INTERACTION BETWEEN SMOOTH MUSCLE CELL PLASMA MEMBRANE AND A FLAT LIPID MEMBRANE

V. K. Rybal'chenko and V. I. Karamushka

UDC 612.734.014.2:612.397.7.014.2]:576.314

KEY WORDS: plasma membrane of smooth muscle cells; flat lipid membrane; ATPase activity; channel conductance.

The study of interaction of cell membranes with artifical membranes can help to solve many problems. These include the processes of endo- and exocytosis, secretion, fertilization, cell division, fusion of lysosomes and their formation *de novo*, and so on. Adhesion and fusion of membrane systems are problems with a direct bearing on lysosome therapy, and on some developments in biotechnology and genetic engineering.

This paper describes an investigation of interaction between flat lipid membranes (FLM) and the plasma membrane (PM) fraction of smooth-muscle cells of the rabbit small intestine in connection with the development of a method of forming a functionally active PM-FLM complex.

EXPERIMENTAL METHOD

The PM fraction was isolated from smooth-muscle tissue of the rabbit small intestine by differential centrifugation in a sucrose density gradient and was characterized biochemically and by an electron-microscopic method [5]. The ATPase activity of PM was recorded by a potentiometric method based on the increase in the number of protons in a weakly buffered medium [4] and on the quantity of inorganic phosphate formed as a result of ATP hydrolysis [9]. Total bovine brain phospholipids, isolated by the method in [10], azolectin (from Sigma, USA), and egg phosphatidylcholine were used as lipids. Liposomes were obtained by sonication of aqueous dispersions of lipids by measn of an ultrasonic disperser [7]. In some cases liposomes were obtained by repeated freezing and thawing of a suspension of lipids in aqueous buffer. The morphological state of the liposomal suspensions was determined by electron-microscopic analysis by a negative staining technique, using 1% ammonium molybdate or uranyl acetate solution as the contrasting agent [11]. FLM were obtained by the usual method [12] on holes in a Teflon beaker. The resistance and electrical capacity of the membranes were measured by an apparatus based on the K 284 UD 1A integrated microcircuit, according to the principle described previously [3, 13]. Solutions of electrolyte in both compartments of the measuring cell were mixed by means of a magnetic mixer, and the pH of the solution was monitored by means of a pH-electrode of the ESL-13-11 type.

EXPERIMENTAL RESULTS

Investigation of the sensitivity of FLM formed from the total fraction of bovine brain phospholipids or azolectin in heptane to addition of the PM fraction did not succeed in demonstrating significant changes in the electrical properties of the artificial membrane. The experiments were done under sparing conditions, and for that reason, of the total number of factors promoting interaction between the membrane surfaces, only two were used. The PM suspension was kept in solutions with sucrose or glycerin, and for that reason the vesicles of the membrane in hypotonic medium were under osmotic pressure. Millimolar $CaCl_2$ concentrations were used as the second factor. Since the predominant components of the lipid mixtures used were acid and neutral lipids, it can be concluded that the surface of FLM has a resultant negative charge. Negative charges also predominate [6] on the surface of cell membranes, and this does not favor their integration with FLM. We attempted to change the surface charge of FLM, by using the cationic detergent cetyltrimethylammonium (CTA). The detergent is readily adsorbed on the membrane surface, and in concentrations of above 10^{-6} M

Department of Biophysics and Laboratory of Membrane Structure and Function, T. G. Shevchenko Kiev University. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 10, pp. 507-509, October, 1985. Original article submitted January 28, 1985.

 0007-4888/85/0010-1454$09.50

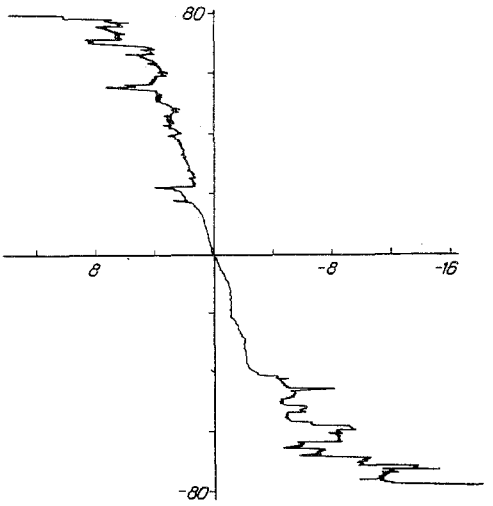

Fig. 1. Current-voltage characteristic curve of FLM, treated with proteoliposomes. Abscissa, strength of current (in pA); ordinate, voltage (in mV). Voltage scanning speed 0.2 mV/sec. Medium: 100 mM NaCl, 2 mM imidazole-HCl, pH 7.2.

