CURARE BLOCKS CATIONIC CONDUCTANCE IN ARTIFICIAL MEMBRANES CONTAINING HYDROPHOBIC PROTEINS FROM CHOLINERGIC TISSUES.

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SUMMARY: The permeability properties of control artificial lipidic membranes containing synthetic lipids were compared with those made with the lipids and hydrophobic proteins(i.e. proteolipids) extracted from electroplax of Electrophorus electricus and cat heart ventricle. While the controls did not show ionic selectivity the others showed a cationic conductance for Na^+ and K^+ giving rise to a diffusion potential, which was inhibited by 10^{-4}Md-tubocurarine. It is suggested that proteolipids are responsible for this cationic conductance.

INTRODUCTION

Previous work has demonstrated that artificial lipid membranes containing a certain hydrophobic protein(i.e. proteolipid) fraction from the electroplax of Electrophorus electricus develop special properties, including a "cholinergic conductance" under the action of acetylcholine, which can be blocked by d-tubocurarine[1,2]. In other laboratories efforts have also been made to incorporate into artificial bilayers other proteins extracted from biological membranes, with the idea of reproducing the ionic permeability properties normally found in such membranes[3,4,5].

In the present paper artificial membranes were made using the total lipid extract from electroplax of Electrophorus electricus and from cat heart. In addition to the lipids of the tissue, the extracts contain all the hydrophobic proteins. It will be shown that in the presence of an ionic gradient such membranes develop a diffussion potential which can be inhibited by d-tubocurarine.

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METHODS

The artificial membranes were made as previously described[2]. The saline bathing solution contained 1 mM NaCl, KCl or Na_2SO_4 with Tris-PO_4 buffer to keep the pH at 7.0 . Increasing voltages were applied with an external D.C. source. The membrane resistance was calculated from the intensity/voltage relationship which was constant, between 0 and 150 mV, for all the membranes studied. After eliminating the applied voltage different ionic gradients were established by increasing the salt concentration on one side of the membrane. The diffusion membrane potential was measured with a Keithley 200 B D.C. Voltmeter via calomel electrodes and visualized with a Keithley 370 recorder. In other experiments the membrane voltage was clamped at zero millivolt during application of the salt gradient and the current flowing through the membrane was measured with a Keithley 150 A Ammeter. Using this parameter and the previously determined membrane resistance the diffusion potential was calculated.

When a NaCl gradient is established across a membrane having the same permeability to Na^+ and Cl^- ions, a diffusion potential due to the difference between the mobilities of the cation (u_c) and the anion (u_a) appears. This diffusion potential can be calculated as[6]:

$$E_{dif} = - \frac{R\,T}{F} \frac{(u_c - u_a)}{(u_c + u_a)} \ln \frac{a''}{a'}$$

where a' and a" represents the salt activity in each side of the membrane.

Three types of membranes of different composition were studied: 1) Control membranes containing 2.3 mg/ml of synthetic dipalmitoyl-lecithin (Sigma Co. standard for chromatography) and 2.3 mg/ml synthetic cholesterol (Sigma Co. 99%) in a solution of chloroform-methanol-tetradecane (1.0 : 0.8 : 0.5 v/v). 2) Membranes made with the total lipid extract from the electroplax of Electrophorus electricus. In this case 0.5 g of lyophilized tissue was extracted with

