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Non-specific currents at fertilisation in sea urchin oocytes

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Summary

Using the whole-cell voltage-clamp technique to clamp sea urchin oocytes we show that the fertilising spermatozoon triggers an inward current of -521 ± 56.7 pA ($n=8$) at activation. Simultaneously, the plasma membrane depolarises and the conductance increases from 23.4 ± 1.4 to 40.6 ± 1.2 nS ($n=8$). The I/V curve for the peak activation current is linear and the current reverses between 0 and +20 mV, suggesting a non-specific ion current. Since injection of inositol triphosphate induced an inward current of -1062 ± 314 pA ($n=4$), and the current was inhibited by preloading oocytes with the calcium chelator BAPTA, the non-specific activation current in sea urchin appears to be calcium dependent.

Keywords: Fertilisation current, Ion currents, Sea urchin

Introduction

One of the first events at activation of the oocyte is a change in plasma membrane conductance (Whitaker & Steinhardt, 1982; Dale, 1983) due to the gating of ion channels by the sperm or intracellular second messengers (Dale, 1983; Chambers, 1989). In lower animals, the opening of ion channels at fertilisation induces a depolarisation of the plasma membrane (Dale, 1983).

The nature of the ion channels that underlie the fertilisation current differs between species, but they share some common properties. In the sea urchin *Paracentrotus lividus*, the ion channels at fertilisation have been estimated to have a single channel conductance of 30–90 pS and to be non-specific for ions (Dale *et al.*, 1978). Other authors have suggested that the channels responsible for the sea urchin fertilisation current are voltage-gated sodium and calcium channels (David *et al.*, 1988; Chambers, 1989). In the ascidian *Ciona intestinalis*, the fertilising spermatozoon gates a population of non-specific, high-conductance channels generating an inward fertilisation current and a depolarisation of the membrane potential. (Dale & De Felice, 1984). These events in the ascidian are not calcium-regulated. (Dale, 1987).

In amphibians the corresponding channels are calcium-gated and are specific for chloride ions (Kline & Nuccitelli, 1985). Mammalian oocytes generate a calcium-dependent hyperpolarisation of the plasma membrane (Miyazaki & Igusa, 1981; Miyazaki, 1993). The channels responsible for this response are probably calcium-activated potassium channels, as suggested also for human oocytes (Gianaroli *et al.*, 1994; Dale *et al.*, 1996).

One of the problems in resolving the ionic basis for this electrical event in the sea urchin oocytes has been the difficulty encountered in patch-clamping this cell. In this paper, we have whole-cell voltage-clamped sea urchin oocytes and show that the ionic current in the sea urchin at activation is non-specific and is regulated by intracellular calcium ions.

Materials and methods

Collection and treatment of gametes

The sea urchin *Paracentrotus lividus* was collected from the Bay of Naples and maintained in natural sea water (NSW). Gametes were obtained by dissection and maintained in NSW for up to 4 h.

