Characterization of specific binding sites of ^3H -labelled platelet-activating factor (^3H PAF) and a new antagonist, ^3H SR 27417, on guinea-pig tracheal epithelial cells

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Binding of ^3H -labelled platelet-activating factor (^3H PAF) to guinea-pig tracheal epithelial cells was time-dependent, reversible and saturable. Scatchard analysis of the saturation-binding data indicated that ^3H PAF bound to one class of specific binding sites with high affinity ($K_D = 4.3 \pm 0.03 \text{ nM}$; $B_{\text{max}} = 0.172 \pm 0.02 \text{ fmol}/10^5 \text{ cells}$; $n = 3$). Unlabelled PAF competitively and selectively inhibited the specific binding of ^3H PAF, with 50% inhibition at $4.8 \pm 0.07 \text{ nM}$ ($n = 3$). SR 27417, the first member of a newly developed PAF antagonist series, competitively displaced ^3H PAF from its binding sites on guinea-pig tracheal epithelial cells with a K_i of $100 \pm 3 \text{ pM}$ ($n = 3$). Studies carried out in parallel demonstrated that SR 27417 was 40 times more potent than C_{16} -PAF itself and more than 100-fold as active as the best synthetic PAF-receptor antagonist yet described. ^3H SR 27417 displayed high-affinity, specific, reversible as well as saturable binding to a single class of binding sites on tracheal epithelial cells ($K_D = 94 \pm 7 \text{ pM}$; $B_{\text{max}} = 0.181 \pm 0.04 \text{ fmol}/10^5 \text{ cells}$; $n = 3$). C_{16} -PAF, lyso-PAF, enantio-PAF, SR 27417 and other PAF-receptor antagonists had K_i values which were nearly identical for both ^3H PAF and ^3H SR 27417, demonstrating that in guinea-pig tracheal epithelial cells they have the same binding sites. In conclusion, these data suggest that tracheal epithelial cells contain PAF-specific receptors and indicate that SR 27417 is an extremely potent PAF-receptor antagonist, as well as being a suitable radioligand for labelling PAF receptors on intact cells.

INTRODUCTION

The alkyl ether phospholipid platelet-activating factor (PAF-acether; PAF), identified as 1-*O*-alkyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholine (Benveniste *et al.*, 1972; Demopoulos *et al.*, 1979) is one of the most potent inducers of platelet aggregation known, inducing shape change, aggregation and secretion (Fouque & Vargaftig, 1984). It is clear now that the action of PAF is far broader than platelet activation only, since PAF has shown a wide variety of potent biological actions: bronchoconstriction, hypotension, increase in vascular permeability and negative inotropic cardiac effects (Braquet *et al.*, 1987a,b; Handley, 1988). Moreover, PAF is believed to be involved in allergic responses, asthma, anaphylactic and septic shocks and inflammation (Braquet *et al.*, 1987a,b; Handley, 1988). Specific PAF-receptor sites have been identified by using radioligand techniques in rabbit (Hwang & Lam, 1986; Robaut *et al.*, 1987), dog (Tahraoui *et al.*, 1988) and human platelets (Klopprogge & Akkerman, 1984; Hwang & Lam, 1986; Tahraoui *et al.*, 1990), human polymorphonuclear leucocytes (O'Flaherty *et al.*, 1986; Marquis *et al.*, 1988) and human lung (Hwang *et al.*, 1985). The development of specific PAF-receptor antagonists is therefore essential for further elucidation of the pathophysiological roles of PAF and for developing new drugs active in these situations. A large number of PAF antagonists have been identified in the past few years (for review see Hwang, 1990). These PAF antagonists appear to compete with specific binding sites present on platelets, lung or brain tissue, neutrophils or eosinophils, and their potencies correlated with their respective abilities to antagonize PAF-induced platelet aggregation, bronchospasm and hypotension (Braquet & Godfroid, 1986; Braquet *et al.*, 1987a,b). Recently Honda *et al.* (1991) described the cloning of the PAF receptor from the guinea-pig lung, but up to now no PAF-binding sites on bronchial or tracheal epithelial cells have been characterized. The aim of this study was therefore to

determine the binding characteristics of ^3H PAF to cultured guinea-pig tracheal epithelial cells and to compare its binding parameters with those of a newly developed PAF-receptor antagonist SR 27417.

