Electrical Responses of Eggs to Acrosomal Protein Similar to Those Induced by Sperm

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The earliest known response of eggs to sperm in many species is a change in egg membrane potential. However, for no species is it known what components of the sperm cause the opening of the egg plasma membrane channels. Protein isolated from sperm acrosomal granules of the marine worm *Urechis* caused electrical responses in oocytes with the same form, amplitude, and ion dependence as the fertilization potentials induced by living sperm. Sperm initiated fertilization potentials in oocytes when sperm-oocyte fusion, but not binding, was inhibited by clamping oocyte membrane potentials to positive values. Acrosomal protein also initiated electrical responses in clamped oocytes. These results support the hypothesis that it is the sperm acrosomal protein that opens ion channels in the oocyte membrane.

THE FIRST KNOWN RESPONSE OF eggs to the fertilizing sperm in a variety of species is a rapid positive-going shift in the egg's membrane potential, which is known as the fertilization potential (*1*). One function of the fertilization potential is to provide a rapid block to polyspermy, protecting the egg from supernumerary sperm entries while the more slowly developing permanent polyspermy block is established (*2, 3*). In all species so far studied, the ion channels responsible for the fertilization potential preexist in the unfertilized egg and are opened by sperm but not by voltage (*4–10*). What components of the sperm cause these channels to open is not known for any species. We investigated this question with a protein fraction isolated from sperm acrosomes of the marine worm *Urechis*. The acrosomal protein is in intimate contact with the oocyte surface at the time of fertilization (*11*). The protein is highly basic (~50% lysine and arginine), has an apparent molecular mass of 25 to 30 kilodaltons, and not only binds sperm to oocytes but also activates the oocytes, causing the same initial morphological changes as sperm (*11*). However, activation of oocytes by acrosomal protein does not necessarily imply a normal electrical response since positive potential is neither necessary (*5*) nor sufficient (*12*) for activation. Therefore, we examined the electrical response of oocytes to acrosomal protein.

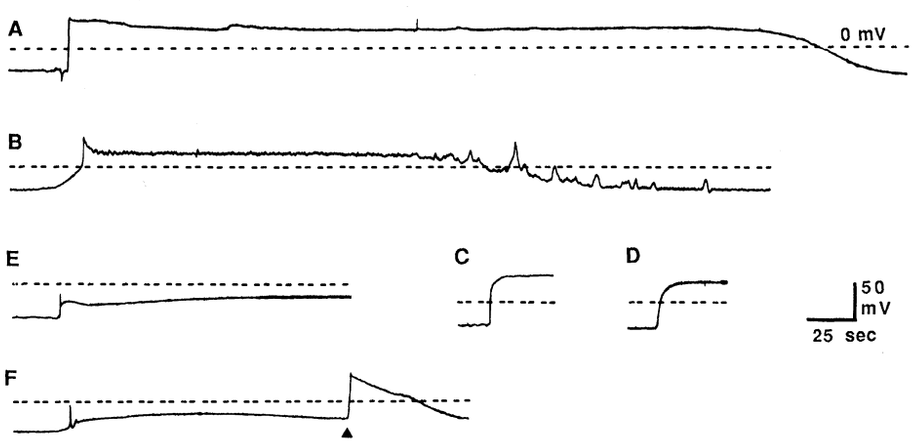

Fig. 1. Comparison of electrical responses induced in *Urechis* oocytes by sperm and acrosomal protein. Methods for handling gametes are in (*19*) and for electrophysiology in (*5*). Microelectrodes of 40 to 80 megohms were filled with 3*M* KCl; a single electrode was used for recording voltage and passing current. The *IR* drop (*I*, current; *R*, resistance) across the electrode was electronically balanced with a bridge circuit in the amplifier (AM-1, Bio-Dyne Electronics Laboratory, Santa Monica, CA). Membrane potentials and applied currents were recorded on a chart recorder (Gould Brush 220, Cleveland, OH). Sperm were diluted 1:600 in the appropriate ASW (*20*) and one drop of sperm suspension was added to ASW in the chamber at some distance from the oocytes (final dilution 1:30,000). Acrosomal protein was isolated as in (*11*), and the dry powder was suspended in the appropriate ASW (*21*). A small amount of the suspension (0.3 to 2 μl) was expelled within 5 mm of the test oocyte from a 10-μl Hamilton syringe or a glass micropipette. Protein concentrations (*11*, *22*) in the suspensions ranged from 0.6 to 10 μg/μl; amounts contacting the test oocyte varied (*23*). (**A**) Fertilization potential in ASW. (**B**) Acrosomal protein potential in ASW (*24*). (**C** and **D**) Electrical responses to sperm and acrosomal protein, respectively, in ASW with 1/10 Ca^{2+}. (**E**) Fertilization potential in ASW with 1/10 Na^+. (**F**) Acrosomal protein potential in ASW with 1/10 Na^+. At the arrow, a small amount of standard ASW (486 m*M* Na^+) was expelled over the oocyte; the potential immediately rose from −25 to +38 mV, confirming that acrosomal protein had opened Na^+ channels. The potential slowly fell again as diffusion reduced the Na^+ concentration. All of the oocytes in (A) to (F) were subsequently activated by the criteria of germinal-vesicle breakdown and surface coat elevation (*11*). See (*25*) for other criteria used in the experiments. Photographs of original chart records are shown in the figures.

