

Changes in airway responsiveness and β - and α -1-adrenergic receptors in the lungs of guinea pigs with experimental asthma

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The effects of inhaled allergen on airway responsiveness and on β - and α -1-adrenergic receptors on lung membrane were investigated in guinea pigs. After measuring the respiratory threshold to histamine (RT-HIS), one group of guinea pigs passively sensitized for ovalbumin was challenged by allergen inhalation (challenged group). Measurement of the RT-HIS 24 h following challenge revealed a significant decrease from 687 μ g/ml (mean, $n = 16$) to 407 μ g/ml ($P < 0.05$). In addition the RT-HIS 24 h after challenge was also significantly lower in the challenged group than in controls ($n = 9$, $P < 0.05$). The density of β -adrenergic receptors on the lung membrane of the challenged group was 594 ± 32 (mean $\pm$ SE) fmol/mg protein ($n = 11$) compared with 712 ± 24 fmol/mg protein ($n = 9$) in the controls, a statistically significant difference ($P < 0.05$). A significant correlation was found between the RT-HIS and density of β -adrenergic receptors. From these results, we concluded that the exaggerated airway responsiveness 24 h after allergen challenge is in part due to a decrease in the density of β -adrenergic receptors. There was no difference in the density of α -1-adrenergic receptors nor a significant correlation between the RT-HIS and the number of α -1-adrenergic receptors in the challenged vs. the control groups.

Key words: airway responsiveness; asthma; alpha-1-adrenergic receptor; beta-adrenergic receptor; guinea pig.

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Patients with bronchial asthma characteristically have a high incidence of atopic diatheses (25). The beta-adrenergic blockade theory (26) has been proposed as one of the pathogenetic mechanisms for their airway hyperresponsiveness. Recently, several reports have linked atopic diatheses to β -adrenergic blockade. Mita et al. (19) reported that the sensitization of guinea pigs by exposure to ovalbumin (OA) aerosol led to a decrease in β -adrenergic receptors with an increase in pulmonary α -1-adrenergic receptors. Barnes et al. observed an increase in pulmonary α -adrenergic receptors and a reduction in β -adrenergic receptors in guinea pig lungs exposed to OA daily for 4 weeks after sensitization

(1). The allergen-induced late asthmatic response (LAR) has reportedly been associated with an increased airway responsiveness to histamine for up to 74 days (3). A decreased number of β -adrenergic receptors and reduced adenylate cyclase activity in lymphocytes have been demonstrated 24 h after the allergen-induced asthmatic response (17).

Based on these reports, we conducted the following experiments in guinea pigs, hypothesizing that the airway hyperresponsiveness obtained after a respiratory anaphylactic reaction may be in part due to changes in the density of β - and α -1-adrenergic receptors in the lung.

