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Evidence for Platelet-Activating Factor Receptor Subtypes on Human Polymorphonuclear Leukocyte Membranes

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Abstract. Platelet-activating factor (PAF) is a potent phospholipid mediator that acts through specific cell surface receptors. The existence of PAF receptor subtypes has been suggested by functional and radioligand binding studies in a variety of cells and tissues. This report addresses this issue more directly and demonstrates differences between specific PAF receptors in human polymorphonuclear leukocytes (PMNs) and COS-7 cells transfected with the cloned human PAF receptor gene. The presence of more than one receptor in human PMNs is supported by three different studies. First, the K_d from the saturation isotherms for the binding of [^{3}H]WEB 2086 on PMNs was 7-fold larger (K_d = 29.2 nM) than the kinetic K_d (4.2 nM). Second, the pseudo-Hill slope determined from the saturation experiments with PMNs was significantly lower than unity (0.69 ± 0.05 SEM), and the saturation K_d values for transfected COS-7 (K_d = 9.6 nM) and PMN membranes were significantly different. These results contrasted with those for the transfected COS-7 cells, which showed a K_d from the saturation isotherms similar to that of the kinetic K_d (3.2 nM) and a pseudo-Hill slope that was not different from 1.0. Third, when the radiolabeled ligand [^{3}H]WEB 2086 was increased in concentration from 10 to 50 nM in inhibition experiments with the human PMN membranes, the K_i increased, indicative of binding mainly to receptors with lower affinity. These results suggest that PAF receptor subtypes exist in human PMNs based on distinct radioligand binding characteristics from the human cloned PAF receptor. BIOCHEM PHARMACOL **54**;9: 1007–1012, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. radioligand binding; platelet-activating factor; G-protein coupled receptor; polymorphonuclear leukocytes

PAF;¶ (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphorylcholine) is a potent phospholipid mediator produced by many cell types, such as PMNs, monocytes, macrophages, mast cells, platelets, and endothelial cells [1]. The effects of this potent autacoid have been shown to go well beyond activation of platelets, and include recruitment and activation of inflammatory cells, bronchoconstriction, anaphylactoid reactions, and increased vascular permeability [2]. Its numerous biologic actions are mediated mainly through specific cell surface receptors. Using radioligand techniques, specific PAF receptor sites have been identified on a variety of inflammatory cells including platelets, eosinophils, and PMNs, as well as lung, brain, heart, and reproductive tissue [1]. A PAF receptor cDNA was cloned from guinea pig lung tissue [3] and was cloned subsequently from human inflammatory cell cDNA libraries and genomic DNA [4–6]. All human clones reported to date have identical sequences throughout the coding region.

PAF receptors are known to transduce their many activities through several different intracellular pathways. It is clear that different aspects of PMN activation, such as enzyme secretion, chemotaxis, generation of superoxide, and "priming," use different signal transduction pathways. The simultaneous activation of two distinct signal transduction mechanisms by PAF has been demonstrated, one (calcium mobilization) being insensitive to pertussis toxin and another (polyphosphoinositol hydrolysis) being sensitive to pertussis toxin [7]. Pinckard *et al.* [8] demonstrated that for PMN priming the agonists C16:0 alkacetyl PAF and C16:0 acylacetyl PAF differ less than an order of magnitude in potency; however, for lysosomal enzyme secretion, C16:0 alkacetyl PAF is very potent, whereas C16:0 acylacetyl PAF is inactive. In addition low concentrations of PAF stimulate Ca^{2+} mobilization, whereas

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¶ *Abbreviations:* B_{max}, density of binding sites; C16:PAF, 1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phosphorylcholine; C18:PAF, 1-*O*-octadecyl-2-acetyl-*sn*-glycero-3-phosphorylcholine; DEAE, diethylaminoethyl; DMEM, Dulbecco's modified Eagle's medium; HBSS, Hanks' buffered salt solution; K_d, dissociation constant; PAF, platelet-activating factor; and WEB 2086, 3-[4-(2-chlorphenyl)-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.

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PAF-induced exocytosis involves high concentrations. Radioligand binding studies using neutrophils, [^{3}H]PAF, and several different antagonists yielded evidence for at least two and possibly three distinct binding sites for PAF with dissociation constants (K_d) of approximately 0.3 nM, 10 nM, and a very low affinity site at >100 nM [9–11]. These findings are alternatively supportive of the possibilities of PAF receptor subtypes or by the same receptor having different membrane orientations and/or associations with intracellular proteins and thereby having different affinities.

