Vol. 127, No. 1, 1985 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 28, 1985 Pages 24-30

DESENSITIZATION OF HUMAN PLATELETS BY PLATELET ACTIVATING FACTOR

Carolyn M. Chesney, D. David Pifer, and Kim M. Huch

Department of Medicine, University of Tennessee Center for the Health Sciences and Department of Pathology, Baptist Memorial Hospital, Memphis, Tennessee 38146

Received December 28, 1984

Human platelets are less responsive to PAF at 37° than at 25°. They can be desensitized to the effects of PAF by pre-exposure to small concentrations. In both cases desensitization appears to be accompanied by a decreased affinity of the high affinity site for PAF rather than loss of binding sites. Alteration of a metabolic step subsequent to binding cannot be excluded, but platelets show normal response to a variety of other agents under the conditions resulting in desensitization of platelets to PAF.

Platelet activating factor (1-O-alkyl-2-acetyl-sn-glyceryl-3-phosphoryl-choline) is a lipid mediator of inflammation which has been shown to elicit platelet aggregation and secretion in various animal species (1) as well as man (2). There is increasing evidence that this lipid mediator has a specific receptor on the platelet. Occupation of this receptor by PAF results in stimulus response coupling. Specific, saturable binding has been demonstrated (3-5). The receptor does not appear to be the classical α_2 receptor, but PAF binding is competitively inhibited by the α_2 antagonists phenoxybenzamine and phentolamine as well as the calcium channel blockers verapamil and nifedipine (3).

The phenomenon of desensitization of platelets to the effects of PAF by prior exposure to subthreshold concentrations has been reported in the rabbit (6). Rabbit platelets were specifically desensitized to PAF, if they had been pretreated with PAF, but reacted normally with other agents. The desensitization was dose-dependent and required the presence of the 2-acetyl group in the correct steric configuration (7).

We have studied desensitization of human platelets by PAF and report the effect of desensitization on both function and binding.

0006-291X/85 $1.50

MATERIALS AND METHODS

Bovine serum albumin (Fraction V), ADP and epinephrine were obtained from Sigma Chemical Co., St. Louis, MO; arachidonic acid from NuChek Preparations, Inc, Elysian MN; and A23187 from Calbiochem-Behring, San Diego, CA. Fibrillar equine tendon collagen (collagen reagent Horm) came from the Hormon-Chemie Co., Munich. Human fibrinogen (grade L, 95% clottable) was a product of Kabi, Stockholm, Sweden. Pure synthetic PAF came from Bachem Feinchemikalien, Bubesdorf, Switzerland. Tritiated PAF was obtained from New England Nuclear, Boston, Mass (specific activity 45 Ci/mmol).

Platelet rich plasma and gel-filtered platelets (GFP) were prepared as previously described (8). Studies on GFP were done in Tyrode's buffer containing 0.5mM $CaCl_2$ and albumin (0.35%). Fibrinogen (final concentration 1.67 mg/ml) was added for aggregation studies but omitted for binding studies. Platelet aggregation was measured on a Payton Dual Channel Aggregometer, Payton Associates, Inc., Buffalo, NY, according to the method of Born (9). Binding studies were carried out on GFP as previously described (3). In brief, gel-filtered platelets in 0.5 ml volume (platelet count 200,000 $\pm$ 50,000 per μl) were incubated with [^{3}H] PAF (40 fmol) at the specified temperature (25° or 37°) 30 or 60 minutes, respectively, to achieve equilibrium binding. Platelets were separated from unbound, labeled PAF by centrifugation at 12,500xg for 2 minutes in an Eppendorf microcentrifuge. The entire supernatant was carefully transferred to a scintillation vial. Scintisol (Isolab, Inc., Akron, Ohio) was added and unbound radioactivity determined in a Searle scintillation counter. The remaining platelet pellet was dissolved in 10% Triton X 100. The bound radioactivity was then assessed in like manner.

RESULTS

Effect of temperature on platelet aggregation induced by PAF

PAF induces biphasic platelet aggregation in platelet rich plasma and in GFP (10). Since plasma contains a hydrolase which rapidly inactivates PAF, a high concentration (in the neighborhood of 800nM) is required (11). However, gel filtration allows preparation of platelets free of this hydrolase. GFP exhibit biphasic platelet aggregation with 80 nM PAF. To test the effect of temperature on PAF induced platelet aggregation, we measured aggregation response to a wide range of PAF concentrations (10^{-12} - 10^{-6}M) at 37° and 25°. As can be seen in Figure 1, lowering the temperature to 25° shifts the dose response curve to the left. The dose of PAF necessary to elicit 50% aggregation at 25° is 10 fold less than that needed at 37°. Therefore physiological temperature appears to desensitize the response of GFP to PAF. No such temperature effect on the aggregation dose response curve for ADP (10^{-7} - 10^{-5}M), epinephrine (10^{-7} - 10^{-5}M), thrombin (0.002 - 0.02 u/ml) and microfibrillar collagen (0.3 - 3.0 μg/ml) is noted (data not shown).

