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Precipitation Membrane Effects in Biologic Membranes: The Role of Calcium

A. Ayalon*, G. Bähr, and P. Hirsch-Ayalon**

Department of Pediatric Surgery, Hadassah University Hospital, Jerusalem, Israel,
and Institute for Biochemistry, University of Köln, Köln, Germany

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Summary. Biologic membranes display rectification of electrical current, as well as other properties, in many respects similar to precipitation membranes. The experiments reported here, performed in frog skin, show that these characteristics are dependent upon the presence of calcium. Upon elimination of calcium from the bathing solution, the property of electrical rectification is lost, the current-voltage relation assuming a linear form. Readministration of calcium brings about complete recovery of the rectification pattern. This behavior is analogous to chemical deconditioning of precipitation membranes. Our findings support the assumption that the binding of calcium in biologic membranes produces electrical effects characteristic of precipitation membranes.

Rectification of electrical current has frequently been observed during the study of the current-voltage ($I,\Delta\Psi$) relations in biologic membranes. Precipitation membranes display rectification, as well as other properties, similar to those of biologic membranes (Hirsch-Ayalon, 1965; Katzir-Katchalsky, Hirsch-Ayalon & Michaeli, 1973). In this study we show that electrical rectification in the frog skin depends on the presence of calcium and reversibly disappears upon its removal from the external bathing solution. In precipitation membranes the characteristic rectification pattern similarly converts into a linear function upon the reduction of the concentration of their generating ions to below a certain minimum (Hirsch-Ayalon, 1979). These findings support the assumption that the binding of calcium in biologic membranes produces electrical effects characteristic of precipitation membranes.

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* *Address for reprint requests:* Department of Surgery, The University of Texas Medical Branch, Galveston, Texas 77550.

** *Present address:* Max-Planck-Institute of Biophysics, Frankfurt, Germany.

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Materials and Methods

The abdominal skin of the frog *Rana esculenta* was mounted between Lucite chambers, with an exposed surface area of 1.5 cm^2. Ringer's solution, containing 110 mM Na, 112 mM Cl, 2.5 mM K, 2.5 mM HCO_3^-, 1 mM Ca, and 10 mM glucose was bubbled with air in gas-lift chambers on both sides of the skin.

Calcium-free conditions were produced by Ca-free Ringer's and the application of EDTA (10 mM) for 1–2 min to the mucosal side.

The potential difference ($\Delta\Psi$) across the skin was measured by two saturated calomel electrodes, and the current was applied by Ag-AgCl electrodes, both through 4.4-M NaCl-agar bridges.

The electronic measuring device has been described previously (Bähr, 1967). $I,\Delta\Psi$-curves were measured in the range from 40 $\mu A/cm^2$ in the depolarizing zone to about 40 $\mu A/cm^2$ of hyperpolarization. The current in this direction was disconnected just before electrical breakthrough occurred. The duration of one sweep was 75 sec.

After the skins had been mounted and allowed to stabilize for 45–60 min, with calcium-containing Ringer's solution, an $I,\Delta\Psi$-curve was taken. The second current-voltage relation was measured immediately after the treatment with EDTA and calcium-free conditions, and the third curve was taken 10–15 min after reapplication of calcium-containing Ringer's.

Results are expressed as mean values ± SE, and statistical significance was determined by the student *t* test.

Results

The initial $I,\Delta\Psi$-curve clearly displays rectification; the current in the depolarizing zone being markedly greater than that at an equal hyperpolarizing potential (Fig. 1). The electrical rectification, expressed as the ratio, Q, of maximal resistance in the hyperpolarizing zone (R_{max}) to the minimal resistance in the depolarizing zone (R_{min}), being 4.89 ± 0.56 ($n = 12$). Skins which did not display a clear rectification pattern were discarded.

