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STIMULATION OF HIGH-AFFINITY HORMONE-SENSITIVE GTPase OF HUMAN PLATELETS BY 1-O-ALKYL-2-O-ACETYL-sn-GLYCERYL-3-PHOSPHOCHOLINE (PLATELET ACTIVATING FACTOR)

P.V. Avdonin, I.V. Svitina-Ulitina, V.I. Kulikov

USSR Cardiology Research Center, Academy of Medical Sciences, 3rd Cherepkovskaya Str. 15A, Moscow 121552, USSR

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1-O-Alkyl-2-O-acetyl-sn-glyceryl-3-phosphocholine (platelet activating factor) inhibits human platelet adenylate cyclase via the GTP-dependent mechanism. Inhibition of adenylate cyclase correlates with the stimulation of high affinity hormone-sensitive GTPase. The half-maximal effects of PAF on both enzymes are observed at concentrations about 10^{-8} M. Phentolamine, an α-adrenergic antagonist, does not abolish the PAF-induced inhibition of adenylate cyclase. The obtained data suggest that PAF receptors are coupled with the GTP-binding inhibitory protein.

The platelet activating factor (PAF) (1-O-alkyl-2-O-acetyl-sn-glyceryl-3-phosphocholine) is a highly potent lipid mediator that stimulates platelets and some other cells (1). Exposure of platelets to PAF results in their shape change, release of vasoactive amines, aggregation factor 4 and ß-thromboglobulin, aggregation of platelets (2-5). Similarly to other aggregation inducers, PAF stimulates inositol phospholipid metabolism in platelets (5-8), increases permeability of platelet membrane for Ca^{2+} (9, 10), and inhibits adenylate cyclase activity (11).

In the present work, we have studied the mechanism of platelet adenylate cyclase regulation by PAF. It is well known that the receptors for hormones and other extracellular agents that inhibit the activity of adenylate cyclase are coupled with the GTP-binding inhibitory protein (N_i) which transmits the ex-

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ternal signal across the plasma membrane to the catalytic component of adenylate cyclase (12, 13). This process requires GTP binding in N_i regulatory site. The signal transmission is accompanied by the stimulation of GTP hydrolysis by this protein (14-18). The aim of the present work was to elucidate whether PAF inhibits platelet adenylate cyclase via this mechanism. For this purpose, we have studied its influence on the high-affinity hormone-sensitive GTPase of human platelet membranes.

MATERIALS AND METHODS: Isolation of human platelet membranes was performed as in (19).

Adenylate cyclase activity was measured in a medium (50 µl) containing 50 mM triethanolamine-NaOH, 30 mM Tris-HCl (pH 7.4), 0.3 mM EGTA, 2 mM $MgCl_2$, 0.1 mM cAMP, 1 mM 3-isobutyl-1-methylxanthine, $3 \cdot 10^{-5}$ M d,l-propranolol, $5 \cdot 10^{-5}$ M ATP, 0.25-0.5 µCi [α-^{32}P] ATP, 0.5 mg/ml of creatine kinase, 5 mM creatine phosphate, 0.2% bovine serum albumin and 1 mM dithiothreitol (30°C, 10 min). Platelet membrane protein content was 5-10 µg per sample. The amount of [^{32}P] cAMP formed was determined by the method of (20).

Activity of high affinity GTPase was measured under the same conditions, except that 0.3 µM [γ-^{32}P] GTP (specific radioactivity 10-20 Ci/mmol) was used as a substrate, and the concentrations of unlabeled ATP, creatine phosphate and creatine kinase were 0.1 mM, 5 mM and 1.2 mg/ml, respectively. Non-specific hydrolysis of [γ-^{32}P] GTP was measured in the presence of 30 µM unlabeled GTP. The activity of high affinity GTPase was calculated from the difference between the total and non-specific hydrolyses. The [^{32}P] H_3PO_4 formed was determined by a modified procedure of Cassel and Selinger (21). For this purpose, 1 ml of chilled 10% suspension of charcoal Norit A in 50 mM KH_2PO_4 (pH 4.5) was added to the incubation sample and thoroughly mixed; the suspension was kept at 0°C for 20-30 min. The charcoal was then precipitated by centrifugation at 1,000 g for 30 min; aliquots of samples (0.5 ml) were collected from the supernatant. Radioactive orthophosphate was determined by the Cerenkov method. Each experimental point represents a mean of triplicate determinations. Protein concentration was determined according to (22).

