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Heterogeneous Platelet-Activating Factor (PAF) Receptors and Calcium Increase in Platelets and Macrophages

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ABSTRACT. We used the increase in cytosolic Ca^{2+} levels, $[Ca^{2+}]_i$, as a way to characterize PAF (platelet-activating factor, 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) receptors in human platelets and rat and human macrophages. $[Ca^{2+}]_i$ was measured by means of the fluorescent probe fura-2/acetoxymethylester. PAF recognized heterogeneous receptors in human macrophages only (curve slope <1). The PAF antagonist SCH 37370 (1-acetyl-4(8-chloro-5,6-dihydro-11H-benzo[5.6]cyclohepta[1,2-*b*]pyridine-11-ylidine)piperidine) abolished $[Ca^{2+}]_i$ elevation in human platelets, while in rat and human macrophages the maximal inhibition was 76% and 85%, respectively. On the contrary, the antagonist WEB 2086 (3-[4-(2-chlorophenyl)-9-methyl-6H-thieno[3,2-f] [1,2,4]triazolo-[4,3-a] [1,4]-diazepin-2-yl]-1-(4-morpholiny)-1-propanon, apafant) totally inhibited the effect of PAF in both platelets and macrophages. The WEB 2086 concentration–response curves had a slope <1 in the three cell types, indicating interaction with heterogeneous receptors. Accordingly, ^{3}H-WEB 2086 bound to two different classes of sites. Both phases of $[Ca^{2+}]_i$ elevation (influx or release) were equally affected by the antagonists. These data support the notions that: 1) PAF receptors are heterogeneous; 2) the two antagonists have a different selectivity toward the receptor subtypes: WEB 2086 recognizes two different receptors both in platelets and in macrophages, while SCH 37370 does not discriminate between receptor subtypes in platelets, and only interacts with one subtype in macrophages; and 3) both SCH 37370 and WEB 2086 display different potencies in rat and human macrophages. BIOCHEM PHARMACOL **57**;3:263–271, 1999.

KEY WORDS. PAF receptors; SCH 37370; WEB 2086; cytosolic calcium; platelets; macrophages

PAF‡ is a potent lipid mediator, generated by a variety of cells. It is involved in various pathophysiological processes, such as platelet activation, reproduction, inflammation, allergy, asthma, ischemia, and shock [1–4]. PAF acts on various cell types, including platelets and macrophages, through specific membrane receptors, coupled to several transduction systems [4, 5]. It has been demonstrated in many cell types that PAF triggers a transient increase in the levels of $[Ca^{2+}]_i$, which depends not only on release from organelles, but also on influx of the ion through the plasma membrane (see [5] for a review).

A single type of PAF receptor has been cloned so far [6–9], but pharmacological studies suggest that the receptors might be heterogeneous: for instance, selected antagonists displayed different potencies or affinities in different cell types [11, 12] or even within the same cell type, when two separate PAF-induced responses were studied [12, 13].

SCH 37370 [14, 15] and WEB 2086 (apafant) [16] are among the most potent PAF antagonists *in vitro* and *in vivo*. SCH 37370 is a dual antagonist of PAF and histamine; in human platelets, it selectively inhibits PAF-induced aggregation with an IC_{50} = 0.6 μM and competes with PAF specific binding (IC_{50} = 1.2 μM) in human lung membranes [14]. WEB 2086 is a selective inhibitor of PAF-induced platelet aggregation (IC_{50} = 0.17 μM) [16]; data obtained in guinea pig macrophages indicate binding to two different classes of receptors (K_{is} = 1.95 nM and 0.30 μM) [17].

We used SCH 37370 and WEB 2086 to investigate the nature and the possible heterogeneity of PAF receptors coupled to cytosolic calcium ion increase in human platelets and in human and rat macrophages.

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‡ *Abbreviations:* $[Ca^{2+}]_i$, cytosolic Ca^{2+} levels; PAF, platelet-activating factor, 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine; SCH 37370, 1-acetyl-4(8-chloro-5,6-dihydro-11H-benzo[5.6]cyclohepta[1,2-*b*]pyridine-11-ylidine)piperidine; WEB 2086, 3-[4-(2-chlorophenyl)-9-methyl-6H-thieno[3,2-f] [1,2,4]triazolo-[4,3-a] [1,4]-diazepin-2-yl]-1-(4-morpholiny)-1-propanon, apafant; K_d, dissociation constant; K_i, dissociation constant for an inhibitory compound; B_{max}, maximal binding; HBS, Hepes-buffered saline; % CV, percent coefficient of variation; and fura-2/AM, fura-2/acetoxymethylester.