After treatment with EDTA in calcium-free conditions, the rectification pattern disappeared in all membranes, giving way to an almost linear $I,\Delta\Psi$-relation (Fig. 2). Membranes in which treatment with EDTA was too short still displayed some rectification. Q decreased from 4.89 ± 0.56 to 1.4 ± 0.08 ($p < 0.001$) (Table 1).

Upon restoration of the initial conditions with calcium-containing Ringer's, the $I,\Delta\Psi$-curve resumed its original form (Fig. 2) and Q regained its initial value of 4.36 ± 0.56 ($p > 0.5$) (Table 1).

Treatment with calcium-free solution plus EDTA could be repeated in the same skin 2–3 times, with recovery of the nonlinear $I,\Delta\Psi$-curve each time, upon readministration of calcium.

Treatment with ouabain (10^{-4} M at serosal side for 1 hr) reduced the short-circuit current (I_{sc}) and $\Delta\Psi_{oc}$ almost to zero, and increased

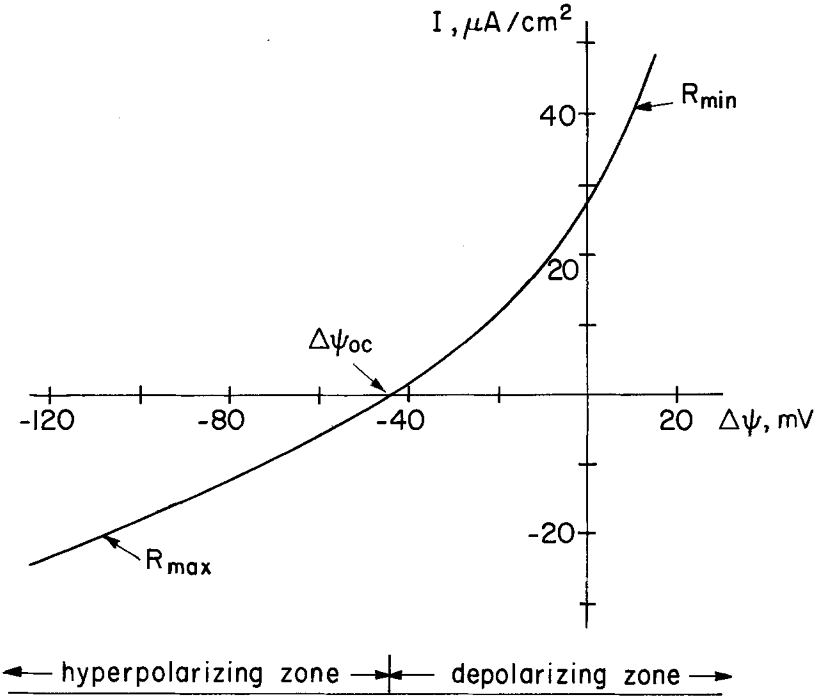

Fig. 1. Typical $I,\Delta\Psi$-relation of the frog skin clearly displaying rectification of electrical current. $\Delta\Psi_{oc}$ = open-circuit potential difference. R_{min} = minimal resistance in the depolarizing zone. R_{max} = maximal resistance in the hyperpolarizing zone

