Biochemical Pharmacology, Vol. 35, No. 4, pp. 621–624, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00

AUTOCOID BINDING TO SERUM PROTEINS

INTERACTION OF PLATELET ACTIVATING FACTOR (PAF) WITH HUMAN SERUM ALPHA-1-ACID GLYCOPROTEIN (AAG)

PATRICK J. MCNAMARA,*† KENNETH R. BROUWER† and MARK N. GILLESPIE‡

†Division of Pharmaceutics and Pharmaceutical Analysis, and ‡Division of Pharmacology and Toxicology, College of Pharmacy, University of Kentucky, Lexington, KY 40536, U.S.A.

(*Received* 14 *February* 1985; *accepted* 19 *July* 1985)

Abstract—Platelet activating factor (PAF; 1-alkyl-2-acetyl-sn-glycero-3-phosphocholine) is a potent bioactive phospholipid released from platelets, neutrophils, basophils and macrophages that has been proposed as a mediator of anaphylaxis, acute lung injury and other disorders. Specific factors which stabilize PAF and/or regulate PAF activity in body fluids are largely unknown. As part of a general autocoid-serum protein binding screen, the platelet activating factor–alpha-1-acid glycoprotein (PAF–AAG) interaction was indirectly characterized by examining the ability of PAF to displace propranolol from AAG. Both PAF and its deacetylated metabolite (lyso-PAF), at 20 μM, doubled the fraction unbound of propranolol (0.4 μM) from purified human AAG (20 μM). None of the other autocoids that were studied (epinephrine, serotonin, spermidine, putrescine, leu-enkephalin or phenethylamine) exhibited any propranolol displacement activity. Scatchard analysis indicated that PAF competitively displaced propranolol from AAG, causing the apparent affinity constant for propranolol-AAG to decrease from 1.8×10^5 M to 6.9×10^4 M. PAF behaved qualitatively like chlorpromazine (a documented inhibitor of propranolol binding to AAG), but PAF was less effective at displacing propranolol. The apparent binding to AAG may help stabilize and transport extracellular PAF. Furthermore, the interaction of PAF and AAG suggests that serum AAG, which fluctuates in a number of diseases, may function to regulate PAF activity during acute and chronic disease states.

Platelet activating factor (PAF) is a potent bioactive phospholipid found in platelets, neutrophils, basophils and macrophages [1–5]. PAF can provoke platelet and neutrophil activation, hypotension and bronchoconstriction [1, 5–7]. It has also been implicated in the pathogenesis of hyperactive airway disease, acute lung injury and other disorders [5, 8]. PAF can be readily metabolized to inactive lyso-PAF by acetylhydrolases located in both intracellular and extracellular (i.e. vascular) spaces [9, 10]. Those factors which stabilize PAF and/or regulate PAF activity in body fluids are largely unknown.

The importance of alpha-1-acid glycoprotein (AAG) in the binding of cationic drugs is widely accepted [11–13]; however, the biological role for AAG has not been established. It would appear likely that, as with serum albumin, AAG may function to transport endogenous substances (e.g. endogenous amines) in serum and extravascular spaces. Serum AAG concentrations have been shown to increase or decrease (2- to 3-fold) in a variety of diseases including inflammation, myocardial infarction, renal and pulmonary disease, and altered physiological conditions, such as surgery, obesity and smoking [14–19]. Neither the underlying causes nor the consequences of these fluctuations have been identified; however, an interaction of AAG with circulating and extravascular autocoids remains an interesting possibility.

During the course of a screening program for autocoid interactions with serum proteins, an interaction between AAG and PAF was observed. The purpose of this study is to characterize the interaction of PAF with AAG, specifically as it relates to the displacement of propranolol from its AAG binding site.

* Correspondence.

METHODS

PAF; 1-alkyl-2-acetyl-sn-glycero-3-phosphocholine was obtained from Calbiochem-Behring (La Jolla, CA) and chlorpromazine was obtained from Smith, Kline & French Laboratories (Philadelphia, PA). Human AAG, lyso-PAF, *d,l*-propranolol, epinephrine, serotonin, spermidine, putrescine, phenethylamine, imipramine and leu-enkephalin were obtained from the Sigma Chemical Co. (St. Louis, MO). Radiolabeled propranolol (*d,l*-[4-^{3}H]propranolol) was obtained from the Amersham Co. (Arlington Heights, IL). Radiochemical purity was greater than 98% when acquired and was verified (via TLC) periodically and following dialysis to ensure stability.

