Reactivity to Acetylcholine Developed by Artificial Lipid Membranes Containing a Proteolipid from *Electrophorus electricus*

We have previously reported on the incorporation into artificial lipidic membranes of a special hydrophobic protein fraction (i.e. proteolipid), separated from the electroplax of *Electrophorus electricus*. The membrane became reactive to the application of acetylcholine as evidenced by a transient change in conductance[1-3].

In this report an exhaustive description is given of the technical approach employed as well as the numerous controls used to rule out the possibility of a non-specific conductance change.

Materials and methods. Control membranes contained synthetic cholesterol (Sigma Co. 99% +) and dipalmitoyl-DL-α-lecithin (Sigma Co.), both at 10 mg/ml. In some cases only cholesterol (20 mg/ml) was used. Early experiments were carried out using a total phospholipid extract of bovine cerebral cortex. As it was difficult to avoid a certain degree of protein contamination, we decided to use only synthetic phospholipids. The membrane-forming solution (MFS) was made in 0.5 ml chloroform, 0.4 ml methanol and 0.3 ml tetradecane. Lipid proteolipid membranes were made using the same solution of lipids into which the proteolipids from *Electrophorus electricus* were added to a final concentration of 20–50 μg/ml. 2 of the 5 proteolipid peaks that are separated from the total lipid extract of the electric organ by column chromatography[4,5] were used: peak I that has no binding capacity for acetylcholine and peak III which binds cholinergic agents[4,5].

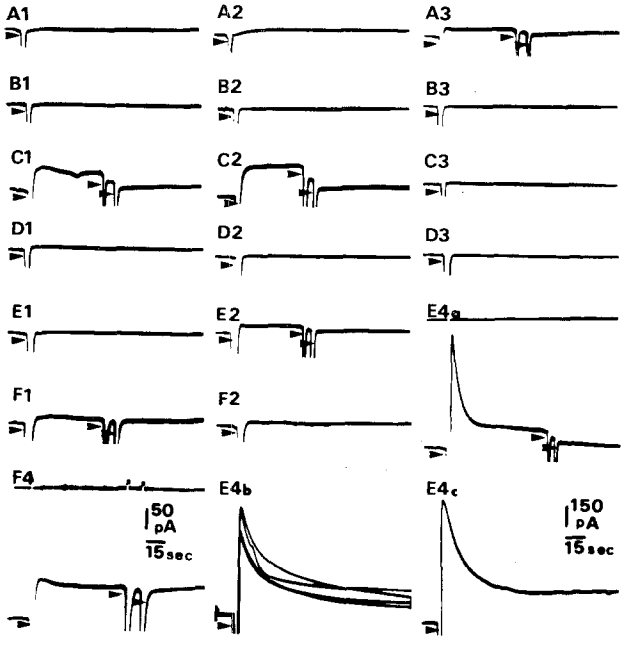
Membranes were made by painting the MFS with a small brush across a 1 mm hole in an horizontal teflon septum, separating 2 chambers containing 100 m*M* NaCl and 50 m*M* *Tris*-Cl buffer (pH 7.00)[2]. This is a modification of the most common set-up in which the septum is vertical. Membranes formed in both types of chambers showed similar conductance properties. However, some differences between horizontal and vertical membranes cannot be discarded and will be analyzed in the future. A potential difference of 100 mV was established with a DC source and measured with a Keithley DC Voltmeter 200 B. The current was determined with a Keithley 150 A Microammeter and displayed on the screen of a Tektronix D11 storage Oscilloscope. The different agents were added in 50 μl aliquots by means of capillary tubes (Kimax, Art. No. 34.500, size 1.5–2.0 × 100 mm). The tip of the tube was situated at 2 mm from the membrane surface at 45° angle, and during the injection the liquid column fell by gravity.