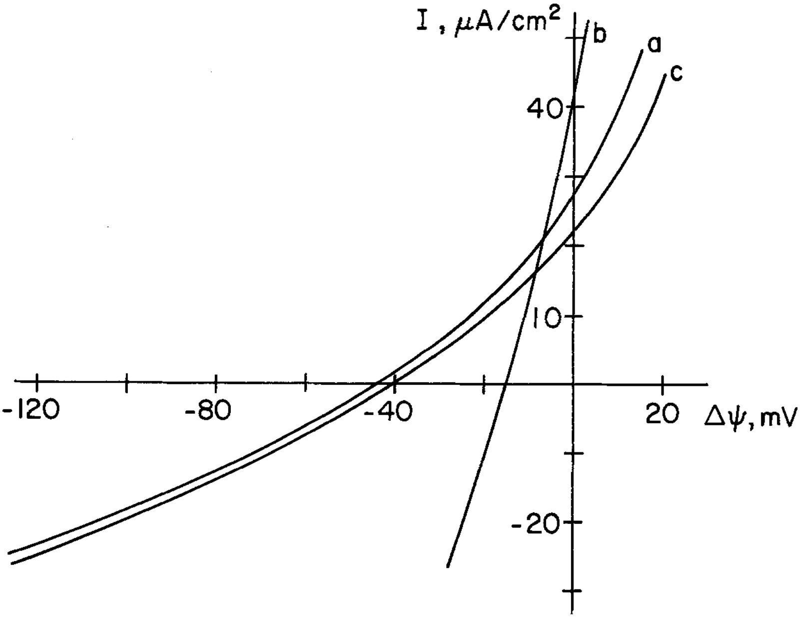

Fig. 2. Representative experiment of current-voltage measurements in the frog skin: a = initial $I,\Delta\Psi$-curve with calcium-containing Ringer's solution, displaying rectification. b = linear $I,\Delta\Psi$-relation after treatment with EDTA and calcium-free solution. c = recovery of the rectification pattern after readministration of calcium-containing Ringer's solution

Table 1. Maximal resistance in the hyperpolarizing zone (R_{max}), minimal resistance in the depolarizing zone (R_{min}) and the rectification ratio (Q)[a]

	Initial with calcium	Calcium-free + EDTA	Recovery with calcium
R_{max}	6088 ±633	1246 ±87	6113 ±605
R_{min}	1272 ± 78	893 ±42	1445 ± 97
Q	4.89 ± 0.56	1.4 ± 0.08	4.36 ± 0.56

[a] Initially in calcium-containing Ringer's solution, then in calcium free conditions after treatment with EDTA, and finally after readministration of calcium-containing Ringer's ($n=12$). ($R = \Omega\ cm^2$.)

R_{max} and R_{min} ($p<0.001$) due to closure of the active pathway. The rectification pattern was maintained; Q in ouabain-treated skins being similar to that of nontreated skins ($p>0.2$). Like in skins without pretreatment with ouabain, calcium-free conditions with EDTA transformed the current-voltage relation into an almost linear function ($p>0.5$), and readministration of calcium reestablished the rectification pattern (Fig. 3, Table 2).