A stock solution containing 20 μM AAG was made up in a phosphate buffer (0.13 M, pH 7.4). Aliquots of this protein stock solution were used to make working solutions containing 50 μM PAF, lyso-PAF, chlorpromazine, imipramine, epinephrine, serotonin, spermidine, putrescine, phenethylamine or leu-enkephalin.

These protein solutions were then used to determine the binding of propranolol in a previously described [18, 19] equilibrium dialysis method. Protein solutions were dialyzed at 37° across a semipermeable (12,000–14,000 mol. wt. cut-off) cellulose

Table 1. Fraction unbound (Fu) for the binding of propranolol (0.4 μM) to AAG (20 μM) in the presence of potential displacers at a concentration of 50 μM

Substance	Fu	Substance	Fu
Control	0.270 ± 0.026		
Epinephrine	0.262 ± 0.016	Serotonin	0.241 ± 0.016
Spermidine	0.245 ± 0.018	Putrescine	0.252 ± 0.017
leu-Enkephalin	0.254 ± 0.014	Phenethylamine	0.260 ± 0.027
PAF	0.543 ± 0.033*	lyso-PAF	0.516 ± 0.014*
Chlorpromazine	0.810 ± 0.031*	Imipramine	0.533 ± 0.016*

Each value is the mean ± S.D. of at least four determinations.
* Statistically significant compared to control, using Student's t-test ($P < 0.01$).

membrane (Spectrapor 2; Spectrum Medical Industries, Los Angeles, CA) against AAG-free buffer containing various concentrations (0.4 to 40 μM) of non-radiolabeled propranolol and a trace amount of radioactive propranolol. At equilibrium (8 hr), propranolol concentrations on both sides of the membrane were quantitated by liquid scintillation counting (model 3255 Tri-Carb, Packard Instrument Co., Downers Grove, IL). Quench correction was performed by the external standard ratio method. Binding constants were obtained by geometric regression analysis [20] of classical Scatchard plots [21].

RESULTS

PAF and lyso-PAF both caused a doubling in the fraction unbound of propranolol (Table 1), similar to the displacement potential of imipramine. Chlorpromazine was somewhat more potent at displacing propranolol from AAG. None of the other autocoids (i.e. epinephrine, serotonin, spermidine, putrescine, leu-enkephalin or phenethylamine) exhibited any propranolol displacement activity.

Scatchard analysis (Fig. 1) indicated that the binding of propranolol to AAG appeared to be to one class of binding sites with an apparent affinity constant of 1.8×10^5 M and a capacity constant of 0.9. Furthermore, Fig. 1 demonstrates that the displacement of propranolol by PAF was competitive in nature, as was the displacement of propranolol by chlorpromazine. The presence of 20 μM PAF decreased the apparent affinity constant of propranolol for AAG by more than 50% (Table 2), whereas the capacity constant remained unchanged. Additional PAF (50 μM) further reduced this affinity

Table 2. Affinity (K_a) and capacity (n) binding constants and correlation coefficients (r) for the binding of propranolol to AAG in a control protein solution and in the presence of chlorpromazine (20 μM) or PAF (20 or 50 μM)

Condition	K_a ($\times 10^{-4}$ M)	n	r
Control	18.13	0.89	0.968
Chlorpromazine (20 μM)	4.23	0.88	0.924
PAF (20 μM)	6.89	0.85	0.942
PAF (50 μM)	2.19	1.11	0.973

