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Fast block to polyspermy in sea urchin eggs is electrically mediated

THE prevention of polyspermy in sea urchin eggs is accomplished in two steps: a "fast" block, occurring within a few seconds of the entry of the fertilising sperm, and a "slow" block, associated with the cortical reaction (compare refs 1–4 with 5 and 6). Results presented here confirm the existence of the fast block and demonstrate that it is caused by the electrical depolarisation of the egg plasma membrane that accompanies the entrance of the fertilising sperm.

Eggs of *Strongylocentrotus purpuratus* were hand centrifuged and agitated mechanically with a jet of seawater, to remove their jelly coats. Some of the dejellied eggs adhered to the bottom of Falcon plastic Petri dishes, such that a microelectrode could be inserted. All experiments involved eggs with a stable resting potential of greater than −60 mV (Table 1). These values of resting potential are close to that calculated from independent ion tracer flux studies done on these eggs (my work in preparation with K. R. Robinson). A single microelectrode was used for both recording voltage and passing current, by means of a conventional bridge circuit. Experiments were done at 15 °C, in natural or artificial seawater, Tris-buffered at *p*H 8. In all experiments, the concentration was about 10^6 sperm per ml, which is within the range that can produce polyspermy

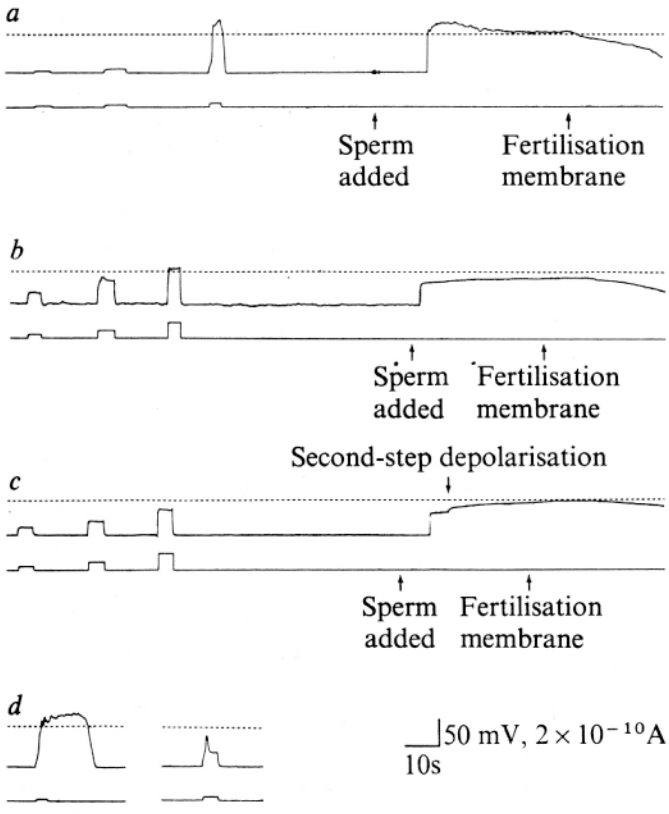

Fig. 1 Action potentials and activation potentials in *S. purpuratus*. The top trace is voltage against time; the bottom trace is current against time. The dotted line indicates 0 mV. Before addition of sperm, each egg membrane was tested for electrical excitability by applying a series of small depolarising current pulses. *a*, Egg with regenerative response to current pulse; monospermic activation potential. *b*, Egg with non-regenerative response; monospermic activation potential. *c*, Egg with non-regenerative response; polyspermic activation potential. *d*, Other examples of the regenerative response; a strong response outlasting the stimulus, a weak response.

in some batches of eggs, but not in most. Polyspermy was determined by observation of first cleavage: monospermic eggs divide into two equal cells, whereas polyspermic eggs cleave abnormally, dispermic eggs usually producing three or four cells at once[7,8].

Figure 1 shows intracellular electrical records from three eggs before, during and after addition of sperm. The fast depolarisation after the addition of sperm has been called the "activation potential"[9–15]. Within 3–30 s after the introduction of sperm near the egg, the egg membrane depolarises to a plateau at −30–+20 mV, and then after about 1 min begins to repolarise.

An interesting correlation was found between the plateau level of the activation potential (measured 3 s after its rise) and the occurrence of polyspermy (Table 1). Eggs with activation potentials which reached a plateau more positive than 0 mV were never polyspermic (none out of eight cases) and those which reached a plateau less positive than −10 mV were sometimes polyspermic (seven out of 13 cases). These results suggest that the entry of extra sperm is prevented by the more positive-going activation potential.