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MATERIALS AND METHODS

Isolation of Rat Peritoneal Macrophages

Resident peritoneal macrophages were obtained from male Sprague–Dawley rats (Charles River) and purified (purity >95%) essentially as described [18]. Briefly, macrophages were collected by peritoneal washing with PBS without Ca^{2+} and Mg^{2+}, pH 7.4. The cells were washed and resuspended to a concentration of approximately 5×10^6 cells/mL in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 2 mM L-glutamine. Macrophages were purified by adherence to glass coverslips, placed in Petri dishes (Ø 35 mm), by seeding 2.5 mL of the peritoneal cell suspension. The dishes were incubated overnight at 37°, in 95% air, 5% CO_2, and nonadherent cells (approximately 40%) were removed by repeated washing with PBS.

Human Monocyte Isolation and Differentiation to Macrophages

Venous blood, from healthy volunteers who gave informed consent, was anticoagulated with 3.8% sodium citrate, and mononuclear cells were separated and cultured as described [19]. Briefly, monocytes were purified from lymphocytes by adherence (2 hr at 37°, 95% air, 5% CO_2) on glass coverslips placed in Petri dishes. Cell preparation was >90% monocytes, as determined by nonspecific esterase staining. Differentiated macrophages were obtained by culturing monocytes for 7 days in Medium 199 with 2 mM of glutamine, 0.5% antibiotics and 10% autologous serum. Macrophages were identified by the presence of the CD68 antigen. Cell viability was >95% (trypan blue exclusion). Experiments were performed in Medium 199 from which serum had been omitted.

Isolation of Human Platelets

Venous blood (10 mL) was anticoagulated with 1.67 mL of ACD (sodium citrate 85 mM, citric acid 78.08 mM, glucose 111 mM) and centrifuged at 200 *g* for 18 min at room temperature. One aliquot of the platelet rich plasma was loaded with fura-2/AM; platelet poor plasma was obtained by centrifugation at 600 *g* for 15 min.

Loading with Fura-2

Adherent rat and human macrophages were incubated with 10 μM fura-2/AM and 0.06% Pluronic F-127 in HBS (145 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM KCl, 10 mM Hepes, and 10 mM glucose at pH 7.4) at 25°, for 45 and 85 min, respectively. Cell monolayers were rinsed three times with 2 mL of HBS and kept in HBS in the dark at 25°, until use. Platelet rich plasma was loaded with 1.5 μM fura-2/AM for 45 min at 37°. Platelets were then diluted 1:2 with ACD Erkfurt solution (sodium citrate 74.8 mM, citric acid 41.9 mM, glucose 136 mM) and centrifuged at 900 *g* for 15 min. The platelets were resuspended with platelet poor plasma. Immediately before fluorescence measurement, platelets were centrifuged and resuspended in HBS at 1.4×10^8 cells/mL. Control cells (used to measure autofluorescence) received DMSO alone.

$[Ca^{2+}]_i$ Measurement

Fluorescence (340 nm excitation, 505 nm emission) was measured both in adherent macrophages and in platelets in suspension under the same conditions, basically as already described [20]. The calibration was carried out by addition of 10 mM of $CaCl_2$, 2.3 μM 4-Br-A23187, and 100 μM digitonin (to obtain maximum fluorescence); 20 mM $MnCl_2$ was added to record the autofluorescence, from which minimum fluorescence was calculated according to Hesketh *et al.* [21]. Values for $[Ca^{2+}]_i$ were calculated from the observed fluorescence (F) according to Tsien *et al.* [22] and Grynkiewicz *et al.* [23].

Selected experiments were carried out on rat macrophages pretreated with 1 mM EGTA in the absence of extracellular Ca^{2+}, according to the Ca^{2+}-free/Ca^{2+}-reintroduction protocol [24, 25] already used by us in rat macrophages [26]. The first phase (release from intracellular stores) was revealed as a rapid and transient increase in $[Ca^{2+}]_i$ upon addition of 36 nM PAF. The antagonist under investigation (or the vehicle) was added either 5 min before or 2 min after PAF. The second more sustained phase (Ca^{2+} influx) was then evidenced by restoring normal extracellular calcium concentration with PAF still present.