Results and discussion. Membranes made of lecithin and cholesterol became black and showed an electrical resistance of about 10^6 ohm cm^2. This value is similar to those reported earlier in the literature[6]. When the MFS contained only cholesterol, the resistance was considerably higher, about 10^8 ohm cm^2.

The addition of the proteolipid, either from peak I or peak III, reduced 3 to 5 times the resistance of the membranes containing lecithin and cholesterol[3]. When the proteolipids were added to the MFS containing only cholesterol, the reduction was about 10 times. The membranes containing proteolipids showed a gray colour, the black appearance developed by patches and it took 10 to 20 min to be completely black. The membranes were also studied by electron microscopy. Under appropriate conditions, images of transverse sections of fixed membranes were obtained. A typical unit membrane of about 50 Å in thickness was observed[7].

A series of experiments was performed to demonstrate that: 1. The reported variation is induced only by acetylcholine, and not by the addition of solutions having similar or even greater ionic strengths. Thus the responses to acetylcholine chloride (ACh-Cl) (5×10^{-2} *M* in the capillar tube) were compared in each case with the response to 10^{-1} *M* choline chloride (Ch-Cl) and 10^{-1} *M* NaCl. 2. The response to acetylcholine only appears when the cholinergic proteolipid (peak III) is added to the MFS. As control, applications of ACh-Cl, Ch-Cl and NaCl were done upon membranes containing either synthetic phospholipids, synthetic phospholipids plus a non-cholinergic protein (peak I) or synthetic phospholipids plus the cholinergic protein. The results obtained are summarized in the Figure in which the current flowing through the membrane is represented. The first arrow indicates the point where the test agent was added. The 2 following arrows, when present, indicate corrections in the volume of one chamber to eliminate differences in hydrostatic pressure.

Membrane made with MFS containing lecithin and cholesterol (A); only cholesterol (B); lecithin, cholesterol and peak I (C); cholesterol and peak I (D); lecithin, cholesterol and peak III (E); and cholesterol and peak III



Original records of membrane current as a function of time. For a description, see results in the text. Calibration shown in E4c is also valid for all A, C and E experiments. Calibration shown in F4 is also valid for all B, D and F experiments. In E4a and F4 the upper trace indicates membrane voltage (100 mV).

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(F) are observed. The applied salts were 10^{-1} *M* NaCl (1); 10^{-1} *M* Ch-Cl (2) and 5×10^{-2} *M* (4) or 10^{-1} *M* ACh-Cl (3). It is clear that only in the case in which ACh-Cl was applied on a membrane containing the cholinergic proteolipid (E4a) a transient change in conductance was observed. In E4b several responses to acetylcholine in the same membrane were superimposed in the screen of the storage oscilloscope. In E4c the response to ACh-Cl was elicited in the presence of Ch-Cl that replaced NaCl in the bath. In F4 the response to ACh-Cl was induced in membranes containing only cholesterol and the cholinergic proteolipid. In this case the response is a sustained one. It is important to remark that membranes containing only cholesterol as the amphipatic molecule (B, D, F) are very stable and in no case showed conductance changes, except when ACh-Cl was added and the MFS contained the peak III protein (F4).

When lecithin was present in the MFS step changes in conductance due to changes in the hydrostatic pressure associated to the drug application were sometimes observed (C1; C2). When the pressure conditions were returned to the initial state, the rise in conductance disappeared. The problem of the conductance fluctuations induced by hydrostatic pressure has been discussed elsewhere[8].

Experiments actually under course in our laboratory are directed to uncover the molecular mechanism underlying the effect of ACh-Cl[9].

Resumen. El objetivo de este trabajo fué dar una exhaustiva descripción de la técnica empleada para estudiar la respuesta a la acetilcolina desarrollada por membranas artificiales que contienen una fracción proteolipídica especial extraída de la electroplaca del *Electrophorus electricus*. Se observó que esta respuesta no se obtiene con otras fracciones de este u otro tejido ni tampoco con differentes soluciones salinas o con cloruro de colina.

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CONGRESSUS

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