CELL MEMBRANE MECHANISMS OF ACTION OF PROSTAGLANDINS E_1 AND $F_{2\alpha}$ ON PLATELET FUNCTION

É. S. Gabrielyan, S. É. Akopov, and S. A. Badzhinyan

UDC 612.111.7.014.46:577.175.859

KEY WORDS: platelets; membranes; Na,K-ATPase; prostaglandins; fluorescence.

Prostaglandins (PG) are universal regulators of platelet function [9]. The antagonistic effect of PG E and F, which constitute the two arms of the mechanism controlling platelet aggregation [9], on platelet function is particularly important. It has been shown that a change in the ratio between concentrations of PG E_1 and $F_{2\alpha}$ is an important factor causing increased aggregation of platelets in circulatory pathology [10]. However, the mechanisms of the action of PG on platelet function are not yet clear. It is suggested that PG exert their action on platelets by changing the levels of cyclic AMP and cyclic GMP inside the platelet, but this hypothesis is not without contradictions [15]. This paper gives data on the effect of PG on the platelet membrane, evidence that they do indeed act on platelet function.

EXPERIMENTAL METHOD

Platelets were isolated from fresh donor's blood, some were used to determine ecto-ATPase activity [13] and some for isolation of platelet membranes [16]. To study Na,K-ATPase activity, during the last hour of centrifugation of the platelet membranes 0.2% Na deoxycholate was present. Na,K-ATPase activity was determined in medium containing 100 mM NaCl, 20 mM KCl, 5 mM $MgCl_2$, 3 mM ATP, and 30 mM Tris-HCl buffer (pH 7.4). Erythrocytic membranes were isolated by the method of Dodge et al [14]. ATPase activity was expressed in nanomoles P_{in}/mg protein/30 min.

Fluorescence spectra were recorded on the MPF-2a spectrofluorometer and absorption spectra on the "Specord UV-VIS" spectrophotometer. In cases when the optical density exceeded 0.1, the effect of the internal filter was allowed for. The change in the fluorescence maximum was assessed by the two-wave method [11].

Platelet aggregation was studied on an aggregatometer based on the "Specol-10" spectrocolorimeter. ADP was used as aggregating agent. Liposomes were obtained by the method in [12]. The results were subjected to statistical analysis using the Wilcoxon–Mann–Whitney nonparametric criterion [4].

EXPERIMENTAL RESULTS AND DISCUSSION

In the first stage of the investigation the effect of PG on activity of ecto- and Na,K-ATPases of platelet membranes was studied. The choice of these enzymes was determined by their important role in the mechanism of platelet aggregation. Ecto-ATPase, which is the ATPase of thrombosthenin [8], reflects its functional state. Contraction and relaxation of thrombosthenin which, according to data in the literature [8], lie at the basis of platelet aggregation, are linked with a change in ecto-ATPase activity. However, only a weak activating effect of PG E_1 on ecto-ATPase was observed, and PG $F_{2\alpha}$ had no effect on this enzyme (Table 1). Conversely, PG E_1 and $F_{2\alpha}$ had a marked antagonistic action on Na,K-ATPase; PG $F_{2\alpha}$ inhibited whereas PG E_1 stimulated its activity (Table 1). Na,K-ATPase is known to regulate Ca^{++} transport through the cell membrane and its liberation from the intracellular depots [1]. Consequently, by changing the activity of transport ATPase, PG can affect the intracellular Ca^{++} distribution inside the platelets, which plays an important role in the regulation of their function [17].

The hypothesis that the influence of PG on Na,K-ATPase is an important factor in the action of these substances on platelet aggregation is confirmed by the fact that ouabain, a specific inhibitor of Na,K-ATPase, like PG $F_{2\alpha}$, stimulates platelet aggregation under the influence of ADP (Fig. 1). The answer to the question whether PG affect platelet aggregation through the same receptor as ouabain or not is interesting. It will be

Erevan Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Zakusov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 5, pp. 515-517, May, 1981. Original article submitted November 11, 1980.

