PERMSELECTIVITY OF A POROUS PHOSPHOLIPID-CHOLESTEROL ARTIFICIAL MEMBRANE. CALCIUM AND LANTHANUM EFFECTS.

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A membrane model prepared by adsorbing phospholipids and cholesterol to the matrix of a millipore filter and introducing an aqueous pore phase, exhibits high cation permselectivity. Association of Ca++ with the fixed negative groups of the phospholipids decreases the cation permselectivity and addition of La+++ changes the membrane from a cation exchanger to an anion exchanger.

Tobias et al. (1962) designed this membrane to study Ca++ regulation of membrane permeability. Observations of biological relevance were that this artificial membrane concentrated cations with selectivity for Ca++ over Na+ and K+; Ca++ reduced membrane hydration and ionic permeability; pH titration curves suggested the fixed negative groups to be phosphoric and carboxyl groups; and an applied voltage gradient caused an electroosmotic water flux across the membrane. (Mikulecky and Tobias, 1964; Leitch and Tobias, 1964; Rojas and Tobias, 1965). Another analogy with cell membranes is that the Tobias type membrane model demonstrates Ca++ stimulated ^{45}Ca flux as has been demonstrated in smooth and cardiac muscle (van Breemen and van Breemen, 1968 a and b; Reuter, 1968).

This communication reports on concentration potentials across this membrane and discusses the parallelism between the depolarizing effect of Ca++ on these concentration potentials and the cell membrane potential depolarization resulting from the interaction of Ca++ with the internal

interface of the axolemma (Grundfest, 1955; Tasaki et al., 1965).

METHODS AND RESULTS

The artificial membranes were prepared according to the method of Tobias et al. (1962). Millipore filters of 100 Å pore size were dipped in an equimolar mixture of phospholipids (Animal cephalin, Nutritional Biochemical Co.) and cholesterol in benzene and subsequently air dried. They were then mounted in a lucite chamber, so that a membrane area of 12.5 cm^2 divided two aqueous solutions each 27 mls in volume. The solutions were in electrical contact with calomel electrodes by means of KCl bridges, and the calomel electrodes were connected to the input and ground leads of a Tektronix Type 503 oscilloscope.

Figure 1 shows the membrane potentials recorded when the chamber connected

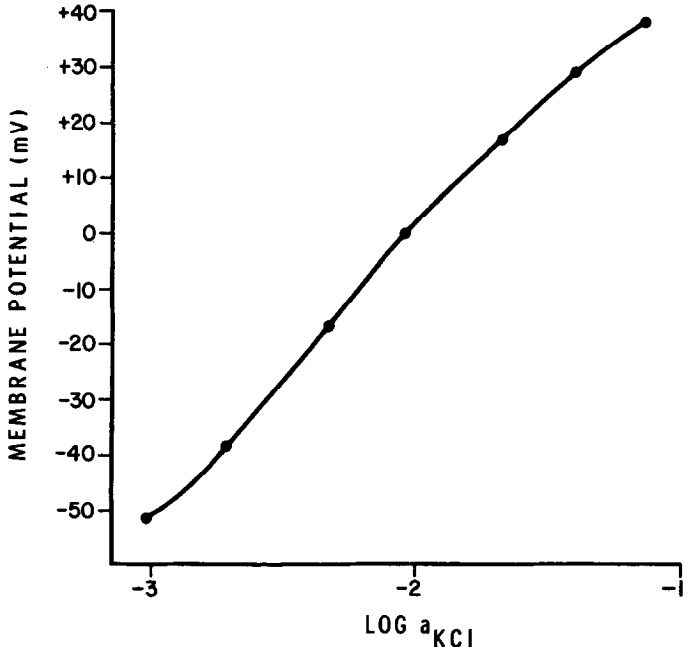

Figure 1. Membrane potential as a function of KCl activity on one side of the artificial membrane. The KCl concentration of the solution on the other side of the membrane and connected to the + scope input was kept constant at 10 mM. Each point is an average of four determinations on different membranes. S.E.M. is in all cases less than 5%.

to the positive scope input contained 10 mM KCl and the chamber connected to the ground lead contained a range of KCl concentrations between 1 and 100 mM. Each point is an average of four measurements on different membranes and concentrations have been converted to activities (Harned and Owen, 1963). Millipore filters not impregnated with phospholipids showed no concentration potentials with KCl gradients. Since the concentration potential is positive on the side of the lower salt concentration, the net negative charge of the hydrophilic portions of the phospholipids serves to create a cation exchange membrane. The cation permselectivity of this membrane is of high magnitude, since the maximum slope of membrane potential vs log KCl activity is 56 mV/10 fold increase in activity. The maximum possible for perfect cation permselectivity calculated from the Nernst equation at 23° C is 58.7 mV/10 fold increase in activity. The potentials at higher concentrations of both solutions were less than at lower salt concentrations, as would be expected from less effective exclusion of the co-ion Cl^- from the ion exchange membrane due to smaller Donnan potentials at the interfaces of the more concentrated solutions (Helfferich, 1962; Sollner, 1955). The same experiments were repeated with NaCl. The same type curve was obtained with a maximum slope somewhat less, 52.5 mV/10 fold increase in NaCl activity. This lesser slope is in agreement with the lower mobility for the hydrated Na ion.

