Short communication

Prostaglandin E_1 Induced Changes in Conductivity of Lipid Bilayers

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Prostaglandins are biologically active substances, representing a group of polyunsaturated fatty acids. They are found in the gastrointestinal tract of man as well as in that of the majority of animals, in approximate concentrations of 10^{-9} g per 1g of fresh tissue (Coceani et al. 1967; Bennet 1971). Obviously, studies of the mechanisms by which prostaglandins influence the digestive system structures, e.g. those of cellular membranes, would be of interest from the viewpoint of conditions of prostaglandin applications in clinical practice.

In a previous work (Ganchurin et al. 1984) it has been shown that prostaglandins of the E group have a pronounced influence upon both non-adrenergic retardation and cholinergic excitation in the smooth muscle; this is manifested by a decrease of post-synaptic retarding potentials in the circular layer of the appendix, and total block of these potential in the circular stomach muscles. Changes in the synaptic transmission in the systems studied were accompanied by cell membrane depolarization in the smooth muscle as well as by a decrease in the amplitudes of electronic potentials. It has been suggested that the above effect was not only associated with effects resulting in the relase of the mediator but also with passive electrical parameters of the membranes. To explain the effects of prostaglandins upon electrical properties in more detail, it would be of interest to study their influence on the electrical conductivity of lipid bilayer membranes and on membranes modified by a channel-forming compound.

Bilayer lipid membranes (BLM) were formed according to Mueller et al. (1962) on a circular hole ($d \sim 0.5$ mm) in a teflon cup wall. Membranes were made using egg lecithin solutions (Kharkov Plant of Chemical Preparations, USSR) either in mixture with cholesterol (Fluka) in a 4:1 weight ratio; or without cholesterol. The purity of the egg lecithin was checked chromatographically. Lipids were dissolved in n-heptane (Kodak), in a concentration of 20 mg/ml. KCl 1 mol/l was used as electrolyte (pH ~ 6.0). In the experiments, prostaglandin E_1 (PGE_1)

(Chinoin, Budapest) was used. It was added to the electrolyte from one side of the membrane in various concentrations (2×10^{-10} through 3×10^{-6} g/ml). The structural formula of PGE_1 is shown in Fig. 1. In one of the experimental series, the membrane was modified using gramicidin D (Dansyl gramicidin C) (GRD) (P-l Biochemicals) added to the electrolyte in a final concentration of 10^{-9} mol/l. GRD, in low concentrations (10^{-13} mol/l) forms ionic channels in BLM (Veatch and Stryer 1977; Ermishkin and Silberstein 1982); in higher concentrations it considerably increases the membrane ionic conductivity. Chemically pure reagents were used throughout. Experiments on 30 membranes were carried out at 25 °C.

Membrane current was measured by the currently used method (Hladky and Haydon 1972). DC voltage of $U_o = 100$ mV was conducted to membranes using calomel electrodes, one of which was connected to the input connector of a WSH 223 electrometer type amplifier (Tesla) (see Dostál 1981) thus enabling recording of the membrane current of $i < 10^{-13}$ A. A TZ 4100 recording unit was connected to the amplifier output (Labor. Instruments, Prague).

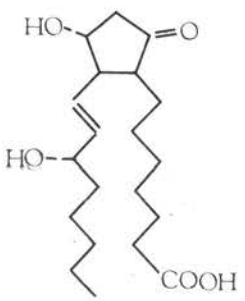

Fig. 1. Structural formula of PGE_1.

The experiments showed that the addition of relatively small amounts ($10^{-10} - 10^{-9}$ g/ml) of PGE_1 into electrolytes, caused membrane current fluctuations. Discrete conductivity was observed, with conductivity peaks reaching values of $g = 44.1 \pm 2.2$ pS; the average duration of individual peaks was $\tau = 1.7 \pm 0.2$ s (Fig. 2*b*). Further increase in prostaglandin concentration, up to 4 to 6×10^{-7} g/ml (Fig. 2*c*, *d*) resulted in a substantial increase in membrane conductivity which could be characterized by at least three conductivity levels: 307, 430, 830 pS. The PGE_1 structure (Fig. 1) does not essentially rule out the possibility of the formation of individual conductivity channels. The polar five-member ring containing one OH group enables interaction with the lecithin polar groups. In addition to this, the polar character of one of the hydrocarbon chains of the molecules (due to the presence of the OH group), enables the formation of a hydrophilic pore during the aggregation of PGE_1 in the BLM monolayer. Similar aggregation in the second membrane monolayer as well as interaction with