The aim of this study was to transfect COS-7 cells with the cloned human PAF receptor gene, establish the ligand binding characteristics for the expressed receptors, and compare the binding characteristics with those for PAF receptors on human PMNs. We reasoned that if the human PMNs express more than one type of PAF receptor, as suggested by functional data, the ligand binding characteristics of these receptors will show evidence of more than one site in contrast to the cloned PAF receptor transfected in the COS-7 cells. On the other hand, if single receptors routinely show multiple affinities based on orientation in the cell membranes, multiple affinities should also be evident by radioligand binding techniques in the transfected COS-7 cells.

MATERIALS AND METHODS

Expression of Human PAF Receptor in COS-7 Cells

To construct the expression plasmid of the human PAF receptor (pBC12BI-PAF), a 1090 bp *Hin*dIII–*Stu*I fragment containing the coding region for the human PAF receptor [5] was cloned into the eukaryotic expression vector pBC12BI (Stratagene, La Jolla, CA). COS-7 cells were grown at 37°, under 5% CO_2 in a humidified atmosphere in DMEM supplemented with 10% fetal bovine serum and 100 U/mL each of penicillin and streptomycin (DMEM complete). The plasmid pBC12BI-PAF was transiently transfected into COS-7 cells by the DEAE-dextran method. Briefly, COS-7 cells were plated at 1.5×10^6 cells/100-mm tissue culture dish. On the following day, the cells were rinsed with PBS and were incubated with DEAE-dextran medium containing pBC12BI-PAF (0.5 mg/mL of DEAE-dextran and 5 μg/mL pBC12BI-PAF) for 20 min at 37°. The DEAE-dextran solution was aspirated off, and 10 mL of DMEM complete containing 0.1 mM chloroquine was added for 2.5 hr at 37°. The chloroquine-containing medium was aspirated off, and the cells were exposed to 10% DMSO solution (1 mL DMSO and 9 mL DMEM complete) for 2.5 min at 37°. After two rinses with PBS, the cells were grown in DMEM complete, and PAF receptor binding was assayed 48 hr after transfection.

PMN Isolation

Blood (200 mL) was obtained from the antecubital vein of healthy, normal, non-smoking adult male and female donors who had not taken any medication for at least 2 weeks and had not donated a unit of blood in the preceding 2 months. The blood was mixed with 1 vol. of ACD (0.65 M citric acid, 0.85 M trisodium citrate, 0.11 M dextrose) to 5 vol. of blood and 2 U heparin/mL. PMNs were then isolated on Ficoll-Hypaque cushions according to established protocols [12]. Briefly, red blood cells were sedimented for 45 min with 4 mL dextran/20 mL blood. The leukocyte-rich upper layer was pooled and underlayered with Ficoll-Hypaque and then centrifuged at 400 *g* for 20 min. The supernatant was discarded, and the granulocyte pellets were suspended in Ca^{2+}/Mg^{2+}-free HBSS and centrifuged at 400 *g* for 12 min. This washing step was repeated. The final pellet was resuspended in 5% (v/v) fetal bovine serum/HBSS (with Ca^{2+}) and diluted to a final concentration of 2×10^7 cells/mL. The cell suspension was overlayed onto Percoll (1.090 g/mL) followed by centrifugation at 700 *g* for 20 min. The cell bands were removed, pooled, and pelleted at 400 *g* for 10 min. Cells were stored in aliquots at −80°. A small sample was removed for cytospin and differential (Diff-Quik, VWR Scientific Products, Westchester, PA). Samples less than 96% PMNs were discarded.

Pharmacologic Agents

The PAF species C16:PAF and C18:PAF were obtained from the Bachem Corp. (Torrance, CA; purity >99%). Tritium-labeled WEB 2086 ([^{3}H]WEB 2086, 10.5 to 14.1 Ci/mmol) was obtained from New England Nuclear (Boston, MA). Unlabeled WEB 2086 was a gift from Boehringer Ingelheim (Ridgefield, CT). All drugs were prepared in the assay buffer.