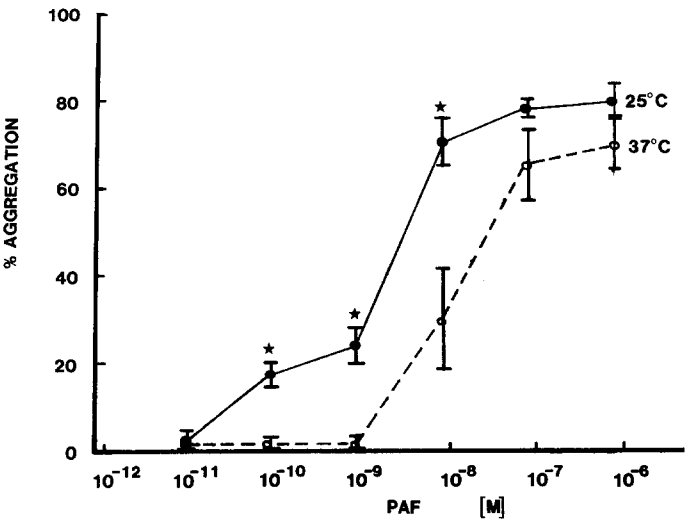

Figure 1. Dose response curve for PAF-induced platelet aggregation in GFP at 25° and 37°. GFP was prepared by filtration of PRP on Sepharose 2B equilibrated with Tyrode's buffer containing $CaCl_2$ (0.5mM) and albumin (0.35%). Fibrinogen (1.67 mg/ml) was added prior to study. Platelet counts of GFP were 200,000±50,000 per µl.
Results are expressed as mean SEM (n = 8)
* $p < 0.001$

Effect of preincubation with subaggregating concentrations of PAF on PAF-induced aggregation. When GFP was preincubated with 0.8 nM PAF at 25° prior to challenge with 8 nM PAF, time dependent desensitization was noted (Figure 2). The desensitizing effect was enhanced at 37°. The desensitization appeared to be specific, since GFP preincubated with PAF showed full aggregatory response when challenged with ADP (10µM), epinephrine (25µM), collagen (8µg/ml), and the ionophore A23187 (8µg/ml), respectively. (data not shown).

Effect of desensitization on specific binding of [^{3}H] PAF to GFP

Platelet rich plasma was preincubated with desensitizing doses of PAF prior to gel filtration. The GFP obtained showed only first wave of aggregation when fibrinogen was added and the GFP rechallenged with aggregating doses of PAF in the aggregometer. Equilibrium binding studies were carried out in parallel as described above in Methods. Figure 3 shows the effect of temperature and prior desensitization with PAF on the binding curves. Analysis of the data by linear regression is consistent with the presence of high

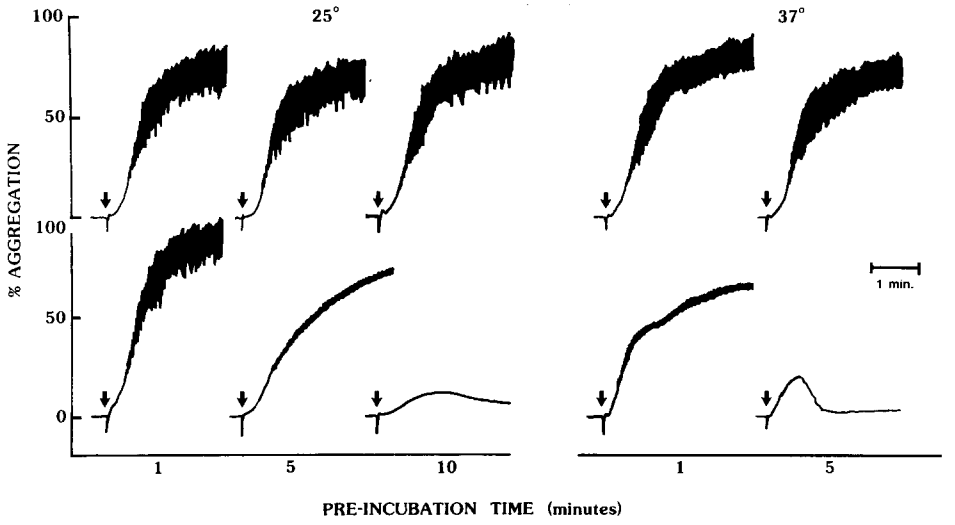

Figure 2. Effect of preincubation with a subaggregating concentration of PAF on PAF-induced aggregation. The GFP were incubated with PAF (0.8nM) for 1, 5, or 15 minutes prior to addition of aggregating dose of PAF, which was 8nM at 25° and 80nM at 37°. The aggregating dose was added at arrow. Control GFP preincubated with saline were tested in parallel.