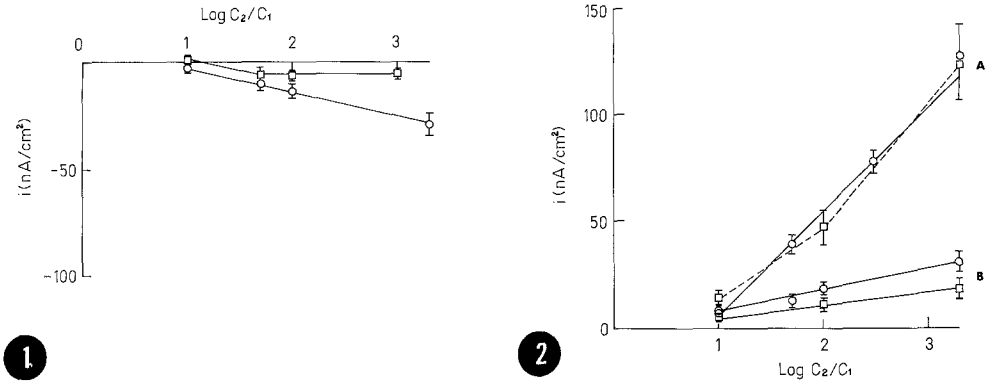

Fig. 1 .- Current flowing across control membranes(detailed composition in the text) as a function of the salt concentration ratio between the two acqueous phases: the effect of dTC. Voltage was clamped at zero mV. The membranes were formed in a solution containing 1 mM of the probe salt and 1 mM Tris-PO_4 buffer, pH 7.0 at room temperature.
Probe salts were NaCl (O) and Na_2SO_4 (□)

Fig. 2 .- Current flowing across proteolipid containing membranes (detailed composition in the text) as a function of the salt concentration ratio between the two acqueous phases. Similar conditions as in figure 1. Probe salts were NaCl (O) and KCl (□). The proteolipids were obtained from Electrophorus electric organ (A) or cat heart tissue (B)

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15 ml of chloroform-methanol (2:1) as previously described[7] and after addition of 7.5 ml of chloroform it was concentrated in a rotatory evaporator at 20°C to a final volume of 3 ml avoiding proteolipid precipitation. The membrane forming solution made with the same solvents as in the controls had 0.7-0.8 mg/ml of protein and 2.3 mg/ml of total phospholipids. 3) Membranes made as in 2) but with total lipid extract of cat heart ventricle. In this case the protein concentration was 0.1 mg/ml.

RESULTS

Fig. 1 shows the results obtained in control membranes. The current flowing is expressed in nA/cm^2 and it varies linearly with the log of C_2/C_1, i.e. the ratio of salt concentration in the two

TABLE I

Diffusion potentials (mV) across control and proteolipid containing membranes in different acqueous media, containing NaCl, KCl or NaCl plus dTC 10^{-4} M (this drug on both sides of the membrane). The salt concentrations were 1 mM on one side and 100 mM on the other side.

	NaCl	KCl	NaCl + dTC
Theoretical expected values for no selective membranes	+ 19.7	+ 1.94	
Membranes containing synthetics lecithin and cholesterol	+ 10.8 $\pm$ 1.0	-	+ 11.4 $\pm$ 2.2
Membranes containing cat heart proteolipids	- 15.4 $\pm$ 1.3	- 12.3 $\pm$ 3.3	- 2.9 $\pm$ 1.3
Membranes containing electric organ proteolipids	- 32.3 $\pm$ 3.8	- 25.1 $\pm$ 4.0	- 0.7 $\pm$ 1.8

chambers, while the voltage is clamped to zero millivolt. In this membrane there is a small "anionic" current which is slightly greater for NaCl than for Na_2SO_4. Such low current values correspond to membranes having poor ionic selectivity; in fact for a 100 fold NaCl gradient there is a diffusion potential of about 10 mV (Table I). The conductance of these control membranes is of about 10^{-7} mho/cm^2 value that is in accordance with previous findings in pure lipidic membranes[8].

The results obtained in the membranes containing material from Electrophorus and cat heart are shown in Fig. 2. It may be noted that the current flowing is of opposite sign and much greater than in the control membranes. This indicates that in this case a cationic permeability predominates. It may also be observed that the extract

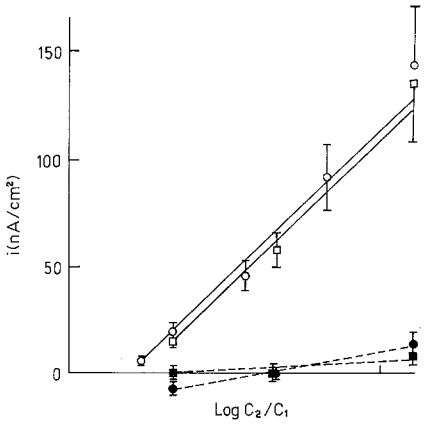

Fig. 3 .- Effects of dTC 10^{-4} M on membrane current when proteolipids from Electrophorus were employed. Similar conditions as in figures 1 and 2. Probe salt were NaCl (○); KCl (□); NaCl in the presence of dTC (●) and KCl in the presence of dTC (■)

from Electrophorus has produced a more intense current than that of heart; however it should be remembered that the amount of protein in the latter is several times smaller.

