Vol. 141, No. 1, 1986 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 26, 1986 Pages 353-359

GTP REGULATION OF PLATELET-ACTIVATING FACTOR BINDING TO HUMAN NEUTROPHIL MEMBRANES

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Received October 22, 1986

Radiolabeled ligand binding studies showed that specific receptors for platelet-activating factor are present in human neutrophil membranes. GTP at 10^{-7} to 10^{-3} M decreased the specific binding of platelet-activating factor to neutrophil membranes in a dose-dependent manner. Inhibition of platelet-activating factor binding was also induced by other guanine nucleotides but not by adenine nucleotides. Our results suggest that platelet-activating factor receptor in human neutrophil membranes may be coupled to a guanine nucleotide binding protein.

Platelet-activating factor (PAF) is a potent phospholipid which is released from many cell types (1-4). It activates platelets (5,6), neutrophils (7,8), macrophages (9), smooth muscle (10) and may function as a mediator of inflammatory reactions (11). Studies with chemically related compounds have indicated that PAF responsive cells may have distinct receptors for PAF (12,13). Indeed, specific binding sites for PAF have been found in platelets (14-16), polymorphonuclear leukocytes (17,18), and human lung tissues (19). However, the mechanism by which PAF binding regulates its biological activities in neutrophils remains to be established. Recent evidence suggests that a G protein may be involved in the actions of PAF since pretreatment of human neutrophils with pertussis toxin inhibited PAF-mediated chemotaxis, superoxide

Abbreviations: PAF, platelet-activating factor (1-O-alkyl-2-O-acetyl-sn-glyceryl-3-phosphorylcholine); G protein, guanine nucleotide binding protein; G_i, the inhibitory G protein associated with adenylate cyclase; G_s, the stimulatory G protein associated with adenylate cyclsae; FMLP, formyl-methionyl-leucyl-phenylalanine; GppNHp, 5'-guanylylimidodiphosphate; GTP-γ-S, guanosine-5'-O-(3-thiotriphosphate); GDP-β-S, guanosine-5'-O-(2-thiodiphosphate); cGMP, guanosine-3'-5'-cyclic monophosphate; cAMP, adenosine-3'-5'-cyclic monophosphate.

0006-291X/86 $1.50

generation, aggregation, and lysozyme release (20). In this communication, we examine this possibility by studying the regulatory effects of GTP and other guanine nucleotides on the binding of PAF to receptors in human neutrophil membranes.

MATERIALS AND METHODS

Materials

[^{3}H]PAF, Alkyl-2-O-acetyl-sn-glyceryl-3-phosphorylcholine-1-O-[alkyl-1',2'-^{3}H(N)]-, with a specific activity of 59.5 Ci/mmol was purchased from New England Nuclear (Boston, MA). GTP was from Calbiochem (La Jolla, CA). The follwing chemicals were from Sigma (St. Louis, MO): PAF, FMLP, leukotriene B_4, arachidonic acid, prostaglandin E_1, GppNHp, GTP-γ-S, GDP-β-S, GDP, GMP, cGMP, ATP, ADP, AMP, cAMP. All other chemicals were of reagent grade.

Preparation of neutrophil membranes

Human neutrophils were isolated as previously described (21). The neutrophils were finally resuspended in 50 mM Tris-HCl, pH 7.7 containing 10 mM $MgCl_2$. The cells were disrupted by sonication for 15 sec on ice. Unbroken cells and nuclei were removed by centrifugation at 1000 g for 10 min at 4°C. The membranes were collected by further centrifugation at 100,000 g for 60 min at 4°C. The final pellet was resuspended in 10 mM Tris-HCl, pH 7.4, with 10 mM $MgCl_2$ and stored at -70°C until use. Protein concentration of membranes was determined by the method of Lowry et. al. (22).

PAF binding assay

Human neutrophil membrane (50 µg) was incubated with [^{3}H]PAF (0.2 nM) and specified inhibitors in 1 ml of incubation buffer containing 10 mM Tris-HCl pH 7.4 and 0.1% bovine serum albumin. The incubation was conducted at 24°C for 30 min unless otherwise specified. The mixture was then filtered through Whatman GF/C glass fiber filter under vacuum and rapidly washed twice with 5 ml of cold incubation buffer. The GF/C filters were presoaked with incubation buffer. The filters were dried and counted by liquid scintillation counting. Specific binding was defined as the difference between total binding and nonspecific binding which was obtained in the presence of 1 µM of unlabeled PAF.

