Pharmacologic Characterization of the Rabbit Neutrophil Receptor for Platelet-Activating Factor(43143)

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Abstract. **The characteristics of receptors for platelet-activating factor (PAF) on rabbit neutrophils are investigated in this report. The presence of PAF-specific binding to rabbit neutrophils was confirmed using radiolabeled ligand binding assays and a rabbit peritoneal neutrophil membrane preparation. Binding of PAF to the neutrophil membranes was reversible and reached equilibrium within 30 min. Scatchard analysis of PAF-specific binding to the rabbit neutrophil membranes revealed a dissociation constant (K_d) for PAF of 0.41 ± 0.045 n*M* and a B_{max} of 0.32 ± 0.11 pmol of PAF receptor/mg of protein. The order of potencies of PAF receptor antagonists to inhibit the binding of ^{3}H-PAF to rabbit peritoneal neutrophil membranes was determined. For the competition assays, 100 μg of neutrophil or platelet membrane protein, 0.18 n*M* ^{3}H-PAF, and varying amounts of PAF antagonist were incubated at room temperature for 1 hr. PAF receptor antagonists tested were ONO-6240, brotizolam, kadsurenone, WEB-2086, L-652,731, BN-52021, CV-3988, triazolam, alprazolam, and verapamil. The orders of potencies of these PAF receptor antagonists were similar for inhibition of ^{3}H-PAF binding to rabbit peritoneal neutrophil and platelet membranes (correlation coefficient, $r = 0.97$). PAF had a significantly higher affinity for rabbit neutrophil membranes (K_d = 0.41 ± 0.045 n*M*), as compared with its affinity for rabbit platelet membranes (K_d = 0.87 ± 0.092 n*M*). In addition, sodium was found to inhibit ^{3}H-PAF specific binding to rabbit platelet membranes and not to affect ^{3}H-PAF binding to neutrophil membranes. These data indicate that, although PAF receptors on rabbit platelets and neutrophils exhibit similar orders of potencies of PAF receptor antagonists to inhibit the binding of ^{3}H-PAF, the disparity in K_d of PAF for the receptors and the effect of NaCl on the binding of ^{3}H-PAF reveal subtle differences between the cell types.** [P.S.E.B.M. 1990, Vol 195]

Platelet-activating factor (PAF; 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphorylcholine) is a potent mediator of cellular functions with a wide variety of physiologic and inflammatory properties. PAF activates platelets, neutrophils, and macrophages, it contracts bronchial smooth muscle, it is hypotensive and ulcerogenic, it enhances microvascular permeability, and it causes anaphylactoid reactions when administered iv (1–3). PAF is believed to activate its target cells by initially binding to a plasma membrane receptor (4, 5) and subsequently modulating adenylate cyclase activity (6), intracellular calcium flux (7, 8), and/or phosphoinositide metabolism (9). Specific binding sites for PAF have been identified on platelets (4, 5) neutrophils (5, 10, 11), smooth muscle (5), mononuclear leukocytes (10), Kupffer cells (12), and the murine macrophage line P388D (13). Compounds have been identified that inhibit both PAF-induced biologic activities *in vivo* and *in vitro* and PAF-specific binding to platelets *in vitro*. These data are evidence that the PAF-induced biologic activities involve a receptor-mediated process (14, 15).

The characteristics of PAF activation of and binding to platelets have been well studied in humans and rabbits. The specific binding of ^{3}H-PAF to whole human platelets (4, 16) and rabbit platelet membranes (5) is saturable, is reversible, and occurs rapidly, reaching equilibrium within minutes at 0°C. Human platelets have about 100–400 PAF receptors/cell, which bind PAF with a dissociation constant (K_d) of 1–3 n*M* (17).

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Received December 18, 1989. [P.S.E.B.M. 1990, Vol 195]
Accepted June 21, 1990.

0037-9727/90/1952-247$2.00/0

In vitro, PAF will stimulate neutrophil degranulation, chemotaxis, adherence, respiratory burst, and arachidonic acid turnover (18, 19). Although PAF-specific binding sites have been described on human, bovine, guinea pig, and rabbit neutrophil membranes (5, 10, 11, 20, 21), the neutrophil PAF receptor has been less well characterized by pharmacologic methods than has the platelet receptor for PAF. Recent data have indicated that the PAF receptor is not the same on the human platelet and human neutrophil. The rank orders of potencies of several PAF analogs and receptor antagonists to inhibit ^{3}H-PAF binding to human neutrophil and platelet membranes were different, suggesting the existence of PAF receptor subtypes in these cells (15). Whether PAF receptor subtypes exist in platelets and neutrophils from the rabbit, a widely used animal model for the study of PAF function, is not known. A species difference in the rabbit and human platelet PAF receptor has been previously demonstrated (22).

Because of the importance of the rabbit as an animal model to study PAF function, we have developed a rabbit neutrophil membrane radioreceptor binding assay for PAF. Characteristics of the rabbit neutrophil membrane receptor for PAF are described in this report. In addition, the rank orders of potencies of known PAF antagonists to inhibit binding of ^{3}H-PAF to the neutrophil and platelet receptors were examined to determine whether the PAF receptors on these two cell types are similar.