[^{3}H]-WEB 2086 Binding in Rat Macrophages

Equilibrium binding experiments were performed on adherent rat macrophages ($6–10 \times 10^6$ cells/Petri dish, 35 mm Ø) at 25° for 1 hr in HBS (final volume 0.75 mL). Binding curves were performed according to a mixed type protocol, combining both saturation (the first 5 concentrations in the curves, 0.03–10 nM) and displacement (the last 4 concentrations, 100–10,000 nM) [27]. WEB 2086 was dissolved in DMSO and diluted in HBS (final DMSO concentration: 0.1% in all samples). The reaction was terminated by rapid aspiration and washing twice with 1 mL of ice-cold PBS. Cells were lysed with 10^{-1} M NaOH containing 0.025% Triton X-100 (0.75 mL). After overnight digestion at 25°, proteins were assayed on an aliquot and the radioactivity was measured in Ultima Gold. Nonspecific binding was calculated by LIGAND [28] (see data analysis) as one of the unknown parameters of the model, and ranged between 55–60% of the total binding of 10 nM [^{3}H]-WEB 2086. Binding is expressed as the ratio of bound concentrations over total concentration. Total concentration is the sum of "hot" and "cold" ligand and includes nonspecific binding.

Data Analysis

The data shown are either means ± SEM or curve parameters ± % CV. The concentration–response curves were analyzed and drawn by means of the computer program ALLFIT [29], which also allows the evaluation of the statistical significance of the difference either between parameters of various curves, or between a parameter and a given value, by means of the F test for the extra sum of squares, taking $P < 0.05$ as statistically significant. Equilibrium binding curves were analyzed and drawn by means of the computer program LIGAND [28], which allows calculation of the parameters (K_d and B_{max}) and selection of the best model (1-site versus 2-sites) by means of the F-test, as well as calculation of the confidence limits.

Materials

Fura-2/AM (Molecular Probes) was stored at −80° in anhydrous DMSO. 4-Br-A23187 (Sigma) and digitonin (Merck), dissolved in anhydrous DMSO, and PAF (Sigma), dissolved in absolute ethanol at 36 μM, were stored at −20°. Pluronic F-127 was from Molecular Probes. SCH 37370 was kindly provided by E. Ongini (Schering-Plough) and WEB 2086 by C. Rizzi, H.M. Jennewein and A. Walland (Boehringer Ingelheim). [^{3}H]-WEB 2086 was from NEN, Ficoll-Paque from Pharmacia Biotech AB, and PBS and Medium 199 from Bio-Whittaker. Dulbecco's modified Eagle's medium, L-glutamine and penicillin–streptomicin were from Sigma, fetal bovine serum from PBI International, Ultima Gold from Packard Instruments Co., and Comassie Protein Assay Reagent from Pierce. Buffer and saline solutions were prepared with water for HPLC.

RESULTS

Concentration–Response Curves for the Effect of PAF on Cytosolic Calcium Levels

The basal $[Ca^{2+}]_i$ values were 130 ± 3 SEM (N = 11), 181 ± 8 (N = 32), and 177 ± 4 nM (N = 13), in human platelets and rat and human macrophages, respectively. Basal values in macrophages, higher than in other cell types, are in agreement with data in the literature and can be explained by cell activation induced by plating and adhesion [30].

PAF (36 pM–0.1 μM) was able to trigger a concentration-dependent elevation of $[Ca^{2+}]_i$. In human platelets, PAF elicited a 4-fold maximal increase over the basal value. The EC_{50} was 1.49 nM ± 33% CV and the slope of the curve was 0.86 ± 29% (not significantly different from 1) (Fig. 1A).

The curve in rat peritoneal macrophages had an EC_{50} of 3.52 nM ± 87% and a slope of 0.81 ± 42% (not significantly different from 1). The maximal $[Ca^{2+}]_i$ increase was 3-fold over basal and the curve was bell-shaped, the inhibitory phase being apparent above 30 nM (Fig. 1B).

In human macrophages, the EC_{50} for the PAF-elicited $[Ca^{2+}]_i$ increase was 9.87 nM ± 41%. In this case, the slope of the curve was 0.58 ± 11% (significantly lower than 1, $P < 0.05$). The maximal $[Ca^{2+}]_i$ increase was approximately 3-fold over basal (Fig. 1C).

