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

Ş ERDOĞAN, G. LOGOGLU and T. OZGÜNEN

*Çukurova University School of Medicine Department of Physiology 01330 Adana, Turkey*

**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 percent 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.

*Correspondence to Şerif Erdoğan M D, Çukurova University School of Medicine Department of Physiology 01330 Adana, Turkey Tel (90) 322-348 68 17 Fax (90) 322-348 65 72 E-mail serdogan@pamuk.cc.edu.tr*
Key words: Membrane potential — Fertilization — Cleavage — Frog egg

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., 1983; Jaffe and Schlüchter, 1985; Jaffe, 1986; Klime et al., 1985; Webb and Nucetelli, 1985a, b; Klime et al., 1986; Bawley, 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 Schlüchter, 1985; Klime et al., 1985; Jaffe, 1986). FP is well documented in various species of animals such as starfish, Urechis and amphibia and in plants like Fucus algae, but its ionic basis is different for different species (Jaffe, 1986; Bawley, 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 Nucetelli, 1985a, b). Woodward (1968) and de Laat and Blumenk (1974) stated that this hyperpolarization might develop due to the addition of a new membrane with a high potassium permeability (Cross and Emson, 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⁺, Ca²⁺ and Cl⁻ together for the return of the membrane potential to a stable value (Klime 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 cameronii frog egg by recording membrane potentials continuously during this early developmental period.

Materials and Methods

Selected frogs of Rana cameronii species were kept until use at +4°C, in plastic boxes which were filled with small amounts of stock solution (Webb and Nucetelli, 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 × 10⁶ 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; CaCl₂, 0.11; MgSO₄, 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'-isothiocyanostilbene-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. [Ca²⁺]₀.₅ solution was prepared by reducing Ca²⁺ to 0.5 of that found in the standard solution. [Ca²⁺]₀ solution, which was free of Ca²⁺. All solutions were adjusted to be isoosmolar with 10% Ringer, by substituting choline Cl for NaCl and MgCl₂ for CaCl₂

To avoid Ca²⁺ contamination, Ca²⁺-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 (Harvard 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 AWB-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 (F₁)
- Peak fertilization potential (FP₀)
- Duration of the fertilization potential (FPₚ)
- Membrane potential of the fertilized egg (MP₀)
- Peak of membrane hyperpolarization observed during the first cleavage (CP₀)
- Time from the onset of membrane hyperpolarization upon the first cleavage, to the return of the potential to a steady value (cleavage cycle C₁)
- Time from peak hyperpolarization to the generation of a steady membrane potential (MP₁)
- Membrane potential after the first cleavage (MP₂)
**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 catesbiana* 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 114 M, D in solution with 0.1 M.
Table 1. Electrical properties of *Rana catesbeiana* eggs during fertilization in different solutions (mean ± S I)

<table>
<thead>
<tr>
<th>Solutions</th>
<th>Membrane potential (mV)</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RMP</td>
<td>FP&lt;sub&gt;p&lt;/sub&gt;</td>
</tr>
<tr>
<td>10% Ringer</td>
<td>-26.3 ± 1.26</td>
<td>6.25 ± 0.79</td>
</tr>
<tr>
<td>(N = 8 n** = 20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na&lt;sup&gt;+&lt;/sup&gt;</td>
<td>-29.5 ± 1.19</td>
<td>2.2 ± 1.21</td>
</tr>
<tr>
<td>(N = 5 n = 20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na&lt;sup&gt;+&lt;/sup&gt;</td>
<td>-29.81 ± 0.81</td>
<td>1.07 ± 0.68</td>
</tr>
<tr>
<td>(N = 3 n = 21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na&lt;sup&gt;+&lt;/sup&gt;</td>
<td>-12.69 ± 1.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.0 ± 0.93&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>(N = 2 n = 16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>-28.37 ± 1.92</td>
<td>9.02 ± 1.23</td>
</tr>
<tr>
<td>(N = 2 n = 21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>-27.81 ± 1.33</td>
<td>7.79 ± 1.21</td>
</tr>
<tr>
<td>(N = 3 n = 19)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>STS</td>
<td>-25.71 ± 1.53</td>
<td>-1.05 ± 1.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(N = 3 n = 21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIA</td>
<td>-20.18 ± 0.86</td>
<td>12.76 ± 0.96</td>
</tr>
<tr>
<td>(N = 1 n = 21)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Number of frogs  ** Number of eggs  <sup>a</sup> Significant vs. the control group  p < 0.05  
<sup>b</sup> Significant vs. other Na<sup>+</sup> 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<sup>+</sup>]<sub>0</sub> solution but generally less negative in TEA solution (Fig 1A B C). The mean RMP values in the control Na<sup>+</sup> and TEA groups were -26.3 ± 1.26 mV -12.69 ± 1.96 mV and -20.48 ± 0.86 mV respectively. The more negative value in the Na<sup>+</sup> 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\textsuperscript{+} groups (mean ± S. L.) (*). Significant vs the control group \((p < 0.05)\)