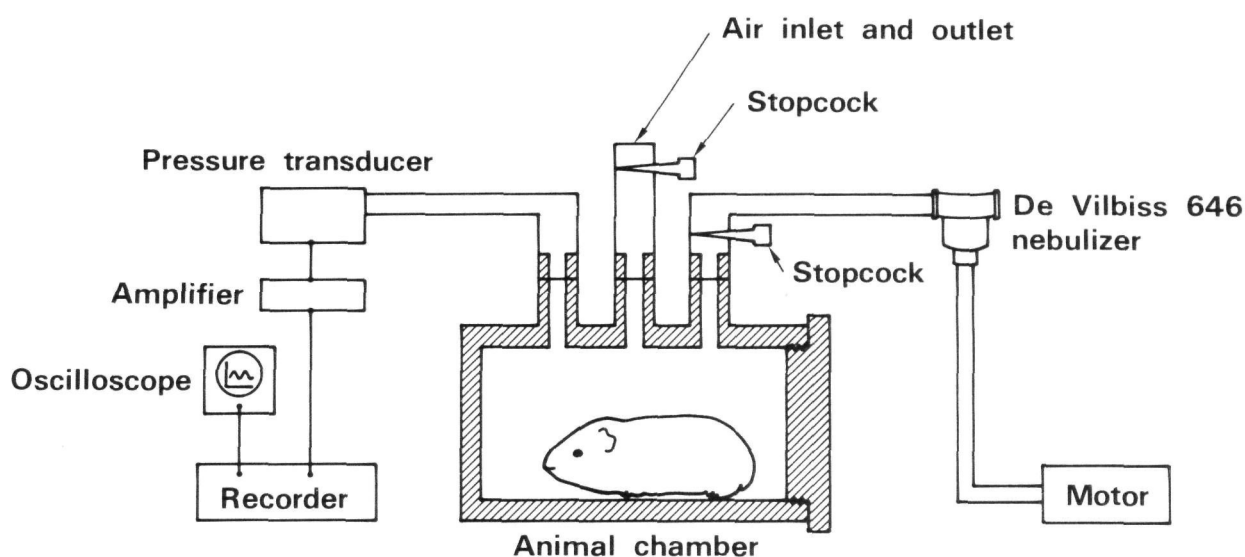


Fig. 1. Diagram illustrating the experimental apparatus for recording pressure changes in the chamber housing guinea pigs. A guinea pig was placed in the chamber. Aerosols were generated by a DeVilbiss #646 nebulizer operated by a compressor (Pulmo-Aide, DeVilbiss Co.). During inhalation, stopcocks on the air outlet and inlet were open and the stopcock to the pressure transducer closed. After inhalation, changes of pressure in the chamber were recorded with the stopcock leading to the pressure transducer open and the others closed.

MATERIAL AND METHODS

Animals and sera used for passive immunization

Hartley male guinea pigs weighing approximately 500 g were studied. Sera for passive immunization were collected from actively sensitized guinea pigs. Active sensitization was done by injecting intraperitoneally 1 μ g OA (Sigma Chemical Co., USA) with 5 mg alumina gel suspended in 0.5 ml physiologic saline. Injections were repeated three times monthly, and 1 week after the third injection the passive cutaneous anaphylaxis (PCA) titer was determined. Sera were collected from guinea pigs with a 48-h PCA titer of more than 1:160.

Experimental protocol

Guinea pigs were passively sensitized to OA by injecting 1 ml of serum obtained as described above. Forty-eight hours after the injection, the histamine inhalation test was conducted to determine the respiratory threshold to histamine (RT-HIS). Two hours after the histamine inhalation test, allergic bronchoconstriction was provoked in one group of guinea pigs (challenged group, $n = 16$) by the inhalation of an

aerosol of OA solution (day 1). Twenty-four hours after the allergen challenge, the histamine inhalation test was repeated. Lungs were removed from the animals 2 h after the second histamine inhalation test for measurement of β - and α -1-adrenergic receptors (day 2). Control guinea pigs ($n = 9$) were treated identically except for the inhalation of OA.

Procedure for inhalation test

A diagram of the equipment used in the inhalation test is shown in Fig. 1. A guinea pig was placed in a cylindrical plastic chamber and the lid was closed. Aerosol were generated by a DeVilbiss #646 nebulizer (DeVilbiss Co., U.S.A.) operated by Pulmo-Aide (DeVilbiss Co.). Pressure changes in the chamber were converted to a electric signal by a pressure transducer (Validyne Engineering Corp., USA) and recorded on a polycorder (Sanei Sokki Co., Japan), the record being used for the determination of RT-HIS. An aerosol of physiologic saline was first administered, followed by an aerosol of histamine dihydrochloride in saline (Sigma Chemical Co.) given at a dose doubled progressively from 39 to 5,000 μ g/ml. After inhalation of histamine for 1 min pressure changes in the chamber were recorded for 1

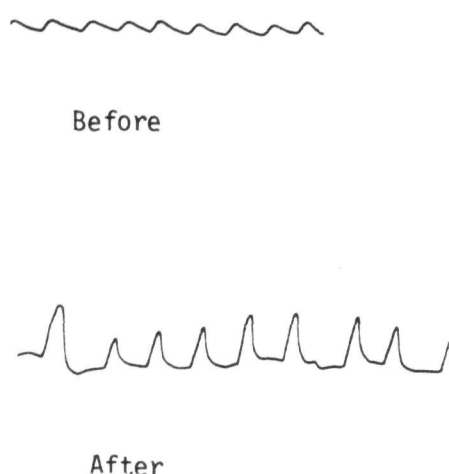


Fig. 2. Pressure changes in the chamber before and after inhalation of 5 mg/ml of OA solution. See text for details of the method of administration.

