The Ionic Basis of Membrane Potential Changes from Before Fertilization Through the First Cleavage in the Egg of the Frog *Rana cameranoi*

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Abstract. Experiments were performed to identify the ionic basis of membrane potential changes in the *Rana cameranoi* egg from prior to fertilization through the first cleavage. The membrane potential was monitored continuously during this period. Ten per cent Ringer was used as the recording solution in the control group. The effects of Na^+ or Ca^{2+} conductances were observed by altering external concentrations of these ions. K^+ and Cl^- conductances were tested by adding channel blockers of these ions (TEA and SITS, respectively) to the extracellular medium.

The resting potential of the unfertilized egg is mainly affected by K^+ conductance. Chloride conductance is responsible for the depolarization phase of the fertilization potential evoked by sperm entry and K^+ conductance is responsible for the repolarization phase of this potential. We suggest that Na^+ permeability does not directly contribute to the fertilization potential, however fertilization potential peak is significantly reduced upon a reduction of extracellular sodium. The fertilization potential is not significantly influenced by extracellular Ca^{2+} and eggs fertilized in calcium-free solutions maintain their normal development, these results suggest that extracellular Ca^{2+} does not significantly contribute to the electrical and mechanical blocks that prevent polyspermy. The membrane potential of the fertilized egg does not alter significantly until the first cleavage. Potassium conductance contributes to hyperpolarization generated upon the first cleavage, whereas sodium is the basic ion responsible for the phase which follows peak hyperpolarization and which plays a role in the return of the post-cleavage membrane potential to a steady level. Cl^- conductance, which is important as the ionic basis of the fertilization potential, does not significantly influence any parameter of the cleavage cycle.

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Introduction

It is essential for the viability of the embryos of many animals and even some plants that only one spermatozoon fertilize the egg. Block to polyspermy is assured in most cases by two or three phenomena. The first block is the fast transient electrical block which is the sudden potential change in the oocyte plasma membrane when the egg is fertilized by a spermatozoon (fertilization potential *FP*) (Charbonneau et al. 1983; Jaffe et al. 1985; Jaffe and Schlichter 1985; Jaffe 1986; Kline et al. 1985; Webb and Nuccitelli 1985a,b; Kline et al. 1986; Brawley 1991). In most species *FP* is the electrical depolarization of the oocyte plasma membrane and it prevents polyspermy until a permanent and a relatively slow mechanical block is established (Jaffe et al. 1985; Jaffe and Schlichter 1985; Kline et al. 1985; Jaffe 1986). *FP* is well documented in various species of animals such as starfish, Urechis and amphibia and in plants like *Fucoid algae* but its ionic basis is different for different species (Jaffe 1986; Brawley 1991).

During early cleavage of frog eggs, electrical hyperpolarization of the fertilized egg plasma membrane is followed by a return of the membrane potential to a stable value (Webb and Nuccitelli 1985a,b). Woodward (1968) and de Laat and Bluemink (1974) stated that this hyperpolarization might develop due to the addition of a new membrane with a high potassium permeability (Cross and Elinson 1980). In another study performed by measuring extracellular ion currents during cleavage of *Xenopus* eggs, it was reported that K^+ might be responsible for membrane hyperpolarization and Na^+, Ca^{2+} and Cl^- together for the return of the membrane potential to a stable value (Kline et al. 1983). Membrane potential changes have been documented from fertilization through the first cleavage stage especially in amphibia such as *Xenopus laevis* and *Rana pipiens*, but the ionic basis of these potential changes has not been evaluated entirely by continuous recordings including this period.

The aim of this study was to determine membrane potential changes and their ionic basis from prior to fertilization through the first cleavage stage in the *Rana cancrivora* frog egg by recording membrane potentials continuously during this early developmental period.

Materials and Methods

Selected frogs of *Rana cancrivora* species were kept until use at +4°C, in plastic boxes which were filled with small amounts of stock solution (Webb and Nuccitelli 1985a).

Obtaining eggs and spermatozoa: insemination procedure

The study was started in November, and terminated in June. The pituitary glands were removed from the female frogs, homogenated, and injected intraperitoneally into the sexually mature female frogs (Perkins et al. 1981). The number of pituitary glands injected to one individual was adjusted according to the season (Rugh 1962).

Progesterone (Sigma, P-0130) was dissolved in vegetable oil at 10 mg/ml by gently heating the test tube, and the stock solution was stored at room temperature. Progesterone was injected into the female's thigh muscle. Pituitary homogenates and progesterone were injected consecutively The amount of the injected progesterone was also adjusted according to the season (Jaffe et al. 1985). The injected frogs were kept at 18 °C for 36 h, or at 25 °C for 24–36 h, and mature eggs were then obtained by squeezing from the cloaca. To obtain mature eggs over 2–3 days, female frogs were kept at +4 °C.

Mature eggs approx. 1.75 mm in diameter, surrounded with vitelline membrane and the jelly envelope, with a pigmented animal moiety and a greyish-white vegetal moiety were included in the study (Rugh 1951).

Sperm suspensions were obtained by macerating frog testes in 10% Ringer, 2–5 h after a male was injected with 300 IU human chorionic gonadotropin (hCG, Sigma CG-2) intraperitoneally For the activation of sperms, the suspensions were kept at room temperature for 5–10 min. To remove debris, sperm suspensions were then filtered through a fine nylon mesh. Sperm was examined for motility and morphology under a microscope at 1000 × magnification.

Five to ten mature eggs were placed in a dry plastic Petri dish; they were immobilized by the natural sticking of their jelly to the plastic. For fertilization, the eggs were covered with a few drops of a sperm suspension (approx. 5×10^6 sperm/ml). After 1 min, the recording solution was added to the dish, and the eggs were impaled with a microelectrode. Fertilization occurred approx. 5 min after the insemination Normally, more than 90% of the inseminated eggs were fertilized. Normal fertilization was scored by a shift in membrane potential towards positive values (about 5 min), rotation (about 30 min), normal first cleavage (about 2.5 h), and embryo formation (Jaffe and Schlichter 1985) Records obtained from eggs that showed membrane depolarization and rotation but did not cleave normally or did not develop until the embryo formation stage, were excluded from the study Experiments were performed at 21–25 °C.