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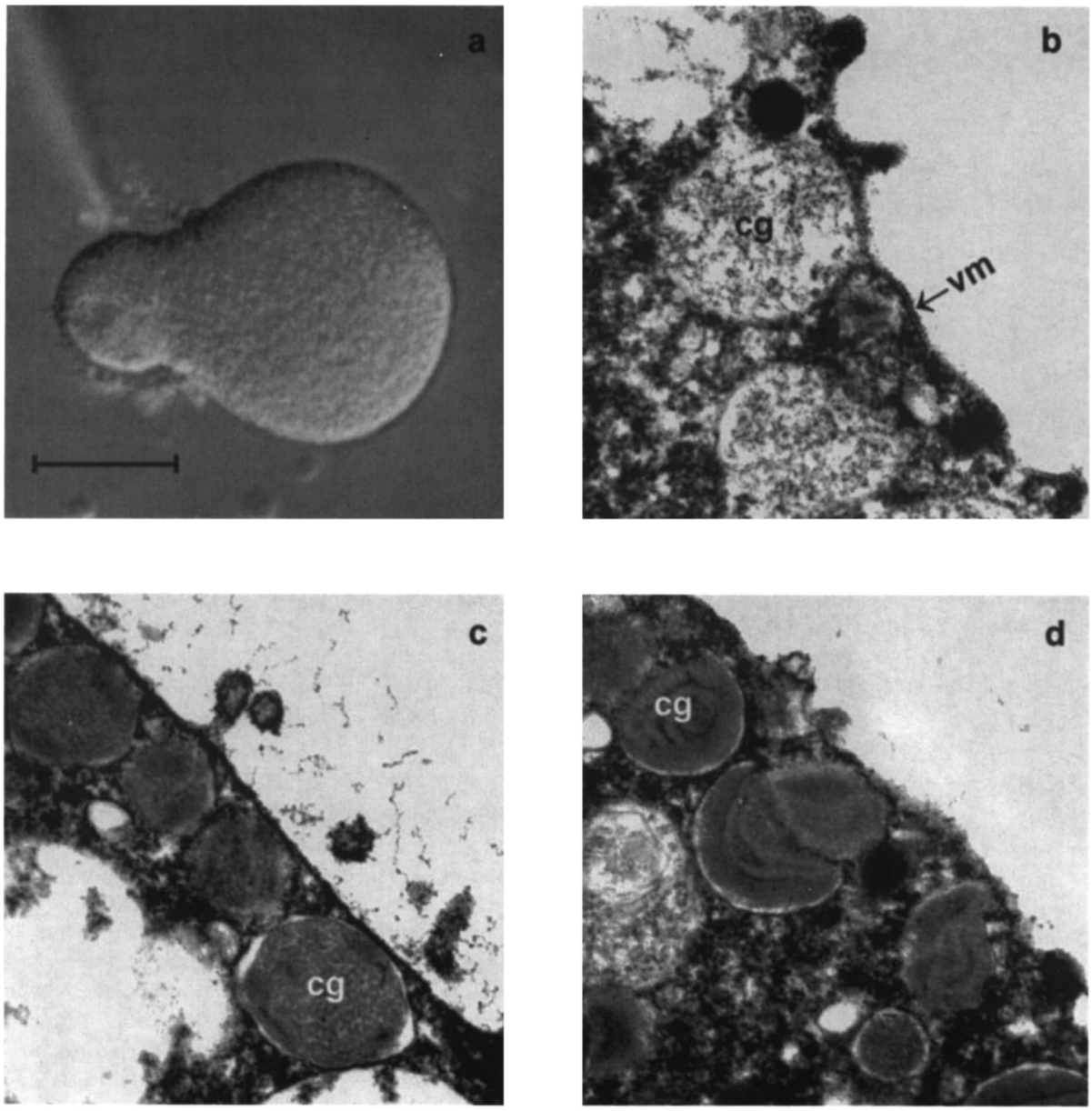

Figure 1 (*a*) Micrographs of DTT-treated control oocyte of the sea urchin *Paracentrotus lividus* showing a single protrusion. (*b*) Transmission electron micrograph (TEM) of an unfertilised control oocyte showing the plasma membrane, the apposed vitelline membrane (vm) and intact cortical granules (cg). (*c*) TEM of intact cortical granules in unfertilised DTT-treated oocytes. (*d*) TEM of cortical exocytosis in a DTT-treated oocyte after fertilisation. Scale bars represent 40 μm in (*a*) and 550 nm in (*b*)–(*d*).

Oocytes were washed free of debris by passing through a 125 μm nylon mesh followed by washing in NSW. The jelly coat was removed by exposing the oocytes to artificial sea water (ASW) containing 500 mM NaCl, 10 mM KCl, 10 mM $CaCl_2$, 50 mM $MgSO_4$ 2.5 mM $NaHCO_3$, pH 4.5, for 1 min followed by three washes in ASW at pH 8.2. The vitelline layer was removed according to Peters & Richter (1981) using a solution containing 20 mM dithiothreitol (DTT; Serva), 500 mM NaCl, 10 mM KCl, 2.5 mM $NaHCO_3$ and 10 mM Tris-HCl, pH 9.1, for 10 min at 18 °C. Oocytes were then repeatedly washed with NSW at pH 8.2. Sperm were obtained by dissection and maintained dry at 18 °C until use. The sperm were diluted 1/1000 in NSW and added to the recording bath to obtain a final concentration of about 1×10^6/ml.

Patch clamp and microinjection

Oocytes that were observed to have a cytoplasmic bleb after treatment with DTT were used for electrophysiology. Micropipettes of 1–2 μm diameter were used to whole-cell clamp the eggs on the cytoplasmic

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bleb. Pipettes were filled with an intracellular-like solution (ICS) containing 200 mM K_2SO_4, 20 mM NaCl, 200 mM sucrose, 10 mM Hepes, pH 7.5. Seals were obtained using conventional methods and the patch ruptured by applying negative pressure. The voltage clamp was set to −40 mV except where stated. Currents were measured with a List EPC-7 amplifier and stored on VHS tape for subsequent analysis. Microinjection was performed through the patch pipette using a high-pressure source (Picospritzer, NJ). The injection volume was estimated by measuring the radius of displacement of cytoplasm. Compounds to be injected were dissolved in ICS. Control injections of ICS had no effect on oocytes even when injected to 10% of the oocyte volume.