min. If no apparent change in flow pattern was observed at the dose administered, twice the concentration of histamine was administered until there was an apparent change in flow pattern, such as an increase in amplitude and prolongation of expiratory phase. We took this concentration as the RT-HIS, considering the report by Yamauchi et al. (28) that apparent flow pattern changes indicate about a three-fold increase of respiratory resistance compared with baseline.

An aerosol of 5 mg/ml OA in saline was administered until we obtained a marked prolongation of the expiratory phase such as that shown in Fig. 2. The duration of administration was 1 to 2 min.

Lung membrane preparations

Adrenergic receptor assay was done for all control guinea pigs ($n = 9$). Assay was done for 11 of the 16 challenged guinea pigs with lungs of five animals used for other experiments.

Lungs were removed and the large vessels and bronchi trimmed. Lungs were chopped and then homogenized in 10 volumes of ice-cold buffer-A (250 mM sucrose, 1 mM MgCl_2 , 5 mM Tris, HCl, pH 7.4) using Disperse Mixer DS-2 (Mitamura Riken Kogyo Co., Japan) for 30 s in ice. The homogenate was centrifuged at $2,000 \times g$, 4°C for 10 min, then the superna-

tant was centrifuged at $39,000 \times g$, 4°C for 15 min. The pellet was resuspended in buffer-B (10 mM MgCl_2 , 50 mM Tris, HCl, pH 7.4), centrifuged at $39,000 \times g$, 4°C for 15 min. After washing three times with buffer-B, the final pellet was resuspended in buffer-B or buffer-C (0.154 M NaCl, 10 mM Tris, HCl, pH 7.4) for measurement of β - and α -1-adrenergic receptors, respectively, in a concentration of 2 mg protein/ml, and kept at -70°C . The membrane suspensions were used for receptor assay after dilution to an appropriate concentration by buffer-B or buffer-C.

Measurement of β -adrenergic receptors

The density of β -adrenergic receptors on lung membrane was determined by using a radio-labeled β -antagonist $(-)^{125}\text{I}$ -cyanopindolol (ICYP, Amersham, UK). The reaction mixture consisted of 400 μl , i.e. 200 μl of lung membrane suspension containing 12–15 μg protein, 100 μl of ICYP solution with concentrations serially doubled from 80 to 1280 pM in buffer-B, and 100 μl of buffer-B or 4×10^{-6} M propranolol hydrochloride (Sigma Chemical Co.) in buffer-B. The reaction was carried out at room temperature (approximately 25°C) for 1 h and was terminated by adding 3 ml of ice-cold buffer-B followed by rapid vacuum filtration through GF/C glassfiber filter (Whatman, UK). After washing with 10 ml of ice-cold buffer-B, the radioactivity remaining on the filter was counted using the Auto Well Gamma System ARC-351 (Aloka, Japan). Non-specific binding was determined by incubation in the presence of propranolol (final concentration, 1 μM). Results were analysed by the method of Scatchard (24).

Measurement of α -1-adrenergic receptors

The density of α -1-adrenergic receptors on lung membrane was measured by using the radio-labeled α -1-antagonist, ^{125}I -BE2254 (IBE-2254, ^{125}I -2-[-beta-(4-hydroxyphenyl)-ethyl-amino-ethyl]-tetralone, New England Nuclear, USA) (5). The reaction mixture consisted of 400 μl lung membrane suspension containing approx-

imately 40 µg protein, 100 µl IBE-2254 solution with the concentration doubled serially from 100 to 1600 pM in buffer-C, and 100 µl buffer-C or 4×10^{-5} M phenotolamine mesylate (courtesy of Japan Ciba-Geigy Co.) in buffer-C. The reaction was processed at room temperature for 15 min. Bound and free ligands were separated by adding 3 ml of ice-cold buffer-C followed by rapid filtration through GF/C fiberglass filter. After washing with an additional 10 ml of buffer-C, the radioactivity on the filter was counted. Non-specific binding was determined by incubation in the presence of phentolamine (final concentration, 10 µM).