MATERIALS AND METHODS

Chemicals

C_{16} -PAF (1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine), enantio-PAF and lyso-PAF were purchased from Bachem (Bubendorf, Switzerland). Stock solutions of PAF or its analogues were prepared in ethanol, and subsequent dilutions were made with Tyrode's solution containing 0.25% BSA. ^3H PAF (sp. radioactivity 80 Ci/mmol) was purchased from Amersham (Les Ulis, France). SR 27417 (fumarate salt) {*N*-(2-dimethylaminoethyl) - *N* - (3 - pyridinylmethyl)[4 - (2,4,6 - triisopropylphenyl)thiazol-2-yl]amine}, ^3H SR 27417 (12.7 Ci/mmol) and WEB-2086 (base, APAFANT) {3-[4-(2-chlorophenyl)-9 - methyl - 6*H* - thieno[3,2 - *f*][1,2,4]triazolo - [4,3 - *a*][1,4] - diazepin-2-yl]-1-(4-morpholinyl)propan-1-one} (Casals-Stenzel *et al.*, 1987) were synthesized in the chemistry laboratories of Sanofi Recherche. The structures of SR 27417 and WEB-2086 were determined by ^1H and ^{13}C n.m.r., m.s. and i.r. spectroscopy. Their purities were measured by h.p.l.c., t.l.c. and elemental analysis and were > 99%. 59227 RP, WEB-2170 and L 659989 were kindly given by Rhône-Poulenc Rorer (Vitry sur Seine, France), Boehringer Ingelheim (Ingelheim am Rhein, Germany) and Merck, Sharp and Dohme Research laboratories (Rahway, NJ, U.S.A.) respectively. For experiments, fresh solutions of the compounds were prepared daily in dimethyl sulphoxide. All tissue-culture reagents and Pronase were from Boehringer Mannheim (Meylan, France). All other chemicals were from Prolabo (Paris, France).

Abbreviations used: IC_{50} , concentration which inhibits 50% of the binding; PAF, platelet-activating factor.

Isolation and culture of tracheal epithelial cells

Epithelial cells were isolated from the trachea of male Hartley guinea pigs (400–500 g) (Charles River, Saint-Aubin Les Elbeuf, France) by enzymic digestion with 0.1 % Pronase (in Ham's F12 medium at 4 °C for 16–18 h) as described previously (Wu *et al.*, 1985). At the end of the incubation period, cells were dislodged from the mucosal surface and centrifuged at 400 *g* for 7 min, washed and resuspended in Ham's F12 medium containing penicillin (100 units/ml), streptomycin sulphate (100 µg/ml), cortisol (0.1 µM), insulin (2 µg/ml), cholera toxin (5 ng/ml), epidermal growth factor (15 ng/ml) and endothelial-cell growth supplement (5.0 µg/ml) (culture medium). The cell pellet was then dispersed by repeated pipetting, and cells were seeded (2×10^4 cells/well) in 24-well cluster plates (Nunc, Roskilde, Denmark) which were incubated in a humidified atmosphere of air/CO₂ (19:1) at 37 °C. At 24 h after plating, the cultures were washed with Ham's F12 medium to remove non-adherent cells and were fed with fresh culture medium. Culture medium was then removed every other day, and epithelial cells were subcultured by treatment with 0.05 % trypsin/0.02 % EDTA. Epithelial cells were used for binding experiments at confluence (6–8 days after plating) [$2\text{--}3 \times 10^5$ cells/well] between passages 1 and 4. Tracheal-epithelial-cell cultures were characterized by immunohistochemistry for cytokeratin as previously described (Churchill *et al.*, 1989).

