observed, when applied to chromaffin cells, that is distinct from its properties as a MAO inhibitor. Its ability to inhibit catecholamine secretion may be the result of non-specific binding at or near the acetylcholine receptor. This effect may have important implications when using this drug for studying the mechanisms of exocytosis in the chromaffin cell system.

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PAF-Receptors on eosinophils: identification with a novel ligand, [^{3}H]WEB 2086

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The eosinophil has been implicated as a major effector cell in asthma. particularly in causing airway epithelial damage through release of proteins such as the eosinophil cationic protein, major basic protein, and eosinophil peroxidase (EPO) [1]. Platelet activating factor (PAF) has a wide range of biological actions that mimic most of the features of asthma, including bronchoconstriction, increased airway microvascular permeability and protein exudation into the airway lumen [2]. In addition, PAF stimulates eosinophil chemotaxis [3] and release of eosinophil peroxidase [4].

Evidence is accumulating that PAF produces its various effects on target cells through activation of specific membrane receptors. Binding studies with [^{3}H]PAF have, however, often proved difficult as a result of the high level of nonspecific binding and the metabolism and uptake of the radioligand. Therefore, labelled PAF antagonists may represent a more suitable means for probing PAF receptors.

In recent years, several radiolabelled PAF antagonists have been developed. Using [^{3}H]WEB 2086 [5], we have recently been able to identify PAF receptors in intact human platelets [6] and neutrophils [7]. The data reported here describe for the first time the characterization of PAF receptors on eosinophils using [^{3}H]WEB 2086. In addition, we have compared these results with the effect of WEB 2086 on PAF-induced EPO release.

Methods and materials

Eosinophils were obtained by intraperitoneal lavage from polymyxin B-treated guinea pigs [8] and purified using a discontinuous Percoll gradient [9]. The purity of the eosinophils was > 97% with a viability of > 99%. EPO release was measured by a colorimetric assay using 1,2-phenylene-diamine as substrate, as described elsewhere [4]. Binding of [^{3}H]WEB 2086 (specific activity 14 Ci/mmol; Boehringer Ingelheim, F.R.G.) was measured at the indicated concentrations using 1×10^7 eosinophils/ml in duplicates at 25° for 90 min. Nonspecific binding was determined in the presence of either 10 μM WEB 2086 or 1 μM C_{16}-PAF (Bachem, Torrance, U.S.A.) which gave the same results. Bound and free radioligand were separated by rapid filtration and the filters washed twice with 4 ml ice-cold Hepes buffer.

Results

Purified guinea pig eosinophils (2×10^6 cells/sample) were incubated with various concentrations of PAF in the presence and absence of WEB 2086 (200 nM). As illustrated in Fig. 1, PAF induced a concentration-dependent release of EPO from eosinophils with an EC_{50} of 1.2 nM (N = 3). The inactive precursor and metabolite lyso-PAF, however, was ineffective at concentrations up to 10 μM (data not shown). The presence of WEB 2086 caused a parallel rightward shift of the concentration response curve for PAF, indicating competitive antagonism. From this shift an affinity constant (K_B) of 7.3 nM was calculated for the antagonist. In contrast, WEB 2086 did not inhibit enzyme release induced by opsonized zymosan or the calcium ionophore A23187 (Table 1).

Initial attempts with [^{3}H]PAF binding to guinea-pig eosinophils were unsuccessful because of a cellular uptake of the radioligand, giving inconsistent binding and high nonspecific labelling. However, binding of [^{3}H]WEB 2086 to guinea-pig eosinophils was specific, reversible and saturable. A representative saturation isotherm is demonstrated