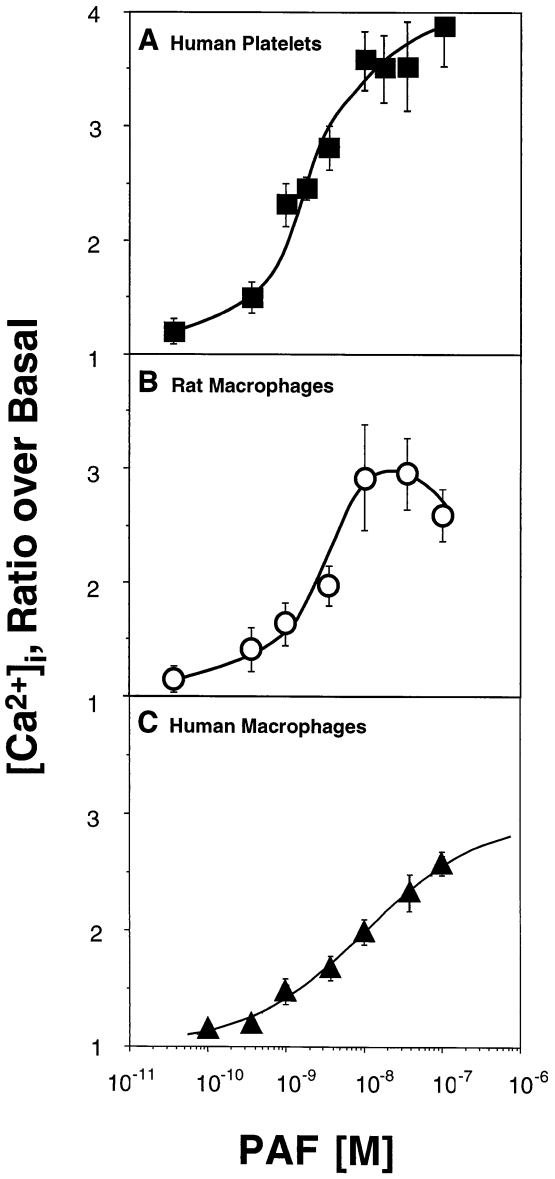

FIG. 1. Concentration–response curves for the effect of PAF on $[Ca^{2+}]_i$ in human platelets (A), rat macrophages (B), and human macrophages (C). Data are means ± SEM of 3 experiments, each run in duplicate or triplicate.

Effect of PAF Antagonists on $[Ca^{2+}]_i$ Elevation

PAF was used at 36 nM, a concentration maximally or near maximally effective in the three cell types (Fig. 1). In human platelets, SCH 37370 dose dependently inhibited PAF-induced elevation of $[Ca^{2+}]_i$ and the inhibition was complete at concentrations higher than 10 μM (Fig. 2A). The slope and IC_{50} are shown in Table 1. Interestingly, the IC_{50} is very close to that reported for inhibition of platelet