Materials and Methods

Chemicals. 1-*O*-Alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine prepared from bovine heart lecithin (mixture of C_{16} and C_{18} chain length) was purchased from Sigma Chemical Co. (St. Louis, MO). 1-*O*-[*hexadecyl*-1′,2′-^{3}H]Hexadecyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine (30–60 Ci/mmol) was purchased from DuPont/New England Nuclear (Wilmington, DE). Kadsurenone (5-propyl-2(3, 4-dimethoxyphenyl-3a,α-methoxy-3-methyl-2,3,3a,6-tetrahydro-6-oxobenzofuran) and L-652,731 (*trans*-2,5-bis(3,4,5-trimethoxyphenyl)-tetrahydrofuran) were gifts from Merck, Sharp & Dohme Research Laboratories (Rahway, NJ). The benzodiazepines triazolam (8-chloro-6-(2-chlorophenyl)-1-methyl-4*H*[1,2,4]-triazolo[4,3-a][1,4]benzodiazepine) and alprazolam (8-chloro-1-methyl-6-phenyl-4*H*[1,2,4]-triazolo[4,3-a][1,4]benzodiazepine) were gifts from the Upjohn Co. (Kalamazoo, MI). CV-3988 (*rac*-3-(*N*-*n*-octadecyl-carbamoyloxy)-2-methoxypropyl-2-thiazolioethyl phosphate) was a gift from Takeda Chemical Industries Ltd. (Osaka, Japan). WEB-2086 (3-(4-(2-chlorophenyl)-9-methyl-6*H*-thieno[3,2-f][1,2,4]-triazolo-[4,3-a][1,4]-diazepine-2-yl]-1-(4-morpholinyl)-1-propanone) and brotizolam (2-bromo-4-(2-chlorophenyl)-9-methyl-6*H*-thieno[3,2,-f][1,2,4]-triazolo [4,3-a] [1 , 4]diazepine were gifts from Boehringer-Ingelheim (Ingelheim an Rhein, FRG). BN-52021 (9*H*-1,7a-(epoxymethano)-1*H*,6a*H*-cyclopenta-[C]furo-(2,3-b)furo-[3′,2′:3,4]cyclopenta-[1,2-d]furan-5,9,12-(4*H*)-trione, 3-*tert*-butylhexahydro-4,7b-11-trihydroxy-8-methyl) was a gift from IHB-IPSEN Institute for Therapeutic Research (Les Plessis Robinson, France). ONO-6240(1-*O*-hexadecyl-2*RS*-*O*-ethyl-3-*O*-(7-thiazolioheptyl)-glycerol methanesulfonate) was obtained from Ono Pharmaceutical Co., Ltd. (Osaka, Japan). The chemical structures of these compounds are shown in Figure 1.

Preparation of Rabbit Platelet Membranes. Female New Zealand White rabbits (Hazleton Research Products, Denver, PA) were bled via cardiac puncture. Blood (400–500 ml) was collected in 50-ml polypropylene syringes containing 4 ml of acid-citrate-dextrose (citric acid, 13.6 g/liter; sodium citrate, 25 g/liter; dextrose, 20 g/liter) solution. Platelet-rich plasma was obtained by centrifuging blood at 700*g* for 20 min at 22°C. To obtain a platelet pellet, platelet-rich plasma was centrifuged at 900*g* for 20 min at 22°C. The platelet

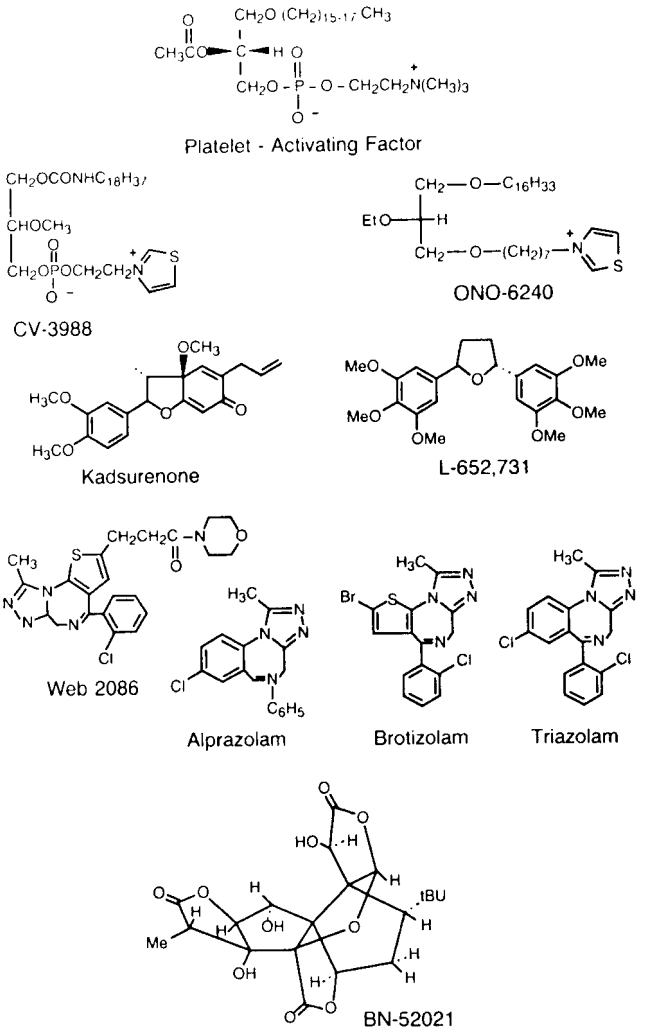

Figure 1. Chemical structures of PAF and PAF antagonists.

