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α_{1L}-, but not α_{1H}-, adrenoceptor antagonist prevents allergic bronchoconstriction in guinea pigs in vivo

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Abstract

α-Adrenoceptors have been classified into α_1- and α_2-adrenoceptors. Recently, the α_1-adrenoceptors were divided into two subtypes: α_{1L} with low affinity and α_{1H} with high affinity for prazosin. Little is known concerning the role of each subtype of α_1-adrenoceptor in asthma. We investigated the effects of specific antagonists of α_1- and α_2-, α_{1H}-, α_{1L}-, and α_2-adrenoceptors, namely moxisylyte, prazosin, 3-{*N*-[2-(4-hydroxy-2-isopropyl-5-methylphenoxy) ethyl]-*N*-methylaminomethyl}-4-methoxy-2, 5, 6-trimethylphenol hemifumarate (JTH-601), and yohimbine, respectively, on antigen-induced airway reactions in guinea pigs. Fifteen minutes after intravenous administration of moxisylyte (0.01, 0.1 or 1 mg/kg), prazosin (0.01, 0.1, 1 or 10 mg/kg), JTH-601 (1, 3, 6 or 10 mg/kg) or yohimbine (0.1 or 1 mg/kg), passively sensitized and artificially ventilated animals received an aerosolized antigen challenge. Bronchial responsiveness to inhaled methacholine was assessed as the dose of methacholine required to produce a 200% increase in the pressure at the airway opening (PC_{200}) in non-sensitized animals. JTH-601 and moxisylyte, but not prazosin or yohimbine, dose dependently inhibited antigen-induced bronchoconstriction. None of the tested drugs altered PC_{200}. JTH-601 significantly reduced leukotriene C_4 levels in bronchoalveolar lavage fluid obtained 5 min after antigen challenge, but prazosin did not. These results indicate that prevention of antigen-induced bronchoconstriction by blockade of α-adrenoceptors is due to the inhibition of mediator release via α_{1L}-adrenoceptor antagonism.

Keywords: α_{1L}-Adrenoceptor; α_{1H}-Adrenoceptor; Bronchial asthma; Leukotriene C_4; Asthmatic response, immediate

1. Introduction

β_2-Adrenoceptor agonists have been widely prescribed as bronchodilators in the treatment of bronchial asthma (O'Donnell, 1970). However, little is known concerning the roles of α-adrenoceptors in the human bronchial tree, despite the presence of α-adrenoceptors in the airways of both animals (Everitt and Cairncross, 1969) and humans (Mathe et al., 1971), and the elicitation of bronchoconstriction by their stimulation (Snashall et al., 1978; Patel and Kerr, 1973). Patel and Kerr (1973) have shown that bronchomotor tone in asthmatics is largely controlled by sympathetic activity. α-Adrenoceptors are present in human airways and are constricted by phenylephrine, a powerful α-adrenoceptor agonist, under conditions of β-adrenoceptor blockade, and the bronchoconstrictor effect is completely inhibited by the α-adrenoceptor antagonists phenoxybenzamine and thymoxamine. Marcelle (1996) has reported that increases in airway resistance are prevented by phentolamine. It has been reported that α-adrenoceptor antagonists inhibit histamine-induced bronchoconstriction (Gaddie et al., 1972) and exercise-induced bronchoconstriction (Bleecker et al., 1983) in asthmatic subjects, suggesting that α-adrenoceptor antagonists are useful for the treatment of asthma. However, Baudouin et al. (1988) failed to find evidence for any therapeutic benefit of prazosin given for 3 weeks to patients with chronic asthma controlled with conventional antiasthma treatment.

α-Adrenoceptors have been classified into α_1- and α_2-adrenoceptors. Recently, the α_1-adrenoceptors were divided into two subtypes: α_{1L} with low affinity and α_{1H} with high affinity for prazosin, the latter consisting of α_{1A}-, α_{1B}- and α_{1D}-subtypes (Muramatsu et al., 1995). Although prazosin, a relatively selective antagonist for α_{1H}-adrenoceptors, has

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no therapeutic potency in asthma, the role of α_{1L}-adrenoceptors in asthma is unknown. The purpose of the present study was to examine the role of the α-adrenoceptor subtypes, especially the α_{1L}-subtype, in the pharmacology of allergic bronchoconstriction in guinea pigs. We studied the effects of the specific α_1- and α_2-adrenoceptor dual antagonist moxisylyte, the selective α_{1H}-adrenoceptor antagonist prazosin, the selective α_{1L}-adrenoceptor antagonist 3-{*N*-[2-(4-hydroxy-2-isopropyl-5-methylphenoxy) ethyl]-*N*-methylaminomethyl}-4-methoxy-2, 5, 6-trimethylphenol hemifumarate (JTH-601) and the selective α_2-adrenoceptor antagonist yohimbine on antigen-induced early-phase bronchoconstriction in passively sensitized guinea pigs and on bronchial responsiveness to inhaled methacholine in non-sensitized animals.