Radioligand Binding Assay

At the time of assay, transfected COS-7 or PMN cells were suspended in cold 50 mM Tris buffer, pH 7.2, and homogenized at 4° with three 15-sec bursts on a Polytron homogenizer at setting 7. Homogenates were centrifuged at 40,000 *g* for 15 min at 4°, and the resulting pellet was resuspended in 9 vol. of 50 mM Tris buffer, pH 7.2. Protein concentrations were determined by the bicinchoninic acid assay (Pierce, Rockford, IL). Specific binding of [^{3}H]WEB 2086 was determined using a rapid filtration assay [13]. The incubation medium for all radioligand binding assays was 50 mM Tris buffer containing 5 mM $MgCl_2$, 125 mM choline chloride, and 2.5 mg/mL BSA at pH 7.2 and was prepared as previously described [13]. Specific [^{3}H]WEB 2086 binding was determined experimentally from the difference between radioligand bound in the absence (total) and the presence (nonspecific) of 10 μM WEB 2086. The radioligand binding assays were performed at 4°. All measurements were made in triplicate in at least three independent experiments. Binding reactions were terminated by filtering the incubation medium over glass fiber filters (Whatman GF/B) using a single filter holder apparatus (model FH 224; Hoefer Scientific Instruments, San Francisco, CA). Each filter was rinsed three times with 4 mL of assay buffer. The

filters were presoaked in assay buffer for 30 min to decrease nonspecific binding to the filter. Receptor-bound radioactivity retained on the filter was extracted for 16 hr with 9 mL of liquid scintillation fluid (Cytoscint; ICN, Aurora, OH). Radioactivity of each sample was determined by liquid scintillation spectrophotometry.

For all studies with [^{3}H]WEB 2086, the assay volume was 1 mL, with a final protein concentration of 20–200 μg protein/mL for transfected COS-7 cells and 40–500 μg protein/mL for PMNs. The incubation time for both transfected COS-7 and PMN membranes was 16 hr for saturation and inhibition experiments. The concentration of [^{3}H]WEB 2086 for kinetic studies was 5 nM and for inhibition studies was 10 or 50 nM.

Data Analysis

Experimental data for the saturation and inhibition studies were analyzed for one-site and two-site binding models according to the law of mass action as described by Unnerstall [14] using the nonlinear fitting method of steepest descent to reach the lowest sum of squares supplied by PRISM (GraphPad Software, Inc., San Diego, CA). Nonspecific binding was calculated as a fitted parameter by linear regression analysis of the nonspecific binding data points. To facilitate comparisons between tissues and literature values, IC_{50} values were converted to K_i values using the Cheng and Prusoff equation (K_i = IC_{50}/1 + [free ligand]/K_d) [15]. Tests of significant difference between means were performed using the *t*-test. The association rate constant, k_{+1}, was calculated from the pseudo-first-order method as described by Bylund and Yamamura [16]. The k_{+1} was derived from the k_{obs} by nonlinear regression of the specific radioligand bound versus time of incubation. This method can be used when less than 10% of the radioligand added is bound. The dissociation rate constant, k_{-1}, was estimated from the linear least-squares regression analysis of the first-order plot of the logarithmically transformed data. The mean values for lognormal distributed data (i.e. K_d, K_i, IC_{50}) are reported as the geometric mean ×/÷ SEM.

RESULTS

The effect of tissue concentration on specific binding of 10 nM [^{3}H]WEB 2086 to transfected COS-7 cell and human PMN membranes was determined in initial experiments. For both cell types, specific binding of the radioligand was linearly dependent on the concentration of tissue from 20 to 200 μg protein/mL for transfected COS-7 cells and 40 to 500 μg protein/mL for PMNs (data not shown). The protein concentrations used for all subsequent binding assays were within these linear ranges.