Control and experimental groups

Control records were carried out in standard 10% Ringer solution which contained (in mmol/l): NaCl, 11.1; KCl, 0.19, $CaCl_2$, 0.11, $MgSO_4$, 0.08; NaOH, 0.4; HEPES, 0.25; pH 7.8 (Jaffe and Schlichter 1985) Solutions to be tested for the effects of ions were prepared by modifying the Ringer solution, and records performed in

these modified solutions served as experimental groups. Fertilization, first cleavage and early embryonal development were normal in all these solutions.

Ten mmol/l tetraethylammonium chloride (TEA, Sigma) and 1 mmol/l 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulphonic acid (SITS, Sigma) were used to block K^+ and Cl^- channels, respectively. The composition of the solutions in the experimental groups were as follows: $[Na^+]_{0.5}$ and $[Na^+]_{0.1}$ solutions were prepared by reducing Na^+ to 0.5 or 0.1 of that found in the standard solution. $[Na^+]_0$ solution, which was free of Na^+. TEA and SITS solutions were prepared by adding blockers of K^+ and Cl^- channels respectively. $[Ca^{2+}]_{0.5}$ solution was prepared by reducing Ca^{2+} to 0.5 of that found in the standard solution, $[Ca^{2+}]_0$ solution, which was free of Ca^{2+}. All solutions were adjusted to be isoosmolar with 10% Ringer, by substituting choline Cl for NaCl and $MgCl_2$ for $CaCl_2$. To avoid Ca^{2+} contamination Ca^{2+}-free solution contained 5 mmol/l EGTA (ethylene glycol bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid, Sigma).

Electrophysiological measurements

Microelectrodes were normally pulled (Harward Microelectrode Puller) from 1.2 mm OD thin glass capillaries (Intracel Ltd) and filled with 3 mol/l KCl. They had tip resistances of 10–20 MΩ and tip potentials of 2–5 mV. These microelectrodes were used to measure membrane potential with respect to the solution bath, which was grounded through an agar bridge that served as the reference electrode. Under a stereomicroscope (Olympus VMZ), the tip of the electrode was gently pressed on the egg's animal moiety by the aid of a hydraulic micromanipulator (Narishige MO-203). The electrode was inserted into the egg by transiently increasing the negative capacitance of the preamplifier (Nihon Kohden MEZ-7200) to produce an oscillating current. Electrical recordings were made continuously until the end of the first cleavage. Membrane potentials were monitored on a storage oscilloscope (Nihon Kohden VC-10) through an amplifier (Nihon Kohden AVB-10) and recorded on a chart recorder (Palmer Bioscience). The following parameters were measured during electrical recordings.

Resting membrane potential of the unfertilized mature egg (RMP), fertilization time (F_t), peak fertilization potential (FP_p), duration of the fertilization potential (FP_d), membrane potential of the fertilized egg (MP_f), the peak of membrane hyperpolarization observed during the first cleavage (CP_p), time from the onset of membrane hyperpolarization upon the first cleavage, to the return of the potential to a steady value (cleavage cycle, C_t; in this cycle phase 1 is the time to the peak hyperpolarization and phase 2 is the time from peak hyperpolarization to the generation of a steady membrane potential), membrane potential after the first cleavage (MP_{pc}).

Statistical analysis

All averages were expressed as mean ± S E M Significance of differences between means was determined using one way Anova test and Student s *t*-test for paired data The criterion for significance was $p < 0\ 05$

Results

Fertilization of the *Rana cameranor* frog egg was accompanied by a sudden and rapid depolarization of the membrane The duration of this potential change was approx 15 min The membrane potential of the fertilized egg which did not change

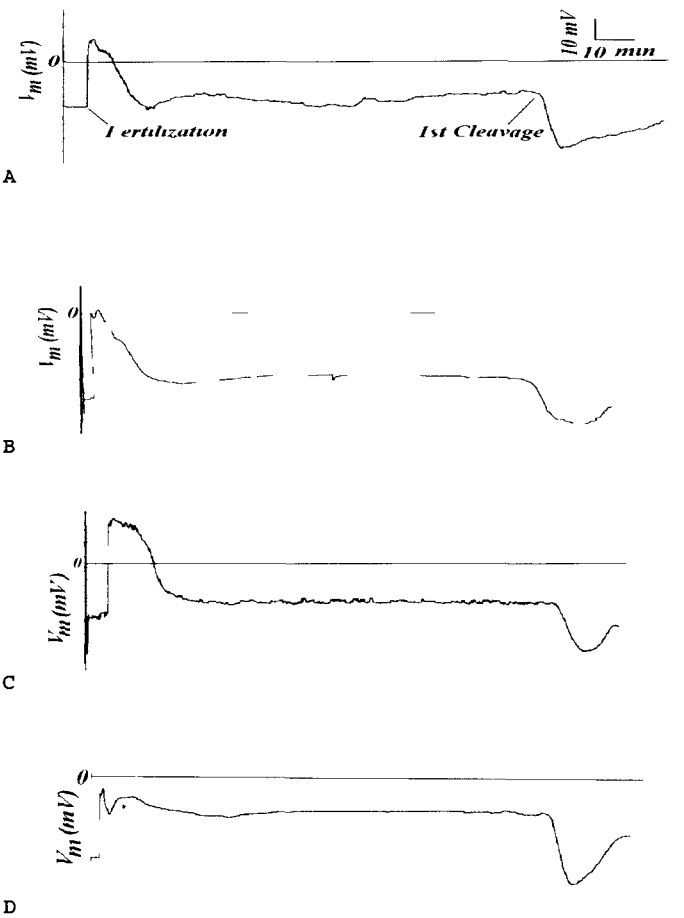

Figure 1. Continuous recordings of the membrane potential from impalement through fertilization to the 1st cleavage in *Rana* eggs *A* In 10 % Ringer *B* In Na^+ free solution *C* In solution with TEA *D* In solution with SITS

