Vol. 143, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 27, 1987 Pages 1-6

EFFECT OF PLATELET-ACTIVATING FACTOR ON BETA-ADRENOCEPTORS IN HUMAN LUNG

Devendra K. Agrawal and Robert G. Townley

Allergic Disease Center,
Creighton University School of Medicine,
Omaha, Nebraska 68178

Received December 10, 1986

We examined the effect of platelet-activating factor (PAF) on beta-adrenoceptors in the membranes of human lung and the functional responses to methacholine, histamine and isoproterenol in human lung parenchyma and tracheal strips. Preincubation of the human lung slices with 0.1 uM PAF decreased the density of beta-adrenoceptors without a change in the affinity. In the functional studies, a subthreshold dose of PAF (0.1 uM) significantly reduced the potency of isoproterenol to reverse methacholine or histamine-induced contraction. BN 52021, a PAF antagonist, protected against these PAF-induced changes. These findings suggest that PAF-induced down-regulation of beta-adrenoceptors could be a mechanism of the non-specific airway hyperreactivity in asthma.

Asthma is characterized by hyperresponsiveness of the airways to both allergic and non-specific stimuli. However, the precise mechanism(s) underlying airway hyperresponsiveness is not known. Recently, PAF has been implicated as a potent mediator of acute allergic reactions and causes airway hyperresponsiveness in man (1). In the lung, PAF causes marked bronchoconstriction, acute inflammation and edema, plaletelet aggregation and degranulation and chemotaxis and activation of neutrophils and eosinophils (2-4). The involvement of the specific receptors of PAF in respiratory anaphylaxis has also been demonstrated using a selective PAF-acether antagonist, BN 52021 (5). However, the mechanism by which PAF causes non-specific bronchial hyperresponsiveness is not known.

Beta-adrenoceptor antagonists cause bronchoconstriction in asthmatics and produce non-specific bronchial reactivity in animals (6). In asthmatic lymphocytes (7) and in the lung membranes of the guinea pig model of asthma (8,9), a downregulation of beta-adrenoceptors and up-regulation of alpha-adrenoceptors by yet unknown mechanism have been reported. Recently, Braquet and colleagues (10) reported that PAF decreased the density of beta-adrenoceptors in the

Abbreviations: PAF, Platelet-activating factor; DHA, dihydroalprenolol; K_D, equilibrium dissociation constant; Bmax, maximum binding capacity.

0006-291X/87 $1.50

homogenate of rat cerebellum. In this study, we therefore tested the hypothesis that PAF directly or indirectly downregulates the beta-adrenergic responses in the airways, which could in turn produce the non-specific airway hyperresponsiveness in asthma.

MATERIALS AND METHODS

Tissue: We used human trachea and lung parenchyma obtained during autopsy (within 5-6 hours after death) from the St. Joseph Hospital and VA Hospital, Omaha. From the lung tissue major bronchi and blood vessels were removed. The viability of the human tissue before it was used in the experiments was verified by the contractile responses to the low doses of methacholine or histamine.

Membrane Preparation: Human lung slices were incubated with PAF (0.1 uM) in the Kreb's buffer (pH 7.4) containing 0.25% human serum albumin at 37°C for 30 minutes in the presence or absence of BN 52021 (0.5 uM). In the control group, the vehicle of PAF and/or PAF antagonist was used. After incubation, human lung tissue was homogenized in Tris buffer (50 mM, pH 7.4) containing 10 mM magnesium chloride. The homogenate was centrifuged at 2,000 x g for 10 min. The supernatant was again centrifuged at 40,000 x g for 30 min. The pellet of this spin was suspended in the Tris buffer and then centrifuged at 2,000 x g for 10 min. The supernatant of the last spin was used in the binding studies for beta-adrenoceptors using (^{3}H)dihydro-alprenolol (DHA) as the radioligand.

(^{3}H)DHA Binding for Beta-Adrenoceptors: Binding assays were performed in freshly prepared lung membranes. (^{3}H)DHA binding was carried out in Tris buffer (50 mM, pH 7.4) containing 10 mM magnesium chloride at 25°C for 40 min in a shaking water bath. Reaction was terminated by adding 4.0 ml of cold (4°C) Tris buffer to the incubation mixture. The bound (^{3}H)DHA was separated by a rapid filtration over Whatman glass fiber GF/B filters. Each filter was then washed with cold Tris buffer three times with 4.0 ml volume each time. Filters were dried and the triton X-100/toluene scintillation solution containing PPO and POPOP was added in each vial. Filters were left to equilibrate in the scintillation solution at room temp. for overnight and then the radioactivity was counted.

