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Contractile Responses to Allergen and Cholinergic Stimulation in Bronchus and Trachea of Cockroach Allergen-Sensitized Guinea Pigs

Abstract

Airway hyperreactivity is a hallmark of asthma. Yet, the role of allergic sensitization in the hyperreactivity remains controversial. This study examined the effects of airborne cockroach allergen (CRa) sensitization on the contractility of bronchial (BSM) and tracheal (TSM) smooth muscle rings to cholinergic stimulations, and to specific antigen, in vitro. Guinea pigs were actively immunized with CRa, 5 mg (high dose, HD), 0.5 mg (low dose, LD), or saline aerosols (2×/day, 5 days/week, for 4 weeks) and challenged by CRa inhalation 5 days after last exposure. Twenty-four hours postchallenge, the contractions of the TSM and BSM to electrical field stimulation (EFS), acetylcholine (ACh), and to CRa were measured in vitro. In addition, the contraction to these stimuli were also examined in the passively sensitized TSM with the sera of CRa-immunized guinea pigs. The contractile responses of actively immunized and passively sensitized tissues to EFS and ACh were very similar to those of control tissues. CRa induced dose-dependent contractions in both actively immunized and passively sensitized, but not in control, tissues. The CRa-induced contraction was stronger in the HD group than in the LD group ($p < 0.0001$). Leukotriene C4/D4 receptor antagonist LY-171883 inhibited the CRa-induced contraction by 86% in actively immunized TSM, and by 9% in the passively sensitized TSM. Pyrilamine inhibited the contraction by 57% in actively immunized TSM and 70% in passively sensitized TSM. The results indicate that CRa sensitization does not cause increased airway smooth muscle contractility to cholinergic stimulations, but induces antigen-specific contractions in vitro. Leukotrienes appear to play a significant role in the CRa-specific contractions, more in the actively immunized than those in the passively sensitized tissues, while histamine exerts a moderate effect on the CRa-induced contractions.

Introduction

Airway hyperreactivity to nonspecific and specific stimuli is a hallmark of asthma and often correlates with the severity of asthma [1]. It is likely that the changes in the

contractility of airway smooth muscles resulted from allergen sensitization and the inflammatory state may contribute to airway hyperreactivity in asthma. Consequently, the contractile responses of the airway smooth muscles to a variety of nonspecific and specific stimuli have been extensively

studied in both humans and animals. However, many conflicting results have been reported in regard to the *in vitro* contractile responses of airway smooth muscles to various agonists, such as histamine, serotonin, acetylcholine (ACh), thromboxane A₂, in asthmatic patients [2–5] and in various animal asthma models [6–15]. Either enhanced [2, 6–9], unaltered [10, 11], or even decreased [4, 12, 15] contractile responses of allergen-sensitized airway smooth muscles to nonspecific stimulation were observed as compared to those of nonsensitized controls. The reasons for such variable results are not apparent and require further clarification. Furthermore, the role of sensitization to indoor allergens, such as cockroach allergen (CRa), in the induction of airway smooth muscle hyperreactivity and its relationship with allergen-specific airway contractions need further investigation.

Previously, our laboratory reported that guinea pigs exposed chronically to airborne CRa developed allergic sensitization and that subsequent CRa challenge induced asthma-like lung function changes [16–18]. As a part of the study to elucidate the mechanisms of CRa-induced asthma, the authors investigated the contractile responses of airway smooth muscles *in vitro* to cholinergic stimulants, ACh and electrical field stimulation (EFS), using tracheal (TSM) and bronchial (BSM) smooth muscle rings, isolated from the guinea pigs chronically immunized by aerosolized CRa or from nonimmunized guinea pigs and passively sensitized with sera of CRa-immunized guinea pigs. In addition, the mechanisms of antigen-specific contractile responses of the airway smooth muscles *in vitro* were examined using antagonists of anaphylactic mediators, pyrilamine (a histamine H₁ receptor antagonist) or LY-171883 [a leukotriene (LT) C₄/D₄ receptor antagonist].

Materials and Methods

Materials

CRa, a lyophilized German cockroach whole body extract, was obtained from Greer Laboratories (Lenior, N.C.) and used for allergic sensitization and challenge after being dissolved in sterile saline. Ovalbumin (OA, grade V), ACh, and pyrilamine were obtained from Sigma Chemical Co. (St. Louis, Mo.) and used after being appropriately prepared. LY-171883 was obtained from Biomol Research Laboratories (Plymouth Meeting, Pa.) and initially dissolved in ethanol; further dilutions were made in Krebs-Ringer solution (K-R solution) of the following composition (in mM): NaCl 118, KCl 4.8, CaCl₂ · 2 H₂O 2.5, MgSO₄ 1.2, NaHCO₃ 25, KH₂PO₄ 1.15, dextrose 5.6 and HEPES 12.6.

