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Single-channel activity in sea urchin sperm revealed by the patch-clamp technique

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Ionic fluxes are deeply involved in the response of spermatozoa to the egg. Using the patch-clamp technique, we show for the first time single ion channel activity in sea urchin spermatozoa and spermatozoa heads. Due to their small size gigaseals were obtained in suspended cells by applying suction through the pipette. The rate of gigaseal formation was very low and improved to 6% ($n = 1145$) when flagella were detached from sperm. Current-voltage curves created from single-channel events showed conductances of approx. 65 and 170 pS, suggesting the presence of two types of channels. At least one appears to be a K^+ channel.

Ion channel; Patch-clamp; (Sea urchin sperm)

1. INTRODUCTION

Membrane ionic fluxes play a fundamental role in sea urchin spermatozoal activation [1], chemotaxis [2] and the acrosome reaction (AR) [3,4]. This latter reaction, which is induced by the egg jelly, is essential for fertilization of the egg and involves a complex series of events leading to exocytosis of the sperm acrosome vesicle. Ca^{2+} and Na^+ influxes and K^+ and H^+ effluxes take place during the AR [4]. There is pharmacological evidence that indicates the involvement of ionic channels in the Ca^{2+} and K^+ fluxes [4–6].

Thus far, because of the small size of sperm, direct demonstration of the presence of ion channels has only been possible in planar bilayers derived from lipids and isolated sperm plasma membranes [7]. Here, we show for the first time direct evidence of single ion channel activity in sea urchin sperm using the patch-clamp technique [8].

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Abbreviation: TEA^+, tetraethylammonium

2. MATERIALS AND METHODS

Strongylocentrotus purpuratus and *Lytechinus pictus* sea urchins (Pacific Bio-Marine Labs) were maintained and sperm obtained as indicated in [6]. Artificial seawater (ASW) contained (mM): ASW A – 486 NaCl, 10 KCl, 2.4 $NaHCO_3$, 56 $MgCl_2$, 10 $CaCl_2$ and 10 Hepes (pH 8.0); ASW B was the same as ASW A except that [$CaCl_2$] and pH were 1 mM and 7.0, respectively. All recordings were made with ASW A unless indicated otherwise.

Briefly, sperm heads were prepared as follows: fresh sperm (100 μl) were diluted in ASW B (5 ml) and centrifuged at $30 \times g$ for 10 min. Thereafter the sperm suspension was passed through a no. 21 gauge needle between 5 and 10 times and centrifuged for 10 min at $270 \times g$. The flagellum-rich supernatant was discarded and the pellet containing the heads was washed by centrifugation ($270 \times g$, 10 min), and resuspended in 500 μl ASW B, which were kept on ice until use. The AR in sperm cells or heads was assayed as in [6].

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The micropipettes were made of pyrex or soft glass. Cells partially immobilized by adding KCl (50 mM) to ASW A or heads were diluted 2500 times in ASW A and added to a 300 μl chamber with a glass bottom on the stage of an inverted phase-contrast microscope (Nikon Diaphoto). Membrane resistance and ion currents were monitored continuously using a home-made patch-clamp with a 10 GΩ in the feed back loop [8]. Currents were low-pass filtered at 1 kHz with a 4-pole bessel filter, and stored on an FM tape recorder (Hewlett-Packard 4970-4) for further off-line computer analysis. All recordings were made at 20–22°C and with ASW A in both the micropipette and bath.

3. RESULTS AND DISCUSSION

Conventional formation of gigaseals for patch-clamping is impractical in the case of cells as small as sperm (1–2 μm) [9]. We approached the problem by forming seals with cells in suspension and by applying negative pressure through the micropipette, thus bringing the sperm into contact with the microelectrode tip (see fig.1).

We used two preparations: intact sperm and heads mechanically separated from the flagella. The viability of the sperm and heads was tested by induction of the AR with egg jelly and high pH as described in table 1. The sperm heads did not lose their responsiveness to egg jelly or high pH, although it was lower than that found in intact sperm.

Gigaseal formation depended on the presence of flagella, being lower in intact cells than in head preparation. Nevertheless, the success rate was very low in all cases as shown in table 2.