Figure 1*c* shows an activation potential in a polyspermic egg. About 5 s after the initial rise, there was a distinct second step depolarisation (see arrow). Eleven polyspermic activation potentials were observed (seven listed in Table 1, four not shown). All 11 showed this second step, and sometimes a third step. Ten monospermic activation potentials, as in Fig. 1*b*, were observed; of these, eight definitely did not show a second step. Two cases did show a second step; their nature is obscure. (In cases like that in Fig. 1*a*, with an oscillating plateau level, the steps could not be counted.) These results indicate that the second step depolarisation is associated with the entrance of a second sperm. The voltage at which this second step occurs is always more negative than −10 mV, suggesting that when the egg membrane is depolarised beyond −10 mV, sperm penetration is less probable.

Figure 2*a* shows that when current was applied to hold the unfertilised egg membrane at a potential more positive than +5 mV, sperm did not fertilise the egg. In spite of the presence of many active sperm adhering to the egg surface, no fertilisation membrane formed, and no electrical event resembling an activation potential occurred. In some experiments, the potential was held for more than 5 min, with repeated additions of sperm; but still no fertilisation occurred in the voltage-clamped eggs. (Surrounding eggs formed fertilisation membranes within 1 min of the first addition of sperm.) As soon as the current was turned off, fertilisation occurred: the egg depolarised, showed a typical activation potential, formed an apparently normal fertilisation membrane with the usual time course, and, if the activation potential was of the one-step form, first cleavage was normal, indicating that only one sperm had entered the egg. The same result was obtained with holding potentials ranging from +5 to +130 mV. In other experiments, the potential was held for about 10 min, without addition of fresh sperm, after which most sperm had become less active and incapable of fertilising eggs. When the current was turned off, the egg membrane returned to its original resting potential and showed the electrical properties characteristic of the unfertilised state. Morphologically, there was no sign of activation, and no development occurred. If more sperm were added, fertilisation and development proceeded in the usual way.

Fertilisation is prevented at +5 mV; but it occurs at

Table 1 Activation potentials for monospermic and polyspermic eggs

Egg	Resting potential (mV)	Regenerative response	Activation potential plateau (mV)	First cleavage	No. of steps in rise of activation potential	Voltage at second step (mV)
A	−75	Strong	+23	M	—	—
B	−69	Strong	+17	M	—	—
C	−68	Strong	+15	M	—	—
D	−76	Strong	+14	M	—	—
E	−77	Strong	+12	M	—	—
F	−74	Strong	+10	M	—	—
G	−71	Strong	+ 6	M	—	—
H	−71	Weak	0	M	—	—
I	−71	Weak	−12	P	2	− 9
J	−74	NT	−13	M	1	—
K	−68	Weak	−14	M	1	—
L	−75	Weak	−15	M	1	—
M	−56	NT	−17	P	2	−15
N	−60	None	−19	M	1	—
O	−63	None	−20	M	1	—
P	−79	Weak	−20	P	2	−14
Q	−71	Weak	−22	P	2	−22
R	−64	None	−23	P	2	−22
S	−70	Weak	−26	M	2	−27
T	−65	None	−29	P	2	−26
U	−78	NT	−35	P	3	−35, −24

The activation potential plateau was measured 3 s after the initial rise. Eggs were classified as monospermic (M) if first cleavage produced two equal cells; otherwise, they were classified as polyspermic (P). The regenerative response of the unfertilised egg was classified as strong if in response to a 5-s current pulse of 3×10^{-11} A, the membrane maintained a positive potential for the duration of the pulse. None means that the response was completely, or almost completely, non-regenerative. Weak means that there was some regenerative response, but it was not strong. NT, Not tested.

−10 mV, as determined by the experiments reported in Table 2. The egg was depolarised enough to block fertilisation, and sperm were added. After 3–5 min, the voltage was lowered to a point where fertilisation occurred. Each experiment sets an upper and a lower limit on the threshold voltage for suppressing sperm entry. This is between +5 and −10 mV; at intermediate potentials, entry of sperm may depend on their concentration and activity, and on individual variation among eggs. This threshold is consistent with the following observations on activation potentials. (1) Eggs whose activation potential plateau (measured 3 s after the initial rise) was more positive than 0 mV, are always monospermic. (2) The entrance of the second sperm in polyspermic eggs occurred at a voltage more negative than −10 mV.