2. Materials and methods

2.1. Animals

Male albino Hartley strain guinea pigs (350–400 g) were obtained from Sankyo Laboratory Service (Toyama, Japan). After arrival at the Institute of Animal Experiments at our university, they were kept in conventional animal housing facilities for 1 week before use. They were allowed food and water ad libitum before experiments. All the animal procedures in this study complied with the standards set out in the guidelines for the care and use of laboratory animals of the Takara-machi campus of Kanazawa University.

2.1.1. Passive sensitization of animals

We used passively sensitized animals because the intensity of sensitization shows a wide range in actively sensitized guinea pigs, and bronchial responsiveness is variable in normal animals. Thus, the degree of antigen-induced bronchoconstriction is more variable and less reproducible in actively sensitized animals than in passively sensitized animals. Guinea pig homocytotropic antiserum was obtained according to the method of Santives et al. (1976). Briefly, 500 μg of ovalbumin was emulsified in Freund's complete adjuvant and injected intradermally (i.d.) in each guinea pig at multiple sites. Boosting was carried out in the same manner 2 weeks later. Serum was collected from each animal 2 weeks after boosting, pooled and kept frozen until use. The antibody titers of this serum were 1:12 800, 1:6400 and 1:512 at 4 h, 24 h and 7 days, respectively, as estimated by passive cutaneous anaphylaxis. Normal guinea pigs were passively sensitized with 1.0 ml of antiserum/kg given intraperitoneally (i.p.).

2.1.2. Preparation of animals

Guinea pigs were anesthetized with sodium pentobarbitone (75 mg/kg, i.p.). They were placed in the supine position and a polyethylene tube (outside diameter, 2.5 mm; inside diameter, 2.1 mm) was inserted into the trachea. The left jugular vein was cannulated for administration of drugs and the left carotid artery for measurement of arterial blood pressure. After surgery, each guinea pig was artificially ventilated with a small animal respiratory pump (Model 1680, Harvard Apparatus, South Natick, MA, USA) adjusted to a tidal volume of 10 ml/kg at a rate of 60 strokes/min. The change in lung resistance to inflation, measured as the lateral pressure of the tracheal tube (pressure at the airway opening, cm H_2O), was determined using a pressure transducer (Model TP-603T, Nihon Koden Kogyo, Tokyo, Japan) by the modified method of Konzett and Rossler, as described by Jones et al. (1982). Since the change in the pressure at the airway opening following inhalation of leukotriene C_4 represents the average of the changes in pulmonary resistance and reciprocal dynamic lung compliance, and the change in the pressure at the airway opening is more reproducible than using a body plethysmography (Fujimura, 1983), we used the pressure at the airway opening as an overall index of the bronchial response to bronchoactive agents.

2.2. Antigen-induced bronchoconstriction

Guinea pigs were used 7–8 days after passive sensitization. Before surgery, the animals were given diphenhydramine hydrochloride (60 mg/kg, i.p.) to block the effect of histamine, and then given sodium pentobarbitone (15 mg/kg, i.p.). The lungs were over-inflated with twice the tidal volume for two breaths by clamping the outlet port of the respirator, to standardize the volume history of the lungs (Fujimura, 1983), and the following drugs were injected into the left jugular vein: moxisylyte at a dose of 0.01, 0.1 or 1 mg/kg dissolved in saline or the vehicle (saline), prazosin at a dose of 0.01 or 0.1 mg/kg dissolved in 10% dimethyl sulfoxide (DMSO) or vehicle (10% DMSO), yohimbine at a dose of 0.1 or 1 mg/kg dissolved in 10% DMSO or vehicle (10% DMSO), or JTH-601 at a dose of 1, 3, 6 or 10 mg/kg dissolved in 10% DMSO or vehicle (10% DMSO). Doses of each test drug in this study were used in the range from one-tenth to 10 times each standard dose used in the guinea pig experiment.

Each experiment with moxisylyte, prazosin, JTH-601 or yohimbine was independently performed using the sandwich method: for example, a set of experiments with vehicle, 0.01 mg/kg moxisylyte, 0.1 mg/kg moxisylyte and 1.0 mg/kg moxisylyte was repeated in this order 10 times in the moxisylyte study (a total of 10 animals for each group).