In the process of checking the inhibition of binding of IBE-2254 to lung membrane by various α -antagonists, a lung membrane suspension containing 160 µg protein and 200 pM of IBE-2254 (as a final concentration) was used. Prazosin hydrochloride was provided as a courtesy of Taito-Pfeizer Co. Japan, and yohimbine hydrochloride was from Sigma Chemical Co.

All receptor assays were carried out in triplicate.

Analysis

Data were analysed by paired or unpaired t-tests. Differences were considered statistically significant when the confidence level exceeded 95 %.

RESULTS

Change in airway responsiveness after OA challenge

Fig. 3 shows the RT-HIS before (day 1) and 24 h after (day 2) inhalation of OA aerosol solution. Following OA challenge, the geometric mean RT-HIS decreased from 687 µg/ml to 407 µg/ml, a statistically significant difference ($P < 0.05$). Eight of 16 guinea pigs showed a decrease of RT-HIS by 2 ranks; however, no difference was seen in the magnitude of allergen-induced bronchoconstriction between the guinea pigs having a decrease in RT-HIS and the same RT-HIS after OA challenge. The mean RT-HIS values in the control group on

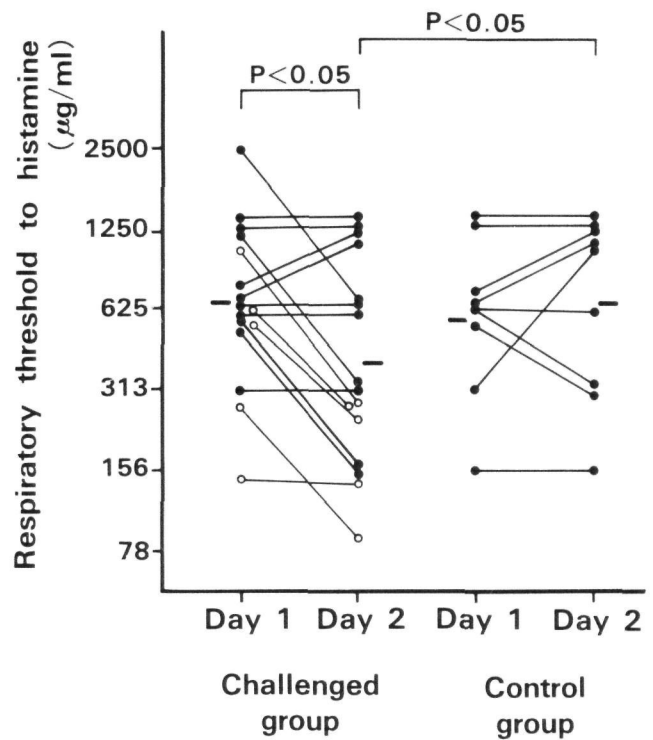


Fig. 3. Changes in the respiratory threshold to histamine before and 24 h after OA inhalation. β - and α -1-Adrenergic receptors on lung membrane were measured in the guinea pigs indicated by closed circles.

day 1 and day 2 were 589 µg/ml and 676 µg/ml, respectively, and did not differ significantly. While no difference was found in RT-HIS between the challenged and the control group on day 1, a statistically significant difference was observed on day 2 ($P < 0.05$).

Density of β -adrenergic receptors

Fig. 4 shows the densities of β -adrenergic receptors on guinea pig lung membrane on day 2. The densities of β -adrenergic receptors in the challenged and control groups were 594 ± 32 (mean $\pm$ SEM) fmol/mg protein ($n = 11$) and 712 ± 24 fmol/mg protein ($n = 9$), respectively. The difference was statistically significant ($P < 0.05$).