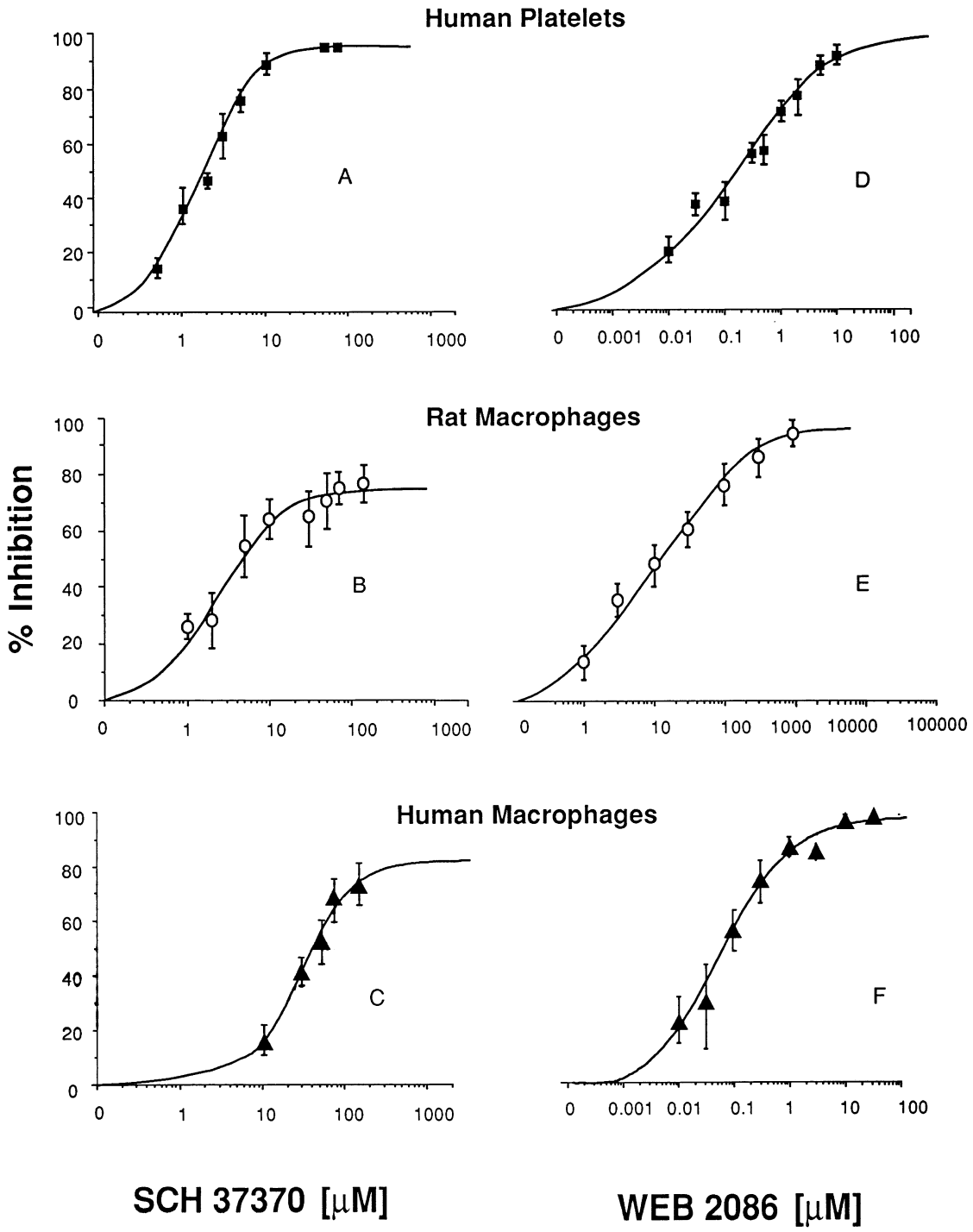

FIG. 2. Concentration–response curves for the effect of SCH 37370 (A–C) and WEB 2086 (D–F) on the $[Ca^{2+}]_i$ increase induced by 36 nM of PAF in human platelets (A and D), rat macrophages (B and E), and human macrophages (C and F). Data are means ± SEM of 3 (A, C, D, E) or 4 (B, F) experiments, each run in duplicate or triplicate.

aggregation and PAF binding. A slope significantly higher than 1 was observed and, although the significance of a high slope value is not known, steep concentration–response curves for both agonists and antagonists are very often observed in these cells.

In macrophages (Fig. 2B and C), SCH 37370 was unable to completely abolish the response to PAF; the maximal inhibition attained was approximately 76% and 85% in rat and human cells, respectively. Furthermore, SCH 37370 was significantly less potent ($P < 0.05$) in human macrophages than in the other cell types. The curve parameters are reported in Table 1.

At variance with SCH 37370, WEB 2086 at the highest concentrations tested totally inhibited the effect of PAF in

TABLE 1. Parameters of the concentration–response curves for the PAF antagonists

Antagonist	Cell type	IC_{50} (μM)	Max Inhibition %	Slope
SCH 37370	Platelets	1.67 ± 6.0%	100	1.24 ± 7.9%§
	Rat macrophages	2.39 ± 19.1%	75.7 ± 5.1%‡	0.98 ± 21.8%
	Human macrophages	29.00 ± 18.3%*	85.6 ± 9.5%	1.39 ± 19.3%
WEB 2086	Platelets	0.14 ± 17.6%†	100	0.49 ± 9.6%§
	Rat macrophages	24.90 ± 20.6%†	100	0.48 ± 11.3%§
	Human macrophages	0.08 ± 12.7%†	100	0.67 ± 8.3%§

Data are expressed as means ± CV % (coefficient of variation).
*$P < 0.05$ with respect to other cells.
†The IC_{50}s for WEB 2086 were significantly different from one another ($P < 0.05$).
‡$P < 0.05$ compared to 100%.
§$P < 0.05$ compared to 1.

all the cell types (Fig. 2D–F). The curve parameters are reported in Table 1. It is interesting to note that the slopes of the concentration–response curves for WEB 2086 were significantly lower than unity ($P < 0.05$) in all the cell types studied. The IC_{50} in platelets is practically superimposable with that obtained for inhibition of aggregation [16].

Effect of SCH 37370 and WEB 2086 on $[Ca^{2+}]_i$ Influx and Release

We investigated which of the two components of the PAF-induced $[Ca^{2+}]_i$ increase (release from intracellular stores and influx through the plasma membrane) was affected by SCH 37370 and WEB 2086. These experiments were performed in rat macrophages, where the two antagonists displayed markedly different behavior.