Table 1. Electrical properties of *Rana camerani* egg during fertilization in different solutions (mean ± SE)

Solutions	Membrane potential (mV)			Time (min)	
	RMP	FP_p	MP_f	T_f	FP_t
10% Ringer ($N = 8$ $n^{**} = 20$)	−26.35 ± 1.26	6.25 ± 0.79	−22.55 ± 0.86	6.30 ± 0.53	15.08 ± 1.03
$Na_{0.6}^+$ ($N = 5$ $n = 20$)	−29.5 ± 1.49	2.2 ± 1.21[a]	−26.35 ± 1.68	5.36 ± 0.28	14.08 ± 0.87
$Na_{0.1}^+$ ($N = 3$ $n = 21$)	−29.81 ± 0.84	1.67 ± 0.68	−23.71 ± 0.99	5.49 ± 0.13	12.51 ± 0.79
Na_0^+ ($N = 2$ $n = 16$)	−42.69 ± 1.96[a,b]	1.0 ± 0.93[a]	−27.81 ± 1.5[a]	5.25 ± 0.57	13.13 ± 0.69
$Ca_{0.6}^{2+}$ ($N = 2$ $n = 21$)	−28.57 ± 1.92	9.62 ± 1.23	−22.85 ± 1.1	1.10 ± 0.1	19.20 ± 1.25
Ca_0^{2+} ($N = 3$ $n = 19$)	−27.81 ± 1.33	7.79 ± 1.21	−21.10 ± 1.05	5.55 ± 0.75	17.52 ± 1.35
SITS ($N = 3$ $n = 21$)	−25.71 ± 1.53	−1.05 ± 1.01[a]	−20.9 ± 1.23	5.10 ± 0.58	7.46 ± 1.12
TEA ($N = 4$ $n = 21$)	−20.48 ± 0.86	12.76 ± 0.96	−15.35 ± 0.81	1.51 ± 0.3	17.05 ± 1.12

* Number of frogs ** Number of eggs [a] Significant vs. the control group $p < 0.05$
[b] Significant vs. other Na^+ groups $p < 0.05$

significantly until the first cleavage showed a slow negative shift at the onset of the cleavage. This potential change in the hyperpolarizing direction lasted approx. 25 min and was followed by a return of the membrane potential toward the level of the unfertilized state (Fig. 1A).

Resting membrane potential of the unfertilized egg and fertilization potential

When the tracing of each egg was examined individually, the resting potential of the unfertilized egg (*RMP*) was more negative in $[Na^+]_0$ solution but generally less negative in TEA solution (Fig. 1A, *B*, *C*). The mean *RMP* values in the control, Na_0^+ and TEA groups were −26.3 ± 1.26 mV, −42.69 ± 1.96 mV, and −20.48 ± 0.86 mV, respectively; the more negative value in the Na_0^+ group and the less negative value in the TEA group were both statistically significant (Table 1, Figs. 2–5).

The mean fertilization time did not differ significantly in modified solutions compared to that in 10% Ringer (Table 1).

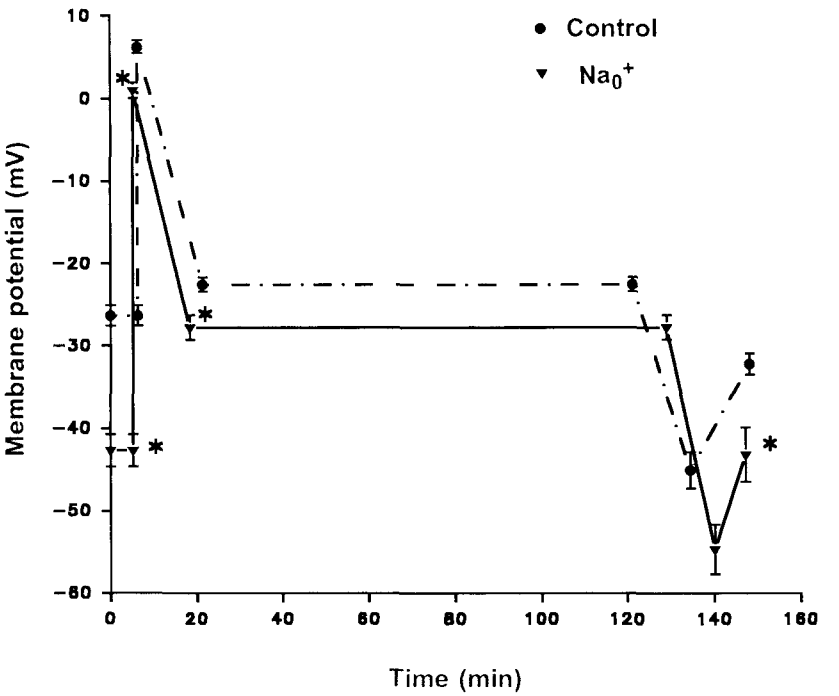

Figure 2. Electrical properties of *Rana catesbeiana* egg from fertilization until the first cleavage: control and Na_0^+ groups (mean ± S.E.). (*) Significant vs. the control group ($p < 0.05$).

Blocking of the K^+ conductance increased the peak of the fertilization potential (more positive FP_f) whereas blocking of the Cl^- conductance reduced it (more negative FP_p) (Figs. 1*C, D*, 4, 5; Table 1). Reducing of extracellular Na^+ reduced the FP_p level in a concentration-dependent manner, and resulted in significantly less positive mean FP_p values (Fig. 2; Table 1). Reducing of extracellular Ca^{2+} did not change the amplitude of the peak significantly (Table 1; Fig. 3).