Relationship between density of β -adrenergic receptors and RT-HIS

The density of β -adrenergic receptors on lung membrane and the RT-HIS on day 2 were found to be significantly correlated ($r = 0.42$, $P < 0.05$) (Fig. 5). When this relationship was

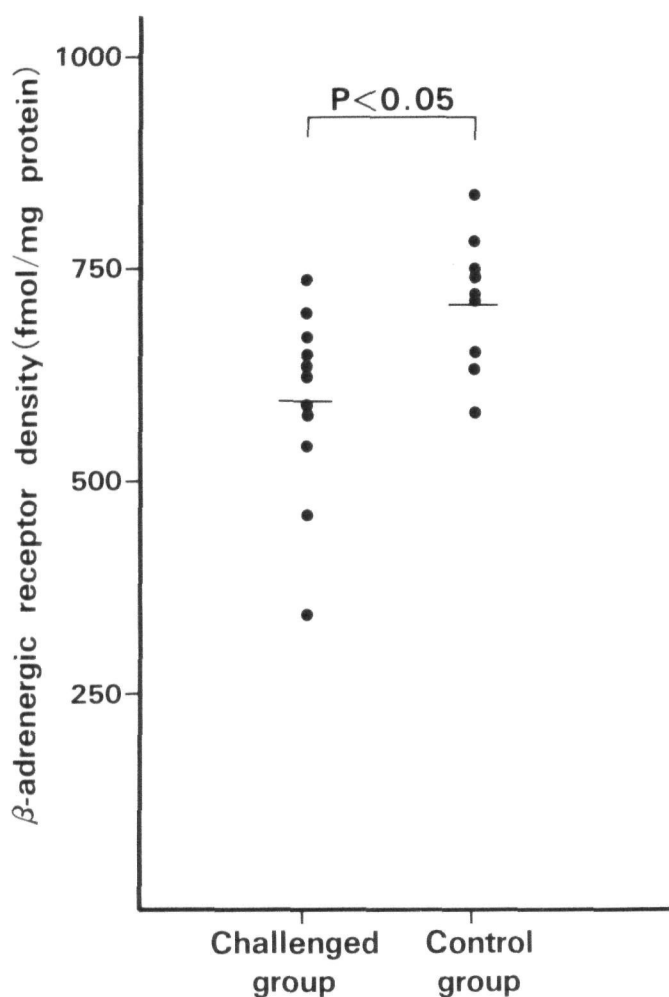


Fig. 4. β -Adrenergic receptor densities on guinea pig lung membrane in each group on day 2. Guinea pig lung particulates containing 12–15 μ g protein were incubated with ICYP (20–320 pM as final concentrations) in the presence or absence of 1 μ M propranolol for 1 h. Reaction mixtures were passed through GF/C glassfiber filter to separate bound and free ICYP, and radioactivities remaining on the filters were counted. Data were analysed by the method of Scatchard.

analysed only in the challenged guinea pigs (indicated by closed circle), the correlation was again statistically significant ($r = 0.63$, $P < 0.05$).

Density of α -1-adrenergic receptors

Fig. 6 shows the saturation curve and the Scatchard analysis of the binding of IBE-2254 to guinea pig lung membrane. The binding of IBE-2254 to lung membrane was saturable, and its binding and dissociation were rapid. Fig. 7 shows the effects of the α -antagonists, prazosin, phentolamine and yohimbine, on the binding of

IBE-2254 to guinea pig lung membrane. In this competitive experiment for IBE-2254 binding, the order of potency in inhibiting IBE-2254 binding was as follows: prazosin, phentolamine and yohimbine. This finding indicates that IBE-2254 possesses the classical binding characteristics of an α -1-adrenergic receptor ligand (5). The densities of α -1-adrenergic receptors in the challenged vs. the control group were 65 ± 9 (mean $\pm$ SEM) fmol/mg protein ($n = 11$) and 52 ± 10 fmol/mg protein ($n = 9$), respectively, not significantly different. No significant correlation was found between the densities of β - and α -1-adrenergic receptors. Also the correlation between the density of α -1-adrenergic receptors and RT-HIS was not statistically significant ($r = 0.31$, $0.05 P < 0.1$).