Electrical responses of oocytes to sperm and acrosomal protein are illustrated in Fig. 1 and summarized in Table 1. The fertilization potential induced by sperm is caused by an increase in Na^+ permeability amplified during the first 15 seconds by a voltage-gated Ca^{2+} action potential (*5*) (Fig. 1A). The Na^+-dependence of the fertilization potential is shown by the lower amplitude in artificial seawater (ASW) with Na^+ reduced to 1/10 (Fig. 1E). The average amplitude during the first minute of the response is reduced by 55 mV, close to the 58 mV predicted by the Nernst equation for a pure Na^+ permeability [see (*5*)]. The membrane potential does not reach threshold [~+10 mV (*5*)] for the Ca^{2+} action potential; the peak at the initiation of the response is due to a second action potential of undetermined ionic basis also present in the oocyte (*5*). The acrosomal protein potential is also Na^+-dependent (Fig. 1F); the average amplitude is reduced by 50 mV in ASW with 1/10 Na^+ (Table 1). That the acrosomal protein indeed opens Na^+ channels in ASW with 1/10 Na^+ is confirmed by the immediate rise in potential when the oocyte is momentarily flooded with standard ASW (Fig. 1F, arrow). In four experiments the potential rose from −36 ± 12 (SD) mV to + 27 ± 6 mV (the potential was measured 7 seconds after the peak of the initial rise to reduce the contribution of the Ca^{2+} action potential). No such effect was observed when standard ASW was introduced over oocytes in ASW with 1/10 Na^+ in the absence of acrosomal protein.

The Ca^{2+}-dependence of the acrosomal protein potential is also the same as that of the fertilization potential: in ASW with 1/10 Ca^{2+} the initial peak disappears but the amplitude is unchanged (Fig. 1, C and D, and Table 1). Thus acrosomal protein potentials are like fertilization potentials in general form, amplitude, and ion dependence.

In the above experiments the entire oocyte surface was exposed to acrosomal protein. Acrosomal protein was also applied

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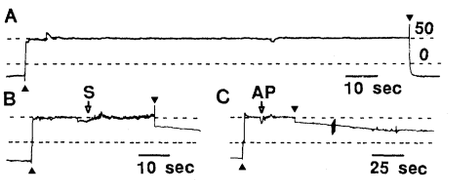

Fig. 2. Induction of an electrical response by sperm and acrosomal protein in oocytes current-clamped to ~+50 mV. Immediately prior to the experiments the *IR* drop across the electrode was subtracted with the bridge circuit in the amplifier. Then current (usually 0.3 nA and never more than 0.6 nA) was applied by manual operation of the stimulator to hold the membrane potential to near +50 mV. Sperm (S) and acrosomal protein (AP) were delivered as described in the legend to Fig. 1. Symbols: (▲) clamp on; (▼) clamp off. Lower dashed line, 0 mV; upper dashed line, +50 mV. (**A**) Control: after 122 seconds at +50 mV the current was turned off, and the membrane potential returned to the resting value. (**B**) The membrane potential was set to +50 mV, and then sperm were added. The arrowhead indicates the time the first sperm were seen arriving at the oocyte. A fertilization potential was initiated during the clamp as indicated by the positive potential after the clamp was turned off. (**C**) Addition of acrosomal protein (arrowhead) to an oocyte clamped at +50 mV. The oocytes in (B) and (C) activated, but the oocyte in (A) did not.

locally from micropipettes with openings 20 to 30 μm in diameter. In four of four experiments, acrosomal protein potentials were like those in Table 1 (all four oocytes activated). In two of the experiments, the oocytes responded to contact with the micropipette without expulsion of solution (concentrations of acrosomal protein were 4.4 and 19 μg/μl). No electrical responses occurred when ASW or mineral oil (which filled the injection system) were expelled onto the oocyte (16 experiments). Oocytes also contracted immediately on exposure to acrosomal protein; the entire oocyte contracted when acrosomal protein was applied as in Fig. 1, and a local contraction occurred at the site of local application. Whether sperm cause a local contraction in *Urechis* oocytes is not yet known.