RESULTS and DISCUSSION

Previous studies with PAF and chemically related compounds have shown that the structural determinants required for the biological activities of this phosphocholine derivative are highly specific (12,13). The data imply that there may be receptors for PAF in the cells it stimulates. Subsequent studies have found specific binding sites for PAF in platelets (14-16) and neutrophils (17,18) and showed that there is a good correlation between the biological actions of PAF and its affinity for the receptors. In this report, we demonstrated in human neutrophil membranes the presence of binding sites which are

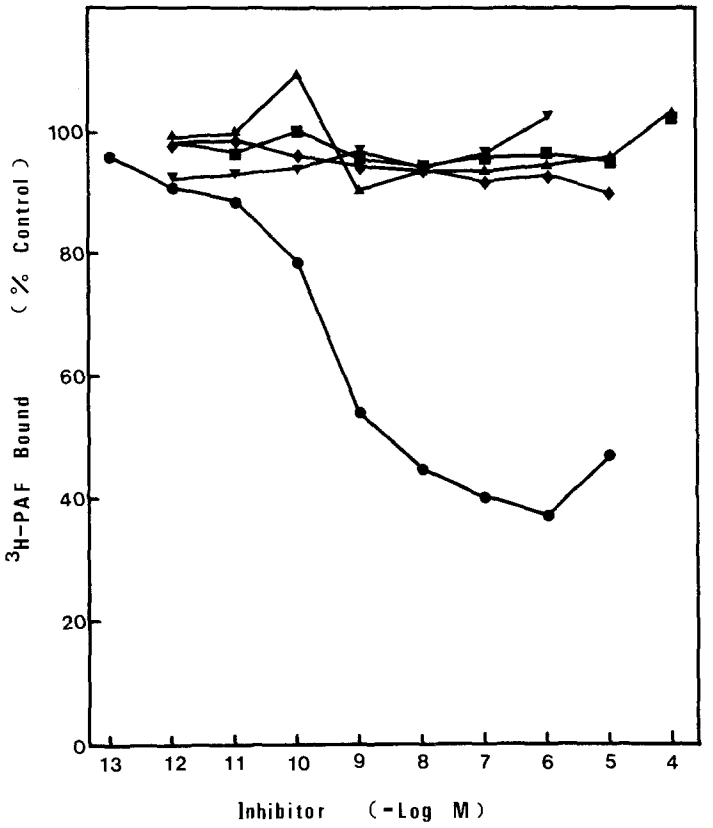

Figure 1. Specificity of [^{3}H]PAF binding to neutrophil membranes. Neutrophil membranes were incubated with [^{3}H]PAF and unlabeled competitors or buffer (control) at 24^{0}C for 30 min. Results are means of duplicates from 2 to 4 experiments. PAF (●), FMLP (■), leukotriene B_4 (▼), prostaglandin E_1 (▲), arachidonic acid (◆).

highly specific for PAF. Figure 1 shows that when radiolabeled PAF was incubated with various competitors, only unlabeled PAF produced a concentration dependent inhibition of [^{3}H]PAF binding to neutrophil membranes. A maximum of 63.0±4.1% (S.E.M.) inhibition was observed at 1 μM of cold PAF. Other agents such as FMLP, leukotriene B_4, prostaglandin E_1, and arachidonic acid, did not affect the binding of [^{3}H]PAF even at high concentrations. Preliminary studies were conducted to determine the affinity of the PAF receptors. Neutrophil membranes were incubated with [^{3}H]PAF over a range of 0.08 to 7 nM and the specific binding of [^{3}H]PAF was found to be saturable. Scatchard analysis of the saturation binding data revealed only one binding site with an equilibrium dissociation constant (K_d) of 0.578±0.033 nM and

the number of binding sites of 0.401±0.094 pmol/mg protein. In intact cells, PAF receptors with dissociation constants of 0.11 and 0.2 nM have been detected. Thus the binding sites examined in our study represent receptors with relatively high specificity and affinity for PAF.