pellet was washed twice by centrifugation in saline at 900*g* for 20 min at 22°C. The final platelet pellet was resuspended in ice-cold buffer containing 150 m*M* choline chloride, 5 m*M* magnesium chloride, 10 m*M* Tris (pH 7.4), and 1 m*M* EDTA. The platelets were lysed by freeze-thawing three times in dry ice and ethanol. A platelet membrane pellet was obtained by centrifugation of the lysed platelets at 40,000*g* for 30 min. The platelet membranes were resuspended in 150 m*M* choline chloride, 10 m*M* Tris (pH 7.4), 1 m*M* EDTA, at a protein concentration of 1 μg/μl, and stored in 2-ml aliquots at −70°C.

Preparation of Rabbit Neutrophil Membranes. Five female New Zealand White rabbits were temporarily restrained on their backs and given an intraperitoneal infusion of 200 ml of 0.1% glycogen in sterile saline. After 16 hr, the rabbits were again temporarily restrained and given an intraperitoneal infusion of 200 ml of sterile saline containing 10,000 units of sodium heparin. After the infusion, the rabbits were inverted and peritoneal fluid was allowed to drain through an 18-gauge needle into 50-ml polypropylene tubes. Approximately 400–500 ml of peritoneal fluid were obtained from five rabbits after such a procedure. The peritoneal fluid contained leukocytes and a few erythrocytes (93–97% polymorphonuclear neutrophils by light microscopy). The 400 ml of peritoneal neutrophil/saline solution collected were centrifuged at 1000*g* for 20 min at room temperature. The peritoneal neutrophil pellet was resuspended in saline and centrifuged at 1000*g* for 20 min at room temperature. The peritoneal neutrophil pellet was resuspended in 20 ml of ice-cold buffer containing 150 m*M* choline chloride, 10 m*M* Tris (pH 7.4), 10 m*M* magnesium chloride, and 1 m*M* EDTA. The total number of neutrophils in two of the receptor preparations was determined with the use of a Coulter counter (model S Plus IV; Curtin Matheson Scientific, Inc., Houston, TX). These data were then combined with the total receptor number, obtained from Scatchard analysis, to determine the total number of receptors per neutrophil (Table I). The cells were broken by sonication with five 5-sec bursts on a cell disruptor (model M220; Heat Systems-Ultrasonics, Inc., Plainview, NY). Verification that the cells were broken was obtained by light microscopy. A peritoneal neutrophil membrane pellet was obtained by centrifugation of the broken cell suspension at 40,000*g* for 30 min at 5°C. The resulting peritoneal neutrophil membrane pellet was resuspended in 150 m*M* choline chloride, 10 m*M* Tris (pH 7.4), 1 m*M* EDTA, at a protein concentration of 0.1 μg/μl, and stored in 2-ml aliquots at −70°C.

Rabbit Neutrophil and Platelet Membrane Radioreceptor Binding Assay. ^{3}H-PAF (0.18 n*M*) and varying concentrations of either unlabeled PAF or PAF receptor antagonists were added to 12- × 75-mm polypropylene assay tubes, in a total volume of 40 μl of ethanol. The addition of 100 μg of membrane protein in 500 μl of 150 m*M* choline chloride, 10 m*M* Tris (pH 7.4), with 1 m*M* EDTA and 0.25% bovine serum albumin (BSA), started the reaction. Tubes were incubated for 1 hr at room temperature. After 1 hr the reaction was stopped with the addition of 4 ml of ice-cold buffer containing BSA (as described above) and the mixture was filtered through presoaked 2.5-cm Whatman GF/C glass fiber filters placed on a Millipore filtration apparatus (Millipore, Bedford, MA) attached to a house vacuum line. The assay tubes were rinsed twice with 4 ml of ice-cold buffer containing BSA. The rinses were also filtered through the GF/C glass fiber filters. The ^{3}H-PAF bound to membrane receptors was retained by the filters. The filters were placed in 20-ml glass scintillation vials containing 10 ml of PCS liquid scintillation cocktail (Amersham, Arlington Heights, IL), and radioactivity was quantitatively determined by liquid scintillation counting. Assays were performed in duplicate or triplicate. Each compound was evaluated in at least three binding assays. Each drug was tested using at least two different receptor preparations.