Blocking of the K^+ conductance prolonged the duration of the fertilization potential (FP_f) whereas blocking of the Cl^- conductance shortened it markedly (Fig. 1*C, D*). FP_d did not change significantly in solutions of different Na^+ concentrations and in Ca^{2+}-free groups. The mean FP_d values in SITS and Ca_0^{2+}-5 groups differed significantly compared to that in the control group (Table 1; Fig. 4).

As also observed with the membrane potential of unfertilized egg, stable membrane potential following fertilization (MP_f) was always more negative in Na_0^+ solutions and less negative in TEA solutions compared to those in the control tracings (Fig. 1*B, C*). The mean MP_f values in all groups were significantly less negative

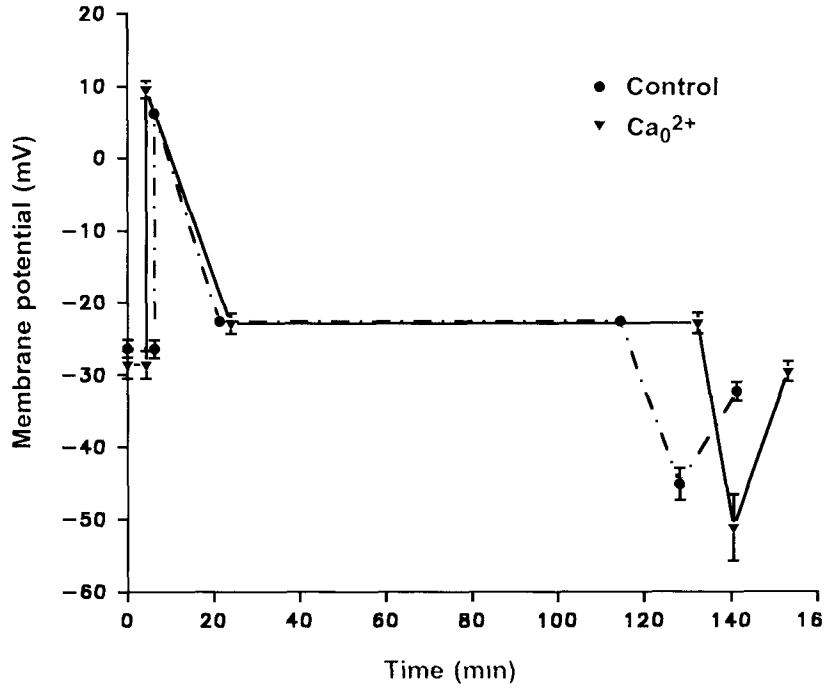

Figure 3. Electrical properties of *Rana camerano* eggs from fertilization until the first cleavage: control and Ca_0^{2+} groups (mean ± S.E.). (*) Significant vs. the control group ($p < 0.05$).

compared to the mean *RMP* levels ($p < 0.05$; Table 1). As also determined for the mean *RMP* value, the mean MP_f levels differed significantly in Na_0^+ and TEA groups compared to that in the control group (Table 1; Figs. 2,3).

Cleavage-associated membrane potential changes

Upon cleavage, first the membrane gradually hyperpolarized (phase 1) and this hyperpolarization was followed by a recovery of the membrane potential to a new steady value (phase 2) (Fig. 1A). This post-cleavage membrane potential (MP_{pc}) was more negative in all groups compared to the membrane potential following fertilization (MP_f) (Fig. 1A, *B, C, D*).

The peak potential of hyperpolarization upon the first cleavage (CP_p) was always less negative in TEA solutions (Fig. 1*C*), whereas it did not markedly differ in the other groups. The mean CP_p value of −45.12 ± 2.23 mV for the control group was significantly less negative in TEA group (−28.63 ± 1.86 mV; $p < 0.05$, Table 2; Fig. 5).

The new membrane potential generated following hyperpolarization (MP_{pc})

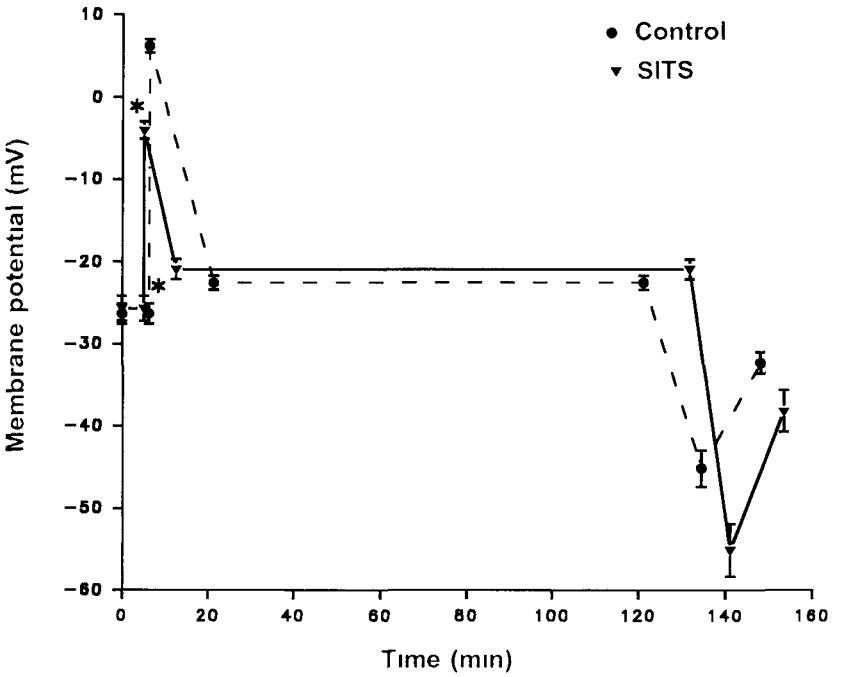

Figure 4 Electrical properties of *Rana temporaria* egg from fertilization until the first cleavage: control and SITS groups (mean ± SE). (*) Significant vs. the control group ($p < 0.05$).

differed significantly in Na_0^+. Na_0^+ and TEA groups compared to that in the control group (respectively −44.79 ± 4.46 mV, −43.25 ± 3.3 mV, −18.44 ± 1.03 mV and −32.24 ± 1.28 mV; Table 2, Figs. 2–5).