Although the receptors for PAF have been identified, the mechanism by which PAF receptor binding modulates its biological functions in different cells is not well understood. In rabbit platelet membranes, PAF decreases the prostaglandin E_1-stimulated, and fluoride-stimulated adenylate cyclase activities and cellular cAMP production (23). A linkage of PAF receptor to the adenylate cyclase system via an inhibitory G protein was suggested by Hwang et. al. (24) who found that in rabbit platelet membranes, PAF stimulates the GTPase activity and the binding of PAF is inhibited by sodium and GTP. In human neutrophil membranes, we found that GTP also inhibited the specific binding of PAF. Figure 2 shows that GTP-induced inhibition increased with concentrations of the nucleotide and this effect appears to be temperature dependent. At 37°C, the inhibitory effect of 1 mM of GTP

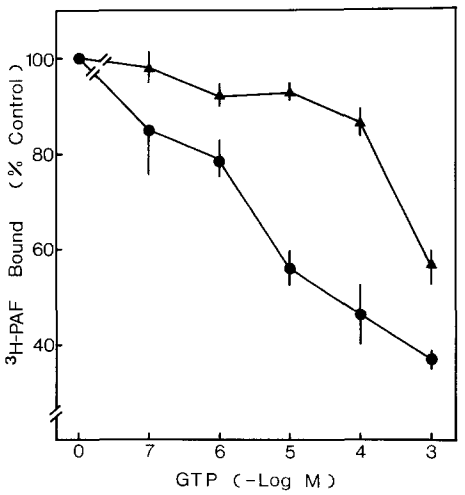

Figure 2. Inhibition of specific binding of [^{3}H]PAF to neutrophil membranes by GTP. Neutrophil membranes were incubated with [^{3}H]PAF and GTP for 60 min at 24°C (▲) and 37°C (●). Results are expressed as percent of specific binding of [^{3}H]PAF to membranes incubated with buffer alone (control). The vertical bars are S.E. of means from 3 to 4 experiments.

Table 1

Effect of nucleotides on [^{3}H]PAF binding to human neutrophil membranes

Nucleotide (1 mM)	% Inhibition	(N)
GppNHp	77.4 ± 6.3	(3)
GTP-γ-S	68.8 ± 2.8	(3)
GTP	63.0 ± 1.9	(3)
GDP	62.3 ± 2.0	(3)
GDP-β-S	60.3 ± 5.4	(3)
GMP	48.9 ± 2.8	(2)
cGMP	21.5 ± 0.6	(3)
ATP	0	(3)
ADP	0	(3)
AMP	0	(3)
cAMP	5.5 ± 3.4	(4)

Neutrophil membranes were incubated with [^{3}H]PAF and nucleotides at 37°C for 60 min. Results are expressed as percent inhibition of specific binding of [^{3}H]PAF to membranes incubated with buffer alone. The values are means ± S.E.M. The effects of GMP and cGMP compared to other guanine nucleotides are significantly different (Student's t-test, $p<0.05$), except there is no signifiant difference between GMP and GDP-β-S.

increased from 43.8±3.5% to 63.0±1.9%. This temperature dependency of GTP inhibition is similar to that observed in rabbit platelet membranes (24). To determine the specificity of the GTP effect on PAF binding, several other nucleotides were tested. Table 1 shows that GTP, GDP, and their nonhydrolyzable analogues significantly inhibited the specific binding of PAF to neutrophil membranes. GMP and cGMP were less effective than other guanine nucleotides ($p<0.05$). In contrast, the adenine nucleotides: ATP, ADP, AMP, and cAMP at similar concentrations had no effect on the binding of PAF to human neutrophil membranes. Studies on human polymorphonuclear leukocyte membranes showed that the inhibitory effect of guanine nucleotide on the binding of FMLP to its receptors was due to conversion of a portion of the FMLP receptors from the high affinity state to the low affinity state (25). Whether GTP has similar effect on the binding of PAF to its receptors remains to be determined.

In summary our data show that guanine nucleotides inhibit the specific binding of PAF to neutrophil membranes and support the notion

that a guanine nucleotide binding protein is important for the signal transduction mechanism of PAF receptor in human neutrophils. However, the identity of this G protein and its relationship, if any, to G_i is unknown. Although studies showing that pertussis toxin inhibits PAF-mediated effects on neutrophil function (20) is suggestive of the involvement of G_i in the actions of PAF on human neutrophils, one cannot exclude the possibility that a G protein other than G_i is involved. Recently Houslay et. al. found that in human platelet membranes, either cholera or pertussis toxin has no or only a small effect, respectively on the GTPase activity stimulated by PAF. They proposed that PAF exerts actions through a G protein which is distinct from G_s and G_i (26). The presence of multiple forms of G proteins in neurtophils is also noted by Gierschik et. al. (27) who found that the predominant pertussis toxin substrate of human neutrophil membranes is a novel protein which is immunochemically distinct from G_i.