DISCUSSION

In this experiment, we observed an increase in airway responsiveness to histamine 24 h after inducing bronchoconstriction by administering an OA aerosol in guinea pigs. Different results have been obtained in other studies. Marsh et al. (16) observed increased airway responsiveness 3 days after the development of LAR in rabbits. Cartier et al. (3) reported that the magnitude of increased airway responsiveness to histamine correlated with that of LAR as measured by the percent fall in FEV₁; the duration of airway hyperresponsiveness also correlated with the magnitude of LAR in patients with asthma. However, Krell et al. (12) found no change in airway responsiveness to various broncho-spasmogenic substances following chronic exposure to *Ascaris* in *Ascaris*-sensitive dogs. Popa et al. (22) showed an exaggerated airway responsiveness to histamine, acetylcholine and propranolol after allergen challenge in guinea pigs; however this was observed only within 2 h of challenge. The reasons for these different results have not been explained. However, a different dose of inhaled allergen may be in part responsible, since the occurrence of a dual asthmatic response in allergic patients is thought to be dose-related. That is, an increase in the amount of inhaled allergen may promote LAR in those who had initially exhibited an immediate reaction to

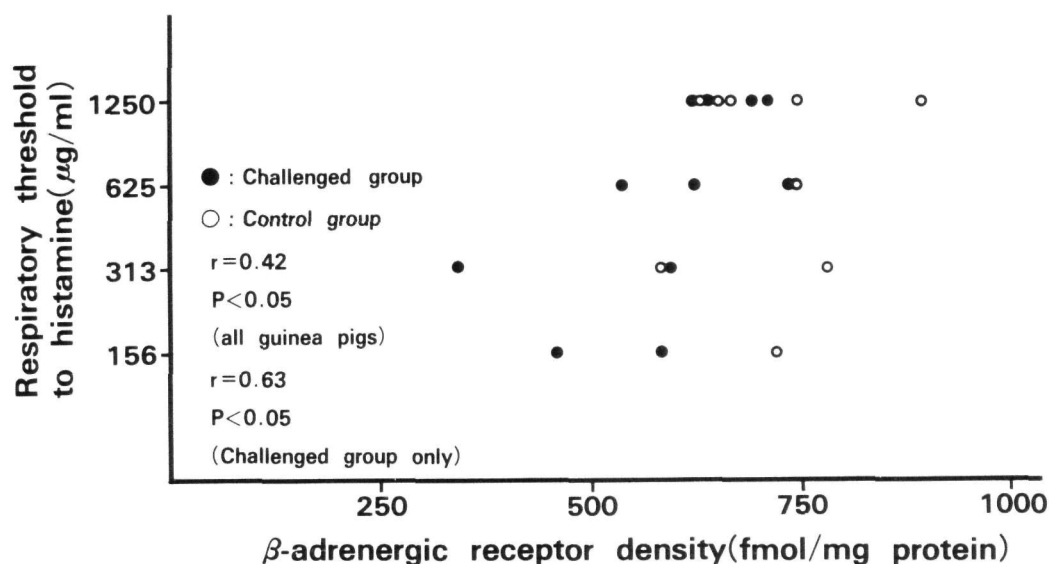


Fig. 5. Relationship between β -adrenergic receptor density on guinea pig lung membrane and respiratory threshold to histamine on day 2.