Electron microscopy

Oocytes and zygotes were fixed in a solution containing 2.5% glutaraldehyde and 1% paraformaldehyde in a buffer composed of 0.2% cacodylate and 20% sea water pH 7.2 for 1 h and post-fixed in 1% osmium tetroxide. The material was then dehydrated in ethanol, embedded in Epon resins and sectioned on a Reichert-Jung ultramicrotome. Sections were examined on a Philips 400 transmission electron microscope.

Results

Exposure of unfertilised oocytes to DTT resulted in several morphological modification of the oocytes, including formation of one surface protrusion and complete extrusion of the oocyte from the vitelline membrane. The percentage of oocytes undergoing each modification varied from batch to batch. We used oocytes that developed an incomplete extrusion of cytoplasm to apply the patch-clamp technique. Examination of these oocytes by light microscopy and electron microscopy suggested that the cytoplasmic extrusions were devoid of vitelline membrane (Fig. 1*a*), and that the DTT treatment did not trigger the exocytosis of the cortical granules (Fig. 1*b–d*).

Patch seals of 0.5 GΩ resistance were routinely obtained. The mean resting potential of unfertilised oocytes was −2.4 ± 1.7 mV (mean ± SEM, $n = 8$), while the membrane conductance was calculated to be 23.4 ± 1.4 nS (mean ± SEM, $n = 8$).

After addition of spermatozoa to the bath an inward current was generated that peaked after 50 s and then slowly inactivated over about 5 min (Fig. 2*a*). The mean peak amplitude of the fertilisation current was −521.4 ± 56.7 pA (mean ± SEM, $n = 8$; Fig. 2*a*). The resting potential depolarized to +4.5 ± 0.5 mV (mean ± SEM, $n = 8$) during the inward current and remained at this level for several minutes, eventually repolarising to the initial value. The inward current was accompanied by an increase in steady-state conductance from 23.4 ± 1.4 nS to 40.6 ± 1.2 nS (mean ± SEM, $n = 8$; Fig. 2*b*).

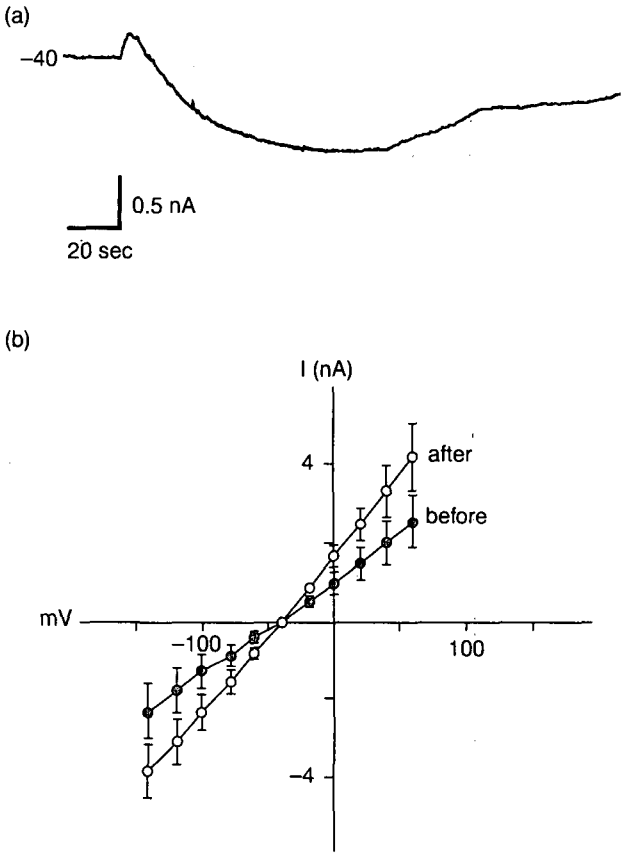

Figure 2 (*a*) Fertilisation current in an oocyte of *Paracentrotus lividus* using the whole-cell voltage-clamp configuration. (*b*) *I/V* curves in a sea urchin oocyte before (filled circles) and (open circles) the fertilisation current.