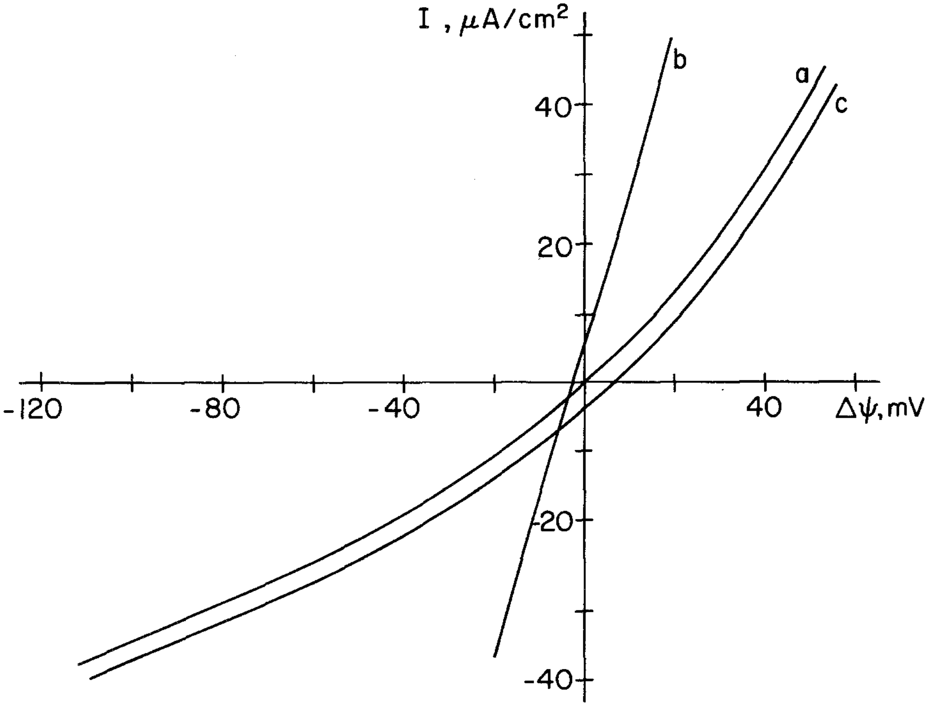

Fig. 3. Current-voltage curves in ouabain pretreated skins: a = initial $I, \Delta\Psi$-curve with calcium-containing Ringer's solution, now passing approximately through $I_{sc}=0$; $\Delta\Psi_{oc}=0$. b = linear $I, \Delta\Psi$-curve after treatment with EDTA and calcium-free solution. c = re-establishment of the rectification pattern after recovery in calcium-containing Ringer's

Table 2. Maximal and minimal resistances and the ratio Q between them, expressing rectification, in ouabain treated skins[a]

	Initial with calcium	Calcium-free + EDTA	Recovery with calcium
R_{max}	8196 ± 1338	1277 ± 217	7084 ± 248
R_{min}	2106 ± 373	901 ± 114	1727
Q	3.92 ± 0.12	1.41 ± 0.16	4.24

[a] Initially in calcium-containing Ringer's, then in calcium-free conditions, and finally after recovery in calcium-containing Ringer's ($n=3$). ($R = \Omega\,cm^2$.)

The resistance at short-circuit current ($R_{I_{sc}}$) in calcium-free conditions with EDTA decreased by 53% ($p < 0.001$), I_{sc} increased by 28% ($p < 0.001$) and $\Delta\Psi$ decreased by 45% ($p < 0.001$) (Table 3).

Table 3. Electrical parameters in calcium-containing Ringer's and in calcium-free conditions ($n=12$)

	Initial with calcium	Calcium-free + EDTA
R_{Isc} ($\Omega\,cm^2$)	1985 ± 207	931 ± 54
I_{sc} ($\mu A\,cm^{-2}$)	15.3 ± 2	21.2 ± 1.5
$\Delta\Psi_{oc}$ (mV)	43 ± 3	23.2 ± 1.6

Discussion

Properties of Precipitation Membranes

Precipitation membranes form spontaneously by diffusion controlled precipitation of sparingly soluble electrolytes within an inert carrier membrane. Counter diffusion of calcium hydroxide *vs.* oxalic acid, or *vs.* phosphate, barium hydroxide *vs.* sulfuric acid, as well as many other combinations, produce precipitation membranes (Hirsch-Ayalon, 1956). They act as ion selective barriers and display rectification of electrical current (Hirsch-Ayalon, 1961*a*, 1965, 1973). The "conditioned" precipitation membrane consists of an electrically charged bilayer; the charges being those of the adsorbed surplus ions of the species that form the precipitate (Hirsch-Ayalon, 1961*b*). These charges are not fixed, however, and can easily and reversibly be desorbed. Desorption, and thus deconditioning, can be brought about by the reduction of the concentration of one of the generating ions to below a certain minimum (Hirsch-Ayalon, 1979), or by the application of a depolarizing potential which draws the charges away from the precipitate (Bähr & Hirsch-Ayalon, 1974). The characteristic rectification pattern, in both instances, transforms into a linear $I,\Delta\Psi$-relation. Re-establishment of the initial conditions brings about reconditioning, with recovery of the rectification pattern.

Current-Voltage Relations in Biologic Membranes

Rectification of electrical current has been demonstrated by Coster (1965) in cell membranes of *Chara australis* and *Nitella species*. This is followed by an electrical breakthrough at high hyperpolarizing potentials, with $I,\Delta\Psi$-curves very similar to those of precipitation membranes. Candia (1970), who studied the $I,\Delta\Psi$-curves in isolated frog skin, obtained comparable results. He further showed that rectification and breakthrough were not affected by inhibitors of the active Na transport mechanism. Fuchs, Larsen and Lindemann (1977), who calculated the $I,\Delta\Psi$-curve of the Na channels in frog skin by subtracting the shunt current from the total current, obtained almost complete rectification.