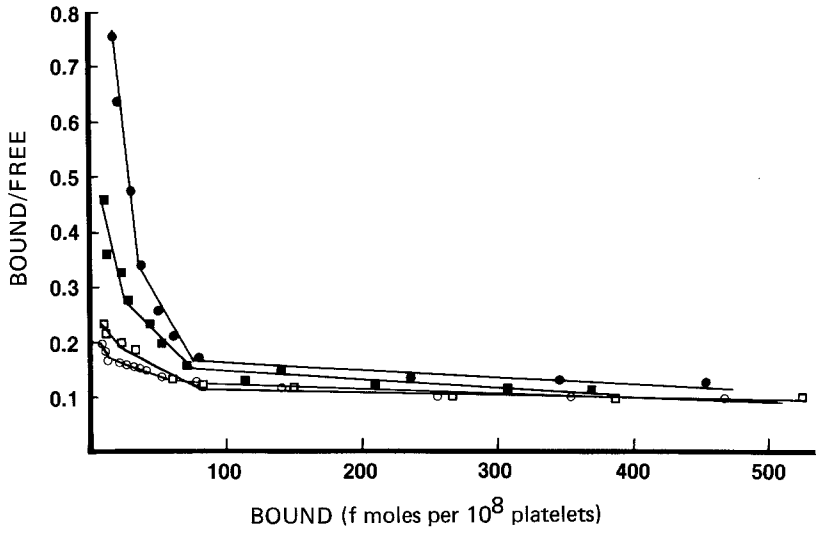

Figure 3. Scatchard analysis of binding of [^{3}H] PAF to GFP. Each data point is the mean of 5 experiments under the following conditions: Control 25° (closed circles), control, 37° (closed squares), desensitized, 25° (open circles), desensitized 37° (open squares). For desensitization studies PRP was incubated with 28 nM PAF at 25° or 16nM PAF at 37° for 5 minutes prior to gel filtration. Resultant GFP was functionally desensitized (no second wave of aggregation when challenged with 8 nM PAF at 25° or 80 nM PAF at 37°). Binding studies were carried out on aliquots of the GFP as described in Methods. Specific binding was calculated by subtracting non-specific binding (100 molar excess unlabeled PAF) from total binding.

The data were analyzed by linear regression analysis. In all cases, the high affinity sites showed good correlation ($r > 0.9$ for H and I for all conditions; r for L ranged from 0.8 - 0.9).

TABLE I

EFFECT OF PRIOR EXPOSURE TO PAF ON RECEPTOR AFFINITY FOR PAF

Temperature	Dissociation Constant(nm)			Estimated binding sites/platelet		
	Kd_H	Kd_I	Kd_L	High	Intermediate	Low
25°						
Control	0.10±0.02	0.55±0.07	19.58± 6.76	320±38	733±49	8841±1855
Desensitized	1.54±0.40	2.43±0.36	25.78*	944±298	1326±196	16899*
P - value	0.007	0.001	NS	0.07	0.02	0.02
37°						
Control	0.35±0.08	0.96±0.23	19.10±7.07	424±8	862±91	7238±3503
Desensitized	0.81±0.18	1.87±0.31	47.28±5.42	579±64	1139±122	16899±1957
P - value	0.06	0.06	0.02	.05	NS	0.05

Dissociation constants and estimated binding sites are the mean ± SEM of 5 separate experiments and compared using student t-test.
*Data from one experiment was compared to the population mean of the control.

(H), intermediate (I), and low (L) affinity binding sites. At 25° the high affinity site has Kd = 0.10 nM, 320 sites per platelet (Table I). At 37° the high affinity site has affinity Kd = 0.35nM, 424 binding sites per platelets. The difference in affinity noted at the two temperatures is significant for the high affinity site ($p = 0.009$). However no significant difference in affinity is noted for the intermediate and lower affinity sites. When the platelets were desensitized by exposure to unlabeled PAF prior to gel filtration equilibrium binding studies on the GFP revealed a significant decrease in affinity for both the high and intermediate sites at 25°. There appears to be less change in the low affinity sites. Estimation of binding sites is consistent with significant increase in sites of intermediate and low affinity. When the studies were carried out at 37°, the same trend was observed, but the differences in Kd for the H and I sites did not reach statistical significance.