For Scatchard analysis to assess the total, specific, and nonspecific binding of ^{3}H-PAF, 0.03–3.0 n*M* ^{3}H-PAF was incubated with 100 μg of membrane protein for 1 hr at room temperature in the presence or absence of a 1000-fold excess of unlabeled PAF. Nonspecific binding was defined as the amount of ^{3}H-PAF bound after incubation of increasing concentrations of ^{3}H-PAF with a 1000-fold excess of unlabeled PAF. Specific

Table I. K_d and B_{max} for Rabbit Peritoneal Neutrophil and Platelet Membrane Protein

Cell membrane preparation	K_d (n*M*)[a]	B_{max} (pmol/mg protein)	No. of receptors/cell
Platelet	0.87 ± 0.092[b] (5)	0.739 ± 0.359 (5)	ND[c]
Neutrophil	0.41 ± 0.045[d] (6)	1.32 ± 0.11 (6)	7354, 4675[e]

[a] Numbers in parentheses, number of receptor preparations tested.
[b] Mean ± SE.
[c] ND, not determined.
[d] Significantly different at $P < 0.05$, using Student's *t* test.
[e] Determined from two different receptor preparations.

binding was computed as the difference between total and nonspecific binding.

Metabolism of ^{3}H-PAF in Rabbit Platelet and Neutrophil Preparations. One-hundred micrograms of rabbit platelet or neutrophil membranes in 500 μl of buffer containing 150 m*M* choline chloride, 1 m*M* EDTA, 0.25% BSA, and 10 m*M* Tris (pH 7.4), were incubated for 1 hr at 25°C with 0.045 μCi of ^{3}H-PAF in 12- × 75-mm polypropylene assay tubes. The reaction was stopped by the addition of 4.5 ml of methanol. Samples were extracted according to the method of Bligh and Dyer (23) and subjected to thin layer chromatography performed on Analtech precoated silica gel G plates with a chloroform/methanol/water (65:35:4, v/v/v) solvent system. Three-millimeter fractions of the silica gel were scraped into scintillation vials and radioactivity was determined in each fraction by liquid scintillation counting. The migration position of PAF was determined using tritiated PAF as a marker. The migration position of γ-*O*-hexadecyl lysophophatidycholine (lyso-PAF) (Sigma) was determined by placing the silica gel G plate in an environment of iodine vapor.

Protein Determination. Protein was determined according to the method of Lowry *et al.* (24)

Data Analysis. Scatchard analysis was performed to determine K_d and B_{max} values for PAF binding in each receptor preparation. K_d values were computed by fitting a linear regression to pooled data from each cell type, with separate intercept values for each experiment but with a common slope. This method controls for experiment to experiment error in intercept value and gives the best estimate of slope using pooled data. The standard error of the K_d was computed as a function of the slope estimate and its standard error. Differences between the two groups were determined with a Student's *t* test.

IC_{50} values were generated from competitive displacement curves as follows. Data from all the experiments of the particular drug tested were pooled. The IC_{50} values were computed by fitting a four-parameter logistic nonlinear regression model that has IC_{50} as one of its parameters:

$$Y = \frac{D - A}{1 + \left(\frac{X}{\log C}\right)_B} + A$$

where D is the maximum, A is the minimum, B is the slope, and C is the IC_{50} of a sinusoidally shaped curve. In this report, X equals the log concentration of the PAF antagonist and Y equals the percentage of inhibition of binding. The software package ALLFIT (25) was used to perform this analysis and returned both parameter estimates and standard errors of those estimates. K_i values were computed as $K_i = IC_{50}/(1 + L/K_d)$, where L is the free tracer concentration.

Results

Metabolism of ^{3}H-PAF in Rabbit Platelet and Neutrophil Membranes. Greater than 92% of the radioactivity from both the neutrophil and platelet membrane extracts added to the thin layer chromatography plates was recovered in the fractions that comigrated with the tritiated PAF standard. These data indicate that minimal metabolism of radioligand was occurring under the standard incubation conditions used in this study.

^{3}H-PAF Binding to Rabbit Neutrophil and Platelet Membranes. The saturation isotherm of ^{3}H-PAF binding to rabbit neutrophil membranes is summarized in Figures 2 and 3. The binding of 0.18 n*M* ^{3}H-PAF to the neutrophil membranes was rapid, reaching equilibrium within 10 min at room temperature (Fig. 2). This equilibrium was maintained for up to 120 min. This experiment was repeated four times with similar results. The binding of ^{3}H-PAF to the neutrophil membranes was reversible, as demonstrated by the decrease in ^{3}H-PAF binding at 60 min after the addition of 1000-fold excess unlabeled PAF 30 min into the incubation (Fig. 2). Subsequent experiments examined ^{3}H-PAF binding at 1 hr of incubation, during the equilibrium period.

Figure 3 shows the saturation isotherm of ^{3}H-PAF to one preparation of rabbit neutrophil membranes. Specific binding of ^{3}H-PAF to the neutrophil membranes was saturable and reached its maximum at about 1 n*M* ^{3}H-PAF. The nonspecific binding was relatively high and linear over the concentration range of ^{3}H-PAF tested. At a concentration of 0.18 n*M* ^{3}H-PAF, the nonspecific binding was approximately 20–25% of the total binding. Scatchard analysis of ^{3}H-PAF binding to rabbit neutrophil membranes from one representa-