Fifteen minutes after preparation, when the pressure at the airway opening had stabilized, passively sensitized guinea pigs were challenged with nebulized ovalbumin solution dissolved in physiological saline (1.0 mg/ml) under constant ventilation. Animals inhaled the aerosol through another tube connected to the ventilator, while they continued to be ventilated. The ovalbumin aerosol was generated for 30 s with an ultrasonic nebulizer developed for

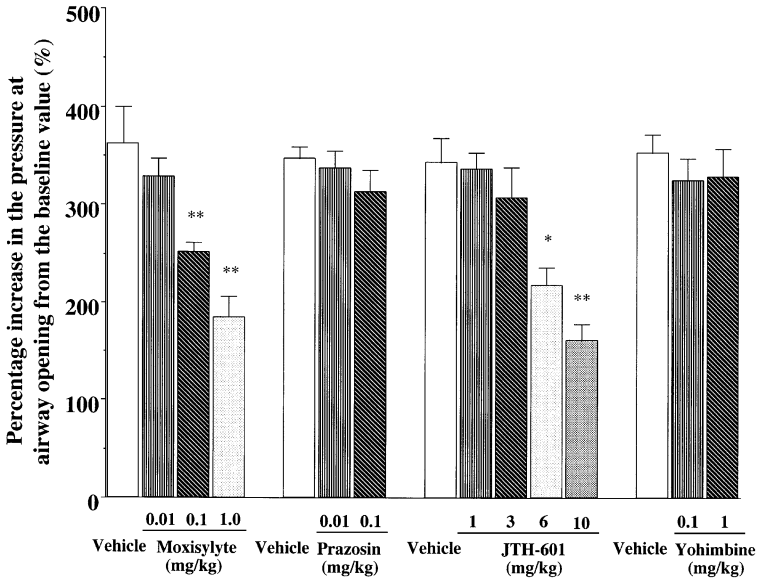

Fig. 1. Antigen-induced bronchoconstriction in passively sensitized guinea pigs. Data are shown as peak increase in pressure at the airway opening from the prechallenge value (mean ± S.E.M.). Each test drug was intravenously administered 15 min before the antigen challenge. Each experiment for moxisylyte, prazosin, JTH-601 or yohimbine was independently performed using the sandwich method: for example a set of experiments with vehicle, 0.01 mg/kg moxisylyte, 0.1 mg/kg moxisylyte and 1.0 mg/kg moxisylyte was repeated in this order 10 times in the moxisylyte study (a total of 10 animals for each group). The vehicle of moxisylyte was saline, and the vehicles of prazosin, JTH-601 and yohimbine were 10% DMSO. Ten animals were used for each group. $*P<0.05$, $**P<0.01$ vs. vehicle.

small animals at our institution (Minami et al., 1983). The rate of aerosol production was 15.2 μl/min and 46.4% of the aerosol was deposited in the lung, as measured by the radioaerosol technique (Minami et al., 1983). The median aerodynamic diameter of the particles of physiologic saline was 3.59 ± 1.96 μm (mean ± S.D.), as measured with a laser particle size analyzer (Kurashima et al., 1997). Changes in the pressure at the airway opening were continuously measured for 20 min after the challenge with ovalbumin.

Considering the results with JTH-601, we confirmed the effects of high doses of prazosin (1 or 10 mg/kg dissolved in 10% DMSO) on antigen-induced bronchoconstriction after the above-described experiments.

2.3. Bronchial responsiveness to inhaled methacholine

Non-sensitized guinea pigs were prepared and given each test drug in the same fashion as described above. In addition, because each drug is known to improve hypertension, in order to assess the vasodilator effect a cannula connected to a pressure transducer (Model AP-601G, Nihon Koden Kogyo) was inserted into the carotid artery. Heparin (20 units/ml) diluted with saline was added ad libitum to the cannula to prevent blood clotting inside it. Ascending doses of methacholine (25, 50, 100 and 200 μg/ml) were inhaled for 20 s via the nebulizer at intervals of 5 min (Fujimura et al., 1997).

2.4. Leukotriene C_4 level in bronchoalveolar lavage fluid

To examine the effect of the selective α_{1H}-adrenoceptor antagonist prazosin or the selective α_{1L}-adrenoceptor antagonist JTH-601 on antigen-induced leukotriene C_4 production, bronchoalveolar lavage was carried out 5 min after ovalbumin challenge in passively sensitized guinea pigs. A 10-ml aliquot of normal saline was injected into the lungs through the tracheal cannula and the lavage effluent was allowed to drain into a 50-ml conical tube. This procedure was repeated twice (Fujimura et al., 1998). The recovered bronchoalveolar lavage fluid was centrifuged at 1000 rpm ($167 \times g$) for 10 min, and the supernatant fluid was collected and frozen at −80 °C until use. Leukotriene C_4