To this aim, the biphasic action of 36 nM of PAF was separated into its components by means of the Ca^{2+}-free/Ca^{2+}-reintroduction protocol (see Methods) [24–26] (Fig. 3). SCH 37370 or WEB 2086 was added either before PAF (Fig. 3C and E) to investigate their effect on both phases, or after the agonist (Fig. 3D and F) but before addition of Ca^{2+}, to investigate their effect on the second phase only. SCH 37370 and WEB 2086 were able to inhibit both the first and second phases markedly when added before PAF. When they were added after PAF, they were able to inhibit the second phase, i.e. Ca^{2+} influx. Such behavior suggests that the influx is at least partly receptor-mediated, although we cannot rule out that store-dependent Ca^{2+} influx is also operative [24, 25]. Quantitative analysis of the data is shown in Table 2.

Binding of ^{3}H-WEB 2086 to Rat Macrophages

To confirm the heterogeneity of PAF receptors, the binding of ^{3}H-WEB 2086 [17] was studied on rat macrophages, selected because they markedly differed in the behavior of the two antagonists, as already mentioned. Figure 4 shows that the mixed-type binding curve for ^{3}H-WEB 2086 is clearly biphasic; indeed, computerized analysis indicated the presence of two different sites with the following characteristics: $K_{d1} = 4.3 \times 10^{-11}$ M ± 48% CV, $B_{max1} =$ 4.2 fmol/mg prot ± 32% CV; $K_{d2} = 2.1 \times 10^{-8}$ M ± 44% CV, $B_{max2} = 512$ fmol/mg prot ± 37% CV.

DISCUSSION

It is widely accepted that PAF elicits most of its cellular effects through interaction with specific receptors [5, 31], usually coupled to $[Ca^{2+}]_i$ elevation. In particular, PAF-induced $[Ca^{2+}]_i$ elevation was reported in human platelets [32] and in rat [33] and human macrophages [34, 35]. The PAF-triggered Ca^{2+} response is considered an ideal index to monitor functional PAF receptors [36]. We have used it as a clear, although indirect, way to investigate possible differences in PAF receptor subclasses.

In human platelets, the PAF concentration–response curve had a slope not significantly different from unity (Fig. 1A), thus suggesting an interaction with a homogeneous class of receptors, in accordance with the data from binding studies [5]. In agreement with this hypothesis, the SCH 37370 curve did not span more than 2 orders of magnitude and inhibition was complete (Fig. 2A, Table 1). Conversely, the slope of the WEB 2086 curve was significantly lower than unity (Fig. 2D, Table 1), indicating interaction with more than one type of receptor. This apparent discrepancy can be reconciled if one hypothesizes that human platelets possess at least two receptor subtypes: WEB 2086, but not PAF or SCH 37370, would be able to discriminate between them.

The existence of heterogenous receptors is also supported by the results obtained in macrophages; in fact, not only are the slopes of WEB 2086 concentration–response curves lower than unity, both in rat and in human cells (Fig. 2E and F, Table 1), but SCH 37370 also displays peculiar behavior, being unable to inhibit the PAF response completely (Fig. 2B and C). Thus, of the two receptors present in macrophages, only one would be recognized by SCH 37370, while WEB 2086 would interact with both, albeit with different affinities. Furthermore, in accordance with this hypothesis, the binding of ^{3}H-WEB 2086 in rat macrophages clearly indicates the presence of two different binding sites (Fig. 4).

