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Short communication

# Prostaglandin $E_2$ and $F_{2\alpha}$ Induced Calcium Transport Across Lipid Bilayers

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Prostaglandins, polyunsaturated derivatives of arachidonic acid, are among biologicaly active compounds that have been intensely studied during the recent years. Coceani et al. (1967) revealed the significance of prostaglandins in stimulating muscle contraction. Ganchurin et al. (1984) showed that the effect of prostaglandins of the group $PGE_2$ and $PGF_{2\alpha}$ is associated with depolarization of smooth muscles cells as well as with a decrease of electrotonic potential amplitude. The authors of the present paper believe that this effect is not only associated with the action of prostaglandins on the release of mediators, but also with that on passive electrical parameters which can affect in particular the transport of calcium. Physiological investigations have stimulated a numerous works dealing with the influnce of prostaglandins on $Ca^{2+}$ transport across the microsomal fraction of the sarcoplasmic reticulum (Carsten and Miller 1977) or across an organic lipid phase and lipid bilayer or liposomes formed from different kinds of diacyl phosphatidyl cholines and cholesterol (Carsten and Miller 1978; Deleers et al. 1985). In the latter works it has been assumed that prostaglandins $PGB_2$, $PGE_2$ and $PGF_{2\alpha}$ are able to form complexes with $Ca^{2+}$ and to transport calcium as ionophores. However, the role of prostaglandins as ionophores (Nayar et al. 1984) in bilayers of different composition containing phosphatidyl acid is questionable.

In our recent work (Hianik et al. 1986) it has been shown that prostaglandin $PGE_1$ can form ionic channels in lecithin and cholesterol (molar ratio 2/1) bilayer lipid membranes (BLM), and several discrete conductivity levels could be observed for $K^+$ within an interval (44—830) pS in dependence on $PGE_1$ concentration in the electrolyte. With respect to the existing ambiguity in the opinions concerning the mechanism of prostaglandin $PGB_2$, $PGE_2$ and $PGF_{2\alpha}$ induced calcium transport across the membranes we tried to establish whether prostaglandins $PGE_2$ and $PGF_{2\alpha}$ can form calcium channels in the membranes.

Prostaglandins $PGE_2$ and $PGF_{2\alpha}$ (Fig. 1) were obtained from the Experi-

mental plant of Organic Synthesis and Biopreparations, Institute of Chemistry, Academy of Sciences of the Estonian SSR (Tallin, USSR). BLM were formed as described by Mueller et al. (1962) on a circular hole ($d \sim 0.5$ mm) in a teflon cup wall. The membranes were made of azolectin (Sigma, USA) diluted in $n$-heptane (Kodak, USA) in (40 mg/ml), and from a mixture of egg lecithin standard (Kharkov Plant of Chemical Preparations, USSR) with cholesterol (Fluka) (molar ratio 2/1) in $n$-heptane (20 mg/ml). Redistilled water was used to prepare $CaCl_2$ or KCl (chemically pure) electrolytes.

Prostaglandin-BLM interactions were studied in two ways: A — measurements of membrane conductivity; B — measurements of current-voltage characteristics of BLM.

