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Transient conductance changes induced by pressure in artificial lipidic membranes

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SUMMARY

The electrical conductance changes induced by pressure in artificial lipidic membranes were studied. A reversible function (third order) between the applied pressure and the increase in conductance was found. When the stimulus reached a threshold value a transient conductance change was observed. These results were strongly dependent on lipid composition and calcium concentration. The experimental data described here may give insight into the mechanism of mechano-electrical transduction and emphasize the role of lipids in transient permeability phenomena.

Hydrostatic pressure, when applied to one side of artificial lipidic membranes, modifies their electrical properties. Conductance becomes a function of pressure and at a given "threshold" value, it produces a transient change in the conductance of the membrane. These effects are strongly dependent on Ca^{2+} concentration and on the origin of the membrane lipidic compounds. Artificial lipidic (*i.e.* black) membranes separating two aqueous phases were made by the method of Mueller *et al.*[1] using a solution of chloroform–methanol–tetradecane (10:8:4, v/v/v) containing different lipidic compounds. The studies were carried out on artificial membranes made of ox brain gray matter lipids, whose properties have been widely studied, and of toad urinary bladder lipids, a tissue where pressure is known to modify the electrical properties[2]. Total lipids were extracted by the method of Folch *et al.*[3] in all cases. Phospholipids were separated by a column of silicic acid and stored under N_2 at $-40°$. In some cases the total lipid extract was used to form membranes (20 mg/ml). In other cases only the phospholipids (10 mg/ml), with the addition of purified cholesterol (10 mg/ml, Sigma 99%) were used. The membranes were made across a hole of 1.0 or 1.5 mm diameter in a teflon septum separating two chambers

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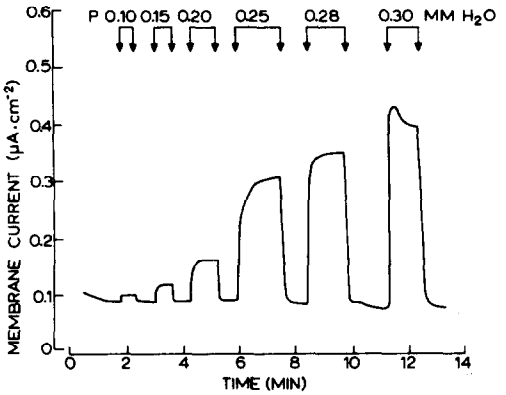
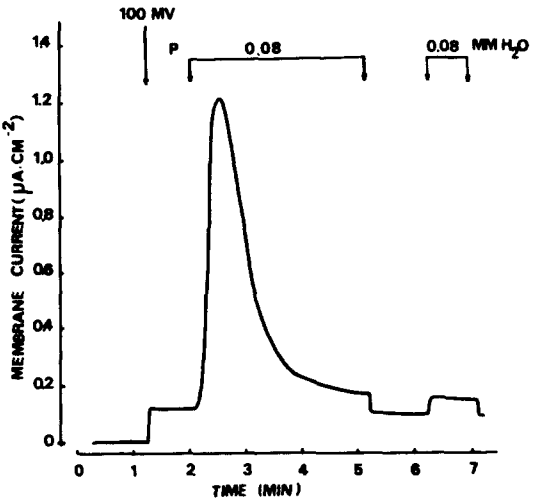
containing 100 mM NaCl and 50 mM Tris (pH 7). The instrumental set up, similar to that of Ehrenstein *et al.*[4], comprised a voltage source establishing a potential difference, which was kept constant across the membrane and measured via calomel electrodes with a Keithley DC voltmeter 200B. The current was measured with a Keithley 150A microammeter connected to a Heat EUW servorecorder. Different voltages were imposed across the membrane. In all types of membranes used the current/voltage curves showed an ohmic relationship between 0 and 100 mV and the resistance was $4.2 \pm 0.6 \cdot 10^5\ \Omega \cdot cm^2$, when membranes were made with brain phospholipids (mean ± S.E.; $n = 10$). This value is similar to that previously reported in the literature[5]. With membranes made of total lipids from brain or bladder the values were between $2 \cdot 10^5$ and $9 \cdot 10^5\ \Omega \cdot cm^2$. These resistance values are lower than those described using absolutely pure phospholipid fractions in some cases. To discard the possibility of the presence of mechanical leaks in our experimental chamber we have made membranes of cholesterol in decane, obtaining resistances of about $10^9\ \Omega \cdot cm^2$ which are similar to those previously described[4]. Pressure variations were produced by adding or subtracting solution with a syringe immersed in the bath at one side of the membrane. The volume of liquid injected or subtracted was between 0.01 to 1.0 ml which produced a level difference of 0.01 to 1.0 mm. The time of injection was about 1 sec. Since in this instance the voltage was maintained constant (100 mV in most cases) current variations reflected changes in conductance. When pressure was applied the conductance increased to a new level and remained constant until the pressure was eliminated (Fig. 1). If the stimulus became greater a delayed conductance-restoring effect appeared (Fig. 1). Finally, when the hydrostatic pressure applied reached a "threshold" value, it produced a transient change in conductance (Fig. 2). This parameter increased 5- to 10-fold and then it returned almost to the initial value. When the stimulus was suppressed, the initial basal line was restored. If in the following minutes we again applied the same hydrostatic pressure a very small non-transient response, similar in magnitude to the residual value of the transient response, was observed. This "refractive period" disappeared after some 15 min. Similar results were obtained with septa having holes 1.0 or 1.5 mm in diameter. Although these results were observed with all the lipids used, the magnitude of the pressure stimulus necessary to elicit this type of response was strongly dependent on the origin of the lipids present in the membrane. In the case of lipids from toad urinary bladder the range of change in pressure used was between 0.01 and 0.08 mm of water, but material from bovine cerebral cortex required a pressure 10 times higher. In this last case we used either a total lipid extract where some proteic material (such as proteolipids) was present, or the phospholipid fraction where protein content had been reduced by several precipitations and purification through a silicic acid column to 1.5% (w/w, related to phospholipids). The results were similar in both cases. This suggests that the difference observed between toad bladder material and ox gray matter might be due to a variation in the lipid composition of the two extracts. The addition or subtraction of solution by the same amount on both sides of the membrane at the same time, which did not involve any change of pressure, did not produce a conductance change. The relationship between applied pressure and change in conductance in the subthreshold zone was not linear (Fig. 3). On the contrary, we had a potential relation of the third order. The sensitivity of lipids from toad urinary bladder was 10 times greater than that of lipids from cerebral cortex but the relationship was exactly the same (Fig. 3). When $CaCl_2$ was spread





