Increasing intracellular pH of sea urchin sperm with NH_4Cl induces Ca^{2+} uptake and acrosome reaction in the absence of egg jelly

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Intracellular alkalinization and Ca^{2+} uptake accompany the egg jelly-induced acrosome reaction of sea urchin sperm. Here, sperm were incubated in NH_4Cl-containing seawater maintained at its physiological pH (8.0). Under these conditions, 20 mM NH_4Cl induced intracellular alkalinization, Ca^{2+} uptake and acrosome reaction in the absence of egg jelly. The NH_4Cl-triggered acrosome reaction required external Ca^{2+}. Our results indicate that internal alkalinization activates Ca^{2+} influx into sperm possibly through a pH sensitive Ca^{2+} channel. Thus egg jelly could first stimulate the sperm plasma membrane Na^+/H^+ exchanger inducing intracellular alkalinization which would then activate Ca^{2+} uptake and acrosome reactions.

Sea urchin sperm *Acrosome reaction* *Egg jelly* *Ca^{2+} uptake* *Intracellular pH*

1. INTRODUCTION

The acrosome reaction of sperm from many species plays a crucial role in fertilization. In sea urchins, this event involves the exocytosis of the acrosomal vesicles located at the anterior region of the sperm head [1], a rapid polymerization of actin [2], and the formation of the acrosomal tubule through which sperm bind and fuse to the egg [3,4].

The acrosome reaction of sea urchin sperm is triggered by a fucose rich polymer, the main component of the outer investment ('jelly') of the egg [5]. During the reaction, sperm take up Ca^{2+} and Na^+ from seawater, release K^+, and undergo a plasma membrane depolarization [6–8]. Additionally, there is a H^+ efflux which induces an intracellular alkalinization [6,9]. Various results indicate that the above ionic movements are interrelated [7,10] and that they modulate the acrosome reaction. Removal of Ca^{2+} or Na^+ from seawater [6] or drugs like D600 [6] and nisoldipine [10] known to block Ca^{2+} channels or tetraethylammonium [6] which blocks K^+ channels inhibit the reaction. The ionophores nigericin and A23187 or increasing external pH to 9, stimulates the acrosome reaction in the absence of egg jelly [6,10,11]. In addition, it is known that Na^+ and H^+ fluxes are coupled through an exchange mechanism which in turn is responsible for internal alkalinization [6,12,13].

We have become interested in investigating if the stimulation of Ca^{2+} uptake, which occurs when the acrosome reaction is triggered by egg jelly is regulated by the Na^+/H^+ exchange via the intracellular pH [10]. Here we have experimentally increased the intracellular pH in the absence of jelly exposing sperm to NH_4Cl, keeping the external pH at its normal value of 8 in seawater. Under

Abbreviations: ASW, artificial seawater; OCaSW, Ca^{2+}-free artificial seawater; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; pH_i, intracellular pH

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these conditions, the percentage of acrosome reactions and the uptake of $^{45}Ca^{2+}$ were determined.

2. MATERIALS AND METHODS

Sea urchins (*Strongylocentrotus purpuratus* from Bio-Marine Laboratories, Venice, CA) were spawned by intracoelomic injection of 0.5 M KCl. Egg jelly was prepared and quantified as described in [10]. ASW was made with: 486 mM NaCl, 10 mM KCl, 10 mM $CaCl_2$, 2.4 mM $NaHCO_3$, 56 mM $MgCl_2$, and 10 mM Hepes, adjusted to pH 8 with 0.1 N NaOH. Where indicated, NH_4Cl was added at concentrations given in the figures. OCaASW was as ASW except that $CaCl_2$ was omitted.

Uptake of $^{45}Ca^{2+}$ (Amersham): Semen ($2–4 \times 10^{10}$ sperm/ml) was first diluted 4-fold in OCaASW. At zero time, an aliquot of this sperm suspension (60–80 μl to give 6×10^8 sperm in 1.2 ml of total volume) was added to seawater containing 7 μCi/ml $^{45}Ca^{2+}$ and NH_4Cl or egg jelly (22 μg of fucose equivalents/ml). At appropriate times, a sample of 0.2 ml was removed from the incubation medium and centrifuged through an oil layer (Dibuthylftalate, Eastman Kodak) for 40 s at full speed in a 152 Beckman microcentrifuge. Radioactivity was then determined in the pellet. Controls were without NH_4Cl or egg jelly in the medium. Incubations were performed at 17°C.

Sperm acrosome reaction was determined in 20 μl aliquots from the incubation medium for $^{45}Ca^{2+}$ uptake by phase contrast microscopy as described in [10].