The kinetics of [^{3}H]WEB 2086 (5 nM) binding to membranes of transfected COS-7 cells and PMNs were examined (Figs. 1 and 2). Steady-state binding of [^{3}H]WEB 2086 at the lowest concentration used in the saturation studies (5 nM) was observed within 6 hr at 4° for transfected COS-7 cell membranes and within 12 and 24 hr at 4° for PMN membranes. The association rate constant (k_{+1}) for transfected COS-7 cell membranes, as determined by pseudo-first-order analysis of association data, was 1.2 × 10^6 M^{-1} min^{-1} for N = 3. A time-dependent

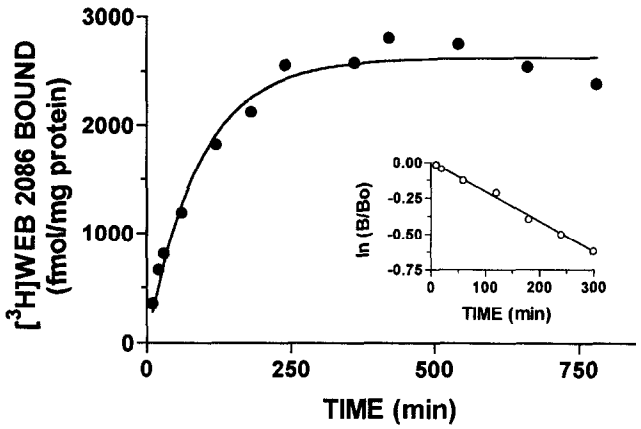

FIG. 1. Representative experiment of the time–course of association of [^{3}H]WEB 2086 (5 nM) binding to transfected COS-7 cell membranes (protein concentration = 34.2 μg/mL). Incubations were performed at 4°. The association rate constant (k_{+1}) determined by pseudo-first-order analysis of the data was 1.3 × 10^6 M^{-1} min^{-1}. Inset shows the dissociation of [^{3}H]WEB 2086 binding. WEB 2086 (10 μM) was added to the assay after preincubation with 5 nM [^{3}H]WEB 2086 for 8 hr. The amount of radioligand bound was then determined as a function of time after addition of WEB 2086. The data are plotted as the natural logarithm of the quotient of the amount (B) of [^{3}H]WEB 2086 bound at each time divided by the initial amount (B_0) of radioligand bound. The dissociation rate constant (k_{-1}) was derived from the slope of this plot as described under Materials and Methods. For this experiment, k_{-1} = 3.6 × 10^{-3} min^{-1}.

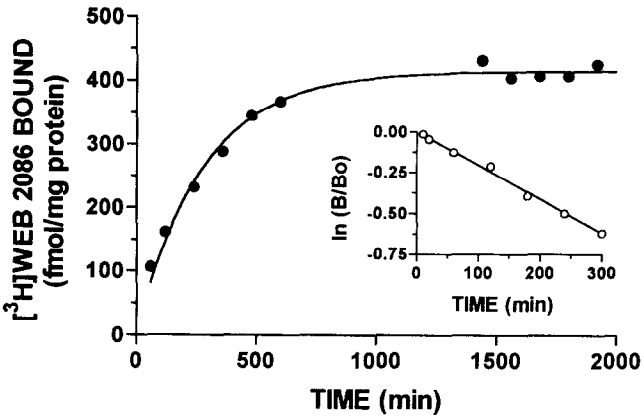

FIG. 2. Representative experiment of the time–course of association of [^{3}H]WEB 2086 (5 nM) binding to human PMN membranes (protein concentration = 92 μg/mL). Incubations were performed at 4°. The association rate constant (k_{+1}) determined by pseudo-first-order analysis of the data was 0.38 × 10^6 M^{-1} min^{-1}. Inset: WEB 2086 (10 μM) was added to the assay after preincubation with [^{3}H]WEB 2086 for 28 hr. The amount of radioligand bound was then determined as a function of time after the addition of WEB 2086. The data are plotted as described in the legend of Fig. 1. For this experiment, k_{-1} = 1.7 × 10^{-3} min^{-1}.