PAF was synthesized by acetylation of lyso-alkyl-phosphatidylcholine with acetic anhydride as described in (2); [α-^{32}P] ATP and [γ-^{32}P] GTP were obtained from Amersham International Ltd; tris, glucose, cAMP, sucrose, charcoal, bovine serum albumin (fraction V), d,l-propranolol, epinephrine, creatine kinase, citrate and EGTA were purchased from Sigma Chemical Co.; EDTA and dithiothreitol were obtained from Serva; triethanolamine and ATP were from Boehringer Mannheim Biochemical Co.; 3-isobutyl-1-methyl-xanthine and GTP were purchased from Calbiochem; creatine phosphate was a product of Reanal.

RESULTS: Fig. 1 shows the effects of PAF on the activity of adenylate cyclase in human platelet membranes. In the pre-

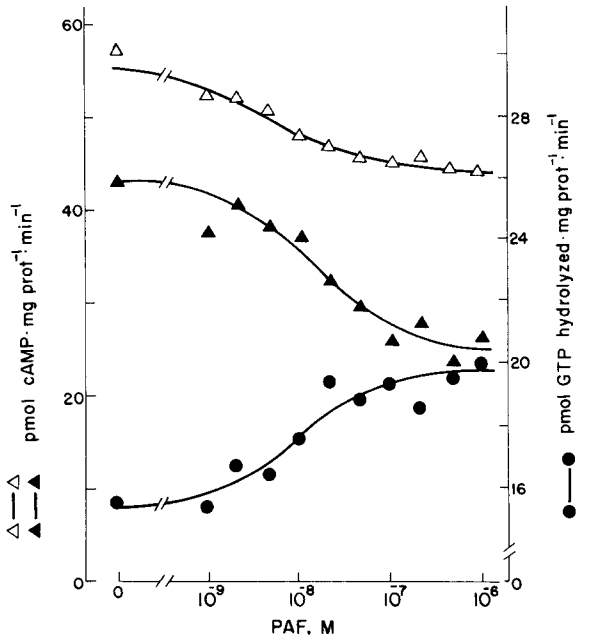

Fig. 1. Dependence of adenylate cyclase and GTPase activity on PAF concentration. Adenylate cyclase activity was measured in the absence (△ — △) and presence (▲ — ▲) of GTP (10^{-5} M). Similar results were obtained in an additional experiment.

sence of GTP, the inhibition of cAMP synthesis by PAF is increased more than 2-fold. GTP, too, inhibits adenylate cyclase. An increase in PAF inhibitory action on adenylate cyclase caused by GTP suggests that N_i participates in this process. It was shown that the receptor-mediated inhibition of adenylate cyclase is accompanied by the GTP hydrolysis by this protein (14-18). In platelets, such mechanism was demonstrated for epinephrine (14, 16), thrombin (17) and PGH_2 analogs, U46619 and U44069 (23). Thus, to elucidate whether PAF exerts its action through the same mechanism, we have studied its influence on the activity of high-affinity hormone-sensitive GTPase of human platelet membranes.

The properties of high-affinity GTPase of platelet plasma membranes used in our experiments are shown in Fig. 2 and Table 1. Fig. 2 shows a double-reciprocal plot of GTP hydrolysis by

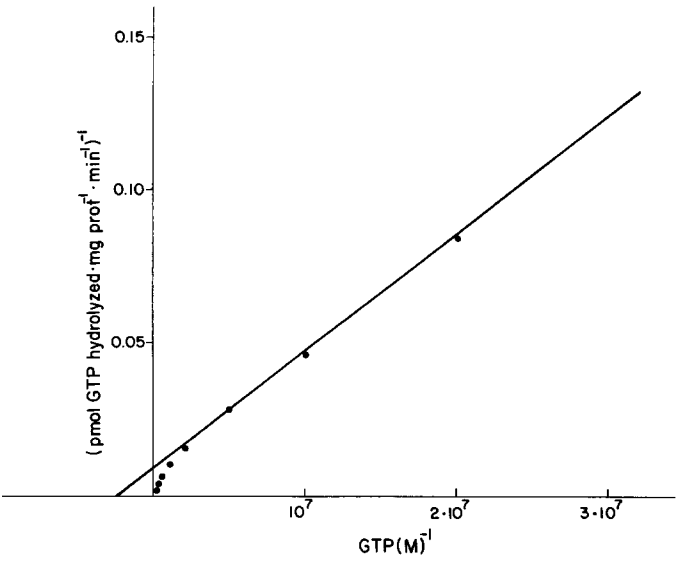

Fig. 2. Lineweaver-Burk plot of GTP hydrolysis by human platelet membranes. The GTP concentration was increased by adding larger amounts of [γ-^{32}P]GTP to the incubation medium. Specific radioactivity of the substrate was constant.

platelet membranes tested over $3 \cdot 10^{-8}$-$3 \cdot 10^{-5}$ M GTP. The presence of two enzymes with different affinity is indicated by the obvious break in this plot. The apparent K_m value of high-affinity GTPase is approximately $0.3\text{-}0.5 \cdot 10^{-7}$ M. The data from Table 1 demonstrate that the high-affinity GTPase is hormone-sensitive since it is stimulated by epinephrine.