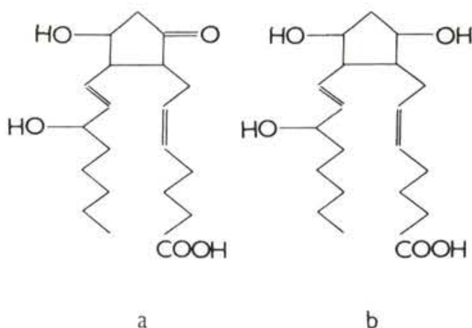


**Fig. 1.** Structural formulae of prostaglandins $E_2$ (*a*) and $F_{2\alpha}$ (*b*).

*A. Measurements of membrane conductivity* were performed using the common technique (Hladky et al. 1972). DC voltage with an amplitude $U_0 = 100$ mV was applied to the membrane by a calomel electrode. The other electrode was connected to the input of WSH 223 electrometric amplifier (Tesla) (see Dostál 1981) allowing to record membrane currents $i < 10^{-13}$ A. Prostaglandins were added directly to the lipid solution to obtain final concentrations of 1 mol $PGE_2$ or $PGF_{2\alpha}$ per 100 moles of lipids. Following membrane formation, prostaglandins diluted in ethanol were also added to the electrolyte to one side of the membrane so as to obtain a final concentration in the electrolyte of $\sim 10^{-6}$ mol/l. The concentration of ethanol in the electrolyte did not exceed 0.4 %.

*B. Current-voltage characteristics* were determined by a new method described in detail by Flerov et al. (1981) and Passechnik et al. (1985). The method of direct measurement of current-voltage characteristics of membranes (CVC), is based on the recording of the third harmonic of current from BLM with a non-linear dependence of the current on voltage U. We shall give the basic formulae only. In the first approximation, membrane CVC can be expressed as:

$$i = gU(1 + \beta U^2), \tag{1}$$

where $g$ is the conductivity and $\beta$ is the non-linearity coefficient. If an alternating voltage $U = U_0 \sin \omega t$ with a sufficiently low frequency is applied to the membrane so as to eliminate the capacity current component, a current component with a frequency $3\omega$ and an amplitude $A_3$ will flow through membrane in addition with that with a frequency $\omega$ and amplitude $A_1$. The non-linearity coefficient is determined from the relationship:

$$\beta = \frac{4}{E_0^2} \cdot \frac{A_3}{A_1(1 - rA_1/E_0)^3}, \tag{2}$$

where $r$ is the resistance of the electrodes and the electrolyte, $E_0$ is the amplitude of the alternating voltage applied to the system electrodes-electrolyte-membrane. The relationship of the total amplitude of voltage $E_0$ and the amplitude of the voltage decrement on the membrane $U_0$ is expressed by: $U_0 = 1 - rA_1/E_0$. Thus, by measuring amplitudes $A_1$, $A_3$ and the resistance $r$, we can determine the shape of the membrane CVC. The method used allows a more accurate and much faster determination of CVC compared with classical methods especially when non-linearity of CVC is weak, which is the case e.g. with BLM modified by gramicidin A (see Passechnik et al. 1985).

Prostaglandins ($1.4 \times 10^{-4}$ mol/l) were added directly to the lipid solution, which BLM were formed from, during the measurement of the CVC coefficient. A voltage $E_0 = 140$ mV with a frequency $f = 40$ Hz was applied to the membrane. The experiments were carried out under the same conditions as those for measuring membrane conductivity (20°C).

The addition of small amounts of $PGE_2$ and $PGF_{2\alpha}$ into the electrolyte (1 mol/l $CaCl_2$) caused the membrane current to fluctuate (Fig. 2). In $PGE_2$-modified azolectin membranes (Fig. 2*a*) current fluctuations with a discrete level $g = 91.3 \pm 3.5$ pS and of a duration of the conductive state $\tau = 0.43 \pm 0.3$ s were observed (number of conductive states measured: $n = 81$). In cholesterol supplemented egg-lecithin membranes an entirely different kinetics could be recorded (Fig. 2*b*). The membrane formed was characterized by a constant conductivity level $g = 440.1 \pm 9.2$ pS, giving rise to current fluctuations with two other clearly distinguishable levels with conductivities $g_1 = 213.9 \pm 4.6$ pS and $g_2 = 346.6 \pm 3.2$ pS and durations of conductivity states $\tau_1 = 17.8 \pm 2.3$ s ($n = 32$) and $\tau_2 = 0.34 \pm 0.01$ s ($n = 333$), respectively. The differences in the individual current levels were statistically significant (by the $\chi^2$-test) at $p < 0.01$. For $PGF_2$ discrete current fluctuations could be recorded only in egg-lecithin/cholesterol BLM (molar ratio 2/1) (Fig. 2*c*). Similarly as in the other BLM (Fig. 2*b*) membrane current conductivity fluctuations originated from a constant level $g = 96.4$ pS to one distinguishable level of conductivity $g = 66.5 \pm 4.6$ pS with a duration of conductive states $\tau = 0.83 \pm 4.6$ s. Let us now analyse the results of CVC measurements of prostaglandin-modified mem-