The cleavage cycle (C.C.) i.e. the time from the onset of membrane hyperpolarization upon cleavage to the return of the potential to a steady value was 26.42 ± 1.73 min in the control group. The duration of this cycle was significantly shorter in TEA and Ca_0^{2+} groups, the mean ± S.E. values being 17.36 ± 1.29 min and 17.38 ± 1.95 min respectively ($p < 0.05$; Tables 2, 3). The durations of the cleavage cycle phases are also shown in Table 3.

Discussion

Resting membrane potential of unfertilized egg

In the present study the resting potential of the unfertilized *Rana temporaria* egg bathed in 10% Ringer was −26.35 ± 1.26 mV. Similar values have been reported for

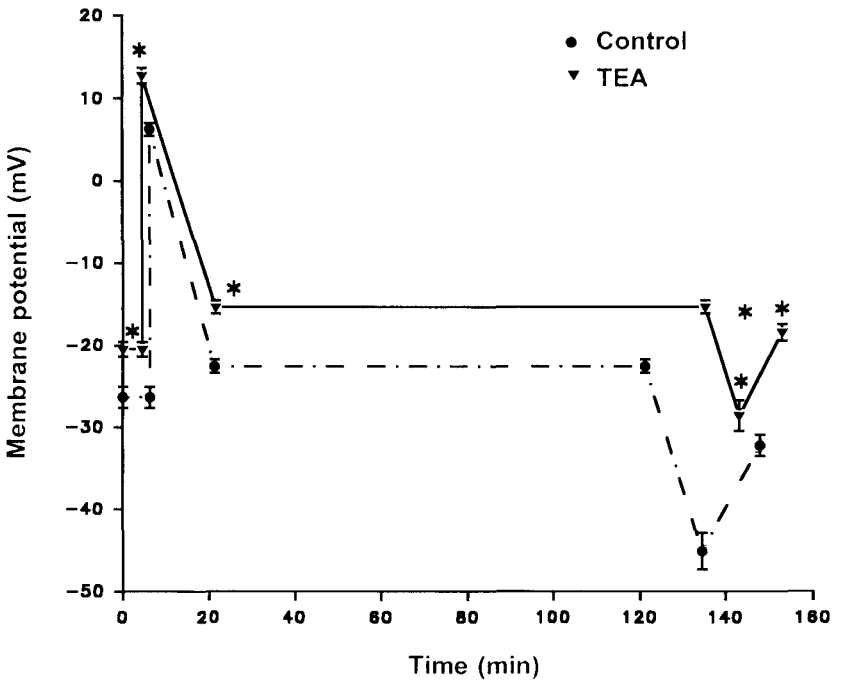

Figure 5. Electrical properties of *Rana catesbeiana* egg from fertilization until the first cleavage in the control and TEA groups (mean ± SE) (*) Significant vs the control group ($p < 0.05$)

Rana pipiens, *Rana esculenta*, and *Rana temporaria* eggs bathed in solutions of ionic content similar to 10% Ringer (Cross and Elinson 1980, Schlichter and Elinson 1981, Jaffe et al. 1985, Jaffe and Schlichter 1985). For *Rana pipiens* and *Xenopus laevis* eggs bathed in solutions of different composition the reported resting potential was approx. −55 mV and −19 mV respectively (Webb and Nuccitelli 1985a).

In solutions of reduced Na^+ ($Na_{0.5}^+$ and $Na_{0.1}^+$ groups) the resting potential was insignificantly more negative whereas hyperpolarization observed in Na^+-free solution (Na_0^+ group) was significantly different from the control and other Na^+ groups (Table 1). Significant hyperpolarization detected in Na^+-free solution was considered to be due to Na^+ efflux through the membrane and this conclusion was supported by insignificantly more negative resting potential values in oocytes bathed in low external sodium (Table 1).

As also reported for *Xenopus laevis* eggs (Peres and Mancinelli 1985), calcium and chloride ions did not significantly affect the resting potential in *Rana catesbeiana* eggs (Table 1).

Table 2. Electrical properties of *Rana cameranoi* egg upon the first cleavage in different solutions (mean ± S E)

Solutions		CP_p (mV)	C_t (min)	MP_{pc} (mV)
10% Ringer	($N = 8$ $n = 17$)	−45 12 ± 2 23	26 42 ± 1 73	−32 24 ± 1 28
$Na_{0.5}^+$	($N = 5$ $n = 14$)	−52 29 ± 4 18	19 56 ± 3 24	−44 79 ± 1 46[a]
$Na_{0.1}^+$	($N = 3$ $n = 19$)	−43 11 ± 2 81	19 36 ± 1 88	−36 47 ± 2 13
Na_0^+	($N = 2$ $n = 12$)	−54 67 ± 3 01	19 00 ± 1 30	−43 25 ± 3 3[a]
$Ca_{0.5}^{2+}$	($N = 2$ $n = 19$)	−51 11 ± 4 56	21 08 ± 1 85	−29 53 ± 1 39
Ca_0^{2+}	($N = 3, n = 14$)	−37 79 ± 3 63	17 38 ± 1 95[a]	−25 29 ± 1 17
SHS	($N = 2$ $n = 22$)	−55 05 ± 3 23	21 13 ± 1 33	−38 11 ± 2 55
HLA	($N = 4$ $n = 16$)	−28 63 ± 1 86[a]	17 36 ± 1 29[a]	−18 44 ± 1 03[a]