[³H]PAF or [³H]SR 27417 binding to tracheal epithelial cells

Experiments studying the specific binding of [³H]PAF or [³H]SR 27417 to guinea-pig tracheal epithelial cells and its inhibition by PAF-receptor antagonists were performed on cells cultured in 24-well cluster plates. Culture medium was removed, and confluent epithelial cells in the well were washed with 2 × 1 ml of ice-cold phosphate-buffered saline (Boehringer Mannheim), containing 1 mM-CaCl₂, 0.5 mM-MgCl₂ and 0.1 % BSA. Incubations were carried out in a total volume of 1 ml of buffer (140 mM-NaCl, 2.7 mM-KCl, 0.4 mM-NaH₂PO₄, 2 mM-MgCl₂, 12 mM-NaHCO₃, 10 mM-Tris/HCl, 6.2 mM-dextrose and 0.25 % BSA, pH 7.4) which contained [³H]PAF or [³H]SR 27417 (0–30 nM for saturation experiments, 0.1 or 0.5 nM for competition studies). Other substances were added in dimethyl sulphoxide as indicated. Triplicate incubations were carried out at 0 °C for the indicated period of time and were terminated by addition of 1 ml of ice-cold assay buffer. Cells were then rapidly washed with 2 × 1.5 ml of ice-cold incubation buffer, and the radioactivity was measured by scintillation counting after digestion of the cell monolayer with 0.5 ml of aqueous 0.3 % Triton X-100 solution. Non-specific binding was defined as the total binding measured in the presence of excess unlabelled C₁₆-PAF or [³H]SR 27417 (1 µM), and specific binding was defined as the difference between total binding and non-specific binding. The percentage inhibition (%I) was expressed as $100 \times (\text{total binding} - \text{total binding with antagonist}) / \text{specific binding}$. The IC₅₀ value was defined as the concentration of inhibitor required to obtain 50 % inhibition of the specific binding and was determined by an indirect Hill plot (Jacobs *et al.*, 1975). The equilibrium inhibition constant (*K*_i) was calculated from the Cheng & Prusoff (1973) equation, $K_i = \text{IC}_{50} / (1 + L/K_D)$, where *L* is the concentration of labelled ligand and *K*_D the apparent dissociation constant of radioligand binding to tracheal epithelial cells. The apparent dissociation constant (*K*_D) and the maximal number of binding sites (*B*_{max}) were calculated by using Scatchard (1949) representation of the experimental data. Data from saturation, competition and association studies were analysed by a non-linear regression program (Munson & Rodbard, 1980). During the incubation period, the stability of [³H]PAF or

[³H]SR 27417 in the binding medium as assessed by h.p.l.c. analysis, and the degradation of the ligands, under our standard conditions, represented less than 2 % after a 2 h incubation period at 0 °C (results not shown).

RESULTS

Characteristics of [³H]PAF binding to tracheal epithelial cells

In a preliminary experiment, when incubated at 25 °C or higher temperatures, the specific binding of [³H]PAF to epithelial cells decreased rapidly (results not shown), thus confirming the metabolic instability of PAF observed in the presence of intact cells (Homma *et al.*, 1987; Churchill *et al.*, 1991). In addition, total, specific and non-specific binding of [³H]PAF to the cells increased linearly over cell concentrations ranging from 1×10^3 to 6×10^5 cells/well (results not shown). Therefore, incubation of $(2\text{--}3) \times 10^5$ cells/well at 0 °C was chosen for all the following experiments.

As shown in Fig. 1(a), specific binding of [³H]PAF to tracheal epithelial cells at 0 °C was time-dependent and reached an equilibrium 20–40 min after the beginning of the experiment. An apparent equilibrium binding constant (*K*_{obs}) of $0.059 \pm 0.001 \text{ min}^{-1}$ and a time of half-maximum association (*t*_{1/2}) of $11.7 \pm 0.5 \text{ min}$ (*n* = 3) were calculated from this time-course binding study.

The specific binding of [³H]PAF was totally reversible, since addition of excess unlabelled ligand (1 µM) dissociated [³H]PAF from the cells. From these kinetics, the apparent dissociation constant was found to be $3.9 \pm 0.7 \text{ nM}$ (*n* = 3), which is in

Fig. 1. Time course of association of [³H]PAF and [³H]SR 27417 to cultured guinea-pig tracheal epithelial cells

Confluent tracheal epithelial cells [$(2\text{--}3) \times 10^5$ cells/well] were incubated for various periods of time with [³H]PAF (a) or [³H]SR 27417 (b) (0.5 nM) at 0 °C. The arrows indicate the time at which unlabelled C₁₆-PAF (■) or SR 27417 (●) (1 µM) was added to initiate the dissociation process. Results represent data from typical experiments which were repeated three times without noticeable modifications.

