromatin within the first polar body. A hollow arrowhead points to the chromosomes showing the metaphase–anaphase transition. (B) An optical section through another oocyte following the photolytic release of InsP3. The cortical granule exocytosis is complete and an aggregate of the cortical granule content (stained with rhodamine-conjugated lens culinaris agglutinin) is visible immediately outside the oolemma (solid arrowhead). The inset shows anaphase chromosomes (stained with 4,6-diamidino-2-phenylindole) from the oocyte in B. (C) An approximately equatorial optical section passing through the metaphase plate of an oocyte which had been injected with the 18A10 mAb (see text) and subjected to photolytic release of InsP3. The arrow points to the first polar body, and the hollow arrowhead points to the oocyte chromosome plate in metaphase. The scale bar in A represents A, B and C and corresponds to 50 µm. (D and E) Extended focus views created from optical sections of oocytes in B and C respectively. D and E are X–Y projections from images in a stack covering about three-quarters of all sections as indicated by arrows at the border of D2 and E2. D1 and E1 are Y–Z projections obtained from the X–Y stack located between the indications on the left border of the image D or E. (D2 and E2) X–Z projections obtained from a stack located between the indications on the bottom border of D and E. Cortical granule exocytosis and patchy aggregations external to the oolemma are evident in D. The oocyte in E had been injected with 18A10 mAb and subjected to photolytic release of InsP3. Cortical granules are intact in E despite the photolytic release of InsP3. Scale bars in D and E represent 50 µm. Note: A is a pseudo-coloured image highlighting the cortical granules (green) and chromatin (red).

The various agonists used by others (Sun et al., 1994; Fissore et al., 1995; Sousa et al., 1996a,b). However, InsP3 in its native form is likely to be rapidly metabolized, thus requiring immediate recording of cellular Ca2+ changes. We avoided this problem by using InsP3 in a caged, inactive form, which was injected into oocytes, whereas the active InsP3 was released from the inactive caged compound after exposure to UV light (Callamaras and Parker, 1998; Leybaert and Sanderson, 2001). This method allowed us to monitor the effects of InsP3 immediately after its release into the oocytes. Furthermore, we used the specific function-blocking antibody 18A10, which was previously shown to block InsP3 thimerosal as well as sperm induced Ca2+ oscillations in hamster oocytes (Miyazaki et al., 1992a,b).

In experiment set A, we investigated the Ca2+ responses in human oocytes that were matured in vitro from the GV stage. We found an instantaneous response to photolytic release of InsP3 in the form of a fluorescence change indicating Ca2+ release. This Ca2+ release signal propagated from the stimulation point to the rest of the oocyte in a wave-like manner, as demonstrated by the fluorescence recordings performed at four different points within the oocyte. The peak Ca2+ change associated with this wave was nearly the same throughout the oocyte, indicating that Ca2+ by itself is not the diffusing ion. It is known that the diffusion of Ca2+ ions in the cytoplasm proceeds at a slower rate as compared to InsP3, because Ca2+ is bound to less mobile cytoplasmic Ca2+ binding molecules (Allbritton et al., 1992). The wave-like propagation of Ca2+ changes is therefore more likely the result of the diffusion of InsP3 through the oocyte cytoplasm. Compatible with this conclusion is the fact that the overall Ca2+ wave propagation velocity of 19.3 µm/s is in the order of the root-mean-square velocity which, assuming diffusion in one direction, was calculated to be in the order of 24 µm/s (square root from 2D/t, with D[InsP3] = 283 µm2/s). The waveform Ca2+ release was subsequently followed by an exponential recovery. Overall, the
characteristics of the Ca$^{2+}$ wave generated in response to photo-
lytically released InsP$_3$ were similar to the initial Ca$^{2+}$ response
occurring at fertilization in human and other mammalian species
(Miyazaki et al., 1993; Taylor et al., 1993). However, in experiment
set A, no subsequent spontaneous transients occurred in any of
the oocytes, which is contrary to the oscillatory Ca$^{2+}$ release
characteristically seen during mammalian fertilization. Oscillatory
Ca$^{2+}$ release in oocytes has also been noted after InsP$_3$ injection.
Thus the subsequent Ca$^{2+}$ responses in oocytes in experiment set A
were different compared to earlier reports (Galiane et al., 1994; Jones
et al., 1998). These differences may be related to the quantity of
InsP$_3$ released after the flash, as we used 250 ms exposure time,
which may cause a release of InsP$_3$ that is inadequate for mounting
secondary responses as the primary response may not reach the
threshold (Miyazaki et al., 1993). Hence we increased the UV flash
exposure time to 500 ms in our subsequent experiment sets. We
found that the initial peak Ca$^{2+}$ response was higher in experiment
sets B and C compared to that in experiment set A (~300 versus
~220 nmol/l respectively). As increased exposure time increases the
relative concentration of photolytically released InsP$_3$, this could lead
to a proportionately higher Ca$^{2+}$ response. Furthermore, increased
exposure time also leads to the occurrence of spontaneous Ca$^{2+}$
transients in some oocytes at a frequency of 1–2/min.