[a] Significant vs the control group $p < 0\ 05$

Table 3. Durations of phases of the first cleavage in different solutions (mean ± S E)

		Phase 1 (min)	Phase 2 (min)	Total (min)
10% Ringer	($n = 17$)	13 21 ± 2 13	13 18 ± 0 92	26 42 ± 1 73
Na_0^+	($n = 11$)	11 32 ± 1 17	8 23 ± 2 23	19 56 ± 3 21
$Na_{0.1}^+$	($n = 19$)	12 18 ± 1 1	7 19 ± 1 48	19 36 ± 1 88
Na_0^+	($n = 12$)	11 06 ± 0 96	7 51 ± 1 23	19 00 ± 1 30
$Ca_{0.5}^{2+}$	($n = 19$)	8 25 ± 0 92[a]	12 13 ± 1 19	21 08 ± 1 85
Ca_0^{2+}	($n = 14$)	8 29 ± 0 78[a]	9 08 ± 1 65	17 38 ± 1 95
SHS	($n = 22$)	9 12 ± 0 97	12 31 ± 0 98	21 13 ± 1 33
HLA	($n = 16$)	7 50 ± 0 76[a]	9 16 ± 0 80	17 36 ± 1 29[a]

[a] Significant vs the control group $p < 0\ 05$

In solution containing K^+ channel blocker the membrane potential decreased significantly compared to the control group (Table 1) It has been reported for *Rana pipiens* eggs that potassium ions are present in higher concentrations in the

ICF (approx. 121 mmol/l) than in the cell exterior (approx. 0.19 mmol/l) and they tend to diffuse out of the cell along this concentration gradient (Jaffe and Schlichter 1985). We suggest that the significant depolarization observed in the TEA group is due to decreased K^+ efflux due to the blocking of K^+ channels.

In summary, the resting potential of the unfertilized *Rana camerani* egg is strongly influenced by the chemical and electrical gradients of K^+ ($E_K = -150$ mV) (Jaffe and Schlichter 1985) and it also differs significantly in sodium-free solutions.

Fertilization time (F_t)

The mean fertilization time was 6.30±0.53 min in the control group and it did not differ significantly in any of the modified solutions (Table 1). Similar findings have been reported for various frog eggs inseminated by similar sperm concentrations (Charbonneau et al. 1983; Jaffe et al. 1985; Jaffe and Schlichter 1985; Webb and Nuccitelli 1985a,b).

The peak fertilization potential (FP_p)

Fertilization is accompanied by membrane depolarization. In 10% Ringer the peak of this potential change was 6.25±0.79 mV (Table 1). For *Rana pipiens* eggs bathed in the same solution it has been reported to be 13 mV (Jaffe and Schlichter 1985); different values have been measured for *Rana pipiens* and *Xenopus* eggs (−5.1 and +3.0 mV, respectively) bathed in solutions of different composition (Webb and Nuccitelli 1985a).

Jaffe and Schlichter (1985) has reported that Cl^- channel blockers (SITS and DIDS) had no effect on the fertilization or activation of *Rana pipiens* eggs, but in another study performed with *Xenopus* eggs DIDS was found to decrease Cl^- conductance which increased upon fertilization (Webb and Nuccitelli 1982). When Cl^- channels were blocked by SITS in our experiments the fertilization potential peak (FP_p) was significantly more negative (−4.05 ± 1.04 mV, Table 1, Fig. 4) compared to the control group; in contrast, blocking of K^+ channels by TEA caused this peak to become significantly more positive (12.76 ± 0.96 mV, Table 1, Fig. 5). In the TEA group FP_p was very close to the Cl^- equilibrium potential (E_{Cl}) which for *Rana pipiens* egg is +18 mV (Jaffe and Schlichter 1985); in the SITS group on the other hand FP_p shifted towards the calculated E_K of −150 mV (Jaffe and Schlichter 1985) but this shift was not as prominent as that seen in the TEA group. These results support the conclusions that K^+ and Cl^- conductances which are both present in higher concentrations in the ooplasm than in the egg exterior (Jaffe and Schlichter 1985) contribute to the fertilization potential and that the depolarization is due to a larger efflux of chloride ions through the *Rana camerani* egg membrane.

In eggs bathed in low $[Na^+]_0$ or in sodium-free solution the fertilization potential peak was found to decrease significantly, Na^+-free solutions did not interfere with the development of the depolarizing fertilization potential (Table 1 Fig 1*B*) Sodium channels which open when the membrane potential is brought to positive values have been reported in eggs of *Rana* and *Xenopus* (Baud et al 1982 Schlichter 1983) in low external sodium as in pond water sodium current through these channels will be outward and therefore it will have a hyperpolarizing effect causing the potential change to become less positive When the results reported in our work are compared with those of other studies, it may be suggested that sodium does not directly contribute to the development of the fertilization potential but Na^+ efflux through the Na^+ channels which are opened by the Cl^--induced depolarization will prevent the peak fertilization potential from reaching more positive values in eggs bathed in low external sodium

We could observe no significant effect of extracellular calcium on the fertilization potential (Table 1 Fig 3)

Duration of the fertilization potential (FP_d)