Fig. 2. Specific binding of [³H]PAF and [³H]SR 27417 to tracheal epithelial cells

Tracheal epithelial cells [(2–3) × 10⁵ cells/well] were incubated for 30 min at 0 °C with increasing concentrations of [³H]PAF (0–30 nM) (a) or [³H]SR 27417 (0–0.5 nM) (b). Specific binding is given by the difference between total and non-specific binding determined in the presence of 1 μM-C₁₆-PAF or SR 27417. Insets: Scatchard plots of the specific binding of [³H]PAF or [³H]SR 27417 calculated from saturation isotherms. Each point is the average of results from at least three independent experiments performed in triplicate.

agreement with the K_D determined in the saturation experiment. On the basis of this finding, equilibrium binding experiments were performed by setting the incubation time at 30 min.

Fig. 2(a) shows the dose-dependent binding of [³H]PAF to tracheal epithelial cells. The non-specific binding as measured in the presence of an excess of unlabelled C₁₆-PAF (1 μM) was linearly dependent on the concentration of [³H]PAF. The specific binding, defined as the total amount of [³H]PAF bound minus the non-specific binding, was saturable, and reached a maximum around 20 nM. At 0.5 nM-[³H]PAF, the specific binding to rabbit platelets varied between 40 % and 50 % of the total binding. The high level of non-specific binding of [³H]PAF to various cell types has previously been reported (Terashita *et al.*, 1985; Robaut *et al.*, 1987). A Scatchard (1949) analysis of the bound/free ratio of the radiolabelled [³H]PAF versus bound [³H]PAF revealed the presence of only one class of binding sites (Fig. 2a, inset). This population of sites exhibited high affinity, with an apparent equilibrium dissociation constant (K_D) value of $4.3 \pm 0.3 \text{ nM}$ ($n = 3$) and a total number of receptor sites (B_{max}) of $0.172 \pm 0.02 \text{ fmol/10}^5 \text{ epithelial cells}$ ($1044 \pm 121 \text{ sites/cell}$) ($n = 3$).

Binding of [³H]SR 27417 to tracheal epithelial cells

The association of [³H]SR 27417 to confluent epithelial cells was rapid, inasmuch as equilibrium was reached within the

Fig. 3. Competition by PAF antagonists for [³H]PAF and [³H]SR 27417 binding on their binding sites on tracheal epithelial cells

Guinea-pig tracheal epithelial cells [(2–3) × 10⁵ cells/well] were incubated with 0.5 nM-[³H]PAF (a) or 0.1 nM-[³H]SR 27417 (b) for 30 min at 0 °C with increasing concentrations of SR 27417 (●), C₁₆-PAF (▲), WEB-2086 (■), WEB-2170 (□), 59227 RP (○) or L 659989 (△). Each data point is the average of results from at least three independent determinations performed in triplicate.

Table 1. Comparative abilities of various PAF antagonists to inhibit [³H]PAF and [³H]-SR 27417 binding to tracheal epithelial cells

Values are means ± s.d. obtained from at least three independent experiments performed in triplicate.

Compounds	IC ₅₀ (nM)	
	[³ H]PAF	[³ H]SR 27417
C ₁₆ -PAF	4.8 ± 0.07	3.9 ± 0.9
Lyso-PAF	2 % at 100 μM	0 % at 100 μM
Enantio-PAF	5 % at 100 μM	3 % at 100 μM
SR 27417	0.12 ± 0.004	0.11 ± 0.01
L 659989	12.3 ± 0.9	16.1 ± 0.9
59227 RP	17.5 ± 1.9	18.9 ± 2.1
WEB-2170	45.9 ± 7.6	56.3 ± 4.3
WEB-2086	60.1 ± 9.7	75.0 ± 8.1

initial 15 min of the incubation time at 0 °C (Fig. 1b). The specifically bound [³H]SR 27417 was totally displaced by 1 μM unlabelled SR 27417 or C₁₆-PAF, indicating that the binding of [³H]SR 27417 was fully reversible. Half-maximum dissociation of [³H]SR 27417 occurred 3.2 ± 0.5 min and 9.7 ± 2.9 min after addition of SR 27417 and C₁₆-PAF respectively.