Another possible contributor to differences in the peak primary
and the subsequent spontaneous Ca$^{2+}$ responses could be related to the
oocyte’s InsP$_3$ sensitivity and ability to generate spontaneous
Ca$^{2+}$ transients. This may be compromised in oocytes matured in vitro
from the GV stage as opposed to being retrieved at the MI or
MII stage. In experiment sets B and C, we included oocytes matured
from MI stage in addition to those matured from the GV stage.
Interestingly, all the oocytes showing secondary spontaneous Ca$^{2+}$
transients had been retrieved at the MI stage and were subsequently
matured in vitro, as compared with the oocytes which were matured
from the GV stage in vitro. These differences in spontaneous
Ca$^{2+}$ transients indicate that these two types of oocytes may differ
in terms of their Ca$^{2+}$ release mechanisms.

In experiment sets B and C, our main objective was to investigate the
contribution of type I InsP$_3$R in controlling intracellular Ca$^{2+}$
release and oocyte activation in oocytes respectively. In both experi-
ment sets B and C, the specific function-blocking antibody to type I
InsP$_3$R completely blocked the InsP$_3$-induced Ca$^{2+}$ release (IICR)
in oocytes that were injected with the antibody prior to flash photolysis.

The 18A10 mAb recognizes an epitope close to the proposed Ca$^{2+}$
release channel region in the COOH-terminus of the receptor protein
and inhibits ICR in mouse cerebellar microsomes (Furuichi et al., 1989;
Nakade et al., 1991). Furthermore, it also blocks the Ca$^{2+}$ release
induced by InsP$_3$ and spermatozoa in hamster and mouse oocytes
(Miyazaki et al., 1992b; Xu et al., 1994).

Our findings in human oocytes are similar to those in hamster oocytes
with regard to ICR, although the peak Ca$^{2+}$ levels and the 18A10 mAb
concentrations required to block Ca$^{2+}$ release were relatively lower
(~300 versus 500–550 nmol/l and ~70 versus 165 µg/ml respectively)
(Miyazaki et al., 1992b). Nevertheless, these differences can be
explained by the difference in the InsP$_3$ injection amount and technique
and possibly by an interspecies difference. Miyazaki et al. (1992b) have
used an iontophoretic technique, in which the relative amount of InsP$_3$
administered is represented by the magnitude of the square of the current
pulse applied. Increasing the magnitude of the pulse led to an increase
in the peak Ca$^{2+}$ rise and also proportionately increased the amount of
18A10 mAb required to block the Ca$^{2+}$ release completely. Therefore
our micromolar concentrations of InsP$_3$ released after photolytic release
cannot be directly compared with those of Miyazaki et al. (1992b).
Nevertheless, the Ca$^{2+}$ responses were completely blocked by the
18A10 mAb concentrations of ~70 µg/ml in human oocytes in our
study. Thus, even presuming that there are no species differences in
terms of InsP$_3$ sensitivity between human and hamster oocytes, it is
possible that the amount of InsP$_3$ released photolytically in our experi-
ments may be lower than those reported by Miyazaki et al. (1992b).

One small finding in our study was that the oocytes injected with the
18A10 mAb had a slightly higher rate of damage than the sham-
injected control oocytes. Although the oocyte numbers were too small
to draw conclusive evidence, considering the role of intracellular
Ca$^{2+}$ in repairing the membrane disruption (McNeil and Terasaki,
2001), InsP$_3$ and its receptor may play a role in membrane wound
repair.

In experiment set C, in addition to monitoring the Ca$^{2+}$ responses,
we also studied two fertilization-related phenomena, namely cortical
granule exocytosis and meiotic progression. Close examination of the
optical sections obtained on the CLSM allowed us to evaluate the
occurrence of cortical granule (CG) exocytosis as well as the chromatin
configurations. All the control oocytes showed signs of CG exocytosis,
although there were minor differences in the degree of its occurrence.
The CG exocytosis in the control oocytes was so distinct that
quantitative studies were deemed unnecessary. On the other hand,
the 18A10 mAb-injected oocytes distinctly revealed intact cortical
granules. Furthermore, study of the chromatin configurations revealed
that in the control oocytes, the chromosome groups separated,
indicating a metaphase–anaphase transition. The 18A10 mAb-injected
oocytes, however, showed that their chromosomes were distinctly in
meiotic metaphase. Thus, these oocyte activation-related phenomena
were related to the Ca$^{2+}$ release that was induced by InsP$_3$ and
mediated via the type I InsP$_3$R. These findings are in agreement with
the occurrence of CG exocytosis and cell cycle progression examined
by others (Whitaker and Patel, 1990; Xu et al., 1994; Macháty et al., 1997).

Thus, photolytic release of InsP$_3$ in MII stage human oocytes
resulted in a Ca$^{2+}$ response, which was blocked by the specific
18A10 mAb. The occurrence and spatiotemporal characteristics of
Ca$^{2+}$ release were dependent on the amount of InsP$_3$ released and
also on the source of the oocyte. Blockade of the Ca$^{2+}$ release in 18A10
mAB-injected oocytes also resulted in the blockade of subsequent
fertilization-related phenomena of CG exocytosis and meiotic exit.
Collectively, these phenomena indicate an active role played by type I
InsP$_3$R and InsP$_3$ in controlling the Ca$^{2+}$ release in human oocytes
and raise the likelihood of InsP$_3$ and type I InsP$_3$R playing an active
role during human fertilization.

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