It is interesting to compare the manner of acrosomal protein delivery by sperm with that in the experiments. In fertilization, the acrosomal protein contacting the oocyte surface is in a ring of about 1.5 μm in diameter (*11*). With 0.5 pg of acrosomal protein per sperm (*11*) and a ring volume of 6×10^{-10} μl [see figure 1b in (*11*)], the sperm could deliver acrosomal protein at a concentration of 850 μg/μl in a patch 1.5 μm in diameter, a higher concentration and smaller patch than in the experimental applications. In fertilization, sperm might also deliver acrosomal protein more rapidly onto the surface of the oocyte. Variations in amplitude and duration and the generally slower rise time of acrosomal protein potentials (Fig. 1 and Table 1) might also be explained by these differences in delivery. Nevertheless it is impressive that responses to isolated acrosomal protein are so similar to those produced by sperm.

Table 1. Comparison of electrical responses induced by sperm and acrosomal protein (AP). The average amplitude during the first minute was calculated by taking the average of the membrane potential values at 12, 24, 36, 48, and 60 seconds after the initiation of the electrical response. The duration was the length of time the membrane potential was more positive than 0 mV; these values are not reported for 1/10 Ca^{2+} ASW since they were quite long and the electrode frequently came out before the response returned to negative values [see also (*5*)]. Methods are in the legend to Fig. 1. Values are means ± SD, with the number of experiments in parentheses. For each type of ASW, the mean values for sperm and acrosomal protein for each parameter did not differ significantly ($P > 0.1$) by the Student's t test.

ASW	Initial peak (mV)	Average amplitude in first minute (mV)	Duration (minutes)
Standard			
Sperm	+42 ± 5 (5)	+33 ± 3 (5)	7.3 ± 0.9 (3)
AP	+38 ± 8 (10)	+27 ± 6 (10)	5.3 ± 3.5 (10)
1/10 Ca^{2+}			
Sperm		+36 (2)	
AP		+28 ± 2 (3)	
1/10 Na^{+}			
Sperm		−22 ± 5 (3)	0 (3)
AP		−23 ± 11 (6)	0 (6)

Table 2. Interaction of sperm and acrosomal protein with oocytes current-clamped to +50 mV. Some oscillations occurred (see Fig. 2 for typical data) but the potential never dropped below +38 mV in any of the experiments reported. The oocyte membrane was clamped to +50 mV; then sperm were added as in the legend to Fig. 1. The time of arrival of sperm was observed with the stereomicroscope and occurred within 10 to 23 seconds after clamping was begun. Acrosomal protein was added (see Fig. 1) at 5 to 57 seconds after clamping was initiated. Controls include three experiments in which drops of ASW were added during the clamp to simulate the disturbance of adding sperm or protein; there were no differences in the results. None of the control oocytes were activated.

Experiment	n	Resting potential before clamp (mV)	Membrane potential at clamp off (mV)	Total time clamped (seconds)
Control	11	−34 ± 12	−21 ± 15	(22–122)
Sperm	6	−40 ± 14	+31 ± 8	(30–47)
AP	4	−37 ± 11	+25 ± 12	(16–100)

If the acrosomal protein on sperm causes oocyte Na^{+} channels to open, sperm-oocyte lipid bilayer fusion should not be necessary. Whether fertilization potentials could be initiated by sperm in the absence of fusion was investigated by clamping the oocyte membrane potential to positive values. The most likely effect of positive potential is to inhibit sperm-oocyte bilayer fusion as such, although a transient fusion insufficient to result in sperm penetration is not rigorously excluded by the data (*12*). In previous experiments, when the oocyte membrane potential was clamped to about −5 mV, sperm failed to penetrate three of six oocytes, although fertilization potentials occurred in all six (*12*). In the present experiments, oocyte membrane potentials were clamped to +50 mV to inhibit sperm penetration more strongly (*12*). Results are shown in Fig. 2 and Table 2. Despite the clamp, fertilization potentials were invariably initiated. Acrosomal protein also produced fertilization potential–like responses in oocytes clamped to +50 mV (Fig. 2 and Table 2). During the clamp, sperm bound to oocytes, and oocytes exposed to acrosomal protein contracted. Evidence against the fusion hypothesis for initiation of fertilization potentials is also available in sea urchin eggs, for which it has been shown that fusion probably occurs after the fertilization current begins (*13*).

We also investigated the possibility that sperm lipids associated with the isolated acrosomal protein could be responsible for its effects. No intact membranes are visible by electron microscopy in samples of acrosomal protein (*11*), but lipid might be present in the acrosomal protein in situ or become associated with acrosomal protein when sperm are lysed in detergent. However, a sample of acrosomal protein subjected to exhaustive extraction with lipid solvents and hydrolyzed to remove covalently bound lipid (*14–17*) retained the ability to induce fertilization potential–like responses and activate oocytes (two experiments in ASW and two in ASW with 1/10 Na^{+} reported in Table 1).