Fig. 4. Correlation between the potency of various PAF antagonists to displace the specific binding of $[^3\text{H}]\text{PAF}$ and $[^3\text{H}]\text{SR 27417}$ to their binding sites on tracheal epithelial cells

IC_{50} values of SR 27417 (●), WEB-2086 (■), WEB-2170 (□), L 659989 (△), 59227 RP (○) and C_{16} -PAF (▲) against binding of $[^3\text{H}]\text{PAF}$ or $[^3\text{H}]\text{SR 27417}$ were determined from an indirect Hill plot. The points reported were fitted with a least-squares linear regression. Statistical parameters of correlation analysis are as indicated.

$[^3\text{H}]\text{SR 27417}$ specific binding to tracheal epithelial cells occurred in a dose-dependent manner which was saturable at higher ligand concentrations (Fig. 2b). With this ligand, non-specific binding determined in the presence of $1\ \mu\text{M}$ -SR 27417 represented less than 5% of total binding. Analysis of the binding data from saturation isotherms revealed one class of apparent binding sites (Fig. 2b, inset). $[^3\text{H}]\text{SR 27417}$ binding to the high-affinity site had a K_D value of $94 \pm 7\ \text{pM}$ and a B_{max} of $0.181 \pm 0.04\ \text{fmol}/10^5$ epithelial cells (1100 ± 243 sites/cell) ($n = 3$).

Inhibition of $[^3\text{H}]\text{PAF}$ and $[^3\text{H}]\text{SR 27417}$ binding to tracheal epithelial cells

As shown in Fig. 3(a), unlabelled PAF displaced $[^3\text{H}]\text{PAF}$ specifically bound to high-affinity receptor sites in guinea-pig tracheal epithelial cells in a dose-dependent manner. IC_{50} was $4.8 \pm 0.07\ \text{nM}$ ($n = 3$) (Table 1). When calculated from the Cheng & Prusoff (1973) equation, the K_i value for the specific binding of $[^3\text{H}]\text{PAF}$ was $4.3 \pm 0.06\ \text{nM}$. Under the same experimental conditions, lyso-PAF and enantio-PAF ($100\ \mu\text{M}$), two close derivatives of PAF devoid of significant biological activity via PAF receptors, inhibited the specific $[^3\text{H}]\text{PAF}$ binding by only 2% and 5% respectively. However, SR 27417 dose-dependently inhibited the specific binding of $[^3\text{H}]\text{PAF}$ to platelets with an IC_{50} value of $120 \pm 4\ \text{pM}$ ($K_i = 100 \pm 13\ \text{pM}$) ($n = 3$). Other PAF-receptor antagonists, including WEB-2086, WEB-2170, 59227 RP and L 659989, could fully displace the $[^3\text{H}]\text{PAF}$ specific binding and showed no effect on the non-specific binding of $[^3\text{H}]\text{PAF}$ (results not shown). For comparative purposes, IC_{50} values of SR 27417 and of these various PAF-receptor antagonists are shown in Table 1. For all compounds, the Hill slope factors (h) were near unity (results not shown), suggesting a bimolecular reaction and indicating that the inhibition of $[^3\text{H}]\text{PAF}$ binding by these compounds was due to an interaction with the receptor itself.

As illustrated in Fig. 3(b), C_{16} -PAF, SR 27417 and other PAF-receptor antagonists attained the same maximal degree of inhibition of $[^3\text{H}]\text{SR 27417}$ binding to its binding sites on epithelial cells. C_{16} -PAF, SR 27417, WEB-2170, L 659989, 59227 RP and WEB-2086 dose-dependently inhibited the specific binding of $[^3\text{H}]\text{SR 27417}$ to its binding sites, with the IC_{50} values indicated in Table 1. Similar values were found for the effect of these compounds on $[^3\text{H}]\text{PAF}$ binding to the same sites (Fig. 3a, Table 1). Indeed, a linear relationship could be established between the ability of these compounds to displace the $[^3\text{H}]\text{PAF}$ binding and their respective potencies to block the $[^3\text{H}]\text{SR 27417}$ binding to tracheal epithelial cells (Fig. 4).