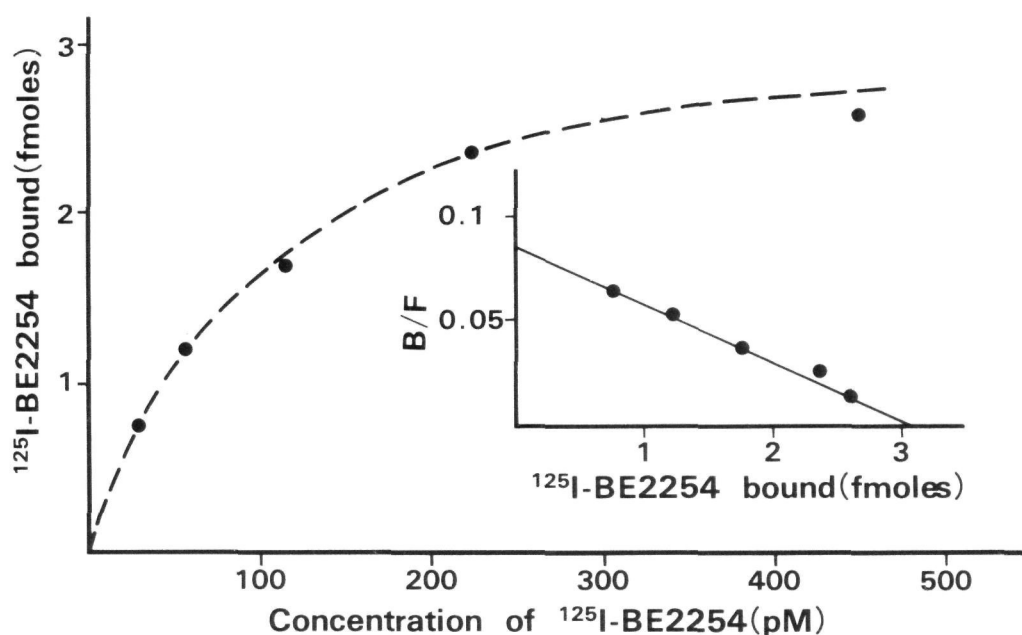


Fig. 6. Saturation curve of IBE-2254 binding to guinea pig lung membrane and the Scatchard analysis of the same data. Guinea pig lung membrane particulates containing approximately 40 μg protein were incubated with IBE-2254 (25–400 pM as final concentrations) in the presence or absence of 10 μM of phentolamine for 15 min. The reaction mixture was passed through GF/C glassfiber filter to separate bound and free IBE-2254. Radioactivity on the filters was counted and analysed by the method of Scatchard.

allergen challenge (23). In our experiment, the concentration of OA solution used in challenge was extremely high and the resulting bronchoconstriction was severe as shown in Fig. 2. Although LAR was not recorded in this experiment, we found a marked infiltration of neutrophils and eosinophils in the bronchial tissue

and injury to the bronchial epithelium 6 to 24 h after challenge (unpublished data). We might have detected changes in pulmonary function 6 to 8 h after allergen challenge if a more sensitive method had been available to measure pulmonary function. Nevertheless, considering our results together with those of others, it seems

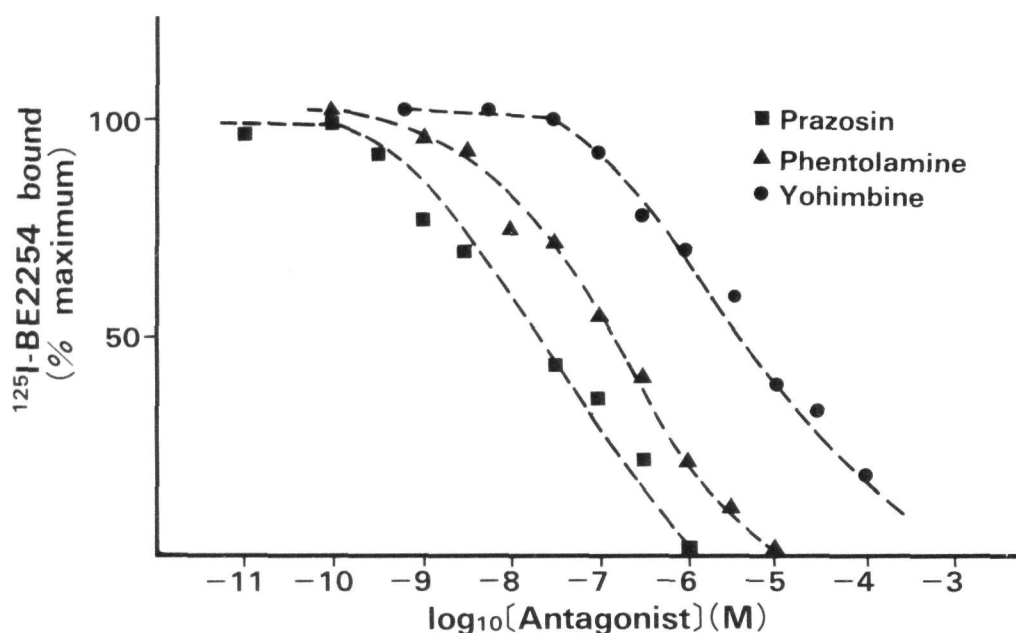


Fig. 7. Competition curves of IBE-2254 (200 pM) with α -adrenergic antagonists. Guinea pig lung particulates containing 160 μ g protein were incubated with 200 pM of IBE-2254 and various concentrations of α -antagonists for 15 min. The reaction mixtures was passed through GF/C glassfiber filter to separate bound and free IBE-2254. Radioactivity remaining on the filters was counted. Results are shown as % of maximal binding obtained in the absence of α -antagonists.

likely that exposure to a large amount of allergen leads to airway hyperresponsiveness.