In rat macrophages, PAF does not clearly discriminate

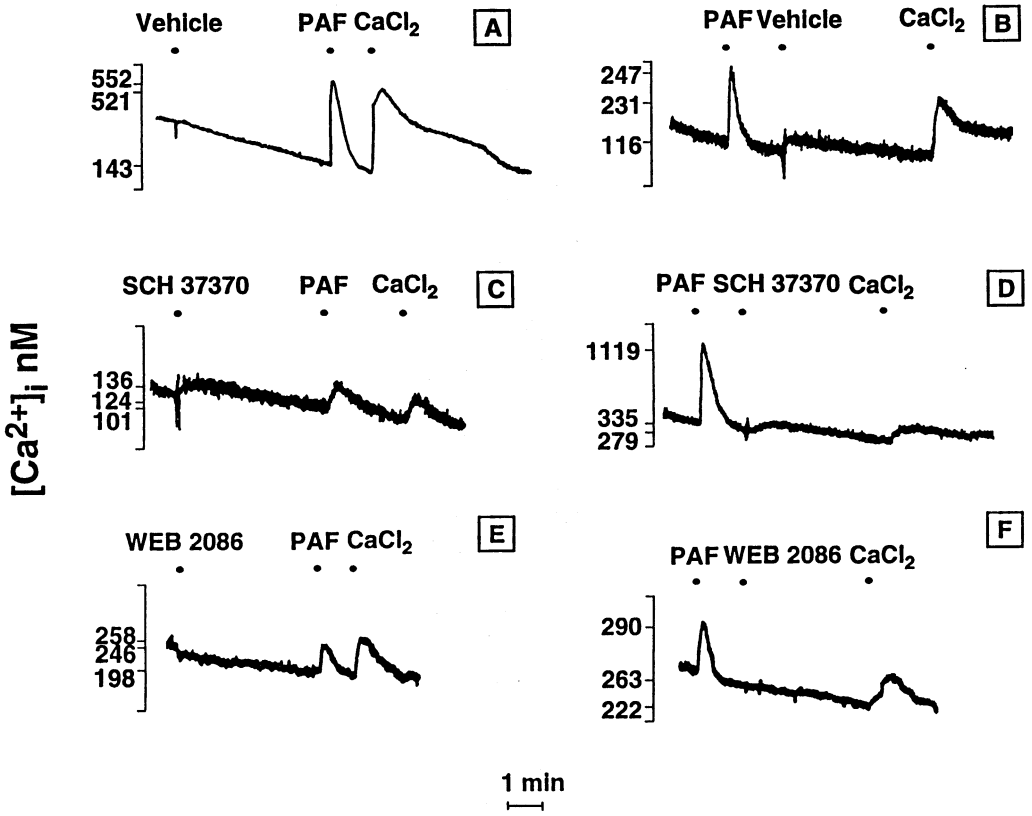

FIG. 3. Representative tracings of the effect of SCH 37370 and WEB 2086 on PAF-induced Ca^{2+} release and influx in rat macrophages. The experiments were carried out in nominally Ca^{2+}-free HBS to which was added 1 mM of EGTA to evaluate Ca^{2+} release; then $CaCl_2$ (2.5 mM) was added after PAF (36 nM) to reveal the Ca^{2+} influx (Ca^{2+}-free/Ca^{2+}-reintroduction protocol). The vehicle (3% DMSO) was added either before (panel A) or after 36 nM of PAF (panel B). Panels C and E: effect of 70 μM of SCH 37370 or 100 μM of WEB 2086, respectively, added before PAF. Panels D and F: effect of 70 μM of SCH 37370 or 100 μM of WEB 2086, respectively, added after PAF.

between the two receptors, similarly to what was observed in platelets: the curve slope is not significantly different from unity, although lower than same. On the contrary, the slope value in human macrophages attains a statistically significant difference from unity, in agreement with the existence of heterogeneous receptors.

In human platelets and in human and rat macrophages, as in other cell types, the PAF-induced $[Ca^{2+}]_i$ increase is attributable both to discharge from intracellular stores and to influx through the cell membrane (Fig. 3). Therefore, we investigated whether the differences observed between the two antagonists might be due to a different effect on the two phases of the PAF-induced Ca^{2+} response. In fact, it has been suggested that some PAF antagonists might have differential effects on Ca^{2+} influx or release [36]. This does not appear to be the case for either SCH 37370 or WEB 2086, because they display approximately the same effect on both components of the PAF-induced $[Ca^{2+}]_i$ increase

TABLE 2. Effect of SCH 37370 and WEB 2086 on PAF-induced calcium mobilization and influx in rat macrophages

	$[Ca^{2+}]_i$, ratio over basal		
Antagonists	**1st phase**	**2nd phase**	**n**
0	1.57 ± 0.15	2.13 ± 0.17	6
Added before PAF			
SCH 37370 (70 μM)	1.10 ± 0.10*	1.30 ± 0.10†	3
WEB 2086 (300 μM)	1.27 ± 0.09*	1.56 ± 0.11*	9
Added after PAF			
SCH 37370 (70 μM)	1.95 ± 0.39	1.50 ± 0.16*	3
WEB 2086 (300 μM)	1.45 ± 0.06	1.68 ± 0.14*	7

Data are means ± SEM.
*$P < 0.05$.
†$P < 0.01$ versus control (Student's *t*-test).