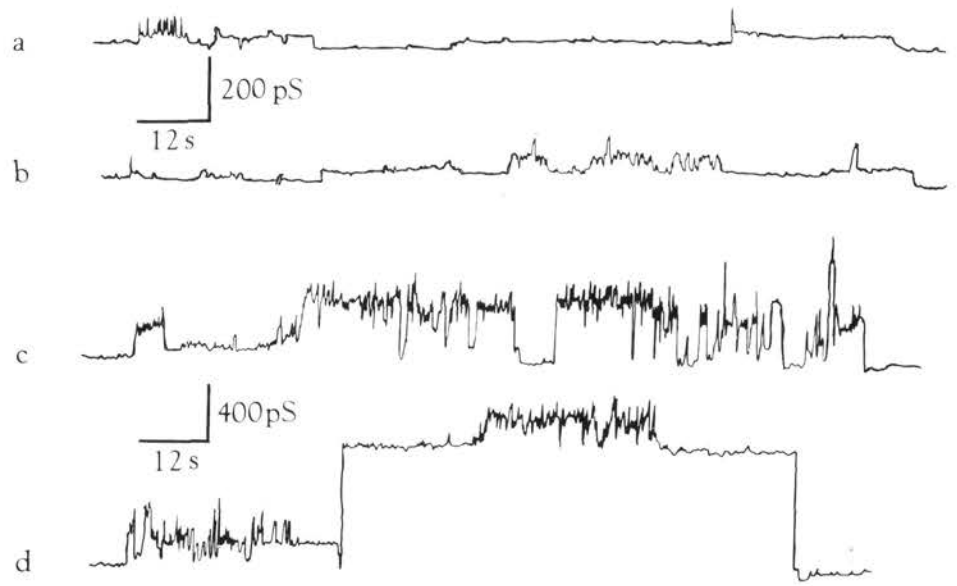

Fig. 2. BLM conductivity kinetics following PGE_1 addition: *a*) 2×10^{-10} g/ml (egg lecithin); *b*) 2×10^{-10}; *c*) 4×10^{-7}; *d*) 6×10^{-7} g/ml (egg lecithin + cholesterol).

semi-pores due to COOH groups of the second, unsaturated hydrocarbon chain in the molecule, present the necessary conditions for the formation of an ionic channel. The formation of ionic channels is independent of the membrane cholesterol contents. Similar conductivity channels as shown in Fig. 2*b*, *c*, *d* were also found in egg lecithin BLM without cholesterol (Fig. 2*a*). The stability of the membranes used, which is indicated by the dielectric dispersion voltage U_p, was similar for BLM with a relatively low cholesterol content (11 mol%) and for that without cholesterol (see Hianik et al. 1984). Different levels of PGE_1 conductivities could be induced by agregate formation with different numbers of PGE_1 molecules.

As already discussed, smooth muscle fibre membranes show changes in their ionic conductivities due to effects of E-group prostaglandins. This is manifested by a decreased stationary potential as well as a decreased resistance of the membrane. With respect to the findings shown, studies of interactions of PGE_1 with BLM with intrinsic ionic conductivity were considered important. Experiments were thus carried out to measure conductivities of BLM modified with a channel-forming compound (gramicidin D) (at a GRD concentration in the electrolyte up to 10^{-9} mol/l). The addition of 10^{-6} g/ml of PGE_1 to electrolytes cause was found to substantially increase membrane conductivities (Fig. 3*a*, *b*). Following the addition of PGE_1 (10^{-6} g/ml) to a membrane with a stable GRD conductivity level the conductivity kinetics (modified by PGE_1) showed a sigmoidal character thus

demonstrating a cooperative process of the PGE_1 interaction with the modified BLM.

With respect to the above results the increased conductivity of smooth muscle cell membranes in the presence of prostaglandins can be explained by the influence of these compounds upon ionic channels, or by the formation of individual ionic channels by prostaglandins themselves.

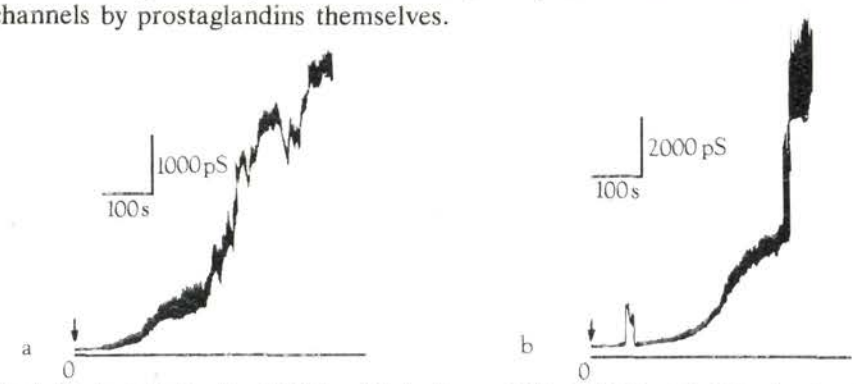

Fig. 3. Conductivity kinetics of BLM modified with gramicidin D (10^{-9} mol/l), following the addition of PGE_1 (arrows): *a*) 10^{-6}; *b*) 3×10^{-6} g/ml.

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References

Bennet A. (1971): Effect of kinins and prostaglandins on the gut. Proc. Roy. Soc. Med. **64**, 12—22

Coceani F., Pace-Asciak C., Volta F., Wolfe L. S. (1967): Effect of nerve stimulation on prostaglandin formation and release from the rat stomach. Amer. J. Physiol. **213**, 1056—1064

Dostál J. (1981): Operational Amplifiers. Elsevier Scientific Publishing Company, Amsterdam—Oxford—New York

Ermishkin L. N., Silberstein A. Y. (1982): Ionic channels formed by antibiotics. Structure and properties. In: Membrane Biophysics v.2 (Ed. P. G. Kostyuk), pp. 82—160, VINITI, Moscow (in Russian)

Ganchurin V. V., Zima V. L., Davidovskaya T. L. (1984): Effect of prostaglandins E_2 and $F_{2\alpha}$ on synaptic transmission in the stomach and large intestine smooth-muscle cells. Fiziol. Zh. **30**, 162—167 (in Russian)

Hianik T., Kvasnička P., Štroffeková K. (1984): Mechanical properties of lipid bilayers with varying cholesterol content. Stud. Biophys. **100**, 23—32

Hladky S. B., Haydon D. A. (1972): Ion transfer across lipid membranes in presence of gramicidin A. I. Studies of the unit conductance channel. Biochim. Biophys. Acta **274**, 294—312

Mueller P., Rudin D. O., Tien H. Ti., Wescott W. C. (1962): Reconstitution of cell membrane structure in vitro and its transformation into an excitable system. Nature **194**, 979—980

Veatch W., Stryer L. (1977): The dimeric nature of the gramicidin A transmembrane channel: Conductance and fluorescence energy transfer studies of hybrid channels. J. Mol. Biol. **113**, 89—102

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