The conductance of the membranes containing the extract from Electrophorus is 5 x 10^{-6} mho/cm^2; that is 5 times greater than that of control membranes; however these membranes did not discriminate between Na^+ and K^+ (Fig. 2). The diffusion potential for a 100 fold salt gradient is about 30 mV (Table I). It is interesting to mention that storage of the extracts containing the proteolipids produce a definite decrease in the above mentioned cationic selectivity.

Other experiments were carried out as indicated above in Figs. 1 and 2 but in the presence of 10^{-4} M d-tubocurarine in the bathing solution. As shown in Fig. 4 this drug did not affect the anionic permeability of the control membranes, while it practically blocked the cationic permeability produced by the addition of the Electrophorus extract (Fig. 3). The effect of d-tubocurarine on the diffusion potential is also reported in Table I.

DISCUSSION

The extracts of the tissues we used, specially the electroplax of Electrophorus, contain cholinergic systems that operate ionic channels[9]. The experiments described here demonstrate that in the presence of an ionic gradient cationic diffusion potentials are produced, which can be blocked by curare.

From the present results and the previous findings of our laboratory in which the acetylcholine conductance was blocked by the association of S-S and SH reagents[10] it is possible to postulate that the observed cationic selectivity may be a function of the protein moiety of the proteolipid. However it is not possible to determine if these hydrophobic proteins produce true cationic pathways through the membrane or induce a reorganization of the lipids with the production of negatively charged areas.

The "cationic" selectivity is apparently not dependent upon the membrane thickness as determined by measurements of capacity. This experimental finding could be explained by an increase in the partition coefficient between the medium and the membrane.

The possible role of the lipids should also be considered, since a considerable amount of lipids is present in these extracts. It has been observed that the polar region of the lipids may play a role in controlling ion selectivity in bilayers[11]. Membranes with

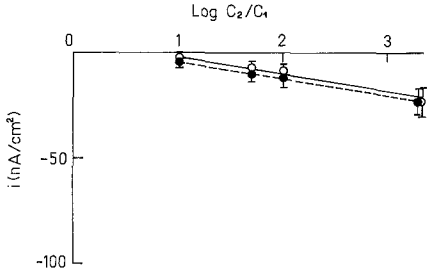

Fig. 4 .- Conditions as in figure 1. Probe salt was NaCl (O) and NaCl in the presence of dTC 10^{-4} M (●)

positive net charge are chloride selective, whereas membranes containing uncharged or amphoteric lipids show only slight cationic selectivity.

In the extract of the electric tissue the main phospholipids are phosphatidylcholine and phosphatidylethanolamine, but it contains also small amounts of phosphatidylinositol and sphingomyelin. The small amounts of negatively charged lipids does not favor the idea that they are responsible for the increase cationic permeability. Nevertheless the existence of negative lipids clustered around the hydrophobic proteins may be important in inducing a localized ionic selectivity.

The effect of d-tubocurarine on the membranes containing the proteolipids is of considerable interest, although the concentration used is much above any physiological action of this cholinergic blocking agent. This drug may act by changing the surface charge or the surface dipole of the membrane as observed with other charged molecules[12,13], probably by interacting with the proteolipid, however the exact mechanism underlying the effect of this drug is not yet established.

We are studying at present the role of the diffusion potentials in the "cholinergic conductance" that we have previously described in artificial membranes containing very small amounts of the specific cholinergic binding protein of Electrophorus[1,2].

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