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A fast transient hyperpolarization occurs during the sea urchin sperm acrosome reaction induced by egg jelly

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The egg jelly-induced sea urchin sperm depolarization, assayed with the membrane potential-sensitive dye diS-C_3-(5), is preceded by a fast quenching, which was initially taken as an artifact. Here we show that part of this quenching results from a K^+-dependent transient hyperpolarization (~4 s). Seawater containing 25–35 mM KCl inhibits this hyperpolarization, the depolarization and the acrosome reaction induced by egg jelly, or by the acrosome reaction inducing factor purified therefrom. These results suggest that egg jelly induces a transient hyperpolarization mediated by an increase in K^+ permeability, which may be involved in triggering the acrosome reaction

Hyperpolarization; Acrosome reaction; Fluorescence; (Sea urchin sperm)

1. INTRODUCTION

To fertilize, sperm of most animals must undergo the acrosome reaction (AR). In sea urchin sperm, the AR is induced by the egg jelly, the outer investment of the egg [1]. This reaction involves the exocytosis of the acrosome vesicle (located at the tip of sperm) [2], which leads to both the release of lytic enzymes [3,4] and exposure of a specialized membrane that is required for sperm-egg binding and fusion [5,6].

In addition, egg jelly induces ionic fluxes in sperm which are essential for the AR. There are Na^+ and Ca^{2+} influxes and K^+ and H^+ effluxes [7–9] that result in an increase in pH_i [10,11] and a membrane potential (V_m) depolarization [11–13].

Since the AR is regulated by ionic fluxes we have become interested in the role of V_m in this process. By using the fluorescent V_m-sensitive probe diS-C_3-(5), we have found, in agreement with previous studies [11,12], a resting membrane potential of ~ −36 mV and a jelly-induced depolarization in *S. purpuratus* sperm. This change in V_m could be explained, at least for the major part, by an electrogenetic, nisoldipine-sensitive uptake of Ca^{2+} [13]. In these experiments we observed that the egg jelly-induced depolarization was preceded by a fast quenching. This quenching was interpreted as an artifact, since egg jelly quenches the fluorescence in the absence of cells. However, here, we present evidence that indicates that in addition to the artifact there is a hyperpolarizing component, probably due to a K^+ efflux, which may be involved in triggering the AR. Some of these results have been presented in an abstract form [26].

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Abbreviations: ASW, artificial seawater; pH_i, intracellular pH; diS-C_3-(5), 3,3′-dipropylthiadicarbocyanine iodide; $[K^+]_{ext}$, external K^+ concentration; V_m, plasma membrane potential

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2. MATERIALS AND METHODS

Lytechinus pictus sea urchin sperm, obtained by intracoelomic injection of KCl (1 ml, 0.5 M) were first diluted (1:10) in seawater at pH 7.0 and 1 mM $CaCl_2$. Then 20 μl of this suspension were added to a cuvette containing 2.5 ml ASW (486 mM NaCl, 56 mM $MgCl_2$, 10 mM $CaCl_2$, 10 mM KCl, 2.5 mM $NaHCO_3$, 0.5 mM EDTA, 10 mM Hepes, pH 8.0), with 2 μM diS-C_3-(5) previously thermostatted at 16°C. The dye takes 2–3 min to equilibrate with the cells. After 5 min incubation egg jelly or egg jelly fucose-rich factor (factor) was added. Egg jelly was obtained from the homologous eggs by an acid treatment [9] and the factor isolated from egg jelly according to [14]. Both egg jelly and the factor were quantified by their fucose content [15].

The cuvette was stirred throughout with a magnetic stirrer and the fluorescence (620–670 nm) recorded continuously (Perkin-Elmer LS-3 spectrofluorometer connected to a recorder). Samples (20 μl) were taken and fixed with 5 μl of 35% formaldehyde 15 s before induction of AR and 1 min after to quantify the percentage of AR by phase-contrast microscopy.

To estimate V_m we used the calibration factor obtained in *S. purpuratus* [13]: $(F-F_0)/F_0$ (mV) = 1.6 ± 0.2, where F_0 is the resting fluorescence value and F its change.

3. RESULTS AND DISCUSSION

Fig.1 shows the quenching of the fluorescence when *L. pictus* sperm are added to the cuvette. This quenching represents an electrophoretic uptake of the dye, which is in an aggregated, non-fluorescent form inside the cell. In 2 or 3 min the fluorescence of the dye equilibrates to a value reflecting the resting V_m, and those of the other compartments such as mitochondria. When the AR is induced by egg jelly there is a fast quenching followed by an increase in fluorescence which is consistent with a depolarization (fig.1a). When the mitochondrial membrane potential was eliminated by adding KCN (2 mM), the egg jelly-induced depolarization was only slightly diminished (~30%) (fig.1b) and the quenching remained unaffected. Thus, these egg jelly-induced changes in the signal are mainly due to the plasma membrane.