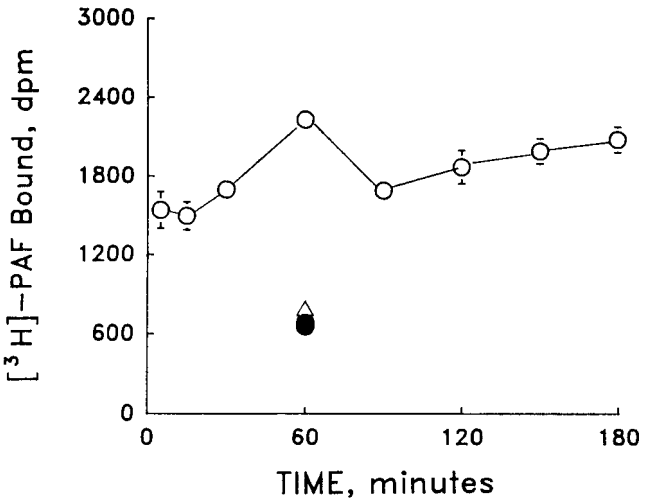

Figure 2. Time course of ^{3}H-PAF binding to rabbit neutrophil membranes. Rabbit neutrophil membrane protein (100 μg) was incubated with ^{3}H-PAF (0.18 n*M*) at room temperature (○). Data are mean ± SE of triplicates. At 0 min (△) or 30 min (●), a 1000-fold excess of unlabeled PAF was added to the incubation mixture and ^{3}H-PAF binding was determined at 60 min.

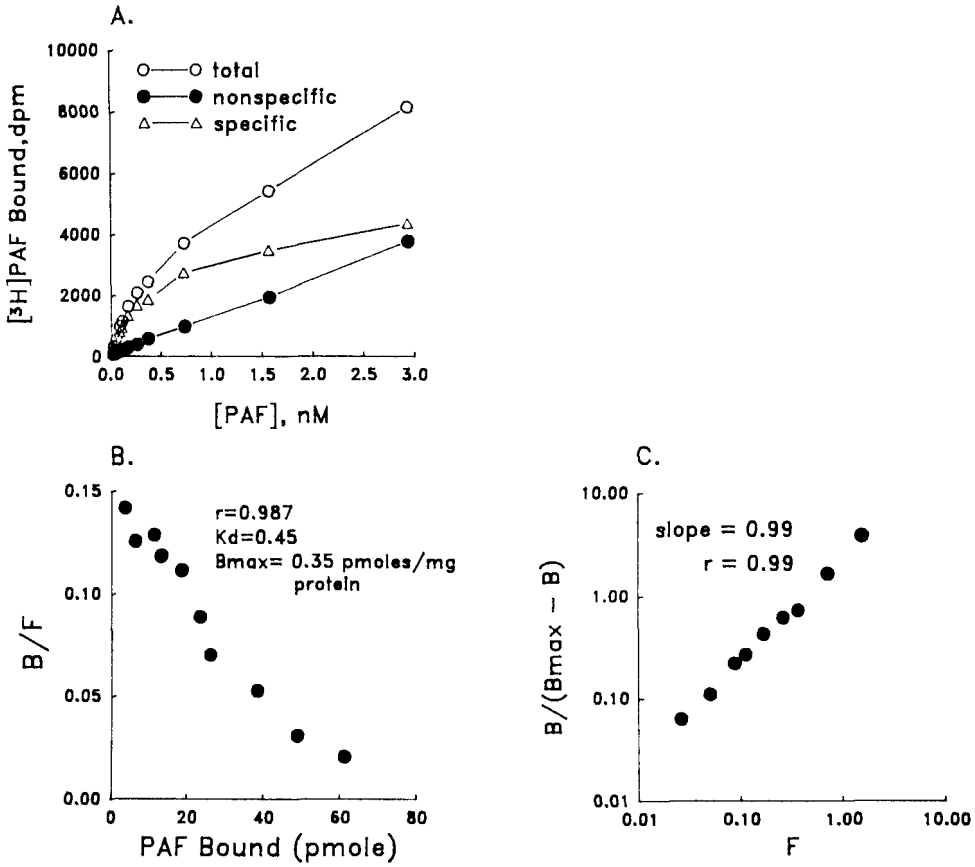

Figure 3. ^{3}H-PAF binding to rabbit neutrophil membranes. (A) ^{3}H-PAF (0.03–3.0 n*M*) was incubated in the presence of 100 μg of membrane protein at room temperature in the presence (●) or absence (○) or a 1000-fold excess of unlabeled PAF (△, specific binding). (B) Scatchard analyses of the specific ^{3}H-PAF binding. (C) Hill plot of ^{3}H-PAF specific binding. *B*, bound; *F*, free.

tive receptor preparation (Fig. 3B) revealed the presence of a single high-affinity binding site for PAF with an apparent K_d of 0.45 n*M* and a B_{max} of 0.35 pmol of receptor/mg of protein. The total number of receptor sites per neutrophil was found to be 4675 and 7354 for two of the receptor preparations tested (Table I). A Hill coefficient was calculated to be approximately 1 (Fig. 3C), indicating that the binding of ^{3}H-PAF to its receptors on rabbit neutrophil membranes does not involve positive or negative cooperativity. The binding of 0.18 n*M* ^{3}H-PAF was linear up to 200 μg of neutrophil membrane protein (data not shown).