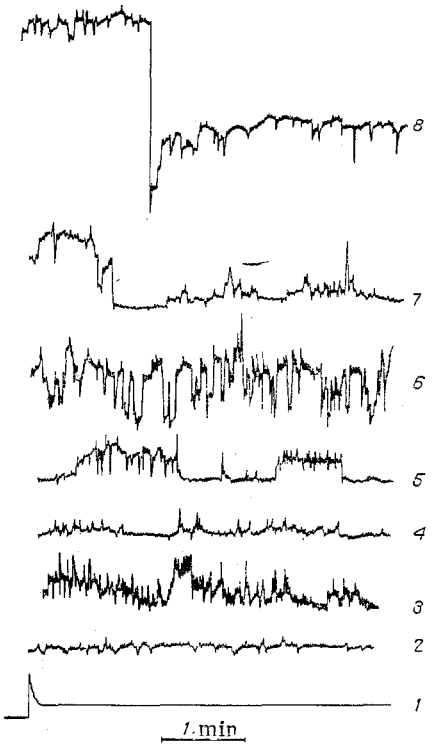

Fig. 2. Traces of fluctuation of FLM current, modified by proteoliposomes after unmodified FLM; 2) fluctuations of current after application of proteoliposomes; 3) fluctuations of current after addition of $MgCl_2$ and ATP; 4-7) fluctuations of current after addition of 0.1 and 1 mM $CaCl_2$ respectively; 8) positive and negative voltage on FLM in the presence of $CaCl_2$. 1-6, 8) 1 pA; 7) 2 pA. Voltage on membrane 50 mV. Medium: 100 mM NaCl, 2 mM imidazole-HCl, pH 7.2.

it leads to the appearance of discrete fluctuations of conductance and to reduction of the barrier properties of FLM [2].

Addition of CTA up to 1.5 μM on one side of PLM did not change the resistance of the membrane, whereas on addition of the PM fraction (final concentration 30 μg protein/ml) lowered the resistance by 50-67% and, at the same time, led to the appearance of stepwise

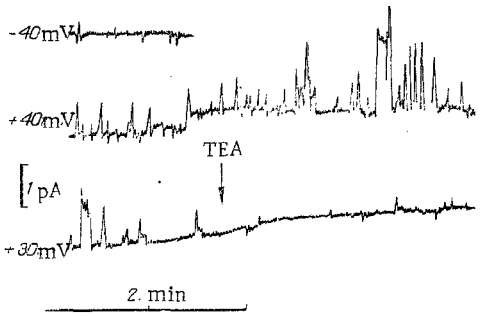

Fig. 3. Fluctuations of current of FLM modified by proteoliposomes. Medium: 100 mM Kcl, 2 mM imidazole-HCl, pH 7.2.

fluctuations of current. These changes are evidence of modification of PLM by material of PM. After addition of $CaCl_2$ (up to 1 mM) the conductance of PLM rose sharply in the presence of a positive voltage on the cis-side, evidence of the predominantly cationic selectivity of the artificial membrane.

More abrupt changes in the electrical characteristics of FLM were recorded after addition of a suspension of PM, incubated beforehand with azolectin liposomes, to the measuring cell. Azolectin liposomes themselves also lead to a fall in resistance of FLM. Usually the resistance of the bilayer was reduced by several times, but the absence of an external voltage can relax it to the original level. The current-voltage characteristic of FLM modified by azolectin liposomes is symmetrical and linear. To modify FLM by the PM fraction, we used mixtures of azolectin liposomes and PM vesicles (conventionally described as proteoliposomes), in the proportions most favorable for manifestation of ATPase activity by the latter [3]. A decrease in the over-all resistance of FLM and modification of the current curves were observed 5-15 min after the addition of proteoliposomes (up to 30 μg protein/ml) to the measuring cell. The latter curve initially showed weak fluctuations, but with time the conductance of FLM changed stepwise. The current-voltage characteristic of such an FLM was nonlinear (Fig. 1) and the membrane lost its resistance at voltages of 80 mV or more. Removal of the external voltage as a rule led to damping of the fluctuations of current and to stabilization of the membrane.