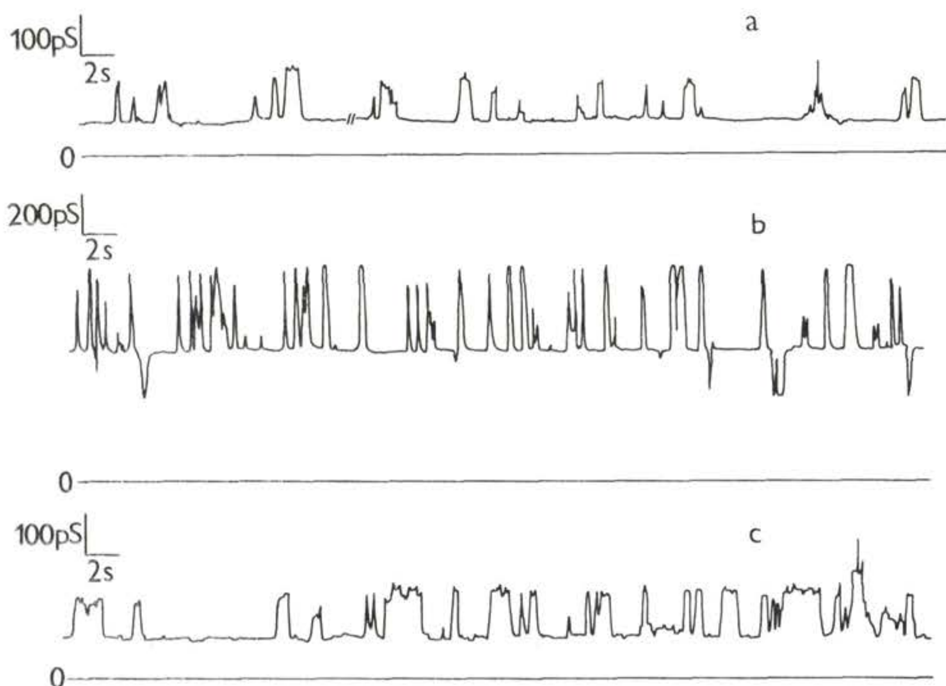


**Fig. 2.** The kinetics of conductivity of $PGE_2$ and $PGF_{2\alpha}$ modified BLM: *a* — $PGE_2$, BLM from azolectin in *n*-heptane (40 mg/ml); *b* — $PGE_2$, BLM from egg lecithin and cholesterol (molar ratio 2/1) in *n*-heptane (20 mg/ml); *c* — $PGF_{2\alpha}$, BLM from egg lecithin and cholesterol (molar ratio 2/1) in *n*-heptane (20 mg/ml). Electrolyte 1.0 mol/l $CaCl_2$, $T = 20°C$.

branes. The shape of CVC depends on the concentration of ions carried from the electrolyte (Passechnik et al. 1985); considerable differences exist between the transport of ions mediated by channels and carriers (Hianik et al. 1987). Fig. 3 (curve 1) shows the relationship of the membrane CVC coefficient for azolectin membranes in *n*-heptane (40 mg/ml) modified by prostaglandin $PGE_2$, on $CaCl_2$ concentration in the electrolyte. The dependence shown is typical of CVC of membranes modified by the channel former gramicidin A or D; it differs conspicuously from that of membranes modified by valinomycin (Passechnik et al. 1985; Hianik et al. 1987). For comparison experiments were performed to study the form of CVC of membranes made of azolectin in *n*-heptane (40 mg/ml) and modified by gramicidin D and valinomycin (P-1 Biochemical, USA) in dependence on the concentration of KCl in the electrolyte since both modifiers are carriers of monovalent cations. The corresponding relationship of the non-linearity coefficient $\beta$ of CVC on the concentration of the electrolyte for