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

Blocking of the K\textsuperscript{+} conductance prolonged the duration of the fertilization potential \(F_{p}\)) whereas blocking of the Cl\textsuperscript{-} conductance shortened it markedly (Fig 1C, D) \(F_{p}\) did not change significantly in solutions of different Na\textsuperscript{+} concentrations and in Ca\textsuperscript{2+}-free groups. The mean \(F_{p}\) values in SITS and Ca\textsuperscript{2+} 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 \(M_{p}\) was always more negative in Na\textsuperscript{+} solutions and less negative in TEA solutions compared to those in the control tracings (Fig 1B, C). The mean \(M_{p}\) values in all groups were significantly less negative.
compared to the mean $RMP$ levels ($p < 0.05$, Table 1). As also determined for the mean $RMP$ value, the mean $MP_j$ levels differed significantly in $Na^+_0$ and TEA groups compared to that in the control group (Table 1 Figs. 2, 3).

**Clearage-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 ($MP_p$) 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. 1C), whereas it did not markedly differ in the other groups. The mean $CP_p$ value of $-45.12 \pm 2.23$ mV for the control group was significantly less negative in TEA group ($-25.63 \pm 1.86$ mV, $p < 0.05$, Table 2 Fig. 5).

The new membrane potential generated following hyperpolarization ($MP_p$)
Membiane Potential from Feitilization

Figure 4. Electrical properties of *Rana camaeroanor* ceb from fertilization until the first cleavage control and SITS groups (mean ± S1) (*) Significant vs the control group (p < 0.05)

![Graph showing electrical properties of Rana camaeroanor egg from fertilization until the first cleavage.](image)

- Control
- SITS

The cleavage cycle (C) is the time from the onset of membrane hyperpolarization upon cleavage to the return of the potential to a steady value. The duration of this cycle was significantly shorter in TEA and Ca**2+** groups, the mean ± SE values being 17.36 ± 1.29 mm and 17.38 ± 1.95 mm 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 camaeroanor* egg, bathed in 10% Ringer, was -26.35 ± 1.26 mV. Similar values have been reported for...
Figure 5. Electrical properties of *Rana cairinae* 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 Elison 1980, Schlichter and Elison 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 Nuccelli 1985a).