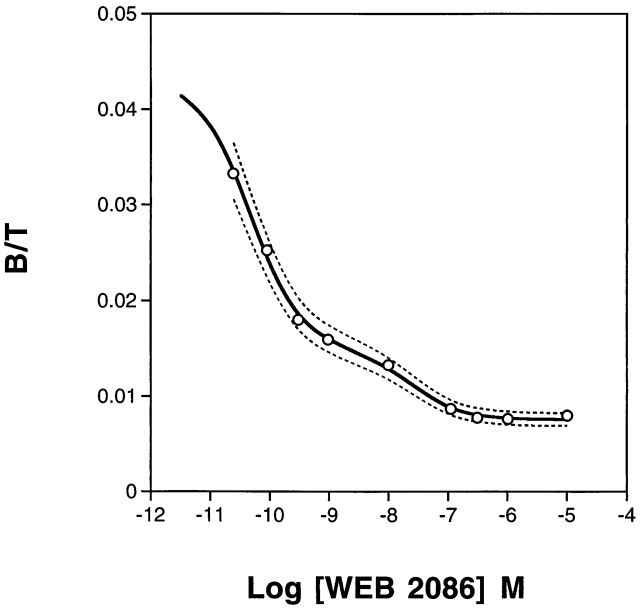

FIG. 4. Binding curve for ^{3}H-WEB 2086 on intact rat macrophages. Data are means of 3 experiments, each run in triplicate. B/T: ratio of bound (B) to total (T) ligand concentration. Dotted lines represent the 95% confidence limits.

(Fig. 3 and Table 2). In addition, at variance with loratadine, which is structurally related to SCH 37370 [26, 37], the effect of both antagonists is selective for PAF, because they are ineffective when ATP is used as agonist (data not shown).

The comparison of data obtained in rat and human macrophages suggests the existence of species differences in PAF receptors. Indeed, the potency of SCH 37370 and WEB 2086 significantly differed in the two cell types (Table 1): SCH 37370 displayed a higher potency in rat than in human cells, while the opposite was true for WEB 2086.

As already mentioned, evidence exists in the literature as to the heterogeneity of PAF receptors, not only in different species [38–40] or cell types [*, 11], but also within the same cell type [12].

It has been suggested that PAF, besides having a receptor on plasma membranes coupled to phospholipase C, might possess an intracellular receptor [5, 41] which can also be recognized by WEB 2086 [42]. We cannot rule out the possibility that one of the receptor subtypes identified here is actually intracellular and/or not accessible to SCH 37370; since the signal transduction for such an intracellular receptor has not been elucidated, our experimental approach does not allow us to address this issue. However, the binding experiments performed with ^{3}H-WEB 2086, which is cell-impermeant [16], suggest that both sites are located on plasma membranes.

* Numao T, Fukuda T, Akutsu I and Makino S, Possible existence of PAF receptor subtypes on human eosinophils and neutrophils. *Third International Conference on Platelet-Activating Factor and Structurally Related Alkyl Ether Lipids*. 1989.

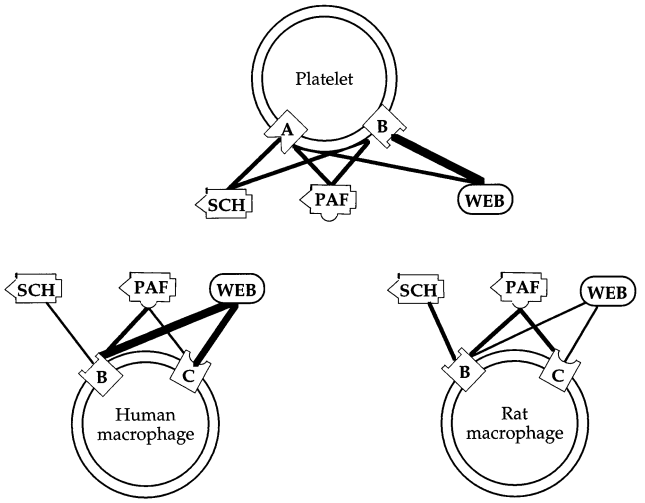

FIG. 5. Hypothetical model depicting the subtypes of PAF receptors in platelets and macrophages, as well as the interaction of PAF itself and the antagonists SCH 37370 and WEB 2086 with each receptor subtype. The thickness of the arrows is meant to be in relation with the ligand affinities.

In conclusion, the data reported here support the existence of multiple PAF receptor types, both within the same cell and in different cells. We cannot rule out that the observed differences might be explained by variations in either bioavailability or metabolism of the agents used in the various cell types; nevertheless, a difference in antagonist behavior or potency in a functional assay has been considered one of the major criteria for defining receptor subtypes [43].

A model that could account for the results we have obtained is shown in Fig. 5 (which is not meant to imply a definite subcellular localization of the receptors): platelets would express at least two receptor subtypes (A and B), while in macrophages the two receptor classes would be only partially coincident with those of platelets (B and C). The thickness of the arrows is meant to be in relation with the ligand affinities.

The heterogeneity of PAF receptors and the existence of selective antagonists might have important implications in the development of anti-PAF drugs with fewer side-effects.

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