gramicidin D (curve 2) and valinomycin (curve 3) are shown in Fig. 3. This Figure illustrates the clear-cut difference between the nature of channel and carrier mediated ion transport. The coefficient $\beta$ for gramicidin D changes with the increase in the electrolyte concentration from negative to positive similar as for $PGE_2$ modified BLM. A sharp decrease of the non-linearity coefficient $\beta$ at high $CaCl_2$ concentrations ($c > 1.5$ mol/l $CaCl_2$) for BLM modified by $PGE_2$ is probably due to an increase in the surface charge of the membrane caused by adsorption of $Ca^{2+}$ ions on the negatively charged surface of the azolectin BLM.

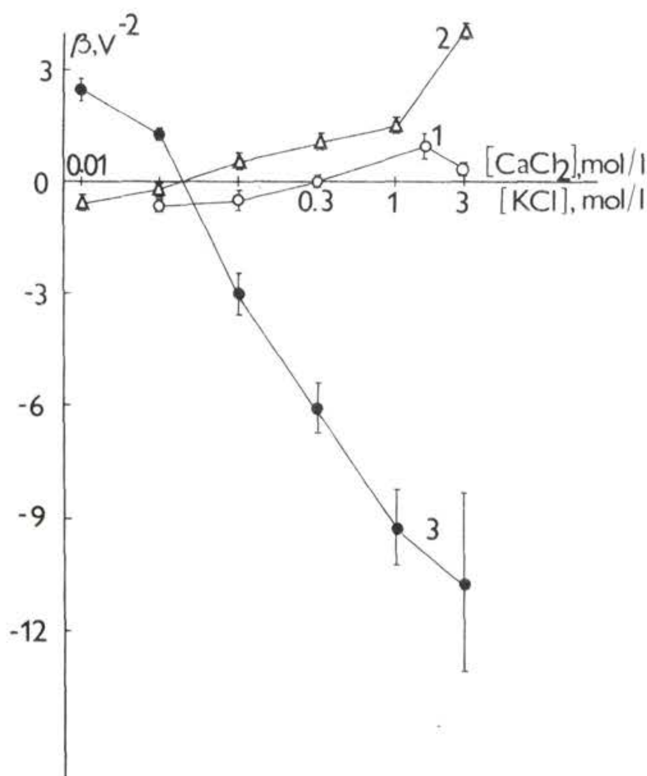


**Fig. 3.** Dependence of the CVC non-linearity coefficient $\beta$ on the electrolyte concentration for azolectin membranes in *n*-heptane (40 mg/ml) modified by: 1 — prostaglandin $E_2$ (electrolyte $CaCl_2$); 2 — gramicidin D; 3 — valinomycin (electrolyte KCl), $T = 20°C$.

No such decrease of $\beta$ is observed in case of $K^+$-transfer; this is consistent with a much stronger influence of bivalent cations on the membrane surface charge compared to that of monovalent cations. In contrast to valinomycin, two limiting transport stages exist for the ionic channels-modified transport: $\beta < 0$

(low concentrations) — the stage of the ion entering the channel; $\beta > 0$ (high ion concentrations) — the transport of ions across the internal part of the channel is the limiting stage (see Passechnik et al. 1985). The situation is different with carrier-mediated ion transport. The explanation for the dependence of coefficient $\beta$ on the electrolyte concentration as observed in our experiments is based on the idea of the existence of a potential barrier for ion transport on the membrane/electrolyte boundary, which grows for valinomycin with the increase in ion concentration (see Lev 1976).