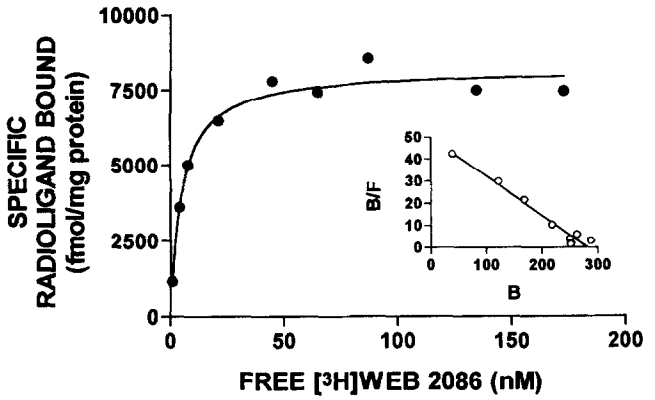

FIG. 3. Saturation isotherm experiment of [^{3}H]WEB 2086 binding in transfected COS-7 cell membranes (protein concentration = 34 μg/mL). Data from one of eight experiments are shown. Assay conditions were as described under Materials and Methods. Specific binding, best described by a one-site fit, was experimentally determined as the difference between total binding and nonspecific binding in the absence and presence of 10 μM WEB 2086. The inset shows a Rosenthal plot of specific [^{3}H]WEB 2086 binding data. Dissociation constant (K_d) = 4.7 nM; receptor density (B_{max}) = 8210 fmol/mg protein, where the abscissa is bound ligand (fmol) and the ordinate is bound over free ligand (fmol/nM).

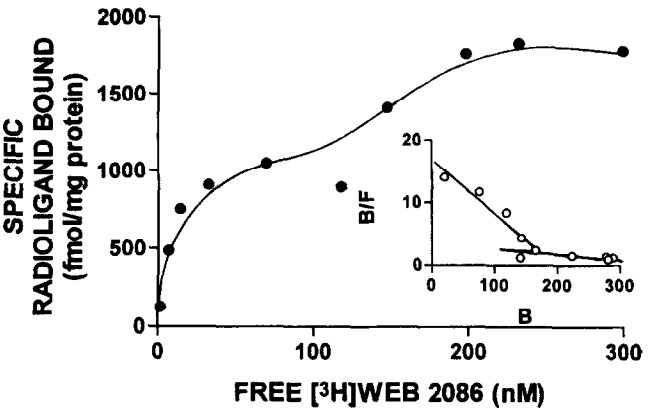

FIG. 4. Saturation isotherm of specific [^{3}H]WEB 2086 binding in PMN membranes (protein concentration = 158 μg/mL). Data from one of eleven experiments are shown. Assay conditions were as described in Materials and Methods. Specific binding, best described by a one-site fit, was experimentally determined as the difference between total binding and nonspecific binding in the absence and presence of 10 μM WEB 2086. The inset shows a Rosenthal plot of specific [^{3}H]WEB 2086 binding data. Dissociation constant (K_d) = 28.0 nM; receptor density (B_{max}) = 1740 fmol/mg protein, where the abscissa is bound ligand (fmol) and the ordinate is bound over free ligand (fmol/nM).

decrease in receptor-bound radioligand was observed after the addition of 10 μM WEB 2086 to COS-7 membranes previously incubated for at least 8 hr with 5 nM [^{3}H]WEB 2086 (Fig. 1, inset). Dissociation of the ligand–receptor complex occurred with a dissociation rate constant (k_{-1}) of 3.8×10^{-3} min^{-1} for N = 3. The kinetic K_d (k_{-1}/k_{+1}) determined from transfected COS-7 cells was 3.2 nM (range 2.3 to 4.5 nM) for a pseudo-first-order association. For PMNs, the association rate constant (k_{+1}) determined by pseudo-first-order analysis of association data was 0.37×10^6 M^{-1} min^{-1} for N = 3. Dissociation of the ligand–receptor complex occurred with a dissociation rate constant (k_{-1}) of 1.6×10^{-3} min^{-1} for N = 3 (Fig. 2, inset). The K_d value for [^{3}H]WEB 2086 calculated from the kinetically derived rate constants was 4.2 nM (range 2.5 to 7.1 nM).

Saturation isotherm experiments with [^{3}H]WEB 2086 binding were performed in both transfected COS-7 cell membranes and PMN membranes (Figs. 3 and 4). Non-transfected COS-7 cell membranes had no detectable specific binding for [^{3}H]WEB 2086, confirming the absence of endogenous PAF receptors (data not shown). The K_d and B_{max} are presented in Table 1. The K_d for binding to COS-7 cell membranes was consistent with the kinetic K_d for these cells. In contrast the saturation K_d for binding to PMN membranes showed a much lower affinity than the kinetic K_d ($P < 0.005$). Also, the pseudo-Hill slope for PMNs, in contrast to that for the COS-7 cells, was significantly less than unity ($P < 0.0005$).