At the time indicated by the arrow in figure 2, Ca++ is added to both sides of a membrane separating a 5 mM from a 50 mM KCl solution. The concentration of Ca++ after addition is 1 mM. Due to the low mobility of Ca++ in membranes containing carboxyl or phosphate groups this relatively small concentration of Ca++ should not affect the K+ concentration potential (Carr, 1968). However the addition of Ca++ is followed by a nearly exponential depolarization to half the original membrane potential. At 124 minutes the solutions are replaced by fresh solutions of 5 and 50 mM KCl each containing also 1 mM $CaCl_2$. This causes practically no repolarization (first open circle on the upper time scale) showing that the depolarization was not due to a

decrease of the KCl gradient. When the solutions are now replaced by a series of fresh solutions of the same KCl concentrations but without $CaCl_2$ the membrane potential returns to its previous level according to a somewhat faster time course (dashed curve and upper time scale). The depolarization indicates a loss in cation permselectivity, which may be due either to a decrease in the fixed negative charge density or to an increase in pore diameter (Helfferich, 1962). The work of Tobias' group (see introduction) established that Ca++ increased the membrane resistance to electric current and to water flow, eliminating the possibility of increased pore size. It thus seems logical that the depolarizing effect of added Ca++ is due to a decrease in the negative fixed charge density resulting from the association of Ca++ with the phosphate and carboxyl groups of the phospholipids. The effect of poly-

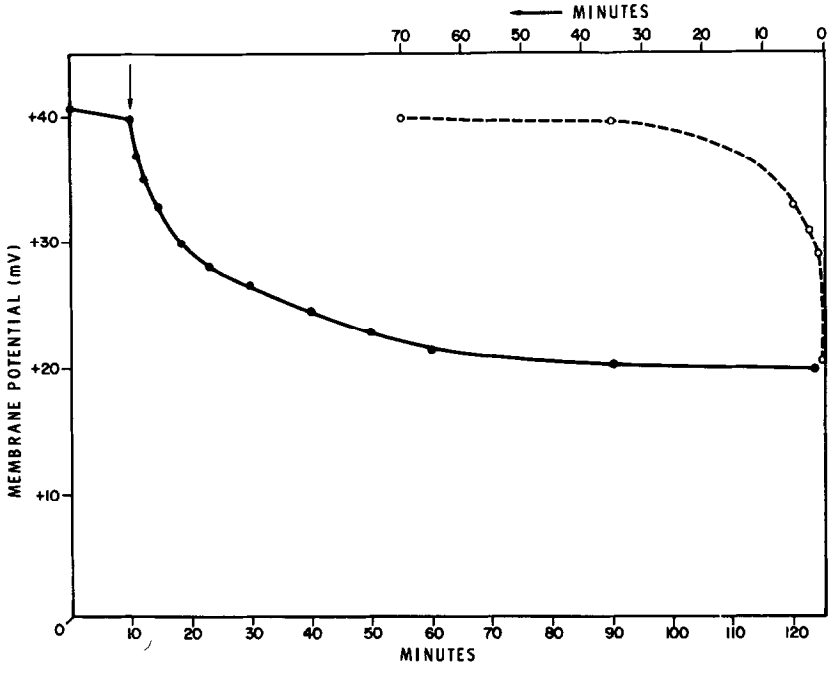

Figure 2. Potential difference across a membrane separating 5 mM KCl from 50 mM KCl. At arrow 1 MM$CaCl_2$ is added to both sides. Dashed curve shows repolarization upon removal of Ca++. The times for this curve are indicated by the top scale.