Using the head preparation no significant difference in success rate was found by using soft or hard glass, by fire polishing the micropipettes or between different sea urchin species (*S. purpuratus* and *L. pictus*). The gigaseal resistance averaged 10.6 ± 12.8 (n = 57) GΩ, with a range of 1–50 GΩ. Single-channel activity could be observed in ~30% of the gigaseals. Single-channel conductance (γ) values of 65 and 170 pS were obtained from the average of two experiments where current transitions were recorded at least at 3 voltages. In these experiments the head probably broke during seal formation leaving a single membrane attached

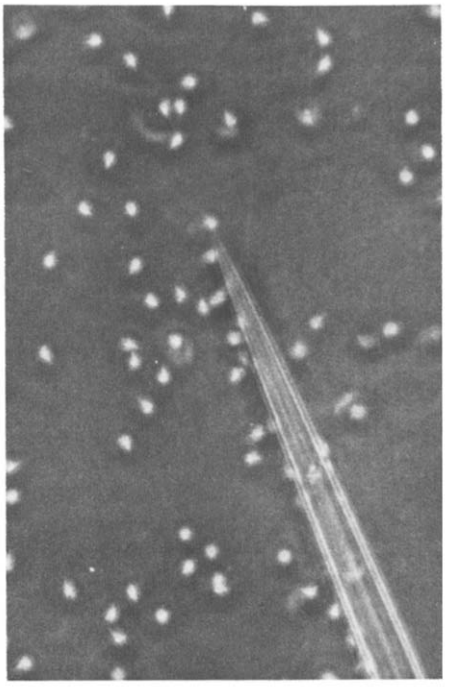

Fig.1. Gigaseal formation procedure in a sea urchin sperm from *S. purpuratus* viewed through phase-contrast microscopy at 700 × magnification (see section 2).

to the pipette, since the I-V curves went through zero. In some experiments the current transitions appeared distorted (see below) or the seal's lifetime was not enough to obtain recordings at several voltages.

The small size of sperm not only prevented a high success rate in making gigaseals, but also complicated the interpretation of membrane current recordings. In fact, Hamill et al. [8] and Fenwick et al. [10] have pointed out that in the cell-attached configuration, the current recordings are distorted when the conductance of both the outer membrane and the ion channel in the patch

Table 1

Percentage of acrosome reaction

Inducer	Sperm	Heads
Control	1.6 ± 0.5 (9)	7.6 ± 3.0 (9)
Egg jelly	89.0 ± 5.2 (9)	58.0 ± 14.3 (9)
pH 9.5	49.3 ± 16.5 (9)	30.6 ± 10.0 (9)

The AR was measured as indicated in section 2. Values are means ± SD (n)

Table 2

Success rate of gigaseal formation

Preparations	Seals/attempts	Percentage
Heads	62/928	6.7
Intact sperm	6/211	2.8

are of the same order of magnitude. A rough calculation indicates that this is the case for sperm. Considering an area for the sperm head [9] of the order of 20 μm^2 and a specific membrane conductance at rest of 3×10^{-5} $S \cdot cm^{-2}$ (as is the case of other cells [10–14]), the conductance of sperm would be ~6 pS. Fig.2A shows a distorted shape in the single-channel current recording when no external voltage was applied. Since symmetrical solutions were used in this experiment, the presence of single-channel currents indicates that sperm have a membrane resting potential as has been suggested by other methods [17,18]. The relaxations of membrane current follow single exponentials (fig.2B). From the time constant of relaxations, when the channel opens or closes, and assuming a specific membrane capacitance of 1 pF/cm^2, the conductance of the sperm head at rest and when the channel opens was calculated to be 2.7 and 6.9 pS, respectively. These values are in close agreement with the estimation of 6 pS mentioned above.

These results suggest that the high rate of 'silent' patches (70%) or distorted current recordings (13%), when gigaseals in the 'on-cell' configuration are obtained may be related to the low conductance of sperm. We tried several strategies to