In inhibition studies, the radioligand binding of [^{3}H]WEB 2086 was inhibited in a concentration-dependent manner by the agonist, C16:PAF, and the antagonist, unlabeled WEB 2086, on both cell types. Inhibition curves are shown in Fig. 5, and pseudo-Hill slopes, IC_{50} values and K_i values are listed in Table 2. The slope on the agonist inhibition curves for PMN membranes was shallow with a Hill coefficient less than unity as expected ($P < 0.05$). In contrast, C16:PAF binding to receptors on transfected COS-7 cell membranes showed relatively steep inhibition curves. This was also true for C18:PAF (pseudo-Hill coefficient = 0.91 for N = 6). In PMNs the K_i increased substantially as the concentration of the antagonist, [^{3}H]WEB 2086, was increased from 10 to 50 nM ($P < 0.025$).

DISCUSSION

The studies presented herein were performed to compare the radioligand binding characteristics of the cloned human PAF receptor transfected into COS-7 cells and PAF receptors produced endogenously in human PMNs. The results of these studies are supportive of the presence of more than one PAF receptor in human PMNs in contrast to the single receptor subtype transfected into COS-7 cells.

The presence of more than one receptor in human PMNs is supported by three different studies. First, the K_d from the

TABLE 1. Saturation isotherm data of [^{3}H]WEB 2086 binding in PMN and transfected COS-7 cell membranes

Membrane	K_d* (nM)	B_{max}† (fmol/mg protein)	Hill slope†	N
COS-7	9.6 (0.6)	4470 (826)	0.86 (0.1)	8
PMN	29.2‡ (0.7)	1300 (120)	0.69§ (0.05)	11

* K_d values represent the geometric mean with geometric SEM in parentheses.
† Values for B_{max} and Hill coefficients represent the arithmetic mean (±SEM).
‡ $P < 0.005$, significantly different from the K_d for transfected COS-7 cells.
§ $P < 0.0005$, significantly different from unity.

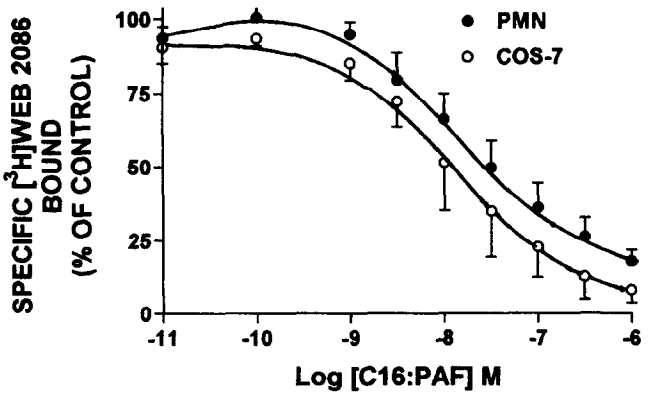

FIG. 5. Inhibition of [^{3}H]WEB 2086 (10 nM) by C16:PAF in transfected COS-7 cell and human PMN membranes. Assay conditions were as stated under Materials and Methods. Each point represents the mean (±SEM) of three experiments for COS-7 membranes and four experiments for PMN membranes. The absolute values corresponding to 100 percent specific [^{3}H]WEB 2086 bound were 950 dpm for PMNs and 1430 dpm for COS-7 cells. Pseudo-Hill slopes and K_i values are presented in Table 2.

saturation isotherms for the binding of [^{3}H]WEB 2086 on PMNs was 7-fold larger (i.e. lower affinity) than the kinetic K_d. This difference would be expected in the case of receptor subtypes because the low concentration of [^{3}H]WEB 2086 (5 nM) used in the kinetic studies would bind primarily to the high affinity site, whereas the K_d from the saturation isotherms would be affected by a second site provided a ligand concentration sufficiently near the K_d of the second site was included in the experiment. Second, the pseudo-Hill slope determined from the saturation experiments with [^{3}H]WEB 2086 on PMNs was significantly lower than unity and the saturation K_d values for this antagonist on COS-7 and PMN membranes were significantly different, also suggestive of more than one receptor subtype. These results contrasted with those for the transfected COS-7 cells, which showed a K_d from the saturation isotherms similar to that of the kinetic K_d and a pseudo-Hill slope that was not different from 1.0. Third, when the radiolabeled ligand [^{3}H]WEB 2086 was increased in concentration from 10 to 50 nM in inhibition experiments with the human PMN membranes, the K_i increased, indicative of binding in the latter case mainly to receptors with lower affinity. Thus, these data provide strong support for additional PAF receptor subtypes in human PMNs. The argument that identical receptors in cells may show varying affinities even for antagonist depending on their orientation in the membrane and/or association with different proteins is weakened by the homogeneity of binding revealed in the transfected COS-7 cells.