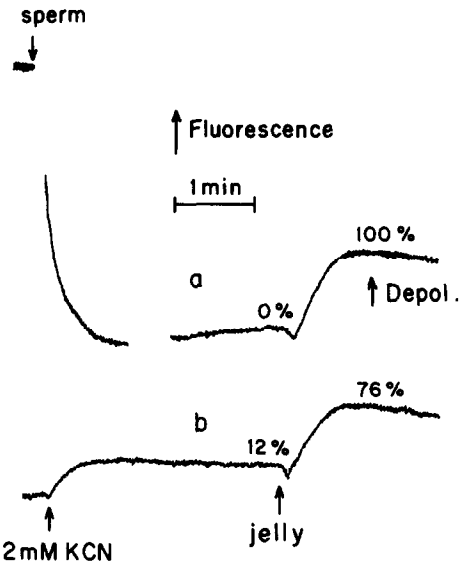

Fig.1. (a) Fluorescence of diS-C_3-(5) (620–670 nm) in the presence of sea urchin sperm and upon addition of egg jelly (120 nmol fucose/ml). An increase in fluorescence indicates a depolarization. Numbers above the curves denote the percentages of AR measured by phase-contrast microscopy. (b) Effect of KCN on the egg jelly-induced depolarization.

According to our previous calibration in *S. purpuratus* sperm [13], this increase in fluorescence corresponds to a depolarization of 20.7 ± 2.8 mV (n = 8).

The initial quenching produced by egg jelly was inhibited by increasing concentrations of KCl in seawater $[KCl]_{ext}$ indicating that a fast hyperpolarizing step, possibly due to opening of K^+ channels, occurs during AR (fig.2). At the normal $[KCl]_{ext}$ in seawater (10 mM), according to our calibration, the hyperpolarization was 8.4 ± 1.0 mV (n = 4). Our membrane potential measurements have ~4 s time resolution, since the hyperpolarization occurs within this time we cannot know its precise kinetics or magnitude. At 25–35 mM $[KCl]_{ext}$, jelly only produced a small quenching that was an artifact since it quenched the signal even in the absence of cells (not shown). Concomitantly with the KCl-induced block of hyperpolarization, the depolarization step and AR were also inhibited.

The acrosome reaction is induced by a component of the egg jelly, a fucose sulfate-rich polymer

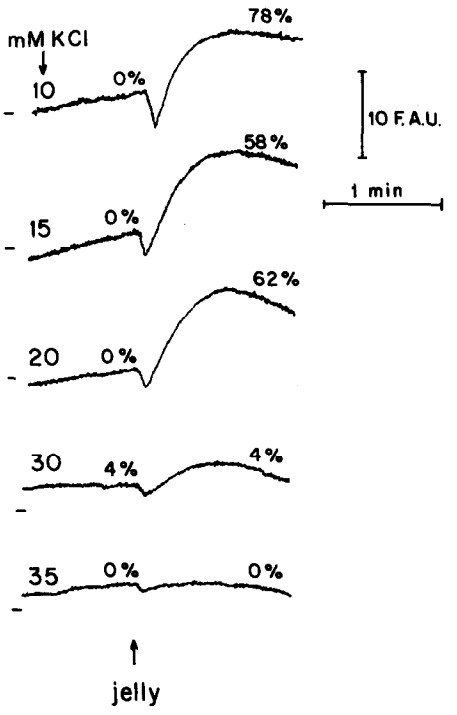

Fig.2. Effect of egg jelly (100 nmol fucose/ml) on the AR and membrane potential in sperm at the indicated concentrations of KCl. Upper trace, control with ASW. Numbers above the curves are percentages of the AR. F.A.U., fluorescence arbitrary units. The hyphen indicates 35 F.A.U.

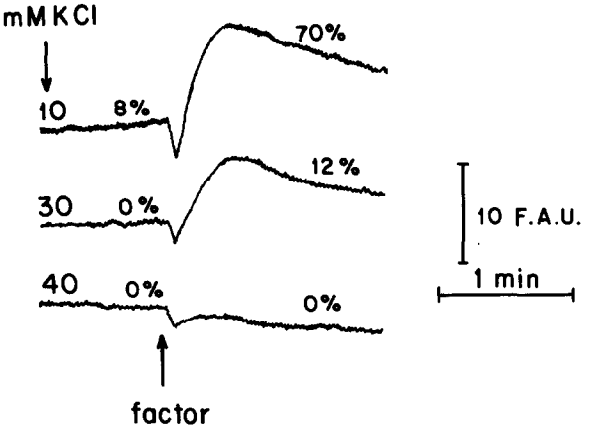

Fig.3. Effect of the egg jelly purified factor (150 nmol fucose/ml) on sperm AR and membrane potential at the indicated concentrations of KCl. Upper trace, control with ASW. Numbers over the curves indicate percentages of the AR.