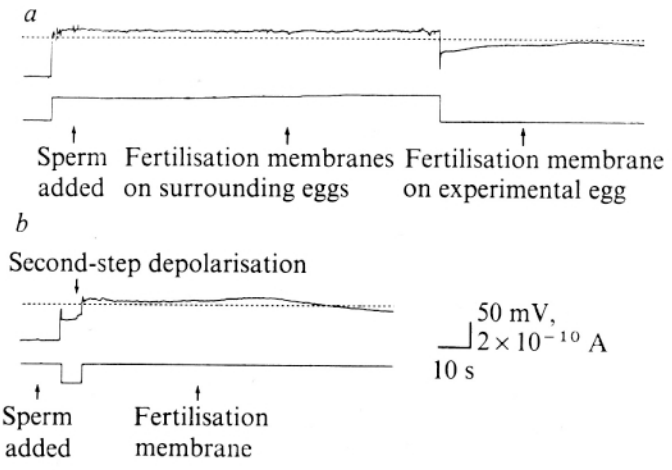

Fig. 2 Demonstration of potential dependence of sperm entrance. The top trace is voltage against time; the bottom trace is current against time. The dotted line indicates 0 mV. *a*, Suppression of fertilisation by positive holding potential. *b*, Induction of polyspermy by negative holding potential. In (*a*) the amount of current required to maintain the holding potential increased about 10% during the first 1–2 min after the current was turned on. This increase was seen even if sperm were not added. In some experiments, sperm were not added until this initial adaptation of the egg membrane was complete. The addition of sperm did not cause any change in the amount of current required to maintain the potential.

Application of current to bring the plateau of the activation potential to a more negative level facilitated entry of additional sperm (Fig. 2*b*). After the activation potential began, current was applied to hold the potential at −30 mV. A second-step depolarisation occurred, then the current was turned off. Observation of first cleavage showed that this egg was polyspermic, whereas 98% of the surrounding eggs in the dish were monospermic. This experiment was repeated seven times, with holding potentials of -30 ± 5 mV. In all cases, experimental eggs were polyspermic and surrounding eggs were monospermic.

These experiments demonstrate that the electrical depolarisation occurring at fertilisation constitutes a fast block to polyspermy. It was frequently observed that when the electrically monitored egg was polyspermic, other eggs in the dish were also polyspermic (except in cases where current was applied to make an egg polyspermic). Therefore, the lack of a fast block to polyspermy is a property of a batch of eggs. Loss of the capacity to block polyspermy might be a symptom of egg deterioration, either in the adult, or after removal from the adult.

The rise of the activation potential can occur within 3 s of insemination. Part of this time is necessary for the sperm to swim to the egg surface and to attach to the vitelline membrane. Only then does the sperm fuse with the egg plasma membrane. Therefore, it can be concluded that the fast block to polyspermy is established in less than 3 s after fusion of the fertilising sperm. Quite possibly, the rise of the activation potential is a direct consequence of the disturbance of the egg membrane by sperm fusion. If this is the case, it follows from the rate of rise of the activation potential that the fast block to polyspermy is established in less than 1 s after fusion. (The rise from resting level to −10 mV takes between 0.1 and 1 s.) The membrane remains in the depolarised state for about 1 min, after which the cortical reaction has occurred, thus providing a permanent block to polyspermy.

In each example in Fig. 1, a series of small depolarising current pulses was applied to the egg before sperm were added. In the case shown in Fig. 1*a*, the stimulus pulse

Table 2 Voltage threshold for suppression of fertilisation

Egg	Suppressing potential (mV)	Non-suppressing potential (mV)
A	+6	−6
B	+3	−11
C	+2	−10
D	+1	−10
E	+5	−7
F	+3	−12
G	+5	−6
H	+6	−5
I	—	−2

The egg membrane was held at the suppressing potential and sperm were added. About 1 min later, all the surrounding eggs had formed fertilisation membranes. 3–5 min after addition of sperm, the potential was reduced to the non-suppressing level: within 1–2 min, the experimental egg formed a fertilisation membrane. After removal of the electrode, balance of the WPI bridge circuit was tested by applying a current pulse of the same magnitude used in the experiment. If the imbalance was greater than 2 mV, data were not used. Error associated with the single microelectrode technique was small, because of the small currents used ($0.3–3 \times 10^{-10}$ A), and the high input resistances of the cells (200–2,000 MΩ, depending on the degree of regenerativeness of the egg's response), compared with the resistances of the microelectrodes (40–80 MΩ).

induced a regenerative response of the egg membrane. This response showed a threshold at about −50 mV, and sometimes outlasted the stimulus (Fig. 1*d*); it has been observed in other echinoderm oocytes and eggs[15–17] and has been studied in sea urchin eggs by K. Takahashi (personal communication). It is an action potential of the sort seen in nerve and muscle (as distinct from the activation potential). Figure 1*b* and *c* illustrates eggs which did not show a regenerative response to depolarisation. These eggs showed less positive-going activation potentials after sperm addition, compared with the egg in Fig. 1*a*. This relationship between the presence of the regenerative response and the height of the activation potential is also shown in Table 1. The correlation indicated that the activation potential is caused partly by a regenerative response of the egg membrane. The data in Table 1 suggest that the sperm fusion depolarises the egg to a level of about −30 to −20 mV. Then, in eggs which have the capacity to produce a regenerative response, the sperm-initiated depolarisation is amplified, bringing the egg membrane potential to a level which excludes additional sperm.

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