Table I summarizes the K_d (equilibrium dissociation constant) and B_{max} (the number of PAF receptor sites) values for the rabbit peritoneal neutrophil and platelet membrane preparations used in this report. The saturation isotherm of PAF binding to rabbit platelet membranes has been reported in detail elsewhere (26). The affinity of PAF for rabbit platelet membranes was less than the affinity of the mediator for rabbit neutrophil membranes. The B_{max} values obtained for the individual peritoneal neutrophil and platelet membrane preparations were not significantly different.

Inhibition of ^{3}H-PAF Binding by PAF and PAF Antagonists. Figures 4 and 5 show the inhibition of ^{3}H-PAF binding to rabbit platelet and neutrophil membranes by PAF receptor antagonists. K_i values are listed in Table II. A strong positive correlation (r = 0.97) existed between the K_i values of the antagonists for inhibition of ^{3}H-PAF binding in the peritoneal neutrophil and platelet membrane preparations (Fig. 6).

Effects of Na^+ on ^{3}H-PAF Specific Binding. The effects of Na^+ on ^{3}H-PAF specific binding to rabbit platelet and neutrophil membranes is shown in Figure 7. ^{3}H-PAF specific binding to rabbit platelet membranes was reduced in the presence of 15–120 m*M* Na^+, whereas minimal effects of Na^+ were observed on PAF specific binding to the neutrophil membranes.

Discussion

PAF is a potent inflammatory agent. Part of the inflammatory activity of PAF may be attributed to its ability to activate neutrophils. PAF causes neutropenia *in vivo* and stimulates neutrophil aggregation, degranulation, chemotaxis, adherence, respiratory burst, and arachidonic acid turnover *in vitro* (1–3). Inhibition of these effects of PAF on neutrophils by PAF receptor antagonists suggests that they occur via a receptor-mediated process (14, 15). The present report describes an assay for and characteristics of a rabbit peritoneal

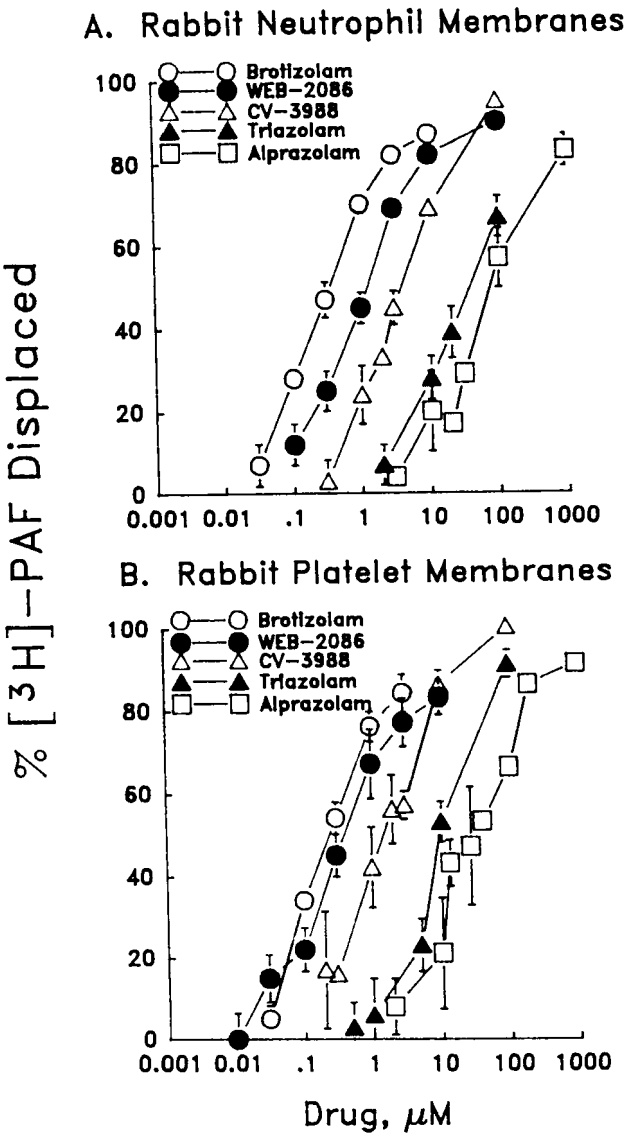

Figure 4. Inhibition of ^{3}H-PAF specific binding to rabbit neutrophil (A) and platelet (B) membranes. Data are the mean of three to five experiments. Each experiment was performed in duplicate or triplicate.