The duration of the fertilization potential was 15 08 ± 1 03 min similar results have been reported for *Rana pipiens* and *Xenopus laevis* eggs (Jaffe and Schlichter 1985 Webb and Nuccitelli 1985a b) Blocking of Cl^- channels by SITS reduced this value significantly (7 46 ± 1 12 min Table 1 Fig 4) whereas blocking of K^+ channels by TEA prolonged this duration insignificantly (17 05 ± 1 42 min Table 1 Fig 5) These findings suggest that when Cl^- conductance responsible from the depolarization phase of the fertilization potential is limited the effect of K^+ conductance becomes more prominent causing the fertilization potential peak to become significantly reduced (Table 1 Fig 4) and the membrane to become polarized again more easily and hence in a shorter time On the contrary when K^+ channels are blocked the more prominent effect of Cl^- conductance causes the fertilization potential peak to increase and the repolarizing forces to become weaker in this condition the membrane will be polarized for a longer time causing an insignificant prolongation of the duration of the fertilization potential The effects of blocking of K^+ and Cl^- channels on the duration of the fertilization potential have been observed clearly in the tracings recorded from each *Rana* egg (Fig 1*C D*)

In eggs bathed in low external calcium the duration of the fertilization potential was significantly prolonged whereas it did not change significantly in calcium-free solutions (Table 1) Moreover Ca^{2+} conductance did not seem to play a significant role in other parameters of the fertilization potential and low extracellular calcium or Ca^{2+}-free solutions did not interfere with the morphologically normal development of fertilized eggs through the neural fold stage These results suggest that extracellular calcium plays no significant role in the generation of the fast

transient electrical block and the relatively slow permanent mechanical block necessary to prevent polyspermy This conclusion is partly supported by the results of Busa et al (1985) who have reported that intracellular calcium stores can be important in producing the permanent mechanical block In the light of all these results we conclude that the significantly long-lasting fertilization potential observed in the $Ca_0^{2+}{}_5$ group can be explained as an effect of a leakage conductance of other ions which may have developed during the experiments

The insignificant effect of Na^+ conductance on the duration of the fertilization potential (Table 1) supports our findings confirming that Na^+ does not directly contribute to the fertilization potential

In summary Cl^- conductance is responsible for the depolarization phase whereas K^+ conductance is responsible for the repolarization phase of the fertilization potential in the egg of the frog *Rana camerano*

Membrane potential of the fertilized egg (MP_f)

In all groups, the membrane potential of the fertilized egg was significantly less negative than the resting potential Similar results were reported for *Rana pipiens* and *Xenopus laevis* eggs and the less negative MP_f values have been explained as an effect of leakage currents which may develop during measurements with an intracellular electrode (Webb and Nuccitelli 1985a, Peres et al 1986)

The membrane potential of the fertilized egg was $-22\ 55 \pm 0\ 86$ mV in the control group In solution with a K^+ blocker this value was significantly less negative ($-15\ 35 \pm 0\ 84$ mV Table 1 Fig 5) leading to the conclusion that K^+ is one of the ions responsible for the repolarization phase of the fertilization potential re for membrane repolarization

In eggs bathed in Na^+-free solution MP_f was significantly more negative ($-27\ 81 \pm 1\ 50$ mV) compared to the control group (Table 1 Fig 2) this result can be explained as an effect of sodium efflux developing in this condition Although sodium-free solution produced such a hyperpolarizing effect the mean MP_f values of the $Na_{0\ 5}^+$, $Na_{0\ 1}^+$ and Na_0^+ groups did not show relative and significant hyperpolarizations when compared to each other (Table 1), moreover Na^+-free solutions did not interfere with the development of the fertilization potential and these solutions did not significantly affect the duration of this potential (Table 1 Fig 1 4 *B*) These findings suggest that Na^+ conductance does not contribute to the generation of the fertilization potential and the membrane potential following fertilization

Our results also show that chloride or calcium conductances do not significantly affect the membrane potential of the fertilized egg (Table 1)

The peak potential upon the first cleavage (CP_p)

In the control group the peak value of membrane hyperpolarization upon the first

cleavage was $-45\ 12 \pm 2\ 23$ mV Upon blocking of K^+ channels, CP_p changed to a significantly less negative value ($-28\ 63 \pm 1\ 86$ mV Table 2, Fig 5), whereas Na^+, Cl^- and Ca^{2+} conductances did not affect it significantly (Table 2) It has been suggested by Woodward (1968), and de Laat and Bluemink (1974) that membrane hyperpolarization observed during the first cleavage is due to the addition of a new membrane with different electrical properties, and a high potassium permeability The increased K^+ conductance upon cleavage will cause the membrane potential to become much closer to E_K (which is approx -150 mV), leading to membrane hyperpolarization Therefore, blocking of K^+ channels by TEA will cause this peak potential to become significantly less negative, as observed in the present study

Cleavage cycle (C_c)

In the control group, the cleavage cycle lasted for $26\ 42 \pm 1\ 73$ min Calcium-free solutions and blocking of K^+ channels by TEA shortened the duration of this cycle compared to the control group (Tables 2,3, Figs 3,5) We suggest that the significantly less negative peak potential measured in the TEA group upon the first cleavage may lead to a shortening of the cleavage cycle and hence to the return of the post-cleavage potential to a steady value in a much shorter time This suggestion is supported by our finding of a significant and strong correlation between the peak potential upon cleavage and the cleavage cycle, when K^+ channels are blocked by TEA ($n = 16$, $r = -0\ 678$ $p < 0\ 01$) In our experiments, phase 1 of the cleavage cycle (the time from the onset of membrane hyperpolarization to the peak value of hyperpolarization) was most strongly affected when K^+ conductance was limited by TEA As shown in Table 3 the duration of phase 1 was significantly shorter in the TEA group whereas the shortening in phase 2 was not significant compared to the control group

In eggs bathed in low external Ca^{2+} or in Ca^{2+}-free solutions we were unable to detect reasonable and significant differences in the mean peak hyperpolarization values (i e relatively more negative peak hyperpolarization with the reduction of extracellular Ca^{2+}) as compared to the control group (Table 2), so, the effect of Ca^{2+} conductance on significant shortening in phase 1 seen in these groups (Table 3), could not be explained by our present knowledge

Membrane potential of the cleaved egg (MP_{pc})