The specific binding of (^{3}H)DHA was defined as the radioactivity displaceable by unlabeled dl-propranolol (1 uM). Quench correction in the counting of tritium was made using the external standard ratio method. Saturation ligand binding curves were analyzed using the IBM computer program (LIGAND and SCAFIT) for single or multiple binding sites.

Functional Studies: Human lung parenchymal and tracheal strips were mounted in an organ bath containing normal Kreb's buffer (pH 7.4) as previously described (11). The human tracheal and lung parenchymal strips were subjected to 2 g and 0.5 g preload tension, respectively. At this tension the tissues generated the maximum contractile response. Tissues were then allowed to equilibrate at 37°C for one hour in the Kreb's buffer. Stabilization of the tissue preparation was checked by two consecutive responses to 1 uM methacholine. Cumulative dose-response curves to methacholine in the trachea and to histamine in the lung parenchymal strips were constructed by a step-wise addition of the drug. When the responses to these agents reached a plateau, a dose-response curve to l-isoproterenol was constructed.

To examine the effect of a sub-threshold dose of PAF (0.1 uM), human lung parenchymal and tracheal strips were preincubated with PAF for 20 min before the construction of the dose-response curves to methacholine or histamine and isoproterenol. In all of the experiments, control tissues were run in parallel in order to determine time dependent changes in agonist sensitivity which were negligible under the conditions employed.

Analysis of the Data: In the binding studies, maximum binding capacity (Bmax) and the equilibrium dissociation constant (K_D) were obtained by the computer analysis. In the functional studies, the pD_2 values ($-\log_{10} EC_{50}$) of the agonists were calculated from the dose-response curves. All the values have been represented as means ± S.E.M. Statistical difference between two means ($p<0.05$) was determined by the Student's t-test for unpaired observations or by testing for overlap of the 95% confidence limits.

Drugs: (^{3}H)DHA was purchased from New England Nuclear (Boston, MA) with specific activity of 55.4 Ci/mmol. C_{16}-PAF (1-O-hexadecyl-2-O-acetyl -sn-glycero-3-phosphocholine) was purchased from BACHEM (Torrance, CA). BN 52021, a potent PAF antagonist, was provided as a kind gift by Dr. P. Braquet (Institut Henri Beaufour, Plessis-Robinson, France).

RESULTS

Binding Studies: The specific binding of (^{3}H)DHA in the human lung membranes was more than 80% of the total binding at 2 nM concentration. Figure 1, which is a representative of four such experiments, shows the Scatchard plots of the saturable binding of (^{3}H)DHA in the presence and absence of PAF. The preincubation of human lung tissue with PAF decreased the density of beta-adrenoceptors significantly without any change in the affinity (Table 1). BN 52021, a potent PAF antagonist, inhibited the PAF-induced downregulation of beta-adrenoceptors.

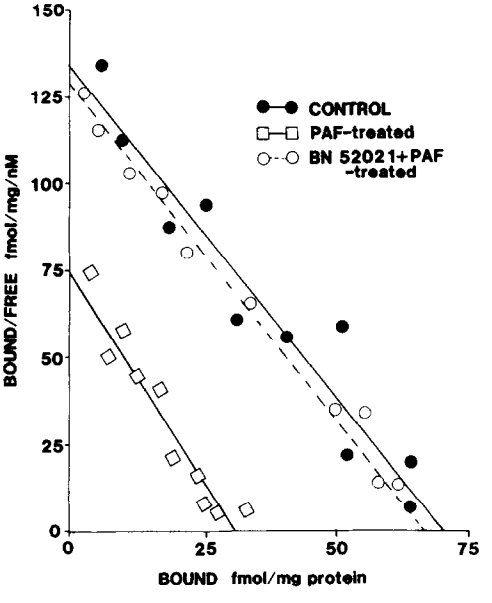

Fig. 1. This is a representative of four such experiments of the saturation curves data transformed into Scatchard plots. Specific binding of (^{3}H)DHA was performed in the human lung membranes of the control, PAF-treated and PAF + BN 52021-treated groups. In this experiment, the Bmax values of the (^{3}H)DHA binding in all the three groups were calculated to be 71.5, 31, and 67 fmol/mg protein, respectively. There was no significant difference in the K_D values.