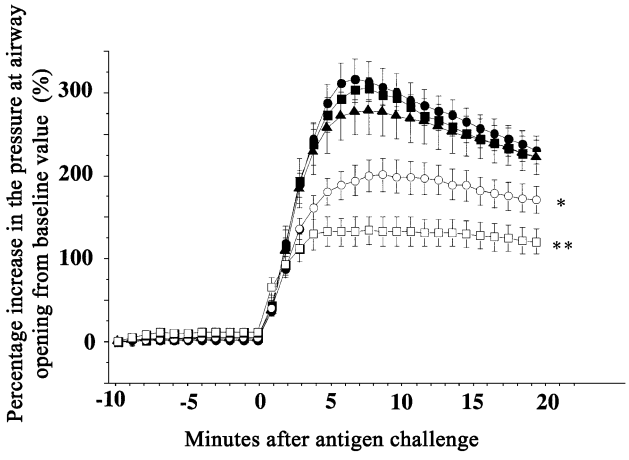

Fig. 2. Time course of percentage increase in pressure at the airway opening from the baseline value in guinea pigs treated with JTH-601 in doses of 1 (closed squares), 3 (closed triangles), 6 (open circles) and 10 mg/kg (open squares), and vehicle (closed circles) (mean ± S.E.M.). Ten animals were used for each group. $*P<0.05$, $**P<0.01$ vs. vehicle by repeated measure ANOVA.

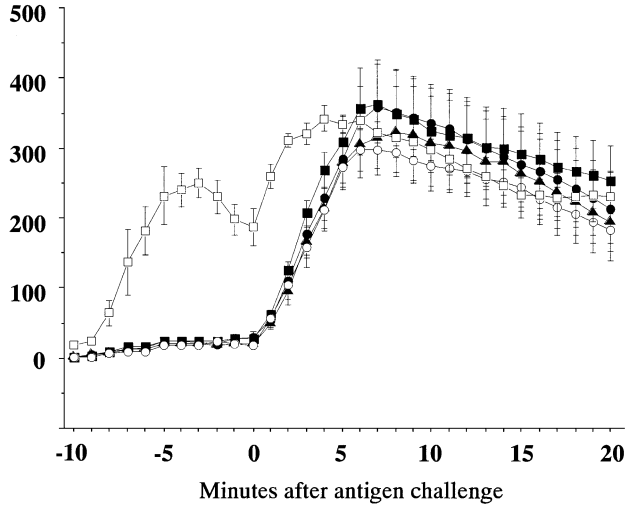

Fig. 3. Time course of percentage increase in pressure at the airway opening from the baseline value in guinea pigs treated with prazosin in doses of 0.01 (closed squares), 0.1 (closed triangles), 1 (open circles) and 10 mg/kg (open squares), and vehicle (closed circles) (mean ± S.E.M.). Seven animals were used for each group. $*P<0.05$, $**P<0.01$ vs. vehicle by repeated measure ANOVA.

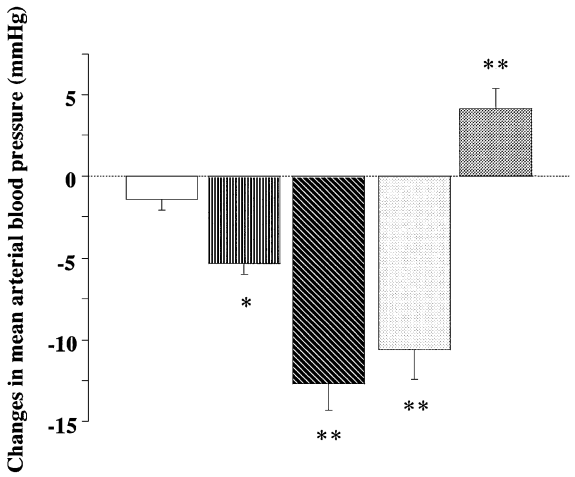

Fig. 5. Change in mean arterial blood pressure in non-sensitized guinea pigs treated with vehicle (open column), 1 mg/kg moxisylyte (striped column), 0.1 mg/kg prazosin (hatched column), 10 mg/kg JTH-601 (stippled column) and 1 mg/kg yohimbine (shaded column) (mean ± S.E.M.). Nine animals were used for each group. $*P<0.05$, $**P<0.01$ vs. vehicle.

concentration in the bronchoalveolar lavage fluid was determined with a commercially available enzyme immunoassay (Cayman Chemical, Ann Arbor, MI, USA). The sensitivity of this assay was 80%.

2.5. Chemicals

The following drugs were used: sodium pentobarbitone (Abbot Laboratories, North Chicago, IL, USA); ovalbumin (Sigma, St. Louis, MO, USA); diphenhydramine hydrochloride (Sigma); methacholine (Wako, Osaka, Japan); moxisylyte hydrochloride (Sigma); prazosin hydrochloride (Sigma); yohimbine hydrochloride (Sigma); JTH-601 (3-{*N*-[2-(4-hydroxy-2-isopropyl-5-methylphenoxy) ethyl]-*N*-methylaminomethyl}-4-methoxy-2, 5, 6-trimethylphenol hemifumarate) (Japan Tobacco, Central Pharmaceutical Research Institute, Osaka, Japan).