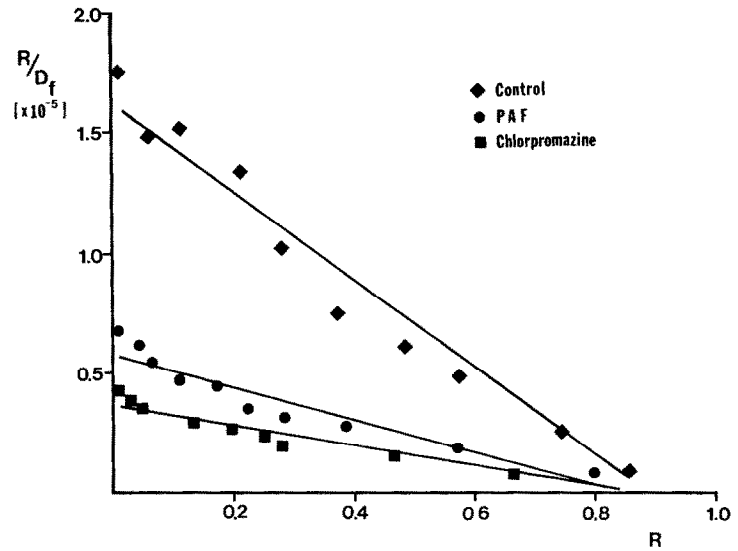

Fig. 1. Scatchard plots for the interaction of propranolol and AAG in the presence and absence (control) of PAF (20 μM) or chlorpromazine (20 μM). Each data point represents the mean of triplicate binding experiments.

constant to approximately one-tenth (2.2×10^4 M) of the original value. Chlorpromazine (20 μM) was more effective than PAF (20 μM) at reducing the apparent affinity constant for propranolol–AAG.

DISCUSSION

The principal observation of this study was that PAF competitively displaced propranolol from AAG. Propranolol, as with other cationic drugs interacting with AAG, binds to one class of binding sites on AAG [22, 23]. Moreover, this binding site appears to be common to all cationic drugs (i.e. they can all readily displace one another) [11–13]. Therefore, it would appear likely that endogenous amines, if they interact with AAG, would also displace propranolol from AAG. Thus, an indirect method, such as the one utilized in the present study, can effectively screen a number of endogenous amines for their abilities to interact with the propranolol binding site of AAG. It should be noted that the concentrations of PAF used in the present study were many orders of magnitude greater than any reported serum levels of PAF in man (<1 nM); therefore, the displacement of propranolol from serum AAG by PAF is unlikely to be of clinical concern. However, the displacement of PAF from AAG by cationic drugs may be pharmacologically significant but cannot be addressed directly from the present study.

The binding of cationic drugs (i.e. propranolol, lidocaine, chlorpromazine, etc.) to AAG has received considerable attention in the therapeutic management of these drugs for several reasons. First, AAG concentrations fluctuate in numerous disease states and stress conditions [14–19] and, as a result, concentrations of free drug may also vary widely within and between individuals. Second, the low concentration of AAG in serum (relative to albumin) makes the binding of AAG more readily saturable, again resulting in fluctuations in free (presumably active) drug. These same concerns may also apply to the interaction of AAG with autocoids (i.e. PAF).

The concept of a common binding site for the binding of cationic drugs to AAG has been reported previously [11–13]; however, the interaction of this protein with endogenous substances has received limited attention. Westphal and co-workers [24, 25] noted the interaction of AAG with several natural steroids (i.e. progesterone) but have concluded that sex-hormone binding globulin and albumin are more important than AAG for the overall serum binding of these hormones. The present study indicates that there are additional endogenous substances which interact with AAG. Moreover, this type of interaction may be of particular importance when the endogenous substance is as potent an autocoid as PAF.

There have been several reports concerning the binding of PAF to serum constituents. Lartigue-Mattel and co-workers [26] utilized radiolabeled PAF and ultrafiltration to determine the binding of PAF to rabbit plasma (>97% bound). Recently, Lichey and co-workers [27] reported on the pharmacological consequences of the interaction between PAF and serum albumin. Various concentrations of bovine serum albumin were added to perfusate in an isolated perfused rat lung preparation and resulted in a decrease in the response (i.e. perfusion pressure) and a diminished uptake of tritiated PAF as the concentration of albumin increased. Therefore, the binding of PAF appears to play a significant role in regulating the pharmacological response and the transport/uptake of PAF.

The apparent binding of PAF to AAG and other serum proteins may help stabilize and transport extracellular PAF. Furthermore, the interaction of PAF with AAG suggests that serum AAG, which fluctuates in a number of diseases, may function to regulate PAF activity during acute and chronic disease states. The physiological and pathological significance of this PAF–AAG interaction and the factors which may affect it need to be evaluated further. In addition, the interaction of AAG with other autocoids should be examined.