Cockroach Allergen Sensitization and Challenge

Male Hartley guinea pigs, weighing 200–250 g, were obtained from Hilltop Laboratory (Scotsdale, Pa.). After 1 week of quarantine at the animal care facility of the University of Kentucky, accredited by

the American Association of Laboratory Animal Care, the guinea pigs were sensitized with CRa as previously described [18]. Briefly, the guinea pigs were randomly divided into four groups (6 animals/group): two CRa groups (high dose, HD; low dose, LD) and two time- and age-matched control groups for each corresponding CRa group. A DeVilbiss ultrasonic nebulizer (Model 100, Somerset, Pa.) was used to generate and deliver the aerosolized CRa (5 mg for HD, 0.5 mg for LD, in 10 ml sterile saline) or saline (for control groups) to a semiclosed acrylic chamber housing guinea pigs to immunize the animals. The aerosol exposure was carried out for a period of 45 min repeated twice a day, 5 days a week, for 4 weeks. After 4 weeks of immunization, development of anaphylactic sensitivity to CRa was confirmed by both allergic skin test and PCA test as described [16–18]. Five days after the last aerosol exposure, the asthmatic responses *in vivo* were measured using a double chamber plethysmograph following CRa challenge (1 mg/ml × 10 ml) in conscious state as described [18]. Immediately after completion of a 24-hour study of the lung function, bronchoalveolar lavage (BAL) was performed to determine inflammatory state of the airway as described [18]. The study was carried out by the protocol approved by Institutional Animal Care and Usage Committee of the University of Kentucky.

Airway Smooth Muscle Ring Preparation

Preparation of the Tissues from Actively Immunized Animals. The guinea pigs were euthanatized by intraperitoneal injection of sodium pentobarbital immediately after completion of the study on asthmatic responses and BAL *in vivo* at 24 h post-CRa challenge. The whole lung along with the trachea was removed from the animals and soaked in pregassed K-R solution (4°C). The trachea and main bronchi were cut into 3-mm rings after removal of the surrounding connective tissues, then mounted on plastic tissue holders and immersed in water-jacketed organ baths containing 5 ml K-R solution (37°C, pH 7.4) with aeration by 95% O₂-5% CO₂. The tissues were equilibrated for 60–90 min at a resting tension of 0.75 g (for trachea) or 0.5 g (for bronchi) and refreshed with K-R solution every 20 min as described.

Preparation of the Passively Sensitized Tissues. Segments of tracheal muscle rings obtained from nonimmunized guinea pigs were passively sensitized by incubation of the tissues in the sera (1:3, sera to K-R solution) collected from the CRa-immunized or from control guinea pigs at 37°C for 3 h. Then the TSM was washed, mounted and equilibrated as described above.

Measurement of Airway Smooth Muscle Contraction to Cholinergic Stimulations

After equilibration, the isometric tension of the TSM or BSM was measured using FT-03C force displacement transducers coupled with a Grass 79D polygraph data recording system (Grass Instrument, Quincy, Mass.). The responses of the tissues were determined in the order of EFS, ACh, and allergen (CRa or OA) challenges with a minimum 30-min interval during which extensive washings were carried out to allow the tissue tension to return to the baseline following each stimulation. The contractile response to EFS was elicited via a Grass SD4 stimulator (Grass Instrument, Quincy, Mass.) using a pair of platinum electrodes positioned on the tissue holder on opposite sides of the tissue with a distance of 6 mm. EFS was applied by square wave of 1 ms duration at 40 V and 10 Hz for 10 s. These parameters were chosen because they produced optimal reproducible contractions in both TSM and BSM of the guinea pigs. The EFS-induced contraction was completely abolished by atropine (10⁻⁶ M) or tetrodotoxin (10⁻⁶ M), indicating its cholinergic and neurogenic nature, i.e., it is elicited via

Table 1. Inflammatory cells in BAL fluid 24 h after CRa challenge ($\times 10^6$)

	Control (n = 11)	LD CRa (n = 5)	HD CRa (n = 6)	p value
Total leukocytes	4.91 $\pm$ 0.46	7.03 $\pm$ 1.37	19.50 $\pm$ 4.31	<0.001 (HD vs. control) <0.01 (HD vs. LD)
Lymphocytes	0.85 $\pm$ 0.11	1.77 $\pm$ 0.39	1.22 $\pm$ 0.28	
Eosinophils	0.17 $\pm$ 0.04	0.88 $\pm$ 0.43	1.83 $\pm$ 0.42	<0.01 (HD vs. control)
Macrophages	2.99 $\pm$ 0.51	3.54 $\pm$ 0.93	5.80 $\pm$ 0.82	
Neutrophils	0.99 $\pm$ 0.13	0.79 $\pm$ 0.20	8.25 $\pm$ 3.53	<0.01 (HD vs. control) <0.01 (HD vs. LD)