There are several possible mechanisms by which allergen challenge increases airway responsiveness. First, infiltration of the airway epithelium by polymorphonuclear leukocytes is believed to play a role (4, 9, 21). Second, allergen challenges reportedly increase the permeability of the airway epithelium leading to a hyperresponsiveness to histamine (2). Third, airway epithelial damages by inflammation induced by an allergen challenge may expose nerve endings, thus tending toward airway hyperresponsiveness (13). Fourth, epithelial damage may perhaps induce airway hyperresponsiveness by a lack of as yet undetermined epithelium derived relaxant factors (6). And fifth, the results of our experiment, i.e. the increased airway responsiveness and the decreased density of β -adrenergic receptors after allergen challenge, and a positive correlation between RT-HIS and the density of β -adrenergic receptors, suggest that a decreased density of β -adrenergic receptors participates in the development of airway hyperresponsiveness. We previously reported a positive correlation between the numbers of β -adrenergic receptors on lymphocytes and respiratory threshold to

acetylcholine (20). A positive correlation between the cyclic AMP response of peripheral lymphocytes to salbutamol and the respiratory threshold to acetylcholine has been shown in another report (15). A significant correlation has also been reported between the respiratory threshold to acetylcholine and the adrenergic hyperglycemic response (14). Kraan et al. (11) noted an increased airway responsiveness after administration of terbutaline for 2 weeks and attributed this phenomenon to β -adrenergic receptor desensitization. Their reports as well as ours support the β -adrenergic blockade theory as one of the pathogenetic mechanisms of airway hyperresponsiveness.

Two mechanisms can be suggested by which the density of β -adrenergic receptors on lung membrane decrease following allergen challenge. While it is well known that the numbers of β -adrenergic receptors decrease following exposure to high concentration of β -adrenergic agonist (8), even the concentration of catecholamine existing in human plasma can influence the number of β -adrenergic receptors on mononuclear cells (8). The dyspneic state induced by allergen challenge seems to increase the concentration of plasma catecholamine. Therefore one mecha-

nism for the decreased density of β -adrenergic receptors is probably an elevated concentration of plasma catecholamine. On the other hand, Taki et al. (27) reported that exposing guinea pig lung membrane particles to phospholipase A_2 induced a reduction in β -adrenergic receptors. Folkerts et al. (7) showed the induction of decrease of β -adrenergic receptor on lungs and airway hyperresponsiveness by injection of 15-HPETE. Since phospholipase A_2 is activated by allergen challenge (23), the consecutive events following phospholipase A_2 activation may have participated in the decrease of β -adrenergic receptors in our experiment.

Although the density of α -1-adrenergic receptor was not significantly altered by the allergen challenge, we noticed a tendency toward an inverse correlation between their density and the RT-HIS ($0.05 < P < 0.1$). Stimulation of α -1-adrenergic receptors can provoke bronchoconstriction, and an α -1-blocker may be effective in treating bronchial asthma. However, the potency of α -1-agonist is so weak as to elicit bronchoconstriction only when the β -adrenergic receptor is blocked (18). Our findings that the correlation between the density of α -1-adrenergic receptors and the RT-HIS is less than that between the density of β -adrenergic receptors and the RT-HIS, and that the density of α -1-adrenergic receptors is approximately 10% of that of β -adrenergic receptors, seem to be consistent with the report by Jenkins et al. (10): that the administration of an α -1-antagonist does not influence the RT-HIS as dramatically as does a β -agonist. Further studies are required to elucidate the role of α -1-adrenergic receptors in airway hyperresponsiveness.

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