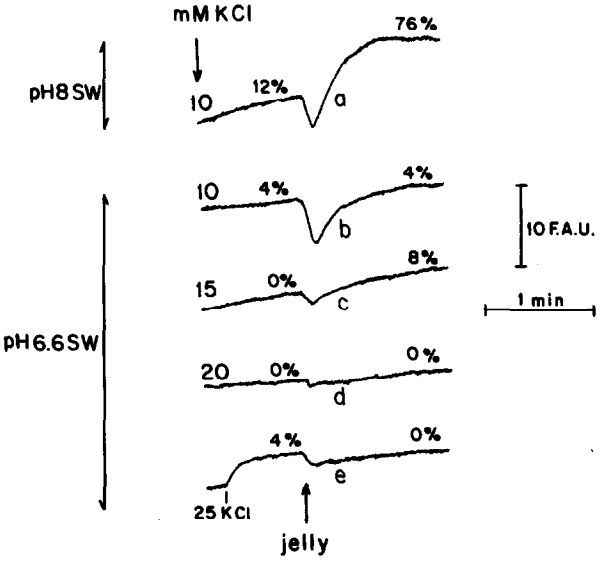

Fig.4. Effect of egg jelly (300 nmol fucose/ml) on the AR and membrane potential of sperm in pH 6.6 sea water. (a) Control with normal pH 8 seawater, (b–d) in pH 6.6 seawater at the indicated concentrations of KCl and (e) in pH 6.6 seawater plus 25 mM KCl added 45 s before egg jelly addition. Numbers over curves are percentages of AR. F.A.U., fluorescence arbitrary units.

of high M_r [14]. In addition, egg jelly contains a small peptide which in *S. purpuratus* has been called speract [16,17]. This polypeptide can stimulate sperm respiration [16–18] and triggers transient Ca^{2+} uptake and an increase in pH_i [19], which may be mediated by an increase in K^+ permeability that hyperpolarizes sperm [20]. Fig.3 shows that the fucose-rich factor isolated from *L. pictus*, which is free of small polypeptides, induces similar changes to those of egg jelly, i.e. a hyperpolarization, preceding the depolarization, that is inhibited by KCl. Therefore, the changes observed in V_m are apparently associated with the specific mechanisms that induce the AR. It is known that low-pH seawater (~7.0) inhibits the AR and the depolarization induced by egg jelly [8,12,13]. As illustrated in fig.4, egg jelly also induced a transient K^+ dependent hyperpolarization when sperm were suspended in seawater at pH 6.6. As expected, under these conditions depolarization and the AR were inhibited.

Our results indicate that a hyperpolarization

caused possibly by opening of K^+ channels could participate in triggering the AR.

During the AR, the increase in pH_i (~7.4 to 7.7) may involve the stimulation of an Na^+/H^+ exchanger [7,8]. A similar increase in pH_i, induced by NH_4Cl in the absence of jelly, can induce the AR, Ca^{2+} uptake [21] and a nisoldipine-sensitive depolarization [13]. Thus, egg jelly could stimulate first an Na^+/H^+ exchanger which would increase pH_i and somehow activate Ca^{2+} channels that contribute to the depolarization [13]. On the other hand, sperm flagella contain a voltage-dependent Na^+/H^+ exchanger which is activated under hyperpolarizing conditions [22]. Considering that pH_i can be modulated by V_m, we believe that the egg jelly-induced hyperpolarization could stimulate the Na^+-dependent increase in pH_i by activating this voltage-dependent Na^+/H^+ exchanger.

This working hypothesis requires the demonstration that the voltage-dependent Na^+/H^+ exchanger found in sperm flagella [22] is present in the head and participates in the AR. The hypothesis is consistent with the presence of K^+ channels in the plasma membrane of sea urchin sperm [23,24], and with the inhibition caused by high K^+ (this paper) and TEA^+, a K^+ channel blocker, of the egg jelly-induced AR [8], the acid release [8] and the depolarization [13,25]. In addition, unlike egg jelly, the NH_4Cl-induced AR and depolarization in *S. purpuratus* are not inhibited by TEA^+ or high K^+ [13], suggesting that these inhibitory conditions affect the mechanisms by which egg jelly induces in sperm an increase in pH_i.

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