Reducing of extracellular Na^+ shifted the membrane potential of the cleaved egg towards more negative values the differences in $Na^+_{0.5}$ and Na^+_0 groups being significant compared to the control group (Table 2) in addition phase 2 of the cleavage cycle was shorter in all Na^+ groups as compared to each of the other groups (Table 3), these findings suggest that sodium is the basic ion contributing to phase 2 of cleavage (i e to the phase which follows peak hyperpolarization, and which plays a role in the return of the post cleavage membrane potential to a steady level)

Kline et al (1983) have reported that Ca^{2+} and Cl^- conductances in addition to that of Na^+, may also be involved in the generation of phase 2 however we could demonstrate no significant effect of Cl^- conductance on any parameter of the cleavage cycle (Tables 2 3 Fig 4), upon changing extracellular calcium composition, we could not explain the contribution of Ca^{2+} conductance to phase 2 by our findings related to cleavage

In all groups the membrane potential of the cleaved egg (the potential which follows the hyperpolarizing cleavage potential) was significantly more negative as compared to the membrane potential of the fertilized egg Similar results have been reported for *Rana pipiens* and *Xenopus laevis* eggs (Webb and Nuccitelli 1985a) We suggest that the addition of a new membrane with a high potassium permeability (Woodward 1968 de Laat and Bluemink 1974) may cause the membrane potential of the cleaved egg to become more negative, this suggestion is supported by our finding that this potential was significantly less negative when a K^+ channel blocker was added to the extracellular medium (Table 2)

The overall comparison of our results with those reported in the literature has led us to conclude that the response to fertilization with a rapid membrane depolarization is an active phenomenon evoked by the sperm entry into the *Rana cam cranor* egg, whereas membrane hyperpolarization observed during the first cleavage appears to be a passive process which develops slowly and lasts longer and which is produced by the addition of a new membrane with different electrical properties

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References

Baud C Kado R T Marchet K (1982) Sodium channels induced by depolarization of the *Xenopus laevis* oocyte Proc Natl Acad Sci U S A **79**, 3188—3192

Brawley S H (1991) The fast block against polyspermy in *Fucoid algae* is an electrical block Develop Biol **144**, 91—106

Busa W B Ferguson L J Joseph S K Williamson J R Nuccitelli R (1985) Activation of frog (*Xenopus laevis*) eggs by inositol triphosphate I Characterization of Ca^{2+} release from intracellular stores J Cell Biol **101**, 677 682

Charbonneau M Moreau M Picheral B Guerrier P Vilain J P (1983) Voltage noise changes during monospermic and polyspermic fertilization of mature eggs of the anuran *Rana temporaria* Develop Growth Differ **25**, 185 191

Cross N L Elinson R P (1980) A fast block to polyspermy in frog mediated by changes in the membrane potential Develop Biol **75**, 187 198

de Laat S W Bluemink J G (1971) New membrane formation during cytokinesis in normal and cytochalasin B-treated eggs of *Xenopus laevis* J Cell Biol **60**, 529—540

Jaffe L A (1986) Electrical regulation of sperm-egg fusion Annu Rev Physiol **48**, 191—200

Jaffe L A Schlichter L C (1985) Fertilization induced ionic conductances in eggs of the frog *Rana Pipiens* J Physiol **358**, 299—319

Jaffe L A Kado R T Muncy L (1985) Propagating potassium and chloride conductances during activation and fertilization of the egg of the frog *Rana Pipiens* J Physiol (London) **368**, 227—242

Kline D Robinson K R Nuccitelli R (1983) Ion current and membrane domains in the cleaving *Xenopus* egg J Cell Biol **97**, 1753—1761

Kline D Jaffe L A Tucker R P (1985) Fertilization potential and polyspermy prevention in the egg of the *Nemertean Cerebratulus lacteus* J Exper Zool **236**, 45—52

Kline D Jaffe L A Kado R T (1986) Calcium activated sodium conductance contributes to the fertilization potential in the egg of the *Nemertean Worm Cerebratulus lacteus* Develop Biol **117**, 184—193

Peres A Mancinelli L (1985) Sodium conductance and the activation potential in *Xenopus laevis* eggs Pflügers Arch **405**, 29—36

Peres A Bernardini G Negrini C (1986) Membrane potential measurements of unfertilized and fertilized *Xenopus laevis* eggs are affected by damage caused by the electrode Exp Cell Res **162**, 159—168

Perkins K W Franks R T Whitten R H (1981) Reptiles and Amphibians Care and Culture Carolina Biological Supply Company USA

Rugh R (1951) The frog Its Reproduction and Development McGraw Hill Book Company USA

Rugh R (1962) Experimental Embryology Techniques and Procedures Third Edition Burgess Publishing Company

Schlichter L C (1983) Spontaneous action potentials produced by Na^+ and Cl^- channels in maturing *Rana pipiens* oocytes Develop Biol **98**, 47—59

Schlichter L C Elinson R P (1981) Electrical responses of immature and mature *Rana pipiens* oocytes to sperm and other activating stimuli Develop Biol **83**, 33—41

Webb D J Nuccitelli R (1982) Intracellular pH changes accompanying the activation of development in frog eggs comparison of pH microelectrodes and ^{31}P NMR measurements In Intracellular pH Its Measurements Regulations and Utilization in Cellular Functions (Eds R Nuccitelli and D Deamer) pp 293—324 Alan R Liss Inc New York

Webb D J Nuccitelli R (1985a) A comparative study of the membrane from before fertilization through cleavage in two frogs *Rana pipiens* and *Xenopus laevis* Comp Biochem Physiol **82A**, 35—42

Webb D J Nuccitelli R (1985b) Fertilization potential and electrical properties of the *Xenopus laevis* egg Develop Biol **107**, 395—406

Woodward D J (1968) Electrical signs of new membrane production during cleavage of *Rana pipiens* eggs J Gen Physiol **52**, 509—531

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