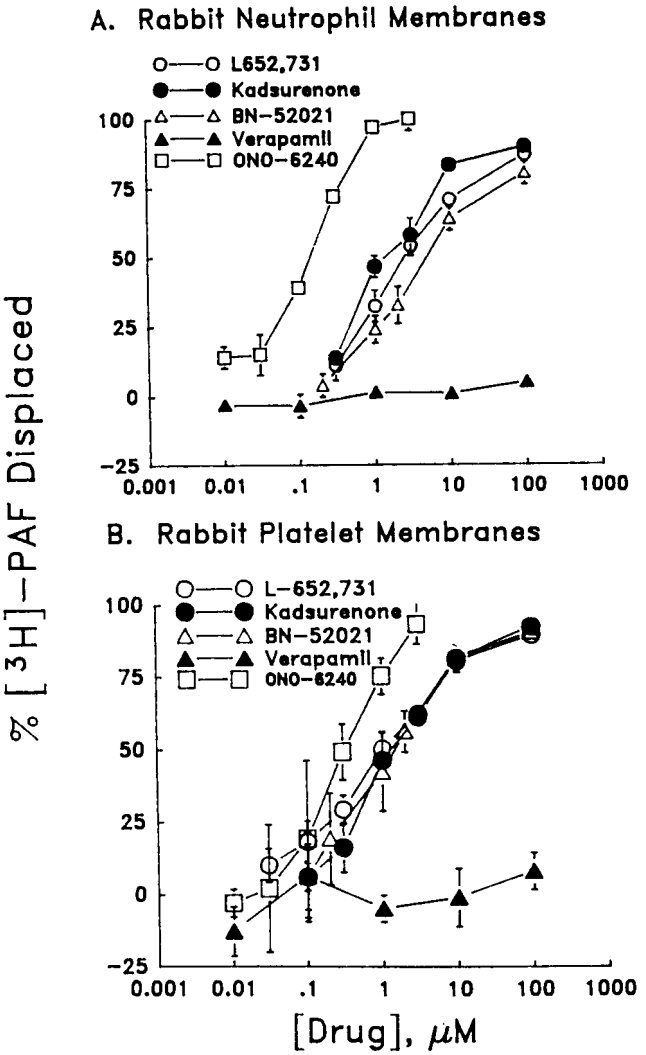

Figure 5. Inhibition of ^{3}H-PAF specific binding to rabbit neutrophil (A) and platelet (B) membranes. Data are the mean of three to five experiments. Each experiment was performed in duplicate or triplicate.

neutrophil membrane receptor for PAF. In addition, with the use of several known PAF antagonists and incubation of membrane preparations with NaCl, data are presented that indicate the rabbit platelet and neutrophil membrane receptors for PAF are different.

Receptors for PAF have been previously identified on human peripheral blood neutrophils (11, 15, 27, 28), guinea pig peritoneal neutrophils (5), and bovine blood neutrophils (5). In this report, we describe the presence of PAF receptors on rabbit neutrophils. We found PAF binding to rabbit peritoneal neutrophils that occurred rapidly, reaching equilibrium within 30 min. The binding of 0.18 nM ^{3}H-PAF was proportional to the amount of neutrophil membrane protein present in the incubation for up to at least 200 μg of membrane protein. Scatchard analysis of PAF specific binding to

Table II. K_i (Mean ± SE) of Several PAF Receptor Antagonists to Inhibit the Binding of [^{3}H]PAF to Rabbit Platelet and Neutrophil Membranes

PAF antagonist	K_i (μM)	
	Platelet membranes	Neutrophil membranes
Brotizolam	0.08 ± 0.05	0.15 ± 0.02
ONO-6240	0.21 ± 0.06	0.10 ± 0.02
WEB-2086	0.23 ± 0.03	0.70 ± 0.08
L-652,731	0.66 ± 0.10	0.86 ± 0.59
Kadsurenone	0.72 ± 0.21	0.39 ± 0.30
BN-52021	1.22 ± 0.19	2.51 ± 0.54
CV-3988	1.73 ± 0.38	2.85 ± 0.26
Triazolam	6.36 ± 0.92	13.46 ± 3.65
Alprazolam	11.85 ± 6.83	4.62 ± 5.29
Verapamil	Inactive	Inactive

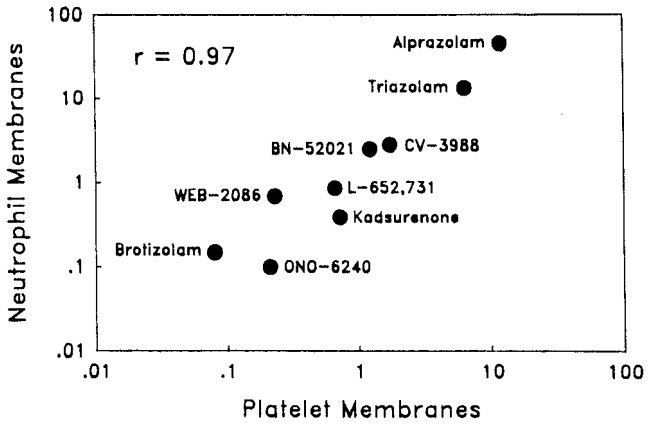

Figure 6. Plot of the log K_i values for PAF antagonist displacement of ^{3}H-PAF binding to rabbit neutrophil versus rabbit platelet membranes.