In solutions of reduced Na⁺ (Na⁺⁺ and Na⁺⁻ groups) the resting potential was insignificantly more negative whereas hyperpolarization observed in Na⁺-free solution (Na⁺⁺ 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 cairinae* eggs (Table 1).
Membrane Potential from Fertilization

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

<table>
<thead>
<tr>
<th>Solutions</th>
<th>CP&lt;sub&gt;p&lt;/sub&gt; (mV)</th>
<th>C&lt;sub&gt;i&lt;/sub&gt; (mm)</th>
<th>MP&lt;sub&gt;pc&lt;/sub&gt; (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10% Ringer (N = 8, n = 17)</td>
<td>-45.12 ± 2.23</td>
<td>26.42 ± 1.73</td>
<td>-32.24 ± 1.28</td>
</tr>
<tr>
<td>Na&lt;sup&gt;+&lt;/sup&gt;(&lt;i&gt;n&lt;/i&gt; = 14)</td>
<td>-52.29 ± 1.48</td>
<td>19.36 ± 1.30</td>
<td>-36.47 ± 2.13</td>
</tr>
<tr>
<td>Na&lt;sub&gt;0&lt;/sub&gt;&lt;sup&gt;+&lt;/sup&gt;(&lt;i&gt;n&lt;/i&gt; = 19)</td>
<td>-44.79 ± 1.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.00 ± 1.30</td>
<td>-32.24 ± 1.28</td>
</tr>
<tr>
<td>Na&lt;sub&gt;0&lt;/sub&gt;&lt;sup&gt;+&lt;/sup&gt;(&lt;i&gt;n&lt;/i&gt; = 12)</td>
<td>-41.67 ± 3.01</td>
<td>19.00 ± 1.30</td>
<td>-43.25 ± 3.34</td>
</tr>
<tr>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt;(&lt;i&gt;n&lt;/i&gt; = 19)</td>
<td>-51.11 ± 4.56</td>
<td>21.08 ± 1.85</td>
<td>-29.53 ± 1.39</td>
</tr>
<tr>
<td>Ca&lt;sub&gt;0&lt;/sub&gt;&lt;sup&gt;2+&lt;/sup&gt;(&lt;i&gt;n&lt;/i&gt; = 14)</td>
<td>-37.79 ± 3.63</td>
<td>17.38 ± 1.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-22.97 ± 1.17</td>
</tr>
<tr>
<td>SHS (&lt;i&gt;n&lt;/i&gt; = 22)</td>
<td>-55.05 ± 3.23</td>
<td>21.13 ± 1.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-38.11 ± 2.55</td>
</tr>
<tr>
<td>HLA (&lt;i&gt;n&lt;/i&gt; = 16)</td>
<td>-28.63 ± 1.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.36 ± 1.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-18.44 ± 1.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Significant vs the control group: p < 0.05

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

<table>
<thead>
<tr>
<th>Solutions</th>
<th>Phase 1 (mm)</th>
<th>Phase 2 (mm)</th>
<th>Total (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10% Ringer (&lt;i&gt;n&lt;/i&gt; = 17)</td>
<td>13.21 ± 2.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.18 ± 0.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.42 ± 1.73</td>
</tr>
<tr>
<td>Na&lt;sup&gt;+&lt;/sup&gt;(&lt;i&gt;n&lt;/i&gt; = 11)</td>
<td>11.32 ± 1.17</td>
<td>8.23 ± 2.23</td>
<td>19.56 ± 3.21</td>
</tr>
<tr>
<td>Na&lt;sub&gt;0&lt;/sub&gt;&lt;sup&gt;+&lt;/sup&gt;(&lt;i&gt;n&lt;/i&gt; = 19)</td>
<td>12.18 ± 1.11</td>
<td>7.19 ± 1.48</td>
<td>19.36 ± 1.88</td>
</tr>
<tr>
<td>Na&lt;sub&gt;0&lt;/sub&gt;&lt;sup&gt;+&lt;/sup&gt;(&lt;i&gt;n&lt;/i&gt; = 12)</td>
<td>11.06 ± 0.96</td>
<td>7.51 ± 1.48</td>
<td>18.00 ± 1.40</td>
</tr>
<tr>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt;(&lt;i&gt;n&lt;/i&gt; = 19)</td>
<td>8.25 ± 0.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.13 ± 1.19</td>
<td>20.08 ± 1.85</td>
</tr>
<tr>
<td>Ca&lt;sub&gt;0&lt;/sub&gt;&lt;sup&gt;2+&lt;/sup&gt;(&lt;i&gt;n&lt;/i&gt; = 14)</td>
<td>8.29 ± 0.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.08 ± 1.65</td>
<td>17.38 ± 1.95</td>
</tr>
<tr>
<td>SHS (&lt;i&gt;n&lt;/i&gt; = 22)</td>
<td>9.12 ± 0.97</td>
<td>12.31 ± 0.98</td>
<td>21.43 ± 1.33</td>
</tr>
<tr>
<td>HLA (&lt;i&gt;n&lt;/i&gt; = 16)</td>
<td>7.50 ± 0.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.46 ± 0.80</td>
<td>17.36 ± 1.29&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Significant vs the control group: p < 0.05