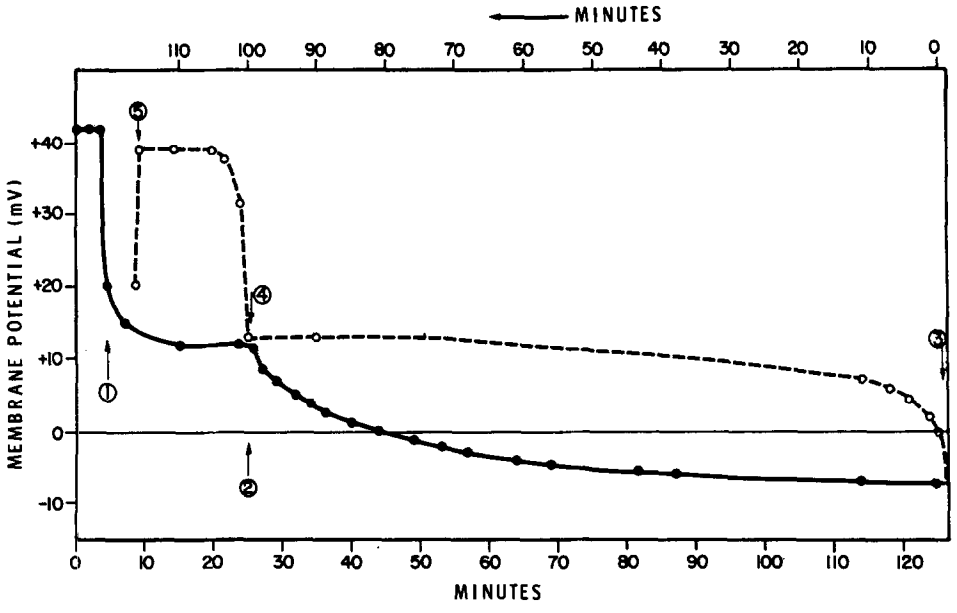

Figure 3. Potential difference across the artificial membrane (solution of lower concentration connected to + scope input) under following conditions: time span 0 - ①, 5 mM CKl|Memb.| 50 mM KCl; ① - ②, 5 mM $CaCl_2$|Memb.| 50 mM $CaCl_2$; ② - ③, 5 mM $CaCl_2$ + 1 mM $LaCl_3$|Memb.| 50 mM $CaCl_2$ + 1 mM $LaCl_3$.

Dashed curve shows reversibility of above changes (upper time scale): ③ - ④, removal of La+++; ④ - ⑤, return to KCl; ⑤, change to $CaCl_2$ gives same initial $CaCl_2$ concentration potential.

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valent cation binding to these negative groups can be seen more dramatically in figure 3. At step ① the solutions in the chambers are changed from 5 and 50 mM KCl to 5 and 50 mM $CaCl_2$. There is an immediate about 50% drop in the membrane potential due to change from monovalency to bivalency of the permeable ion (maximum concentration potential is $\frac{RT}{zF}\ln\frac{a_{cation'}}{a_{cation''}}$ [z = valency of permeable ion, a' and a" = activities of permeable ion on both sides of membrane, RT and F have their usual meaning]. The immediate change is followed by an exponential decrease of the potential, which can again be best explained by a decrease in fixed negative charge density due to association with Ca++. At step ②, 1 mM $LaCl_3$ is added to both sides of the membrane. This causes no immediate change in the potential as would be expected from its relatively low concentration and mobility. However there is a slow exponential decline

in potential resulting in a reversal of polarity. The addition of 1 mM La thus changed the membrane from cation permselective to anion permselective. Such a change is due to the association of the trivalent cation with the fixed negative groups, leaving a net positive fixed charge in the ion exchange membrane (Helfferich, 1962). The dashed curve of figure 3 indicates the reversibility of these effects, see legend.

DISCUSSION

The results emphasize that the Tobias type membrane is an interesting model for pores lined by hydrophylic groups of phospholipids and/or proteins as postulated to exist in cell membranes by Danielli and Davson (1934). Very briefly, the following evidence suggests the presence of phospholipid lined aqueous pores in cell membranes. Globular substructures 50 to 60 Å in diameter have been demonstrated in biological membranes (Sjostrand, 1963). The stability of globular phospholipid micelles (hydrophilic groups pointing outward) has been demonstrated, and pores between junctions of such micelles estimated as having 5 Å radii (Lucy and Glauert, 1964; Stein, 1967). Vast evidence from different experimental and theoretical approaches points to water filled pores of this size in cell membranes (Solomon, 1968). Recent demonstrations of electroosmosis and streaming potentials in squid axons indicate aqueous pores lined by fixed negative sites (Stallworthy and Fenson, 1966; Vargas, 1968). From comparisons of sequences of affinity of cations for the squid axonal membrane with those for phosphate and carboxyl colloids, Tasaki et al. (1965) concluded that the fixed membrane charges on the internal side were due to phosphoric groups and those on the external side due to carboxyl groups. In a study of axolemmal conductance over a wide pH range Rojas and Atwater (1968) found an inflection of the curve pH vs conductance around pH 4.5 suggesting the membrane fixed charges to be carboxyl and phosphoric groups.

Electronmicrographs of the membranes we used showed highly irregular channels lined by a layer of phospholipids and in clear communication with

the external solutions (Feldherr, 1968). The narrowest portions of these channels would allow for the high charge density necessary for effective co-ion exclusion (Sollner, 1955).

The preceeding paragraphs make it plausible that similarities exist between the ion exchange nature of the pores in the artificial membrane and cell membranes. By analogy the conclusion may be drawn that Ca++ injected intracellularly associates with the negative groups on the inner portion of the membrane pores. The resulting loss of cation permselectivity would lead to depolarization.

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