0007-4888/81/9105-0559$07.50

TABLE 1. Effect of PG $F_{2\alpha}$ and PG E_1 on Membrane ATPase Activity of Platelet and Erythrocyte Membranes

Type of PG	Concentration, M	Platelet membranes		Erythrocytic membranes	
		Ecto-ATPase	Na,K-ATPase	Mg^{2+} ATPase	Na,K-ATPase
$F_{2\alpha}$	10^{-6}	152,4	52,1	100,8	110,7
	P	>0,05	<0,05	>0,05	<0,05
	10^{-3}	167,8	42,1	108,7	91,4
	P	>0,05	<0,01	>0,05	<0,05
E_1	10^{-6}	172,4	77,9	110,5	132,5
	P	>0,05	>0,05	>0,05	<0,05
	10^{-3}	178,8	98,6	108,4	118,4
	P	<0,05	<0,05	>0,05	<0,05
Control		160,5	72,4	101,4	148,4

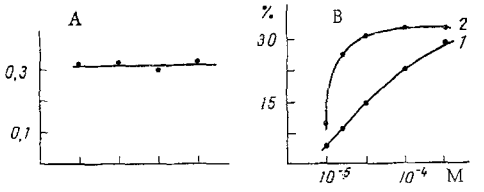

Fig. 1

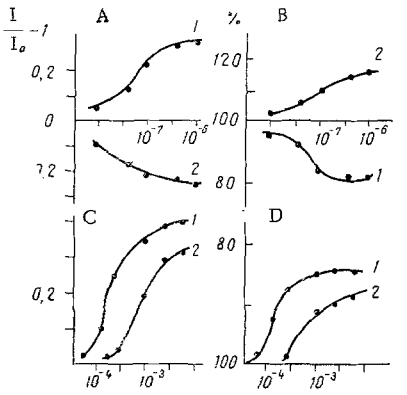

Fig. 2

Fig. 1. Partial inhibition of platelet aggregation by PG E_1 (10^{-5} M) in the presence of different concentrations of ouabain (A) and effect of ouabain on platelet aggregation in the absence of PG $F_{2\alpha}$ (1) and in the presence of PG $F_{2\alpha}$ (2) in a concentration of 10^{-8} M (B). A) Ordinate, value of $\Delta = 1 - (V_1/V_0)$, rate of aggregation; B) abscissa, ouabain concentration; ordinate, change in degree of aggregation (in % of control).

Fig. 2. Effect of PG $F_{2\alpha}$ (1) and PG E_1 (2) on intensity of fluorescence (A, C) and maximum of fluorescence (B, D) of tryptophanyl groups of proteins of platelet (A, B) and erythrocytic (C, D) membranes. Intensity of fluorescence estimated in relative units, maximum of fluorescence in % of change in value $\Delta = I_{365}/I_{320}$. Abscissa, concentrations of PG $F_{2\alpha}$ and PG E_1 (in picograms); ordinate, $\Delta = I_{365}/I_{320}$.

clear from Fig. 1B that the addition of PG $F_{2\alpha}$ to a platelet suspension leads to a fall in $[A_{0.5}]$ of ouabain, evidence of the synergism of their action. Such a change in $[A_{0.5}]$ is interpreted in the literature as evidence of the existence of separate receptors for these two effects [6]. Other evidence in support is given by the graph of partial inhibition of platelet aggregation by PG E_1 in the presence of different concentrations of ouabain (Fig. 1A). Since the graph is a straight line, the presence of separate points of application for PG E_1 and ouabain on the platelet membrane can be postulated.

It is possible that PG influence transport ATPase of the platelets without binding with its active center, and that they exert their action through a generalized membrane transformation, which the PG can induce by binding with the specific receptor. There are all the more grounds for this suggestion because such a mechanism is characteristic of many biologically active substances [5]. In order to study the membrane component of the action of PG on platelets, their effect on protein fluorescence of platelet membranes was investigated.

It will be clear from Fig. 2 that PG E_1 and F_2 induce opposite changes in tryptophan fluorescence in the platelet membrane. PG $F_{2\alpha}$ caused an increase in the intensity of fluorescence of tryptophanyl groups and a short-wave shift of the maximum of their fluorescence, evidence of an increase in rigidity and hydrophobicity

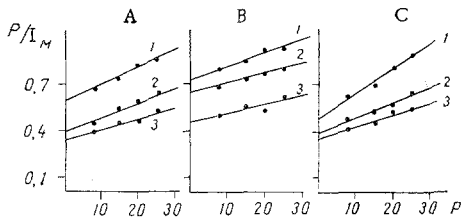

Fig. 3. Effect of PG $F_{2\alpha}$ and E_1 (10^{-3} M) on the diffusion constant of pyrene in platelet (A), erythrocytic (B), and liposomal (C) membranes. 1) Control, 2) PG $F_{2\alpha}$, 3) PG E_1.