Others have suggested that more than one PAF receptor subtype may exist [9–11, 17–21], with supportive evidence from comparing various cells expressing endogenous receptors. However differences noted in K_d values might be attributable to inherent differences in the distribution of receptors associating with different proteins in the cells. Therefore, the strategy employed here provides stronger evidence of subtypes in that a direct comparison of cells with endogenous receptor was made with cells that have been transfected so as to express only one PAF receptor subtype. Indeed, these COS-7 cells transfected with a single cloned receptor showed binding curves indicative of ligand interacting with a single homogeneous receptor for both agonist and antagonist. The studies with agonist thus suggest that the PAF receptor in transfected COS-7 cells is not interacting with a variety of different membrane proteins that alter its affinity for ligand. It was our prediction that transfected COS-7 cells would show a shallow pseudo-Hill slope with PAF inhibition of [^{3}H]WEB 2086, especially given the high density of transfected receptors and thus increasing the possibility of many being uncoupled. However, this was not the case, and the data suggest that the PAF receptors in transfected COS-7 cells are in a single conformational state. A similar pseudo-Hill slope was determined by Plassat *et al.* [22] for the 5HT5 receptor with the agonist serotonin.

Several studies have reported cloning the same human PAF receptor as that reported initially by Honda *et al.* [3] and analogous to that originally reported in the guinea pig [4–6]. No clones for distinct additional PAF receptor subtypes have been reported. Nevertheless, this fact does not preclude the possibility that additional PAF receptors exist. It only argues against the possibility that, if other receptor subtypes exist, they are likely not closely homologous in DNA sequence to the one already identified. The data presented here support the possibility that the high

TABLE 2. Inhibition of [^{3}H]WEB 2086 binding in transfected COS-7 and PMN membranes

	[^{3}H]WEB 2086 (nM)	COS-7 membranes				PMN membranes			
		IC_{50}* (nM)	K_i* (nM)	Hill slope†	N	IC_{50}* (nM)	K_i* (nM)	Hill slope†	N
C16:PAF	10	14.1 (2.2)	6.9 (2.2)	0.98 (0.13)	3	21.2 (1.3)	10.4 (1.3)	0.77‡ (0.08)	4
WEB 2086	10	12.7, 14.9	6.2, 7.3	0.83, 0.85	2	9.2 (1.4)	4.5 (1.4)	0.80 (0.13)	4
WEB 2086	50	ND§	ND	ND	ND	182.0 (0.9)	29.5‖ (0.9)	0.93 (0.09)	3

* Inhibitory constant values were calculated using the Cheng and Prusoff formula [15] and are given as the geometric mean with geometric SEM in parentheses.

† Hill slope values represent the arithmetic mean of the Hill slope (±SEM) unless N = 2 for which individual values are reported.

‡ $P < 0.05$, significantly less than unity.

§ ND, not determined.

‖ $P < 0.025$, significantly different from the K_i for PMNs at 10 nM [^{3}H]WEB 2086.

affinity receptor on PMNs may be analogous to the cloned receptor and that at least one other lower affinity receptor appears to be present.

Thus, we report several differences in radioligand binding characteristics for endogenously produced PAF receptors in human PMNs when compared to COS-7 cells transfected with the one PAF receptor cloned to date. These findings do not provide definitive proof for the existence of additional PAF receptor subtypes in human PMNs, but the antagonist binding studies, in particular, provide strong support for that possibility. Given the usual cautions in interpretation that derive from small sample size and the indirectness of the radioligand approach, we nonetheless suggest that the agonist affinity data in transfected COS-7 cells suggest that the single receptor type in high density appears to be present in a single conformational state. Our results emphasize that continued efforts to identify additional PAF receptor subtypes by cloning techniques are important in order to increase our understanding of the interaction of proinflammatory mediators, their receptors, and the pathways used for intracellular signaling and subsequent biologic functions.

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