TABLE 1

Effect of PAF on the Specific Binding of (^{3}H)dihydroalprenolol in the Human Lung Membranes

Experimental Group	K_D, nM	Bmax, fmol/mg protein
Control	0.5 ± 0.1	65 ± 12
PAF-treated	0.48 ± 0.08	32 ± 11*
PAF + BN 52021 -treated	0.52 ± 0.12	68 ± 14

Values are mean ± S.E.M. (n= 4). Asterisk indicate a significant difference ($p < 0.05$) from the control value.

Functional Studies: In the functional (isometric tension) studies, PAF did not affect the pD_2 values or the maximum responses to methacholine or histamine significantly. PAF in the concentration of 0.1 uM did not elicit any response in the human lung parenchyma or tracheal strips. However, the pD_2 values of isoproterenol to relax the tissue were significantly decreased in the tissues which have been pretreated with 0.1 uM PAF for 20 min. (Table 2). In the presence of 0.5 uM BN 52021, the effect of PAF in decreasing the potency of isoproterenol was abolished.

DISCUSSION

We have shown that PAF decreased the density of beta-adrenoceptors in human lung without any change in the affinity. Furthermore, in the functional study PAF shifted the dose-response curve to isoproterenol to the right indicating a decrease in the potency of isoproterenol to produce relaxation in the human trachea and lung parenchyma. A downregulation of beta-adrenoceptors has been reported in the asthmatic lymphocytes (7) and in lung membranes of the guinea pig model of asthma (8,9). However the mechanism of this was unknown. This study suggests that PAF could be a mediator which alters beta-adrenergic

TABLE 2

Effect of PAF on the pD_2 Values ($-\log_{10} EC_{50}$) of Isoproterenol in Human Tracheal and Lung Parenchymal Strips

Experimental conditions	pD_2 values	
	Trachea	Lung parenchyma
Control	6.8 ± 0.1	6.56 ± 0.06
PAF-treated	6.1 ± 0.2*	6.03 ± 0.11*
PAF + BN 52021-treated	6.9 ± 0.3	6.7 ± 0.2

Values are mean ± S.E.M. (n= 4-6). Asterisks indicate a significant difference from the control values, $p < 0.05$.

responses. Our data are in agreement with those of Braquet and colleagues (10) who previously reported that PAF downregulated the beta-adrenoceptors in the membranes of rat cerebellum. The specific receptors for PAF in the human lung membranes have been characterized in our laboratory (12) and by other investigators (13). A selective PAF receptor antagonist, BN 52021, inhibited the PAF-induced changes in the beta-adrenoceptor density and also in the isoproterenol-induced relaxatory responses. These evidence together with the observation that human lung contains specific PAF receptors suggest that PAF-induced downregulation of beta-adrenoceptors is mediated through specific PAF receptors.

Among the various mediators of inflammation only PAF causes an increase in non-specific airway hyperresponsiveness in non-asthmatics and the pattern of increased responsiveness was similar to that seen after allergen challenge in asthmatic subjects (1). It is therefore possible that PAF-induced downregulation of beta-adrenoceptors in human lung could possibly induce the non-specific airway hyperresponsiveness in asthma. However, the mechanism by which PAF causes a downregulation of beta-adrenoceptors is not clear. Its interaction directly as a beta-adrenoceptor antagonist is highly unlikely. This is supported by a study where salbutamol (a beta-2 adrenoceptor agonist) protected the PAF-induced hypotension and extravasation in rats (14) and a similar effect was also seen with BN 52021. BN 52021 did not have any effect on beta-adrenoceptors directly, and its beneficial effects were partially antagonized by beta-adrenergic blocking agents such as propranolol or butoxamine (14). However, the possibility of the action of PAF on some post-receptor component of beta-adrenoceptors cannot be excluded.

Recently, Taki et al. (15) reported that increased levels of phospholipase A_2 reduced the beta-adrenergic responsiveness in the airways of the guinea pig model of asthma. Exogenous addition of phospholipase A_2 decreased the density of beta-adrenoceptors in guinea pig lung membranes (15). Since PAF has been reported to induce activation of phospholipase A_2 in rabbit platelets (16, 17), it is therefore possible that the activation of this enzyme by PAF in the human lung and trachea could be a mechanism underlying PAF-induced downregulation of beta-adrenoceptors. Further studies are required to support this argument.

In summary, our studies showed that PAF decreased the beta-adrenergic responses in the human airways through a downregulation of beta-adrenoceptors. This could possibly be a mechanism underlying airway hyperresponsiveness in asthma.

ACKNOWLEDGEMENTS

We thank Dr. P. Braquet for supplying BN 52021. This work was supported by the grants from the Biomedical Research Support and Health Future Foundation of the Creighton University.

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