TABLE 2. Conductivity of Bilayer Lecithin Membranes (in $\Omega^{-1} \cdot cm^2$), Modified by PG E_1 and $F_{2\alpha}$ in a Concentration of 10^{-4} M (M ± m)

Lipid bilayer membrane	K^+	Na^+	Ca^{2+}
Without modifier	$(3,1\pm0,4)\cdot10^7$	$(3,5\pm0,3)\cdot10^7$	$(4,1\pm0,5)\cdot10^7$
With PG E_1	$(1,3\pm0,4)\cdot10^5$	$(3,1\pm0,4)\cdot10^6$	$(3,5\pm0,5)\cdot10^5$
With PG $F_{2\alpha}$	$(7,2\pm0,5)\cdot10^6$	$(3,6\pm0,6)\cdot10^6$	$(3,1\pm0,5)\cdot10^6$

of the environment of the tryptophanyl groups in the membrane [2]. It can be tentatively suggested that this is linked with withdrawal of the tryptophanyl groups into the deeper layers of the membrane. Conversely PG E_1 caused a long-wave shift of the maximum of fluorescence and quenching of fluorescence of the tryptophan residues of the membrane proteins, reflecting transfer of the tryptophanyl groups into a more hydrophobic environment, probably closer to the surface of the membrane. Tryptophan residues are known to play an important role in the active centers of transport ATPase [7]. Conformational changes recorded in the protein phase of platelet membranes thus probably lie at the basis of their action on Na, K-ATPase. The hypothesis that the type of membrane transformation is closely connected with the character of the effect of PG on transport ATPase is confirmed by comparison of the effect of PG on Na,K-ATPase activity and on the protein fluorescence of erythrocytic membranes. Figure 2 shows that PG E_1 and $F_{2\alpha}$, by contrast with their action on platelet membranes, caused conformational shifts of tryptophan fluorescence of erythrocytic membranes in the same direction; these transformations, moreover, were similar to the effect of PG $F_{2\alpha}$ on the platelet membrane, and both PG E_1 and PG $F_{2\alpha}$ simultaneously inhibit Na,K-ATPase activity without affecting the Mg-ATPase of erythrocytic membranes (Table 1).

By changing the protein conformation of platelet and erythrocytic membranes, PG also affect their lipid phase. It will be clear from Fig. 3 that both PG E_1 and PG $F_{2\alpha}$ cause an increase in the viscosity of the lipid bilayer of erythrocytic, platelet, and liposomal membranes, which is manifested concretely by a change in the diffusion constant of pyrene in the membrane. In Fig. 3 the diffusion constant of pyrene is proportional to the tangent of the angle of slope of the curve [3]. The fact that in this case the character of the effect of PG E_1 and PG $F_{2\alpha}$ was the same on different membranes, and also that it exerts its action in much higher concentrations than on protein fluorescence, suggests that PG realize their conformational effect on membrane lipids not through a specific receptor, but unselectively, by infiltration into the lipid bilayer with its hydrophobic part. Nevertheless, modification of the lipid bilayer may be one of the mechanisms of action of PG on platelet function, for PG cause an increase in permeability of the lipid membrane of one or two orders of magnitude for K^+, Na^+, and Ca^{++} ions (Table 2).

This investigation thus demonstrates the complex and many-sided effect of PG on the membrane; this effect probably plays an important role in the mechanism of their physiological action on platelet function.

LITERATURE CITED

1. A. A. Boldyrev and V. A. Tverdislov, in: Progress in Science and Technology. Series: Biophysics [in Russian], Vol. 10, Moscow (1978).
2. É. A. Burshtein, in: Progress in Science and Technology. Series: Biophysics [in Russian], Vol. 7, Moscow (1977).