DISCUSSION

Human platelets in plasma or Tyrode's buffer can be specifically desensitized to PAF. Platelet response to PAF is temperature dependent. Platelets in

the aggregometer are 10-fold more responsive to PAF at 25° than at 37°. No such temperature differential is noted in platelet response to other agonists. Equilibrium binding studies carried out at 25° and 37° show decrease in affinity of the high affinity site for PAF at 37° compared to 25°.

Exposure of platelets to small amounts of PAF prior to challenge with higher doses prevents the second wave of aggregation. This effect is observed at 25° and 37°. Platelets desensitized to PAF are capable of normal response to other agonists such as ADP, collagen, thrombin, A23187, and arachidonic acid, implying that a specific receptor and/or pathway has been desensitized. Equilibrium binding studies are consistent with shift in the affinity of the high and intermediate affinity sites to lower affinities.

The exact nature of the PAF receptor is not yet known. However, it has similarities to the α-adrenergic receptor. The α-adrenergic antagonists phentolamine and phenoxybenzamine competitively inhibit specific binding of [^{3}H] PAF to GFP (3). Yohimbine, a selective α_2 antagonist, exhibits only partial antagonism, as do the α_1 antagonist prazosin and the β-adrenergic antagonist propranolol (3). The α_2 agonist epinephrine shows no inhibition of specific binding of PAF to GFP. Since epinephrine (12) and PAF (13) both increase the influx of external calcium into the platelet, these effects may be related to influence on the calcium channel rather than the α-adrenergic receptor. The calcium channel blockers verapamil and nifedipine (3), as well as diltiazem (14), competitively inhibit binding of [^{3}H] PAF by human platelets.

The role of guanine nucleotides in PAF receptor regulation has not yet been explored but is obviously of key importance in understanding regulation of the PAF receptor and its relationship to the α-adrenergic receptor. PAF has been shown to inhibit the prostaglandin E_1 induced rise in c-AMP in intact rabbit platelets and to inhibit basal, prostaglandin E_1-stimulated, and fluoride-stimulated adenylate cyclase activities in rabbit platelet particulate fractions (15). The effect of guanine nucleotides was not assessed in these studies. The reduction in affinity of the high affinity sites by

desensitization is consistent with an uncoupling mechanism, as has been described for the α_2 receptors in platelets (16). Further studies on intact platelets as well as particulate fractions will be needed to clarify this important point.

REFERENCES

1. Benveniste, J., Henson, P.M., and Cochrane, C.G. (1972) J. Exp. Med. 136, 1356-1377.
2. Benveniste, J., Le Couedic, J.P., and Kamoun, P. (1975) Lancet 1, 344-345.
3. Chesney, C.M., Pifer, D.D., and Huch, K.M.(1983) Platelet-Activating Factor INSERM Symposium No. 23, Benveniste, J., Arnoux, B., Eds., Elsevier Science Publishers, Amsterdam, 177-184.
4. Kloprogge, E., and Akkerman, J.W.N. (1983) Platelet-Activating Factor INSERM Symposium No. 23, Benveniste, J., and Arnoux, B., Eds. Elsevier Science Publishers, Amsterdam, 153-159.
5. Valone, F.H., Coles, E., Reinhold, V.R., and Goetzl, E.J. (1983) J. Immunol. 129, 1637-1641.
6. Keraly, C. L., and Benveniste, J. (1982) Br. J. of Haematol. 51, 313-322.
7. Keraly, C.L., Coeffier, M., Tence, M., Borrel, M.C., and Benveniste, J. (1983) Br. J. of Haematol. 53, 513-521.
8. Chesney, C.M., Pifer, D.D., and Colman, R.W. (1981) Proc. Nat'l Acad. Sci. USA, 78, 5180-5184.
9. Born, G.V.R. (1963) J. Physiol. 168, 178-195.
10. Chesney, C.M., Pifer, D.D., Byers, L.W., and Muirhead, E.E. (1982) Blood, 59, 582-585.
11. Pinckard, R.N., Farr, R.S., Hanahan, D.J. (1979) J. Immunol. 123, 1847-1857.
12. Owen, N.E., Feinberg, H., and LeBreton, G.C. (1980) Am. J. Physiol. 239, H483-488.
13. Hallam, T.J., Sanchez, A., and Rink, T.J. (1984) Biochem. J. 218, 819-827.
14. Tuffin, D.P., Wade, P.J., Lunt, D.O., and McCullagh, K.G. (1983) J. Pharmacol. 14 Suppl. 1, 67.
15. Haslam, R.J., and Vanderwel, M. (1982) J. Biol. Chem. 257, 6879-6885.
16. Tsai, B.S., and Lefkowitz, R.J. (1979) Molec. Pharmacol. 16, 61-68.