2.6. Statistical analysis

Airway responsiveness to inhaled methacholine is expressed as the dose of methacholine required to provoke a 200% increase in the pressure at the airway opening (PC_{200}). Values for PC_{200} were logarithmically transformed

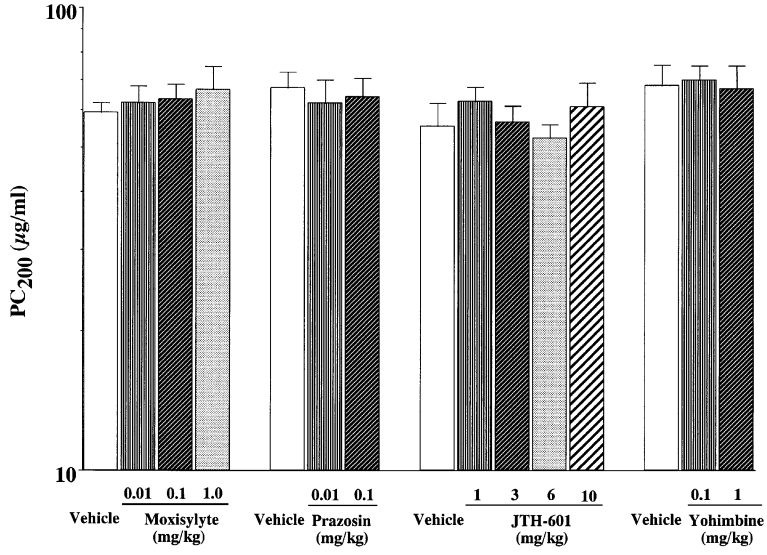

Fig. 4. Bronchial responsiveness to inhaled methacholine in non-sensitized guinea pigs. Columns and vertical bars present geometric mean and geometric standard errors of the mean. PC_{200}: concentration of methacholine required to cause a 200% increase in pressure at the airway opening. Each tested drug was intravenously administered 15 min before the methacholine challenge. Nine animals were used for each group.

for analysis and are reported as the geometric mean with geometric standard error of the mean. All measurements except those for PC_{200} are expressed as means ± S.E.M. Differences among three or more groups were tested for significance by non-parametric analysis of variance (ANOVA), while those between two groups were tested by the Mann–Whitney's *U*-test. Differences in time-course curves for percentage increase in the pressure at the airway opening from the baseline value after ovalbumin or methacholine provocation were examined by two-factor repeated-measures ANOVA. *P* values of 0.05 or less were considered significant.