We measured the reversal potential of this sperm-induced current by varying the voltage clamp between −20, 0 and +20 mV before the addition of spermatozoa. The inward currents decreased in peak amplitude to −225 ± 35.3 pA (mean ± SEM, $n = 3$; Fig. 3*a*) at −20 mV, and −150 ± 141.4 pA (mean ± SEM, $n = 3$; Fig. 3*a*) at 0 mV. The inward current was reversed at +20 mV with a peak amplitude of +350 ± 212 pA (mean ± SEM, $n = 3$; Fig. 3*a*, *b*). The resting potential showed a 3 mV hyperpolarisation when oocytes were clamped to +20 mV. The membrane conductance also increased from 28.6 ± 1.3 nS (mean ± SEM, $n = 3$) to 35.4 ± 3.3 nS (mean ± SEM, $n = 3$).

Injection of inositol triphosphate (IP_3; Sigma, St Louis, MO) (100 μM in the pipette) into voltage-clamped sea urchin oocytes induced an immediate inward current of −1062 ± 314 pA (mean ± SEM, $n = 4$) at −40 mV (Fig. 4). The peak current reduced

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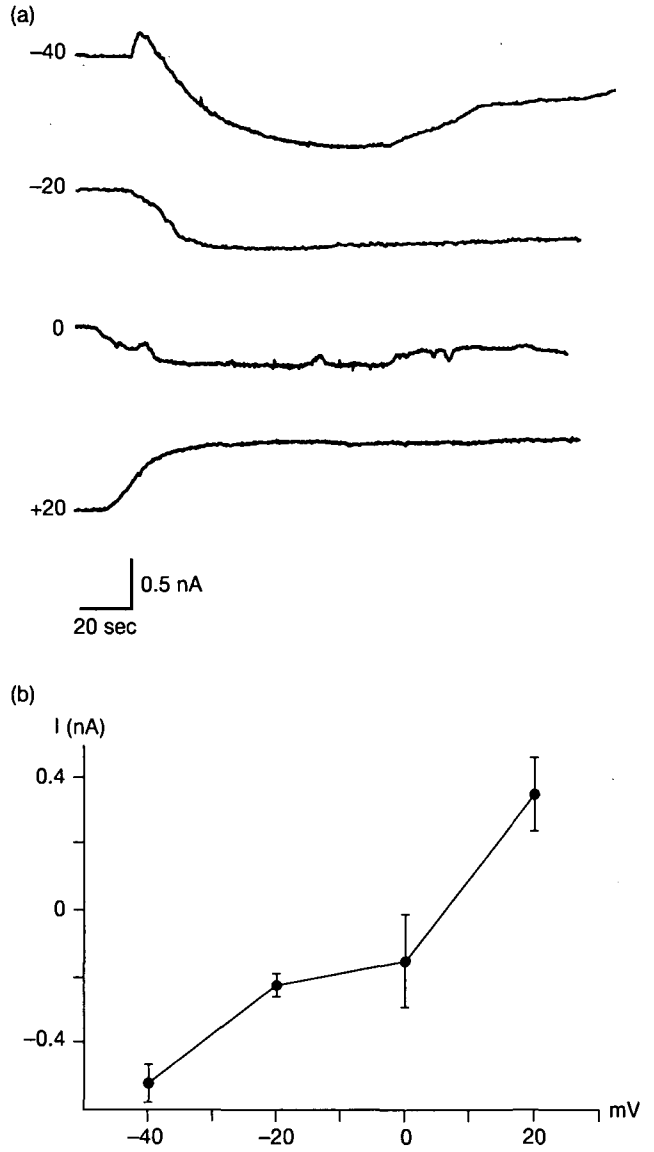

Figure 3 Properties of the fertilisation current in a *Paracentrotus lividus* oocyte. (*a*) The fertilisation current reverses between 0 and 20 mV. (*b*) The *I/V* curve for the peak fertilisation current.