In solution containing K<sup>+</sup> 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 catesbeiana* 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 $6.30 \pm 0.53$ 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 Nuñetelli 1985a,b).

**The peak fertilization potential ($FP_p$)**

Fertilization is accompanied by membrane depolarization. In 10% Ringer, the peak of this potential change was $6.25 \pm 0.79$ 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 Nuñetelli 1985a,b).

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 Nuñetelli 1982). When Cl⁻ channels were blocked by SITS in our experiments, the fertilization potential peak ($FP_p$) was significantly more negative ($-4.05 \pm 1.04$ 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 ($12.76 \pm 0.96$ mV, Table 1, Fig. 5). In the TEA group, $FP_p$ was very close to the Cl⁻ equilibrium potential ($E_i$) 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 catesbeiana* 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 1B). 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 (FPd)*

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 Nucatolli 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 1C–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\textsuperscript{2+} 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\textsuperscript{+} conductance on the duration of the fertilization potential (Table 1) supports our findings confirming that Na\textsuperscript{+} does not directly contribute to the fertilization potential.

In summary Cl\textsuperscript{−} conductance is responsible for the depolarization phase whereas K\textsuperscript{+} conductance is responsible for the repolarization phase of the fertilization potential in the egg of the frog *Rana catesbeiana*.

Membrane potential of the fertilized egg (MP\textsubscript{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\textsubscript{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 ± 0.86 mV in the control group. In solution with a K\textsuperscript{+} blocker, this value was significantly less negative (−15.35 ± 0.84 mV, Table 1, Fig. 5) leading to the conclusion that K\textsuperscript{+} is one of the ions responsible for the repolarization phase of the fertilization potential in the egg of the frog *Rana catesbeiana*.

In eggs bathed in Na\textsuperscript{+}-free solution MP\textsubscript{f} was significantly more negative (−27.81 ± 1.50 mV) compared to the control group (Table 1, Fig. 2) as 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\textsubscript{f} values of the Na\textsuperscript{+5}, Na\textsuperscript{+1} and Na\textsuperscript{+} groups did not show relative and significant hyperpolarizations when compared to each other (Table 1). Moreover, Na\textsuperscript{+}-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. 14 B). These findings suggest that Na\textsuperscript{+} 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\textsubscript{p})

In the control group, the peak value of membrane hyperpolarization upon the first
Membrane Potential from Fertilization

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\(^{+}\) 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).
Klme 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 not demonstrate a 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 Nuñez 1985a). We suggest that the addition of a new membrane with a high potassium permeability (Woodward 1968; de Laat and Blumenk 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 cctus* 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.

**Acknowledgements.** This work was supported by Grant 1193 E 39 from Gukmova University Research Council.

**References**


Membiane Potential from Fertilization


Jaffe L A Kado R 1 Muny M I (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 Jaffe L A Kado R I (1986) Calcium activated sodium conductance contributes to the fertilization potential in the eggs of the Neomuran Waiter Ceratobranchus laevis Develop Biol 117, 181–193

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

Peres A Bernardini G Negri I 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 I Whitten R I (1981) Reptiles and Amphibians Care and Culture Carolina Biological Supply Company USA


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

Final version accepted October 24. 1996