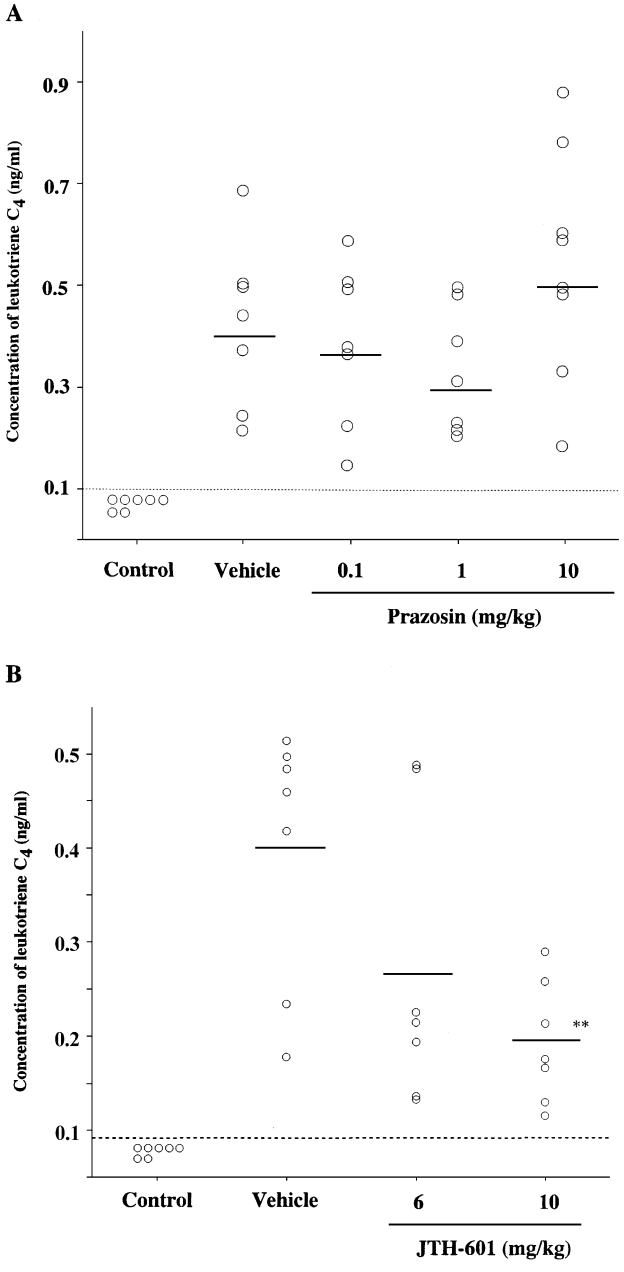

Fig. 6. Concentration of leukotriene C_4 in bronchoalveolar lavage fluid recovered 5 min after antigen challenge in passively sensitized guinea pigs treated intravenously with prazosin in doses of 0.1, 1 and 10 mg/kg, and vehicle (10% DMSO) (A), and with JTH-601 in doses of 6 and 10 mg/kg, and vehicle (10% DMSO) (B) (mean ± S.E.M.). Control means non-sensitized guinea pigs. Leukotriene C_4 concentration in the control animals was below the detection limit of this method. Seven animals were used for each group (nine animals were used for 10 mg/kg prazosin). Vertical bars represent S.E.M. $^{**}P < 0.01$ vs. vehicle.

3. Results

No significant change in baseline pressure at the airway opening was observed after administration of moxisylyte, prazosin, JTH-601 or yohimbine.

Aerosolized antigen caused acute bronchoconstriction that peaked at nearly 10 min after inhalation in all guinea pigs treated with each vehicle. Moxisylyte and JTH-601, but not prazosin or yohimbine, dose dependently prevented the antigen-induced bronchoconstriction (Fig. 1). The time courses of changes in pressure at the airway opening in animals treated with JTH-601 are shown in Fig. 2. Considering the results of JTH-601, we confirmed the effects of high dose of prazosin (1 or 10 mg/kg) on antigen-induced bronchoconstriction on another day (Fig. 3). The amount of 1 mg/kg prazosin did not obviously decrease the pressure at the airway opening compared with vehicle ($P = 0.079$), and 10 mg/kg prazosin increased the pressure at the airway opening before inhalation of ovalbumin.

Moxisylyte, prazosin, JTH-601 or yohimbine did not alter the bronchoconstrictor responses to methacholine (Fig. 4). Moxisylyte, prazosin and JTH-601 decreased mean arterial blood pressure while yohimbine increased it (Fig. 5).

Mean arterial blood pressure after the inhalation of 200 μg/ml methacholine was decreased compared with that at the time of administration of each test drug (methacholine-induced hypotensive effects). The decreases in blood pressure induced by 200 μg/ml methacholine inhalation were 6.96 ± 1.72, 8.74 ± 3.71, 6.56 ± 2.28, 8.33 ± 2.54 and 2.78 ± 1.57 mm Hg when vehicle, 1 mg/kg moxisylyte, 0.1 mg/kg prazosin, 10 mg/kg JTH-601 and 1 mg/kg yohimbine were administered, respectively, and these values were not significantly different, showing that methacholine decreased blood pressure despite the test drugs. Although yohimbine appeared to reduce the hypotensive response to methacholine compared with vehicle, the difference did not reach statistical significance ($P = 0.241$).

JTH-601 dose dependently decreased leukotriene C_4 levels in bronchoalveolar lavage fluid, recovered 5 min after the antigen challenge, in sensitized guinea pigs (Fig. 6B), but prazosin did not (Fig. 6A).

4. Discussion

The present animal study showed that intravenously administered moxisylyte and JTH-601 prevented antigen-induced bronchoconstriction in sensitized guinea pigs with-

out having any direct effect on bronchial responsiveness to methacholine in non-sensitized animals. Neither prazosin nor yohimbine affected antigen-induced bronchoconstriction or methacholine responsiveness. JTH-601 decreased leukotriene C_4 levels in bronchoalveolar lavage fluid recovered 5 min after antigen challenge in sensitized guinea pigs, but prazosin did not. These findings clearly indicate that α_{1L}-, but neither α_{1H}- nor α_2-, adrenoceptors are involved in antigen-induced leukotriene production but not in non-specific bronchial responsiveness. This study is the first to evaluate the role of α_{1L}-adrenoceptors in the airways.