Thus we have four lines of evidence that sperm initiate the fertilization potential by means of acrosomal protein: (i) acrosomal protein is in intimate contact with the oocyte surface at the time of fertilization (*11*), (ii) the isolated protein causes a fertilization potential–like response, (iii) sperm initiate fertilization potentials in the probable absence of fusion, and (iv) sperm lipids are not required. It will be interesting to determine whether blocking the acrosomal protein on sperm will prevent induction of fertilization potentials.

If sperm-egg fusion does not open the fertilization potential channels, the most likely mechanism is that molecules on the sperm surface (for example, the acrosomal protein in *Urechis*) interact with egg surface components to open the channels. This interaction could be of a specific receptor-ligand nature, although other possibilities can be envisaged. Sperm surface molecules could open egg membrane channels by modifying the egg surface charge or by inserting into the egg bilayer, changing its structure. Both possibilities are reasonable for acrosomal protein which is a polycation and has hydrophobic properties (*11*). In *Urechis* it is also known that the fertilizing sperm opens only a localized patch of Na^+ channels (*18*); thus the acrosomal protein could even be acting directly on the egg membrane without the involvement of a second messenger system.

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21. Both the hot trichloroacetic acid–extracted acrosomal protein and the major band acrosomal protein extracted from acetic acid–urea polyacrylamide gels (*11*) were used in the experiments; no differences were seen in the oocytes' responses.
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23. When oocytes are mixed with a uniform concentration of acrosomal protein, 0.03 mg/ml is sufficient to activate them (*11*).
24. Oscillations in membrane potential during the falling phase of the response are occasionally observed in both fertilization and acrosomal protein potentials.
25. Other criteria for acceptable data were (i) that the Ca^{2+} action potential lasted longer than the stimulus at the beginning of the experiment [when ASW with 1/10 Ca^{2+} was used oocytes were first penetrated with the microelectrode in standard ASW then the Ca^{2+} concentration was changed by perfusion (*5*)] and (ii) that at the termination of the experiment when the electrode was removed from the oocyte into the seawater bath, the zero potential on the chart recorder had not changed by more than ±3 mV from the value before the electrode penetrated the oocyte. These criteria apply to all data in the article.

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Detection of Rift Valley Fever Viral Activity in Kenya by Satellite Remote Sensing Imagery

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Data from the advanced very high resolution radiometer on board the National Oceanic and Atmospheric Administration's polar-orbiting meteorological satellites have been used to infer ecological parameters associated with Rift Valley fever (RVF) viral activity in Kenya. An indicator of potential viral activity was produced from satellite data for two different ecological regions in Kenya, where RVF is enzootic. The correlation between the satellite-derived green vegetation index and the ecological parameters associated with RVF virus suggested that satellite data may become a forecasting tool for RVF in Kenya and, perhaps, in other areas of sub-Saharan Africa.

OUTBREAKS OF RIFT VALLEY FEVER (RVF) disease in domestic animals (epizootics) in sub-Saharan Africa are clearly correlated with widespread and heavy rainfall associated with the intertropical convergence zone (*1–4*). It is thought that such rainfall can flood mosquito breeding habitats, known in Kenya as "dambos" (*5*), which contain transovarially infected *Aedes* mosquito eggs and subsequently serve as an excellent habitat for the development of other mosquito vectors (*4, 6, 7*). The introduction of the virus into susceptible vertebrates by *Aedes* and the tremendous increase in numbers of secondary mosquito vectors can create an epizootic of this disease in sub-Saharan Africa. If the rains that evolve from the intertropical convergence zone are not widespread, heavy local rainfall may flood infected mosquito habitats and introduce the virus into domestic vertebrate populations enough to replenish the dambo habitats with infected eggs, but possibly not enough to sustain an epizootic.

To prevent or lessen the impact of RVF disease in Africa, known parameters of epizootic viral activity are monitored so that control efforts can be implemented. Satellite remote sensing technology is the newest and possibly the only method available to conduct surveillance activities over such a large and diversified area as sub-Saharan Africa. Remote sensing of green vegetation dynamics is a well-developed technique and one for which several satellites collect data world-

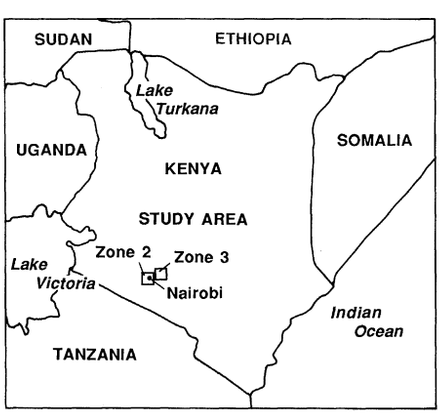

Fig. 1. Location of study areas in Kenya.

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