REFERENCES

1. J. Benveniste, P. M. Hensen and C. G. Cochrane, *J. exp. Med.* **136**, 1356 (1972).
2. J. Benveniste, *Nature, Lond.* **249**, 581 (1974).
3. J. Benveniste, J. P. Le Couedic and P. Kamoun, *Lancet* **1**, 344 (1975).
4. M. C. O'Donnell, P. M. Hensen and B. A. Fiedel, *Immunology* **35**, 953 (1978).
5. B. B. Vargafitg, J. Lefort, M. Chignard and J. Benveniste, *Eur. J. Pharmac.* **65**, 185 (1980).
6. J. O. Shaw, R. N. Pinckard, K. S. Ferrigni, L. M. McManus and D. J. Hanahan, *J. Immun.* **127**, 1250 (1981).
7. M. L. Blank, F. Snyder, L. W. Byers, B. Brooks and E. E. Muirhead, *Biochem. biophys. Res. Commun.* **90**, 1194 (1979).
8. M.-C. Prevost, C. Cariven, M.-F. Simon, H. Chap and L. Douste-Blazy, *Biochem. biophys. Res. Commun.* **119**, 58 (1984).
9. M. L. Blank, M. N. Hall, E. A. Cress and F. Snyder, *Biochem. biophys. Res. Commun.* **113**, 666 (1983).
10. M. L. Blank, T.-C. Lee, V. Fitzgerald and F. Snyder, *J. biol. Chem.* **256**, 175 (1981).
11. W. E. Muller and A. E. Stillbauer, *Naunyn-Schmiedeberg's Archs Pharmac.* **322**, 170 (1983).
12. W. E. Muller, A. E. Stillbauer and S. El-Gamal, *J. Pharm. Pharmac.* **35**, 684 (1983).
13. F. M. Belpaire, M. G. Bogaert and M. Rosseneu, *Eur. J. clin. Pharmac.* **22**, 253 (1982).
14. K. M. Piafsky, O. Borga, I. Odar-Cederlof, C. Johansson and F. Sjoqvist, *New Engl. J. Med.* **299**, 1435 (1978).
15. D. V. Edwards, D. Lalka, F. Cerra and R. L. Slaughter, *Clin. Pharmac. Ther.* **31**, 62 (1981).
16. S. K. Lee, D. W. Thibeault and D. C. Heiner, *Pediat. Res.* **12**, 775 (1978).
17. D. Fremstad, K. Bergerud, J. F. W. Haffner and P. K. M. Lunde, *Eur. J. clin. Pharmac.* **10**, 441 (1976).
18. I. H. Benedek, R. A. Blouin and P. J. McNamara, *J. Pharm. Pharmac.* **36**, 214 (1984).
19. I. H. Benedek, W. D. Fiske, W. O. Griffen, R. M. Bell, R. A. Blouin and P. J. McNamara, *Br. J. clin. Pharmac.* **16**, 751 (1983).
20. D. S. Riggs, J. A. Guarnieri and S. Adderlman, *Life Sci.* **22**, 1305 (1978).
21. G. F. Scatchard, *Ann. N.Y. Acad. Sci.* **51**, 660 (1949).
22. G. Sager, O. G. Nilsen and S. Jacobsen, *Biochem. Pharmac.* **28**, 905 (1979).

23. S. Glasson, R. Zini, P. D'Athis, J.-P. Tillement and J. R. Boissier, *Molec. Pharmac.* **17**, 187 (1980).
24. U. Westphal, *J. Am. Oil Chem. Soc.* **41**, 481 (1964).
25. M. Ganguly and U. Westphal, *Biochim. biophys. Acta* **170**, 309 (1968).
26. C. Lartigue-Mattel, D. Godeneche, J.-L. Chabard and J.-A. Berger, *Agents Actions* **12**, 703 (1982).
27. J. Lichey, T. Friedrich, J. Franke, S. Nigam, M. Priesnitz and K. Oeff, *J. appl. Physiol.* **57**, 1039 (1984).