There is evidence that adrenaline secretion may be impaired in patients with asthma (Ind et al., 1985). Since the adrenoceptors were classified into two classes, α and β, based on the effects of a series of catecholamines, many studies of adrenoceptors have been reported. These days, β_2-adrenoceptor agonists are widely used for the treatment of asthma. α-Adrenoceptors are classified into α_1- and α_2-adrenoceptors, the former being distributed postsynaptically on the effector site and the latter presynaptically on the sympathetic nerves (Langer, 1974). Recently, the existence of postsynaptic α_2-adrenoceptors has been reported (Timmermans and Van Zwieten, 1981). Our question was why α-adrenoceptor antagonists have limited clinical efficacy: their proven efficacy is limited to partial prevention of exercise-induced bronchoconstriction (Patel et al., 1976), despite the broad clinical efficacy of β-adrenoceptor agonists.

β-Adrenoceptors, almost entirely β_2-adrenoceptors, are localized on various cells in the airways and β_2-adrenoceptor agonists have beneficial effects in the treatment of asthma, not only by directly relaxing airway smooth muscle, but also by inhibiting mediator release from mast cells (Hughes et al., 1983). In contrast, α-adrenoceptor agonists cause bronchoconstriction in asthmatic subjects, but not in normal subjects (Black et al., 1982). It is known that the release of chemical mediators by stimulation of α_1-adrenoceptors involves phosphatidylinositol, a membrane phospholipid. Phospholipase C in the cell membrane is activated by stimulation of α_1-adrenoceptors, followed by formation of hydrolyzed phosphatidylinositol, diacylglycerol and inositol trisphosphate (IP_3). IP_3 is a second messenger that controls many cellular processes by generating internal Ca^{2+} signals (Berridge, 1993). These responses increase intracellular Ca^{2+} concentrations, resulting in the synthesis and release of prostanoids and leukotrienes. These findings led us to study the role of α-adrenoceptors, especially α_1-adrenoceptors, in asthma.

Moxisylyte was used as an antagonist of both α_1- and α_2-adrenoceptors in the present study. Phentolamine is commonly used as an α-adrenoceptor antagonist and has been shown to prevent the bronchoconstrictor responses to histamine, allergen and exercise (Barnes, 1984). However, phentolamine lacks pharmacological specificity: it could prevent induction of bronchospasm by its antihistamine effect, potentiation of β-adrenergic effects or α-adrenergic blockade (Bleecker et al., 1983). Thus, we performed this study using moxisylyte, a specific antagonist of both α_1- and α_2-adrenoceptors (Pierre et al., 1993). Although moxisylyte had no effect on the bronchoconstriction induced by methacholine in non-sensitized guinea pigs, it prevented antigen-induced bronchoconstriction. In addition, although it has been reported that α_2- rather than α_1-adrenoceptors mediate contractile responses to α-adrenoceptor agonists in dog airways (Barnes et al., 1983a), the α_2-adrenoceptor antagonist yohimbine altered neither the methacholine-nor the antigen-induced bronchoconstriction in this study, suggesting that α_1-adrenoceptors, but not α_2-adrenoceptors, play a role in antigen-induced mediator release in guinea pigs.

Recently, the existence of α_1-adrenoceptors with low affinity for prazosin (α_{1L}-subtype) has been proposed in addition to α_1-adrenoceptors with high affinity for prazosin (α_{1H}-subtype), the latter consisting of α_{1A}-, α_{1B}- and α_{1D}-subtypes (Muramatsu et al., 1995). Muramatsu et al. (1995) have presented a wealth of detailed evidence that α_{1L}-adrenoceptors are present in many tissues in many species. For example, α_{1L}-adrenoceptor subtypes have been identified in the prostate of humans, dogs and rabbits, the femoral artery of dogs, the heart of rats, and other tissues of other species. However, α_{1L}-adrenoceptors have not yet been identified in the respiratory tract of any species. JTH-601 is a newly synthesized α_{1L}-adrenoceptor antagonist which exhibits approximately 10 times higher affinity for the α_{1L}-subtype, similar affinity for the α_{1A}-subtype, but more than 10 times lower affinity for the α_{1B}- and α_{1D}-subtypes compared with prazosin (Muramatsu et al., 1996). Our results showed that the α_{1L}-adrenoceptor antagonist JTH-601 prevented antigen-induced bronchoconstriction at the maximal dose and decreased leukotriene C_4 levels in bronchoalveolar lavage fluid after antigen challenge without having any direct bronchoprotective effect against methacholine.