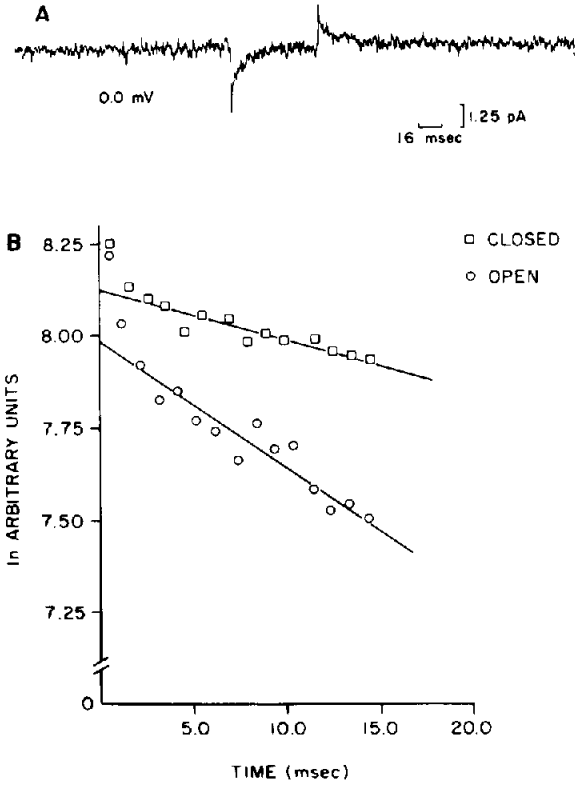

Fig.2. (A) Distortion of single-channel currents in whole-cell recordings from *S. purpuratus* sperm. ASW in bath and pipette solutions in all experiments. (B) Time course of the relaxations; time constants were 29 and 72 ms during opening and closing of the channel, respectively. *S. purpuratus* sperm.

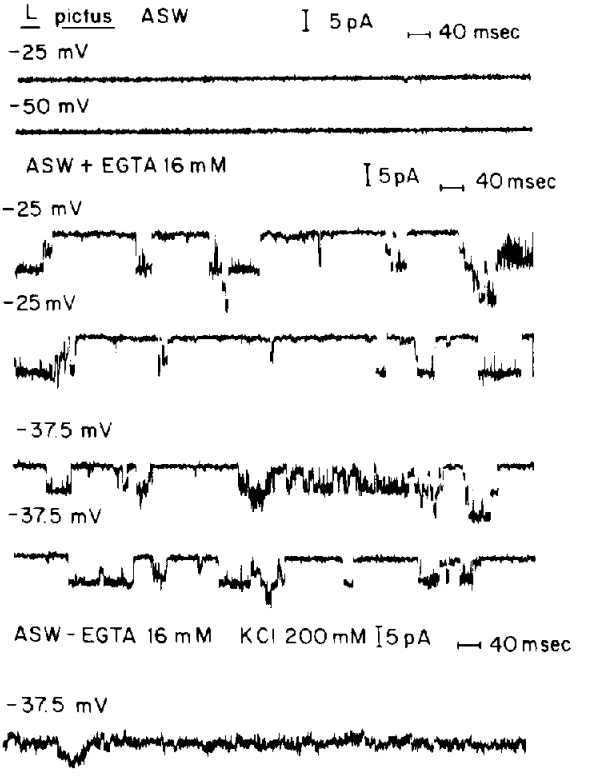

Fig.3. Transformation of a silent on-cell gigaseal to an active inside-out patch from a spermatozoon of *L. pictus*. Seal resistance was ~9 GΩ throughout. Two upper recordings in intact sperm at the indicated potentials. Middle 4 recordings obtained after EGTA (16 mM) addition to the bath. Last recording after KCl (200 mM) addition to the bath.

decrease the outer membrane resistance such as adding valinomycin, removing the pipette through the air-water interface, and decreasing external [Ca^{2+}] with EGTA. The last procedure gave the best results.

Fig.3 shows one of the three experiments where single-channel activity was observed (middle recordings) at the potentials indicated when the 'inside-out' configuration was obtained after adding EGTA to an on-cell patch that was previously silent (upper recordings). In two experiments we measured $\gamma = 170$ pS. K^+ is likely to permeate through these channels, since addition of 200 mM KCl to the bath produced a shift of ~ 40 mV in the current-voltage relation measured at 0 mV. A shift of ~ 78 mV would be expected for a purely selective K^+ channel.

The K^+ channels recorded with the bilayer technique were of 22, 46 and 82 pS [7]. We are unable at this point to compare the results of both techniques, since bilayers were made from flagellar plasma membranes and recorded in 100 mM KCl, while we recorded from the head in ASW A. However, the conductance values we observed fall within the range of K^+ channels that have been observed in several cell preparations [18].

The likely presence of K^+ channels in sperm cells certainly has deep physiological implications, since its resting potential is affected by external K^+ [16,18]. Also, the activity of an Na^+/H^+ exchange mechanism depends on membrane potential [19,20]. This exchanger may play an essential role during the AR [4,21,22]. Raising K^+ in seawater to 20 mM, or adding TEA^+ (5 mM) inhibit the AR [3,4]. In addition, it has been suggested that K^+ channels may play a role during the sperm response to Speract [23].

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