3. Yu. A. Vladimirov and G. E. Dobretsov, Fluorescent Probes in the Investigation of Biological Membranes [in Russian], Moscow (1980).
4. E. V. Gubler, Computer Methods of Analysis and Diagnosis of Pathological Processes [in Russian], Leningrad (1978).
5. S. V. Konev and I. D. Volotovskii, in: Biomembranes [in Russian], Riga (1977), pp. 42-77.
6. B. I. Kurganov, Allosteric Enzymes [in Russian], Moscow (1978).
7. N. M. Mirsalikhanova, E. E. Gussakovskii, S. Burkhanov, et al., Biofizika, 20, 980 (1975).
8. T. A. Odesskaya, Probl. Gematol., No. 2, 37 (1976).
9. I. S. Azhgikhina (editor), Prostaglandins [in Russian], Moscow (1978).
10. Z. A. Suslina, Zh. Nevropatol. Psikhiat., No. 11, 1635 (1978).
11. K. K. Turoverov and B. V. Shchelchkov, Boifizika, 15, 965 (1970).
12. A. D. Bangham, M. M. Standish, and J. C. Watkins, J. Mol. Biol., 13, 238 (1965).
13. J. George, R. Morgan, and P. Lawis, J. Lab. Clin. Med., 92, 430 (1978).
14. G. Dodge, C. Mitchell, and D. Hanahan, Arch. Biochem., 11, 100 (1963).
15. B. Samuelsson, M. Goldyre, E. Granström, et al., Annu. Rev. Biochem., 47, 997 (1978).
16. T. J. Wang, C. Hussey, E. Sasse, et al., Am. J. Clin. Pathol., 67, 528 (1977).
17. M. B. Feinstein, Bibl. Haemat., 45, 1 (1978).

MECHANISM OF THE RELAXING ACTION OF NORADRENALIN ON SMOOTH MUSCLE CELLS OF THE CORONARY ARTERIES

E. I. Nikitina, N. G. Kochemasova, V. M. Taranenko, and M. F. Shuba

UDC 612.181.612.172.1].014.46:615.357.452

KEY WORDS: smooth muscles; coronary arteries; basal tone; noradrenalin; calcium channels.

The basal tone of the coronary vessels and of the vessels of skeletal muscles, the mesentery, etc. is determined mainly by the inflow of external calcium ions into the smooth-muscle cells (SMC). Previously the writers showed that the inflow of calcium takes place through potential-dependent slow calcium channels [2, 11]. The aim of the present investigation was to continue the study of the membrane mechanisms of regulation of basal tone and, in particular, to examine the role of mediators (noradrenalin – NA) in these processes.

EXPERIMENTAL METHODS

Experiments were carried out on circular strips of the anterior descending branch of the bovine left coronary artery with an external diameter of 1.0-1.5 mm by the double sucrose gap method, with simultaneous recording of electrical and contractile activity of the SMC. Electrical activity was derived and the SMC stimulated by Ag–AgCl electrodes. Contact between muscle and electrodes was effected through agar bridges. The original Krebs' solution had the following composition (in mM): NaCl 120.4; KCl 5.9; $NaHCO_3$ 15.5; $MgCl_2$ 1.2; NaH_2PO_4 1.2; $CaCl_2$ 2.5; glucose 11.5 mM, made up in bidistilled water. Potassium-enriched solution (80 mM KCl) was prepared by adding the dry KCl salt to Krebs' solution; calcium-free solution was prepared by removing calcium ions from Krebs' solution by the addition of 0.5 mM EGTA. To stabilize the membrane, the $MgCl_2$ concentration in the calcium-free Krebs' solution was increased to 12 mM. NA was added in a concentration of 10^{-5} M and the testing solutions were aerated with a gas mixture containing 95% O_2 and 5% CO_2. The temperature of all solutions was maintained at 35-36°C. The preparations in the chamber were subjected to a load of 7×10^{-3} to 10×10^{-3} N. The experiments began 60-90 min after loading the preparations in the chamber.

Department of Neuromuscular Physiology, A. A. Bogomolets Institute of Physiology, Academy of Sciences of the Ukrainian SSR, Kiev. (Presented by Academician of the Academy of Medical Sciences of the USSR A. M. Chernukh.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 5, pp. 517-520, May, 1981. Original article submitted July 17, 1980.