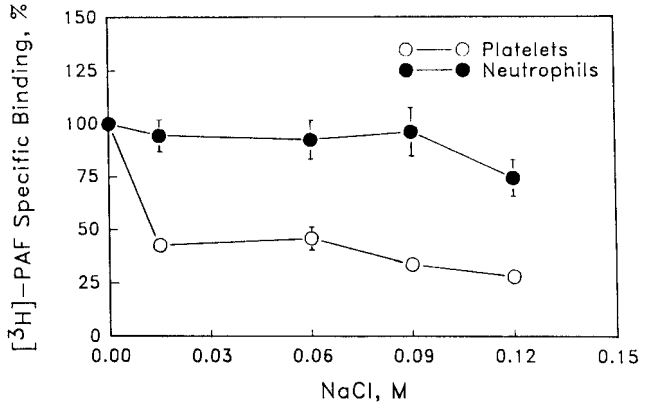

Figure 7. Effect of 15–120 m*M* NaCl on ^{3}H-PAF specific binding to rabbit neutrophil (●) and rabbit platelet (○) membrane. The incubation conditions were the same as described in Materials and Methods, except receptor preparations were incubated in the presence of the designated concentration of sodium chloride instead of choline chloride. Each curve is the mean of three different experiments. Each experiment was performed in duplicate or triplicate. Two-way analysis of variance revealed a significant effect of both Na^+ ($F = 71.76$, $P < 0.0001$) and dose ($F = 10.81$, $P < 0.0001$).

rabbit peritoneal neutrophils revealed the presence of a single high-affinity binding site, with an affinity (K_d) of 0.41 ± 0.45 n*M*. There were 0.33 ± 0.11 pmol of PAF receptor/mg of neutrophil membrane protein or approximately 4600–7000 binding sites/cell. Human neutrophils have been reported to have 1000–2000 binding sites/cell (28). Similar to our findings, most reports concerning neutrophils describe the presence of a single high affinity binding site, with a K_d between 0.3 and 3 n*M*, and approximately 0.1 to 10 pmol of PAF receptor/mg of protein (5, 11, 15). One study (28) has described the presence of two binding sites on human blood neutrophils. The presence of two binding sites for PAF has also been reported on human platelets by some investigators (29), but others disagree (5, 26).

We found the binding of PAF to its rabbit neutrophil membrane receptor to be reversible. PAF binding to human whole platelets (8, 30) and rabbit platelet membranes (5) is also reversible. Previous reports have observed PAF binding to intact human neutrophils (27) and P388D$_1$ macrophages (13) to be irreversible. One possible explanation for these differences is that, in intact neutrophils and macrophages, ^{3}H-PAF may be susceptible to internalization and metabolism, whereas less metabolism of PAF occurs in intact platelets. The metabolism of PAF is less likely to occur in isolated membrane preparations, as demonstrated with the neutrophil and platelet membrane preparations used in the present study.

Because of the multiple molecular species of PAF that have been identified (1, 2), the possibility of the existence of subclasses of PAF receptors has been suggested (15). Kadsurenone, a PAF receptor antagonist, was observed to distinguish between the functional activities of the guinea pig peritoneal macrophage and the pig blood neutrophil (31), suggesting the existence of receptor subtypes. However, from those data, it is difficult to discern whether the discrepancies are related to cell type or to species differences. More recent evidence indicates that PAF receptor subclasses exist within a single species (15). The rank orders of potencies of PAF antagonists and analogs to inhibit ^{3}H-PAF binding to human platelets and neutrophils have been found to differ (15). In addition, PAF-stimulated GTPase activity in human leukocytes, but not in human platelets, is sensitive to pertussis and cholera toxins (15). These data provide strong evidence that subclasses of the PAF receptor exist in these two human cell types. In the present study, we found a strong correlation in the rank orders of potencies of PAF antagonists to inhibit ^{3}H-PAF binding to rabbit platelet and peritoneal neutrophil membranes, indicating that the PAF receptors in these two cell types are similar. Interestingly, we find the affinity of PAF for rabbit neutrophil membranes to be higher than that for rabbit platelet membranes. These data suggest that there are subtle differences in the rabbit platelet and neutrophil PAF receptors. In addition, the presence of 15–120 m*M* Na^+ in the incubation mixture inhibited specific ^{3}H-PAF binding to platelet membranes but had minimal effect upon ^{3}H-PAF binding to the neutrophil preparation. Similarly, in humans, Na^+ inhibits ^{3}H-PAF specific binding to platelet membranes and has no effect upon ^{3}H-PAF specific binding to neutrophil membranes (15). Whether this effect of Na^+ on PAF binding in the present report is an ionic effect or an effect on a signal transduction system (adenylate cyclase) requires further study. However, Na^+ has been shown to be required for PAF-stimulated GTPase activity in rabbit platelets (32). Also, Na^+ has been shown to decrease ligand binding affinity to membrane receptors that are linked to adenylate cyclase (32).

It should be noted that "elicited" peritoneal neutrophils were used in the study instead of the circulating

"nonelicited" cells. It is possible that the PAF receptor in elicited and nonelicited neutrophils may be different. Because PAF is released during inflammation, the PAF receptor on the elicited neutrophil may be a more "physiologically functional" receptor than the receptor on the blood-borne cell.

In conclusion, we find a single class of high affinity binding sites for PAF on rabbit neutrophil membranes. ^{3}H-PAF bound to rabbit neutrophil membranes can be readily displaced with known PAF receptor antagonists, suggesting that these binding sites are receptors. In addition, similar to observations in humans, we find differences between the rabbit platelet and neutrophil PAF receptors. This observation is important, considering that the rabbit has been used as a model in the development of PAF receptor antagonists.

We are grateful to Ms. Nancy Oliver, Ms. Janet Bojan, and Ms. Monica Velgos for the typing of this manuscript. We appreciate the technical assistance of Ms. Geri Kocan.

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