Time variant changes in resistance have been described to occur in frog skin, when $I,\Delta\Psi$-measurements are rapidly run through in the depolarizing zone. When the measurements are carried out at a slower rate, a flat traject appears, followed by a breakthrough at high depolarizing potentials (Finkenstein, 1964; Fishman & Macey, 1969*a*). Analogous electrical phenomena can be obtained in precipitation membranes (G. Bähr, *unpublished*), with $I,\Delta\Psi$-curves very similar to those reported in frog skin (Fishman & Macey, 1969*a*). The flat traject in the depolarizing zone is a steady-state condition, due to diffusion-limited currents.

The Role of Calcium

Calcium plays a major role in the determination of the passive electrical characteristics of biologic membranes. Mandel (1978) pointed out that calcium increases the entry barrier of Na into the cells. The decrease in resistance, and increase in I_{sc}, observed in our study, correspond well with Mandel's findings. Mandel and Curran (1972) suggest that rectification depends on the presence of calcium in the external bathing solution of the frog skin. Fishman and Macey (1969*c*) showed that the time variant resistance changes were also dependent upon the presence of calcium. Upon removal of calcium, a state of low resistance is entered with a resultant collapse of the *N*-shaped $I,\Delta\Psi$-relation to a nearly linear function. Readministration of calcium quickly reverts the $I,\Delta\Psi$-curve to the original form.

The reversible changes in the rectification pattern observed in this study are in striking analogy to chemical deconditioning of precipitation membranes, caused by reducing the concentration of their generating ions to below a certain minimum. In this respect, the biologic membrane behaves like a calcium-precipitation membrane, which becomes reversibly deconditioned by removing calcium, one of its generating ions, from the bathing solution.

Application of depolarizing potentials causes similar effects, the skin entering a low resistance state, with a nearly linear $I,\Delta\Psi$-relation. Upon removal of the applied potential, the initial state is re-established (Fishman & Macey, 1969*b*). This, again, is analogous to electrical deconditioning of precipitation membranes (Bähr & Hirsch-Ayalon, 1974). These phenomena correspond well with Fishman and Macey's suggestion that this is due to unbinding and subsequent rebinding of calcium to membrane sites (1969*c*).

Rectification of electrical current was observed in artificial membranes produced by polyglutamic acid and calcium (Huang & Spangler, 1977). In gramicidin channels in lipid bilayers, calcium and barium produced similar electrical effects, with $I,\Delta\Psi$-curves similar to those of precipitation membranes (Bamberg & Läuger, 1977).

Theoretical Considerations

Various theoretical explanations for the rectification phenomena in biologic membranes have been proposed. Coster (1965) carried out a theoretical analysis of the current-voltage relations, based on a membrane consisting of two fixed-charge regions of opposite sign in contact. He considered the ionic pn-junction as a basic structure of biologic membranes and derived its electrical characteristics in close analogy to those of the electronic pn-junction of semiconductor physics. Adam (1970) proposed a modification of the above model with an ionic psn-junction as a model for the squid axon membranes, based also on layers of fixed charges. Stark (1973) tried to explain electrical rectification on the basis of a simple carrier model.

Precipitation membranes, with calcium as the precipitating cation, may serve as a model for some of the passive electrical properties of biologic membranes. A quantitative theoretical analysis of the rectification behavior of these membranes has been formulated by Katzir-Katchalsky *et al.* (1973). In contradistinction to the previous models, consisting of fixed charges, the electrical bilayer of precipitation membranes is formed by charges which are not fixed, and can easily and reversible be desorbed, thus making deconditioning and subsequent reconditioning possible.

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