Intracellular alkalinization was monitored by using the fluorescent probe 9-aminoacridine (Sigma). The experimental procedure and calculation of pH_i was as in [14].

3. RESULTS AND DISCUSSION

In solution NH_4^+ dissociates into NH_3 and H^+ (pK 9.25). In many cells, NH_3 diffuses across the plasma membrane and alkalinizes the intracellular milieu [15]. Thus various concentrations of NH_4Cl in seawater at constant pH 8 were used to artificially increase pH_i in sea urchin sperm and investigate their effect on the acrosome reaction. Fig.1A shows that less than 5 mM NH_4Cl had no inducing effect whereas 10 mM elicited 49% of acrosome

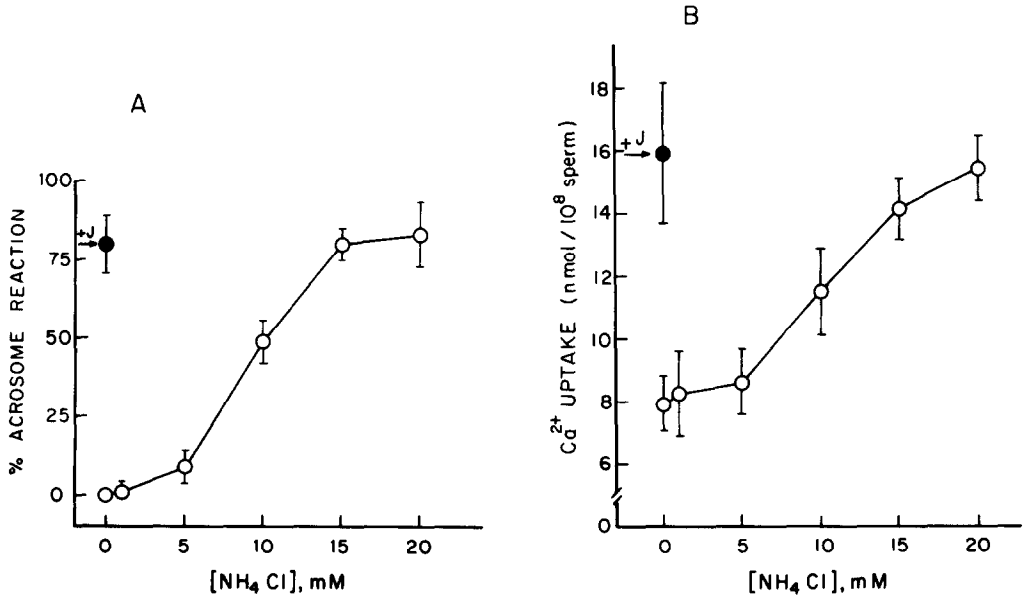

Fig.1. Percentage of sperm acrosome reaction and Ca^{2+} uptake as a function of NH_4Cl concentration in seawater. Sperm (5×10^8 cells/ml) were incubated in seawater with (●) and without (○) egg jelly at pH 8, after 1 min, acrosome reactions and Ca^{2+} uptake were determined as described under section 2. Values represent the mean ± SD of 6 measurements made with 2 sperm batches.

reaction. 15 mM of NH_4Cl was enough to trigger maximal percentage of reacted sperm (80%). Activating concentrations of NH_4Cl in various sperm batches ranged between 15 and 20 mM, and in a few up to 40 mM was needed to induce the reaction.

Ca^{2+} uptake into sperm accompanies the jelly-induced acrosome reaction [6]. Fig.1B illustrates that NH_4Cl stimulated Ca^{2+} uptake at the concentrations that triggered acrosome reactions (fig.1A). The amount of Ca^{2+} uptake and percentage of acrosome reactions induced by NH_4Cl were about the same as with egg jelly (cf. fig. 1A and B). The time course of Ca^{2+} uptake induced by 20 mM NH_4Cl indicated that at 30 s Ca^{2+} entry was already increased with respect to the control (fig.2). Once the reaction was completed further increments in Ca^{2+} uptake were observed as found in the presence of egg jelly [6]. Fig.2 also shows a close parallelism in the Ca^{2+} uptake kinetics triggered by NH_4Cl or egg jelly.

If the medium was poorly buffered (<2.5 mM Hepes), neither Ca^{2+} uptake nor acrosome reactions could be observed (not shown). This can be explained considering that, upon dilution, sperm rapidly release acid into seawater leading to a reduction of the external pH from 8 to ~7.7. Concomitantly, the effective concentration of NH_3 in seawater would decrease to levels below those required to induce the acrosome reaction. In the presence of 10 mM Hepes addition of sperm did not change the external pH (8.0) during incubation.