The character of changes in the conductance of FLM in the presence of proteoliposomes depended on the composition of electrolytes in the medium (Fig. 2). Weak fluctuations of current were virtually unchanged after addition of $MgCl_2$ (up to 2 mM) to the cell, but their frequency and amplitude rose sharply after addition of 3 mM ATP, and after a few minutes excitation disappeared. Small doses of $CaCl_2$ (0.1 mM) did not stimulate the appearance of the current (Fig. 2, 4), but addition of 1 mM $CaCl_2$ caused several short jumps of conductance, followed by a general decrease of resistance and the development of fluctuations of considerable amplitude (Fig. 2, 5, 6). These changes, characterized by decay (Fig. 2, 7), could be prevented by subsequent addition of $CaCl_2$.

Considering that the same additions to the medium surrounding the unmodified FLM did not lead to the appearance of fluctuations of current, and the control curve was similar in appearance to the curve of the current through the original FLM, it can be concluded that the material of the proteoliposomes penetrated into the lipid bilayer and definitely changed its properties.

In potassium medium FLM whose conductance curves are shown in Fig. 3 were formed with the aid of proteoliposomes. As Fig. 3 shows, with a positive voltage on FLM (+ on the side of addition) fluctuations of conductance became regular, whereas if the voltage was negative fluctuations were absent.

The asymmetry of FLM and the character of the current curve are evidence that permeability of the proteoliposome-modified lipid membrane is regulated by structures of channel type, capable of being in open and closed states. The life span of such a structure in the open state (determined from the duration of the conductance jump) did not exceed 25 sec. Current fluctuations due to a positive voltage only were abolished after addition of tetraethylammonium (TEA) in a concentration of 1 mM on the cis-side, when a smooth fall of resis-

tance and often rupture of the membrane were observed. TEA is a specific blocker of K-channels of excitable membranes in a cell-free system [1] and of anionic channels formed in lipid bilayers by polyenic antibiotics [8]. With this in mind, it can be postulated that in the present experiments the K-conducting structure of PM of smooth-muscle cells of the rabbit small intestine is restored in FLM.

Incorporation of PM vesicles and restoration of some properties in FLM can thus be achieved by pretreating biological membranes with azolectin.

LITERATURE CITED

1. M. K. Malysheva and V. K. Lishko, in: Molecular Biology [in Russian], No. 25, Kiev (1980), pp. 66-67.
2. A. M. Omel'chenko, "Investigation of mechanisms of induced conductance of bilayer membranes in the presence of detergents," Dissertation for the Degree of Candidate of Chemical Sciences, Dnepropetrovsk (1978).
3. V. K. Rybal'chenko, V. I, Karamushka, T. G. Gruzina, et al., Ukr. Biokhim. Zh., No. 5, 552 (1983).
4. V. K. Rybal'chenko, P. V. Pogrebnoi, T. G. Gruzina, and V. I. Karamushka, in: Abstracts of Proceedings of the 1st All-Union Biophysical Congress [in Russian], Vol. 4, Moscow (1982), Appendix, p. 4.
5. V. K. Rybal'chenko, P. V. Pogrebnoi, V. I, Karamushka, and T. G. Gruzina, Byull. Éksp. Biol. Med., No. 1, 106 (1984).
6. N. S. Sergeeva, A. F. Poglazov, and Yu. A. Vladimirov, Biofizika, No. 6, 1029 (1975).
7. A. D. Bangham, M. W. Hill, and N. G. Miller, Methods Membr. Biol., 1, 1-68 (1974).
8. L. N. Ermishkin, Kh. M. Kasumov, and V. M. Potzeluev, Nature, 262, 628 (1976).
9. C. H. Fiske and Y. Subbarow, J. Biol. Chem., 66, 375 (1925).
10. J. Folch, M. Lees, G. H. Sloane-Stanley, et al., J. Biol. Chem., 191, 884 (1951).
11. P. R. Lewis and D. P. Knight, in: Staining Methods for Sectioned Material, Amsterdam (1977), pp. 44-62.
12. P. Mueller, D. O. Rudin, H. T. Tien, and W. C. Wesscott, J. Phys. Chem., 67, 534 (1963).
13. A. E. Shamoo and D. Goldstein, Biochim. Biophys. Acta, 472, 13 (1977).