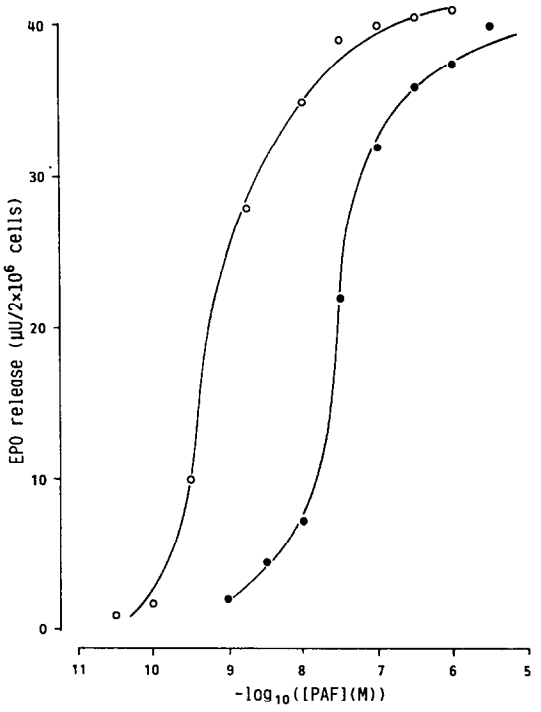

Fig. 1. The effect of WEB 2086 upon PAF-induced eosinophil peroxidase (EPO) release from purified guinea pig eosinophils. 2×10^6 cells (97.8% purity) were incubated (500 μl) with different concentrations of PAF (10 pM–1 μM) in the presence (●) and absence (○) of WEB 2086 (200 nM) for 15 min at 37°C. EPO was measured using with a specific colorimetric assay [4]. The results were expressed as Units referring to a standard curve using horseradish peroxidase, type 1 (hydrogen-peroxide oxireductase, EC 1.11.1.7). One unit of this enzyme will form 1.0 mg of purpuogallin from pyrogallol in 20 sec at pH 6 at 20°. The apparent dissociation constant (K_B) of the receptor antagonist complex was calculated by the method of Furchgott [11].

in Fig. 2(A). Specific binding was saturable, whilst non-specific binding increased linearly with radioligand concentration. The derived Scatchard plot of the data is linear (Fig. 2B), indicating a homogeneous population of non-interacting binding sites. A dissociation constant (K_D) of 16.1 ± 0.6 nM (mean ± SE; N = 3) and a binding capacity (B_{max}) of 580 ± 40 fmol/10^7 cells (N = 3) were obtained. Under the assumption that one molecule binds to one receptor, this B_{max} corresponds to approximately 35,000 binding sites per cell. In competition experiments the K_i values of PAF and WEB 2086 were 0.23 nM (geometric mean, 95% confidence limits 0.12–0.45, N = 3) and 13.4 nM (8.4–21.5), respectively. The maximal displacement of [^{3}H]WEB 2086 binding by unlabelled PAF or WEB 2086 was similar with approximately 85% of total binding. Lyso-PAF did not compete for [^{3}H]WEB 2086 binding sites at concentrations up to 10 μM (9% inhibition at 10 μM).

Discussion

Bronchial asthma is now recognized to be a chronic inflammatory disease characterized by eosinophil infiltrates into the airway mucosa [1] and in which eosinophils may play a critical pathogenetic role. Furthermore, PAF is known to be the most potent eosinophil chemotactic mediator [3] and may also represent a potent stimulus for eosinophil degranulation [4]. However, specific binding sites for PAF on eosinophils have not yet been described. Because of the very high nonspecific binding of PAF, PAF antagonists have proved to be better tools to be used in such experiments as well as elucidating the role of PAF in diseases such as bronchial asthma. Recently, the hetrazepine WEB 2086 has been shown to be a potent and specific PAF antagonist [5]. WEB 2086 potently inhibits PAF-stimulated human platelet aggregation *in vitro* and PAF-induced microvascular leakage in the airways [10].

Table 1. Comparison of eosinophil peroxidase release by PAF, opsonized zymosan, and calcium ionophore from 2×10^6 guinea-pig eosinophils

Stimulus	Concentration	Eosinophil peroxidase (μU)	% Total activity
Platelet activating factor	100 nM	69 ± 8	16.6 ± 1.9
Opsonized zymosan	2 mg/ml	34 ± 5	8.2 ± 1.2
Calcium ionophore A23187	1 μM	52 ± 6	12.5 ± 1.4

Results are expressed as mean ± SE; N = 3.