External Ca^{2+} is required to trigger the acrosome reaction with either jelly, pH 9 or A23187 [6,10,11]. Fig.3 shows that Ca^{2+} must also be present in ASW to induce the reaction with NH_4Cl. The half-maximal Ca^{2+} concentration required for the NH_4Cl induced acrosome reaction ranged between 2 and 3 mM. This was slightly lower than that needed for the jelly induction, 4 mM [6].

The fluorescent probe 9-aminoacridine was used to confirm and monitor intracellular alkalinization induced by NH_4Cl. The percentage of fluorescence

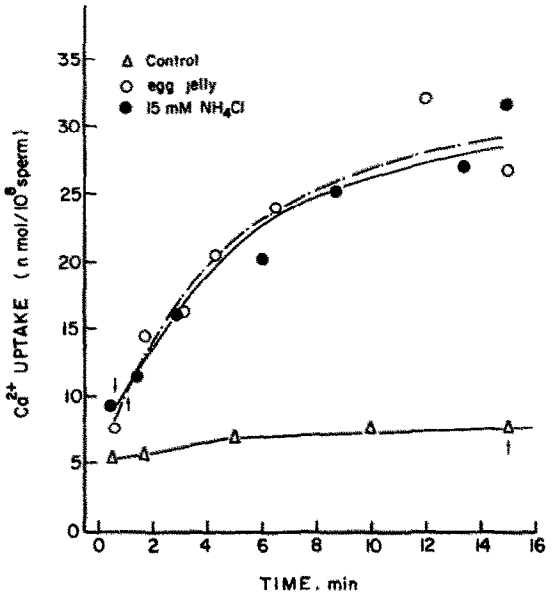

Fig.2. Kinetics of Ca^{2+} uptake by sea urchin sperm suspended in seawater with NH_4Cl or egg jelly. At the indicated times, Ca^{2+} uptake was determined as described under section 2. Symbols are: (Δ) control, no additions; (●) with egg jelly; (○) with 15 mM NH_4Cl. Acrosome reactions were determined (indicated by arrows) at each condition and values were: 80% with jelly; 72% with NH_4Cl; 4% control. Four other experiments made with different sperm batches showed similar results.

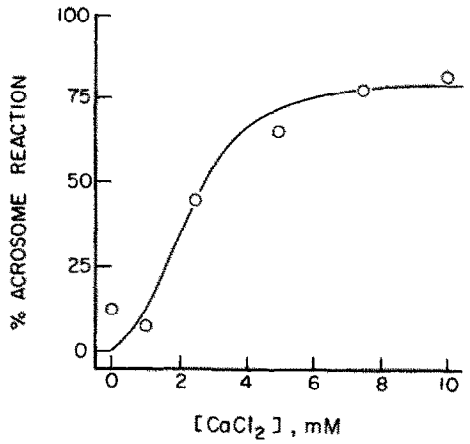

Fig.3. Dependence of the NH_4Cl-induced acrosome reaction on the presence of Ca^{2+} in seawater. After 1 min of incubation in seawater with 20 mM NH_4Cl, sperm (5×10^8/ml) were fixed with 6% formaldehyde and acrosome reactions measured as described under section 2. The medium without Ca^{2+} contained 5 mM EGTA. 81% of acrosome reactions were obtained with egg jelly (without NH_4Cl). Values represent the mean of 2 determinations.

quenching that the probe undergoes in the presence of cells is an index of the pH gradient and pH_i [16]. Sperm quenched 60 and 46% the fluorescence of 9-aminoacridine in ASW without (control) and with 20 mM NH_4Cl, respectively. From the difference in quenching it can be calculated that 20 mM NH_4Cl indeed increased pH_i from 7.24 to 7.58 ($\Delta pH = 0.34$).

Therefore our results indicate that the induction of the acrosome reaction by NH_4Cl involves an internal alkalinization which activates the influx of extracellular Ca^{2+} into sperm. Possibly the interaction of egg jelly with a sperm receptor [17,18] would first promote the activation of the Na^+/H^+ exchanger. As a result of the pH_i increase, Ca^{2+} entry into the sperm could then take place. Blockers of Ca^{2+} channels such as D600 and nisoldipine inhibit the acrosome reaction induced by jelly or pH 9, indicating that a Ca^{2+} channel probably participates in the process. Considering our results with external pH [10] and NH_4Cl, and the physiology of sea urchin sperm, it is worth to speculate that this Ca^{2+} channel is modulated by the internal pH. Recent reports have shown the intracellular pH dependence of Ca^{2+}-sensitive K^+ channels [19,20].

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