Acknowledgements: This work was supported by the Alberta Heritage Foundation for Medical Research and the Arthritis Society of Canada.

REFERENCES

1. Benveniste, J., Chignard, M., LeCouedic, J.P., and Vargaftig, B.B. (1982) Throm. Res. 25, 375-385.
2. Mueller, H.W., O'Flaherty, J.T., and Wykle, R.L. (1984) J. Biol. Chem. 259, 4554-4559.
3. Albert, D.H., and Snyder F. (1983) J Biol. Chem. 258, 97-102.
4. Prescott, S.M., Zimmerman, G.A., and McIntyre, T.M, (1984) Proc. Natl. Acad. Sci. USA 81, 3534-3538.
5. Demopoulos, C.A., Pinckard, R.N., and Hanahan, D.J. (1979) J. Biol. Chem. 254, 9355-9358.
6. Hanahan, D.J., Demopoulos, C.A., Liehr, J., and Pinckard, R.N. (1980) J. Biol. Chem. 255, 5514-5516.
7. Shaw, J.O., Pinckard, R.N., Ferrigni, R.S., McManus, L.M., and Hanahan, D.J. (1981) J. Immunol. 127, 1250-1255.
8. O'Flaherty, J.T., Wykle, R.L., Miller, C.H., Lewis, J.C., Waite, M., Bass, D.A., McCall, C.E., and DeChatlet, L.R. (1981) Am. J. Pathol. 103, 70-79.
9. Hartung, H.P. (1983) FEBS Lett. 160, 209-212.
10. Stimler, N.P., and O'Flaherty. J.T. (1983) Am. J. Pathol. 113, 75-84.
11. Page, C.P., Archer, C.B., Paul, W., and Morley, J. (1984) Trends Pharmacol. Sci. 5, 239-241.
12. Hanahan, D.J., Munder, P.G., Satouchi, K., McManus, L., and Pinckard, R.N. (1981) Biochem. Biophys. Res. Commun. 99, 183-188.
13. Satouchi, K., Pinckard, R.N., McManus, L.M., and Hanahan, D.J. (1981) J. Biol. Chem. 256, 4425-4432.

14. Shaw, J.O., and Henson, P.M. (1980) Am. J. Pathol. 98, 791-810.
15. Valone, F.H., Coles, E., Reinhold, V.R., and Goetzl, E.J. (1982) J. Immunol. 129, 1637-1641.
16. Hwang, S.B., Lee, C.S.C., Cheah, M.J., and Shen, T.Y. (1983) Biochemistry 22, 4756-4763.
17. Valone, F.H., and Goetzl, E.J. (1983) Immunology 48, 141-149.
18. O'Flaherty, J.T., Surles, J.R., Redman, J., Jacobson, D., Piantadosi, C., and Wykles, R.L. (1986) J. Clin. Invest. 78, 381-188.
19. Hwang, S.B., Lam, M.H., and Shen, T.Y. (1985) Biochem. Biophys. Res. Commun. 128, 972-979.
20. Lad, P.M., Olson, C.V., and Grewal, I.S. (1985) Biochem. Biophys. Res. Commun. 129, 632-638.
21. Wong, K., and Freund, K. (1981) Can. J. Physiol. Pharmacol. 59, 915-920.
22. Lowry, O.H., Rosenbrough, N.J., Farr, A.L., and Randall, R.J. (1951) 193, 265-275.
23. Haslam, R.J. and Vanderwel, M. (1982) J. Biol. Chem. 257, 6879-6885.
24. Hwang, S.B., Lam, M.H., and Pong, S.S. (1986) J. Biol. Chem. 261, 532-537.
25. Koo, C., Lefkowitz, R.J., and Snyderman, R. (1983) J. Clin. Invest. 72, 748-753.
26. Houslay, M.D., Bojanic, D., and Wilson, A. (1986) Biochem. J. 234, 737-740.
27. Gierschik, P. Falloon, J., Milligan, G., Pines, M., Gallin, J.I., and Spiegel, A. (1986) J. Biol. Chem. 261, 8058-8062.