Fig. 5. Scatchard analysis of the specific binding of $[^3\text{H}]\text{PAF}$ or $[^3\text{H}]\text{SR 27417}$ performed from saturation isotherms

Tracheal epithelial cells $[(2-3) \times 10^5\ \text{cells/well}]$ were incubated for 30 min at 0°C with increasing concentrations of $[^3\text{H}]\text{PAF}$ (a, c) ($0-2\ \text{nM}$) or $[^3\text{H}]\text{SR 27417}$ (b, d) ($0-0.5\ \text{nM}$) in the absence (○) or presence of C_{16} -PAF (a, b: ●, 2.5 nM; ■, 10 nM; ▲, 25 nM) or SR 27417 (c, d: ●, 0.05 nM; ■, 0.25 nM; ▲, 0.5 nM). Each point is the average of results from at least three independent experiments performed in triplicate.

Nature of the antagonism of PAF and SR 27417 on [^3H]PAF and [^3H]SR 27417 binding

Tracheal epithelial cells were incubated with increasing concentrations of [^3H]PAF (0–2 nM) in the absence and in the presence of three concentrations of C_{16} -PAF (2.5, 10 and 25 nM). The Scatchard analysis indicated a competitive antagonism (Fig. 5a). Indeed, the maximal number of receptor sites (B_{max}) was not affected by increasing concentrations of PAF, whereas the apparent dissociation constant (K'_D) values increased from a control value of 4.3 nM to 6.07, 8.94 and 17.9 nM for 2.5 nM, 10 nM and 25 nM- C_{16} -PAF respectively. Scatchard analysis of binding data revealed that SR 27417 was also a competitive antagonist of [^3H]PAF binding to tracheal epithelial cells (Fig. 5c). Similarly, the Scatchard-plot analysis of [^3H]SR 27417 binding in the presence of various concentrations of SR 27417 or unlabelled C_{16} -PAF demonstrated that both compounds competitively inhibited [^3H]SR 27417 binding to tracheal epithelial cells (Figs 5b and 5d).

In a Schild analysis, by plotting $\log [(K'_\text{D}/K_\text{D}) - 1]$ where K'_D and K_D are apparent and equilibrium dissociation constants of [^3H]PAF and [^3H]SR 27417 in the presence and absence of antagonist respectively versus $\log [\text{antagonist}]$, a slope of unity was found for all competition experiments (results not shown). Therefore the blockage of [^3H]PAF and [^3H]SR 27417 binding by unlabelled C_{16} -PAF and SR 27417 on tracheal epithelial cells is consistent with simple competitive antagonism. The equilibrium dissociation constants (K_D) of SR 27417 can be obtained from the inhibition of [^3H]PAF and [^3H]SR 27417 binding, and were found to be 150 ± 3 pM and 90 ± 7 pM ($n = 3$) respectively. With regard to the inhibitory effect of C_{16} -PAF on [^3H]PAF and [^3H]SR 27417 binding to epithelial cells, the K_B values of PAF obtained from the Schild plots were found to be 4.3 ± 0.9 and 4.1 ± 1.1 nM ($n = 3$) respectively.

DISCUSSION

Along with several other mediators, it has been proposed that PAF is intimately involved in the etiology of a variety of disorders, including asthma (Page, 1988), and it is expected that the development of potent and specific PAF-receptor antagonists may offer a rational and novel therapeutic approach to this disease. Furthermore, selective PAF-receptor antagonists will provide an invaluable experimental tool with which to elucidate the actual involvement of PAF in the pathophysiology of various diseases.

In the past few years, specific PAF-receptor sites have been identified by using radioligand techniques in platelets from various origins, human polymorphonuclear leucocytes, human lung and brain, and a large number of PAF-receptor antagonists have been identified (for review see Hwang, 1990). But, although one of the major impacts of PAF is on the broncho-alveolar tree, no PAF-binding sites on bronchial or tracheal epithelial cells have been characterized so far. Herein, we describe the specific binding of [^3H]PAF to guinea-pig tracheal epithelial cells.

The binding of [^3H]PAF to guinea-pig tracheal epithelial cells showed high affinity, specificity and saturability. These PAF receptors appeared to be of a single class and exhibited high affinity and high binding capacity, with an apparent K_D of 4.3 ± 0.3 nM and a B_{max} of 1044 ± 121 sites/cell. This K_D value was almost comparable with the affinity reported for other tissues or intact cells, such as platelets or polymorphonuclear leucocytes (Hwang, 1990). To our knowledge, this is the first description of [^3H]PAF binding sites on guinea-pig tracheal epithelial cells.