The α_{1H}-adrenoceptor antagonist prazosin had no effect on either antigen-or methacholine-induced bronchoconstriction. Actually, high-dose prazosin (1 mg/kg) did not obviously decrease the pressure at the airway opening compared with vehicles or leukotriene C_4 levels in bronchoalveolar lavage fluid after antigen challenge in sensitized guinea pigs. Furthermore, 10 mg/kg prazosin did not inhibit the antigen-induced increase in leukotriene C_4 levels. Rather, the dose of prazosin seemed to increase the leukotriene C_4 levels compared with vehicle. In addition, this dose happened to increase the pressure at the airway opening before antigen inhalation. Accordingly, we thought that the dose of 10 mg/kg prazosin was so high that it might have caused a partial agonist reaction. Therefore, this dose would not be clinically viable. Pharmacologically, prazosin exhibits approximately 10 times lower affinity for the α_{1L}-subtype compared with JTH-601; however, it is unclear whether 1 mg/kg prazosin fully inhibits α_{1L}-adrenoceptors in vivo and retains receptor selectivity. In our study, 1 mg/kg prazosin might not inhibit these receptors sufficiently or retain

receptor selectivity. It is the difficult problem to confirm, because a higher dose of prazosin caused unstable changes before inhalation of antigen. However, at least α_{1L}-adrenoceptors would be involved in antigen-induced bronchoconstriction.

These findings suggest that α_{1L}-adrenoceptors are localized in the airways and play a role in activating phospholipase C to release mediators in the airways, at least guinea pig airways. The site of action cannot be determined from the results of the present study. Since JTH-601 prevented the immediate asthmatic response, it is possible that the site of action is on mast cells and/or basophils. Further studies are required to determine the site of action.

It has been shown that the generation of leukotriene C_4 by bronchoalveolar lavage cells is significantly increased in asthmatic patients (Mitsunobu et al., 1998). Our study revealed that JTH-601 inhibits antigen-induced leukotriene C_4 release in guinea pigs. Phillips et al. (1985) have reported that dog mastocytoma cells have a high density of β-adrenoceptors, the predominant subtype of which appears to be β_2, but they found no evidence for the presence of α-adrenoceptors in their binding study using prazosin. On the other hand, Kaliner et al. (1972) reported that the immunologic release of histamine and slow-reacting substance of anaphylaxis from human lung tissue could be enhanced by stimulation with α-adrenoceptor agonists in the presence of propranolol. It has been demonstrated, by autoradiography that β-adrenoceptors are present in high density throughout the airways, whereas α-adrenoceptors are sparse in large airways and numerous in small bronchioles (Barnes et al., 1983b). Marquardt et al. (1982) have shown that α_1-adrenoceptors are present in rat lungs, but that β-adrenoceptors are 14 times more prevalent, as determined by [^{3}H] prazosin and 1-[propyl-2, 3-^{3}H] dihydroalprenolol ([^{3}H] DNA) binding. In the respiratory tract of sensitized guinea pigs exposed to antigen, the ratio of α- to β-adrenoceptors is increased (Barnes et al., 1980). Barnes et al. (1981) have proposed that α_1-adrenoceptors may play a role in the pathogenesis of exercise-induced asthma in humans, by facilitating mast cell mediator release. Our findings that JTH-601, but not prazosin, decreased leukotriene C_4 levels in bronchoalveolar lavage fluid following antigen-challenge pharmacologically support the hypothesis that α-adrenoceptors play a role in mediator release and suggest the predominant contribution of α_{1L}-subtypes.

Although Suzuki et al. (1999) have shown that JTH-601 is less potent than prazosin in decreasing systemic blood pressure, little is known about the feasibility of using the drug. In the present study, JTH-601 had vasodilator activity nearly equipotent to that of prazosin. This will be the subject of future studies concerning the clinical use of this compound. Methacholine provocation has been thought to cause drug-induced hypotension by a muscarinic effect (Stang et al., 1982). However, Boulet et al. (1990) showed that methacholine challenge was clinically safe in 18 patients with mild-to-moderate primary hypertension, because no significant effects on blood pressure were observed after methacholine inhalation. Our data showed a decrease in blood pressure after the inhalation of 200 μg/ml methacholine; however, the change was slight and no significant differences were apparent among the groups.

In conclusion, our findings suggest that inhibition of antigen-induced bronchoconstriction by blockade of α-adrenoceptors in sensitized guinea pigs is due to the blockade of α_{1L}-adrenoceptors. The inhibition of antigen-induced leukotriene C_4 release may prevent antigen-induced bronchoconstriction by blocking α_{1L}-adrenoceptors. The aim of future studies will be to confirm the existence of α_{1L}-adrenoceptors on mast cells in the respiratory tract of guinea pigs, and to elucidate the role of α_{1L}-adrenoceptors in other specific asthmatic responses such as exercise-, cold air-, ultrasonically nebulized distilled water-, propranolol-induced bronchoconstriction, and so on. Finally, this is the first study to reveal possible antiasthmatic effects of α_{1L}-adrenoceptor antagonists. Progress in evaluating the clinical use of α_{1L}-adrenoceptor antagonists in the treatment of asthma is of great importance.

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