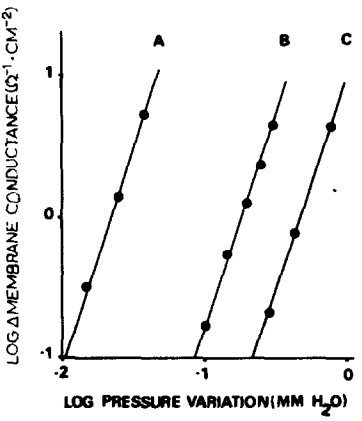


Fig. 1. Changes in current induced by hydrostatic pressure. A 100 mV potential difference is clamped across the membrane. Pressure is applied from the positive to the negative side. Membranes made of phospholipids from ox gray matter and purified cholesterol in chloroform–methanol–tetradecane. P, pressure applied between arrows. "Subthreshold zone" (see text).

Fig. 2. Similar to Fig. 1. The first arrow shows the potential difference application. Membrane made of total lipids from toad urinary bladder. In this case the stimulus is sufficiently important to discharge a transient response. A second stimulus did not elicit a transient response ("refractive period").

Fig. 3. Relationship between the logarithm of pressure applied and the logarithm of change in conductance. Subthreshold zone. Each point is the mean of 6 experiments. A, total lipids from toad urinary bladder; B, total lipids from ox gray matter; C, total lipids from ox gray matter treated with Ca^{2+}.

(a 20 mM solution in Ringer's solution injected through a polyethylene cannula) the sensitivity to the hydrostatic pressure was strongly reduced, but the function relating conductance and pressure remained the same (Fig. 3). The current/potential relationship remained linear in the presence of hydrostatic pressure. Because of the reversibility of the phenomena, we discarded the development of a permanent low resistance leakage pathway as an explanation of our results. Andreoli *et al.*[6] have observed that in spontaneously

bowed membranes, voltage and current declined reversibly and concluded that there is a reversible change, either in molecular architecture or composition in the membrane, which alters its ionic permeability. Under our conditions when pressure was applied to one side the membrane bowed. Therefore the conductance change that we observed in the "subthreshold" zone could just be related to an increase in the membrane area. However, it would be very difficult to explain the transient conductance change on the basis of a geometrical change.

The evidence of the present study indicates that artificial lipidic membranes can exhibit transient electrical behaviour in response to hydrostatic pressure gradients. One may speculate on the physiological implications of this property. Some type of mechano-electrical transductions seem to be the key feature in the physiological response of different biological structures, such as the baroceptors in the carotid sinus, certain sensory receptors like the Pacinian corpuscle, the stretch receptors in muscle, *etc.*[7], Moreover, pressure stimulus develops a transient change in conductance lasting 20 sec in the isolated squid axon which is similar to the results we have described here[8]. In a recent review on mechanoceptor function W.T. Catton[7] says: "The most fundamental question, that of the transducer process converting mechanical energy into the initial electrical change in the membrane has not been answered". Teorell[9] obtained electrical transient changes that could become rhythmical in a porous membrane containing fixed charges. Our results show that a relatively simple lipidic membrane can modify its structure in a dynamic way, producing electrical changes that may be related to those described in biological membranes. Mueller and Rudin[10], among others, have shown that by adding macrocyclic antibiotics or the "excitation inducing material" it is possible to obtain electrical fluctuations in artificial lipidic membranes. In the discussion of their findings they analysed "the possibility of a reversible micellization of the bilayer structure forming channel domains or clusters that can be at the origin of the permeability changes". Results presented here show that transient conductance can also be obtained in artificial membranes where no specific proteins are present. The important role of lipid composition in this type of phenomenon is emphasized by the change in sensitivity to pressure observed with lipids from different sources. The calcium effect could be explained by the known interaction with the negatively charged polar groups of neighbour lipid molecules making bridges that may be at the origin of the effect produced by this ion here[11,12]. Luzzati *et al.*[13] have demonstrated that lipids in water can take different structures according to the thermodynamic conditions in the medium. The highly developed polymorphism reflected in transitions between different phases in the lipid structure may be strongly related to transient changes in membrane permeability.

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