SR 27417, the first member of a newly developed PAF-

antagonist series, which has been shown to be a highly potent, specific and competitive PAF-receptor antagonist by its inhibitory actions on the PAF-induced effect in various systems either *in vitro* or *in vivo* (Herbert *et al.*, 1991), competed with [^3H]PAF for its binding sites, with a K_i in the sub-nanomolar range. Most interestingly, SR 27417 was more potent in inhibiting [^3H]PAF binding than was C_{16} -PAF itself, exhibiting a K_i 40-fold lower than that of C_{16} -PAF. This observation confirms previously published results on intact rabbit platelets (Herbert *et al.*, 1991). In the same experiment, other PAF-receptor antagonists competed for [^3H]PAF binding with the same magnitude of potency (K_i) as that observed for their respective abilities to displace [^3H]PAF from its binding sites on other cell types (Robaut *et al.*, 1987; Casals-Stenzel *et al.*, 1987; Marquis *et al.*, 1988; Ponpidom *et al.*, 1988; Hwang, 1990).

Although intact-cell preparations appear to be more suited for the physiological characterization of radiolabelled-PAF-binding sites, PAF can activate the cells, undergo degradation to inactive metabolites such as lyso-PAF (Kramer *et al.*, 1984), or be taken up in the outer layer of the plasmalemma (Kramer *et al.*, 1984; Homma *et al.*, 1987). The [^3H]PAF-binding procedure is therefore characterized by high non-specific binding and inter-subject variability. For these reasons, specific PAF-receptor antagonists such as [^3H]WEB-2086 or [^3H]52770 RP have been described as useful markers of PAF-receptor sites in intact human platelets (Ukena *et al.*, 1988a,b) and neutrophils (Marquis *et al.*, 1988; Ukena *et al.*, 1989) and in guinea-pig eosinophils (Ukena *et al.*, 1989). Indeed, the use of radiolabelled PAF-specific receptor antagonists may be a better means of studying PAF receptors on cells, since excessive levels of non-specific binding of [^3H]PAF may interfere with measurement of specific [^3H]PAF binding. Hence, although experiments were performed at 0 °C, preventing [^3H]PAF from being degraded into lyso-PAF, in order to study more accurately the PAF-binding sites on tracheal epithelial cells, we used the radiolabelled PAF antagonist, [^3H]SR 27417.

On tracheal epithelial cells, [^3H]SR 27417 bound in a reversible and dose-dependent manner to one population of binding sites of high affinity. K_D and B_{max} values were 94 ± 7 pM and 0.181 ± 0.04 fmol/ 10^5 cells (1100 ± 243 sites/cell) ($n = 3$). The maximum number of PAF-receptor sites determined by using [^3H]PAF in matched parallel studies was virtually identical with that obtained for [^3H]SR 27417. These findings, in addition to the high level of correlation between the capability of various PAF antagonists to displace [^3H]PAF and [^3H]SR 27417 from their respective binding sites (Fig. 4; Table 1), favour the conclusion that both agents interact with the same sites on epithelial cell membranes. On this population of receptors, SR 27417 displaced the specific [^3H]SR 27417 binding in a competitive manner and exhibited high affinity, since the value of its inhibitory constant was in the sub-nanomolar range ($K_\text{i} = 53 \pm 5$ pM; $n = 3$). As shown above for [^3H]PAF, SR 27417 was 35-fold more potent in inhibiting [^3H]SR 27417 binding to epithelial cells than was C_{16} -PAF, and exhibited a K_i more than 150-fold lower than that of the best synthetic PAF antagonist tested. The present [^3H]SR 27417-binding procedure was characterized by an extremely low non-specific binding of the ligand (less than 5%), making it a more suitable tool for labelling PAF-binding sites than [^3H]PAF, which displays such a high non-specific binding that it is often problematic to use it as a routine research tool.

In conclusion, we have demonstrated for the first time the presence of high-affinity PAF-binding sites on the surface of intact tracheal epithelial cells. These sites could also be labelled by [^3H]SR 27417, the first member of a newly developed PAF-antagonist series, which can be considered as one of the most potent antagonists of PAF yet described. Its unique feature of

having a higher affinity for PAF receptors than has unlabelled PAF itself makes it a very interesting tool for furthering our understanding of the mediating role of PAF in certain physiological and pathological situations.

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