Our observation of discrete conductance of prostaglandin-modified membranes as well as a qualitative comparison of ion transport in modified membranes (as measured by CVC) suggest that the PG-induced transport mechanism of calcium ions operate on the channel principle. Also, this idea is supported by the structure of the prostaglandins studied (Fig. 1). The polar five-member ring containing one ($PGE_2$) or two ($PGF_{2\alpha}$) OH-groups enables the interaction of the molecules with the lipid polar headgroups. In addition, the polar nature of one of the hydrocarbon chains of prostaglandins (due to the OH-group) facilitates the formation of hydrophilic pores during the aggregation of prostaglandin molecules in BLM monolayer. A similar aggregation which occurs in the other BLM monolayer, and the interaction between the semi-pores, mediated by the COOH group of the other, unsaturated chain, enables the formation of an ionic channel. The considerable increase in conductivity formed by $PGE_2$-induced ionic channels in egg lecithin and cholesterol BLM as compared to that of azolectin membranes may be due to the interaction of cholesterol with the unsaturated hydrocarbon chain of the prostaglandin molecule. The presence of cholesterol may produce an increase in the effective diameter of the ionic channel and may raise the channel stability as suggested by the considerable increase in the duration of one of the conductance state of the $PGE_2$-induced channel.

Contradictory results were obtained in our work and by Carsten and Miller 1978; Deleers et al. 1985. Therefore it should be noted that the temperature--dependent prostaglandin-induced changes in $Ca^{2+}$ transport through lipid bilayer (Deleers et al. 1985) cannot be considered as being an evidence in favour of a diffuse ionophoric mechanism of ionic transport. For example, the potential barrier to ionic transport at the membrane/electrolyte boundary may become changed due to phase transitions in the lipid matrix (Lev 1976). It nevertheless cannot be ruled out that the mechanism of the prostaglandins-mediated calcium transport through the lipid bilayer can also be dependent on a number of other factors, such as the lipid composition of the membrane, the mediator concentration or the phase stat of the membrane. Thus, we cannot speak definitely about

the ionophoretic or the channel mechanism of $Ca^{2+}$ transport by prostaglandins, without allowing for the above facts.

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## References

Carsten M. E., Miller J. D. (1977): Effect of prostaglandins and oxytocin on calcium release from uterine microsomal fraction. J. Biol. Chem. **252,** 1576—1581

Carsten M. E., Miller J. D. (1978): Comparison of calcium association constants and ionophoretic properties of some prostaglandins and ionophores. Arch. Biochem. Biophys. **185,** 282—283

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

Deleers M., Grognet P., Brasseur R. (1985): Structural consideration for calcium ionophoresis by prostaglandins. Biochem. Pharmacol. **34,** 3831—3836

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

Flerov M. N., Passechnik V. I., Hianik T. (1981): Study of current-voltage characteristics of ionic channels by the transmembrane current harmonics. Biofizika **26,** 277—283 (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)

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

Hianik T., Bajči A., Davidovskaya T. L., Laputková G. (1986): Changes in the lipid bilayer conductivity due to influence of prostaglandin $E_1$. Gen. Physiol. Biophys. **5,** 445—448

Hianik T., Bajči A., Laputková G., Paveleková J. (1987): Gramicidin D and valinomycin in bilayers with different cholesterol content. Biofizika **32,** 458—461 (in Russian)

Lev A. A. (1976): Modelling Ionic Selectivity of Cell Membranes. Nauka, Leningrad (in Russian)

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

Nayar R., Mayer L. D., Hope M. J., Cullis P. R. (1984): Phosphatidic acid as a calcium ionophore in large unilamellar vesicle systems. Biochem. Biophys. Acta **777,** 343—346

Passechnik V. I., Flerov M. N., Hianik T. (1985): The study of volt-ampere characteristics of ionic channels formed by gramicidin A. Gen. Physiol. Biophys. **4,** 35—54

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