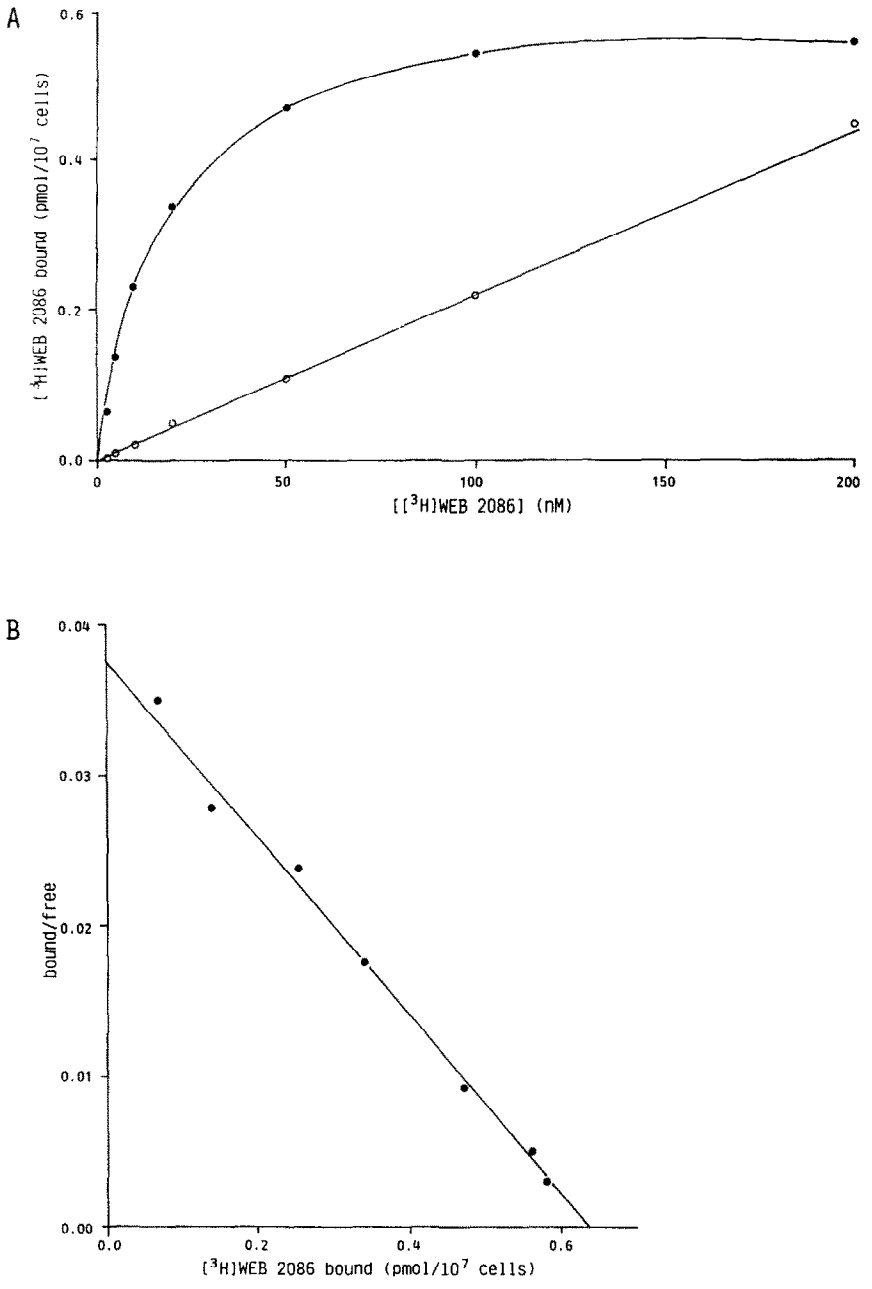

Fig. 2. [^{3}H]WEB 2086 binding to guinea pig eosinophils. (A) Saturation of [^{3}H]WEB 2086 binding to guinea pig eosinophils. Specific (●) and nonspecific (○) binding determined in the presence of 1 μM PAF was measured at 25° for 90 min. (B) Scatchard plot of the same data. In this experiment K_D was 17.2 nM and B_{max} 635 fmol/10^7 cells, corresponding to 38,000 binding sites per cell. Data shown are from one representative experiment.

Moreover, in its radiolabelled form WEB 2086 has been shown to be a useful antagonist ligand for PAF receptors in human platelets [6] and neutrophils [7].

The present study demonstrates that PAF induces eosinophil peroxidase (EPO) release in guinea-pig eosinophils in a dose-dependent fashion with an EC_{50} of 1.2 nM (Fig. 1). In addition, WEB 2086 specifically inhibited this PAF effect in a competitive manner with a K_B of 7.3 nM, while it has no effect on EPO release induced by other stimuli such as opsonized zymosan or calcium ionophore A23187. Our results indicate that WEB 2086 is also a potent PAF antagonist in eosinophils. In our radioligand binding studies, [^{3}H]WEB 2086 bound specifically to eosinophils with a K_D of 16.1 nM. The profile of the binding sites is consistent with that of PAF receptors. Furthermore, the effects of WEB 2086 in the functional and radioligand studies are in good agreement.

The K_D for [^{3}H]WEB 2086 binding on guinea-pig eosinophils is about 2-fold higher than that for binding in human platelets [6]. In contrast, the density of PAF binding sites in eosinophils (35000/cell) is approximately 200-fold higher than in human platelets [6]. This is consistent with the

potent effects of PAF on eosinophils. Recently, a similar B_{max} value has been described for the binding of the pyrrolothiazole derivative [^{3}H]52770 RP in human polymorphonuclear cells [10].

In summary, WEB 2086 is a potent, competitive PAF receptor antagonist in guinea-pig eosinophils. [^{3}H]WEB 2086 labels PAF receptors in these cells with a K_D of 16.1 nM. There is good agreement between the effects of WEB 2086 in functional and binding studies. WEB 2086 should be of value in the elucidation of the role of PAF in the eosinophilic inflammation in bronchial asthma.

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