As can be seen from Fig. 1, PAF, just as epinephrine, increases the activity of high-affinity GTPase in platelet mem-

Table 1. Regulation of adenylate cyclase and high-affinity GTPase in platelet membranes

	Adenylate cyclase, pmol cAMP/min/mg protein	GTPase, pmol $^{32}P_i$/min/mg protein
No additions (control)	43.5±2.3	20.4±0.6
Epinephrine, 10^{-4} M	22.7±1.7*	28.5±1.1*

The values listed are means±SEM. *$p < 0.01$ compared with the control. Adenylate cyclase activity was measured in the presence of 10^{-5} M GTP. Similar results were obtained in the two additional experiments.

branes. The non-specific hydrolysis of GTP measured at nucleotide concentration of 30 μM does not depend on PAF (data not shown). The affinity of the GTPase for PAF coincides with that of adenylate cyclase, i.e. the half-maximal effects of PAF on both enzymes are observed at the effector concentration of 10^{-8} M (Fig. 1). At 10^{-7} M, the plot of the dose-dependent effects of PAF shows a plateau. Identical affinity of adenylate cyclase and high-affinity GTPase with respect to PAF indicates that its action on these enzymes is mediated by the same binding sites.

The effects of PAF on platelet adenylate cyclase system are similar to those exerted by epinephrine. Both agonists inhibit adenylate cyclase and stimulate high-affinity GTPase (Fig. 1 and Table 1). The inhibitory effect of epinephrine on platelet adenylate cyclase is mediated by α_2-adrenoreceptors (14). It was shown in binding experiments that α-adrenoreceptor antagonists competitively block [^{3}H]PAF interaction with platelets (24, 25). These data gave rise to the hypothesis that the effects of PAF on human platelets can be mediated by the α_2-adrenoreceptors (24). The data listed in Table 2 show that phentolamine, an α-adrenergic antagonist, does not suppress the PAF-induced inhibition of adenylate cyclase and completely blocks epinephrine

Table 2. The absence of phentolamine effect on adenylate cyclase inhibition by PAF

	Adenylate cyclase activity, pmol cAMP/min/mg protein	
	without phentolamine	with 10^{-5} M phentolamine
No additions (control)	39.0±1.8 (100%)	35.4±0.8 (100%)
Epinephrine, 10^{-5}M	26.0±2.4 (67%)*	36.0±3.6 (102%)
PAF, 10^{-7} M	31.8±1.3 (81%)*	29.0±1.4 (82%)*

The values listed are means ± SEM. *$p < 0.05$ compared with the control. The incubation medium contained 10^{-5} M GTP. Similar results were obtained in an additional experiment.

action. Thus, it can be concluded that the effects of PAF and epinephrine on platelets are mediated by separate receptors. This conclusion is proved by different physiological platelet responses induced by PAF and epinephrine (8).

DISCUSSION: It has been established in the present study, that PAF stimulates the activity of high-affinity hormone-sensitive GTPase of human platelet membranes (Fig. 1). The kinetic parameters of the PAF-stimulated GTPase (K_m and V_{max}) are close to those published for epinephrine - and thrombin-stimulated GTP hydrolysis by N_i (14, 16, 17). Half-maximal and maximal stimulation of GTP hydrolysis by PAF occurs at identical concentrations of phospholipid causing half-maximal and maximal inhibition of adenylate cyclase. Thus, proceeding from these data, we assume that PAF action on platelet adenylate cyclase system is realized via the inhibitory GTP-binding protein N_i.

Besides adenylate cyclase inhibition and GTPase stimulation, PAF regulates several other processes occuring in platelet plasma membranes. This phospholipid induces calcium inward current (9, 10), stimulates polyphosphoinositide hydrolysis by phospholipase C (5-8), and induces arachidonic acid formation (8). It was hypothesized that the GTP-binding protein participates in calcium gating and polyphosphoinositide metabolism (26-29). Further studies demonstrated that GTP analogs stimulate polyphosphoinositide hydrolysis (30). Future investigations are required to answer a question whether there is a relationship between the high-affinity GTPase stimulation and these PAF-regulated processes in platelet membranes.

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