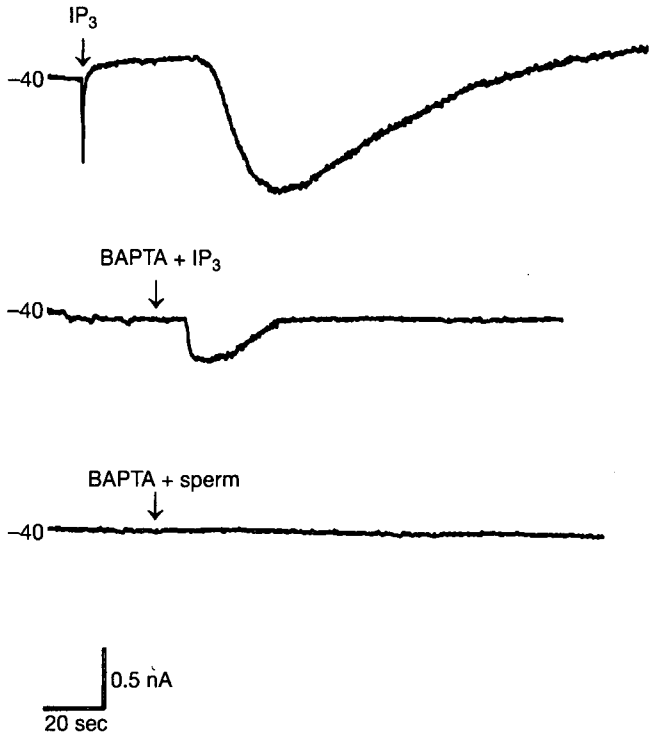

Figure 4 Top trace shows the activation current induced by microinjection of IP_3 into a voltage-clamped sea urchin oocyte. The arrow shows time of injection. The middle trace shows the activation current generated by IP_3 injection in sea urchin eggs preloaded with BAPTA. The bottom trace shows that intracellular BAPTA completely inhibits the sperm-induced fertilisation current.

in amplitude as the membrane clamp became more positive, falling to -525 ± 318 pA at -20 mV (mean $\pm$ SEM, $n=3$) and -350 ± 212 (mean $\pm$ SEM, $n=3$) at 0 mV. The current was outward at +20 mV, reaching $+900 \pm 141$ pA (mean $\pm$ SEM, $n=3$). Furthermore, injection of IP_3 generated an increase in conductance from 29.4 ± 1.4 nS (mean $\pm$ SEM, $n=4$) to 70 ± 0.9 nS (mean $\pm$ SEM, $n=4$) at -40 mV. The properties of this current were comparable to the current induced by sea urchin sperm. To test whether the inward current induced by the injection of IP_3 was due to the release of intracellular calcium, oocytes were preloaded with the calcium chelator BAPTA (1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid) to 10 mM and IP_3 was injected as above. A small inward current of -125 ± 10 pA (mean $\pm$ SEM, $n=2$; Fig. 4) was observed. This suggests that the inward current generated by IP_3 was triggered by the release of intracellular calcium and not by a direct interaction between IP_3 and the plasma membrane ion channel.

To test whether sea urchin sperm induced the inward current at fertilisation through the release of intracellular calcium, oocytes were preloaded with 10 mM BAPTA and then inseminated. No activation current was observed (Fig. 4). These data suggest that sea urchin sperm trigger an inward current at fertilisation through an ion channel that is activated by calcium ions.

Discussion

The first change in the sea urchin oocyte during interaction with a viable spermatozoon is a small 1–2 mV step-like depolarisation accompanied by a decrease in membrane resistance (Dale *et al.*, 1978). This small step-like electrical event lasts about 5–10 s,

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after which time the second larger depolarisation is triggered. Shortly afterwards there is a massive release of intracellular calcium (Dale & DeFelice, 1990). Voltage-clamp studies have confirmed that this sperm-induced electrical event is the result of an increase in membrane conductance and capacitance (McCulloh & Chambers, 1992).

In this study we used DTT to remove the vitelline membrane without damaging the plasma membrane and underlying cortical granules. This permitted us to apply the whole-cell voltage-clamp technique for the first time in unfertilised sea urchin oocytes. With this technique, we recorded a lower resting potential than that previously observed (McCulloh & Chambers, 1992). This may have been due to the DTT treatment; however, treated oocytes developed normally after insemination, and in any case in this study we have measured currents not voltages. Finally, our measurements of plasma membrane conductance before and after fertilisation (Fig. 2*b*) are comparable to those of previous authors using intracellular electrodes (McCulloh & Chambers, 1992).

The fertilisation current reported here suggests that in the sea urchin egg, the fertilising spermatozoon induces an inward current that leads to a depolarisation of the plasma membrane and an increase in conductance as previously reported. The holding voltage used in our experiments precludes the possibility that the observed currents were due to activation of voltage-gated calcium and sodium channels. Since the reversal potential for the activation current is between 0 and +20 mV it is probable that the channels underlying the activation current are non-specific as suggested for the ascidian by DeFelice & Kell (1987). Furthermore, it is interesting that the sea urchin spermatozoon can trigger the fertilisation current at positive clamp potentials as previously shown in ascidians by Pecorella *et al.* (1993).

Microinjection of IP_3 into sea urchin oocytes induces both cortical granule exocytosis and a fertilisation-like potential (Whitaker & Irvine, 1984). Here we show that the inward activation current induced by the spermatozoon can be mimicked by microinjection of IP_3 and is inhibited by clamping intracellular calcium levels with BAPTA. These data suggest that calcium ions may regulate this non-specific activation current in sea urchin oocytes.

Acknowledgements

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