Data are expressed as mean $\pm$ SE. The BAL fluid was obtained from saline (control)-, LD CRa- or HD CRa-sensitized guinea pigs at 24 h after CRa challenge.

release of ACh onto muscarinic receptors on airway smooth muscles. After EFS, a cumulative dose-response curve of ACh was constructed for each airway ring by addition of a series of concentrations of ACh (10^{-7} to 3×10^{-5} M in final bath concentration) with a 0.5 log unit increment [19].

Measurement of Airway Smooth Muscle Contraction to Antigens

To determine the airway smooth muscle contractile responses to CRa, we initially used a series of CRa doses from 0.0001 to 300 μ g/ml (final bath concentration) to establish a full dose-response curve in a preliminary experiment. We found that a detectable level of contractions in the muscle preparations occurred at the concentration of CRa 1 μ g/ml or more. Therefore, the allergen-induced contraction of the airway smooth muscles was measured and expressed as a cumulative CRa dose-response curve for each ring between CRa doses 1–100 μ g/ml with a 0.5 log unit increment. In a separate experiment the airway smooth muscles, TSM and BSM, isolated from CRa-immunized animals were challenged with OA, a non-specific allergen, in a single dose (30 μ g/ml, final bath concentration).

The antigen-specific contractions were studied further to determine the roles of LTs and histamine in the CRa-induced airway contraction. Three tissue rings from the same guinea pig in the HD group were preincubated with LY-171883 (10^{-5} M), pyrilamine (10^{-6} M), or in K-R solution (a vehicle) for 20 min in the organ bathes prior to the CRa challenges. Another tissue ring obtained from a control guinea pig was also preincubated with the vehicle simultaneously as a nonimmunized control. The passively sensitized TSMs were also studied in the same processes as the actively immunized tissues were studied. The antigen-induced smooth muscle contraction was expressed as a percentage of the maximal contraction induced by ACh (3×10^{-5} M) in the same airway muscle ring. The viability of the muscle rings was confirmed by the response to the ACh challenge at the end of each experiment.

Data Analysis and Statistics

Data are expressed as mean $\pm$ SE. Statistical analysis was performed using analysis of variance (ANOVA), corrected with repeated measurement when appropriate. The main effect was considered significant at $p < 0.05$. Further post hoc analysis was conducted using the Student Newman-Keuls test. Student's t test was used for a simple mean comparison between two groups. The difference between means was significant at $p < 0.05$. Since the tissues from two control groups (HD control and LD control) had the same responses to ACh and CRa

($p < 0.05$), both sets of control data were pooled for comparison with those from HD or LD CRa-sensitized guinea pigs.

Characteristics of the CRa-Immunized Guinea Pigs

All CRa-immunized guinea pigs, both LD and HD, developed anaphylactic antibody to CRa confirmed by allergy skin test and passive cutaneous anaphylaxis (PCA) assay. The antibody IgG1 with PCA titer ranged from 1:2 in LD and 1:40 in HD group. The control-immunized guinea pigs showed negative skin reaction and PCA to CRa. In addition, a great majority (10/12) of the CRa-immunized animals, both LD and HD, developed increased specific airway resistance upon CRa challenge. The increases of the specific airway resistance in the individual guinea pigs, however, were variable in its presence as well as in its lapsed time from the point of CRa challenges. These increases were suggestive of early asthmatic response, late asthmatic response or dual responses [18]. Moreover, the CRa-immunized guinea pigs showed significant leukocytosis and eosinophilia in the BAL fluid at 24 h post-CRa challenge as compared to those in control-immunized animals (table 1).

Results

Nonspecific Contractile Responses

EFS induced contractions of the airway smooth muscles. The degrees of contractions of airway smooth muscles of the CRa immunized guinea pigs, both LD and HD, were the same as those from control animals (fig. 1). ACh challenges also induced airway smooth muscle contraction in a dose-dependent manner. Figure 2 shows the cumulative dose-response curves of the BSM isolated from the CRa-immunized guinea pigs as compared to those of control animals. The TSM showed similar contractility to ACh challenge, yet were slightly less sensitive than those of BSM. The mean maximum contractions induced by ACh at 3×10^{-5} M were 1.3 g in BSM and 1.0 g in TSM. In addition, the contractile responses of the passively sensitized tissues to EFS or to ACh were also similar to those of nonsensitized tissues (data not shown).

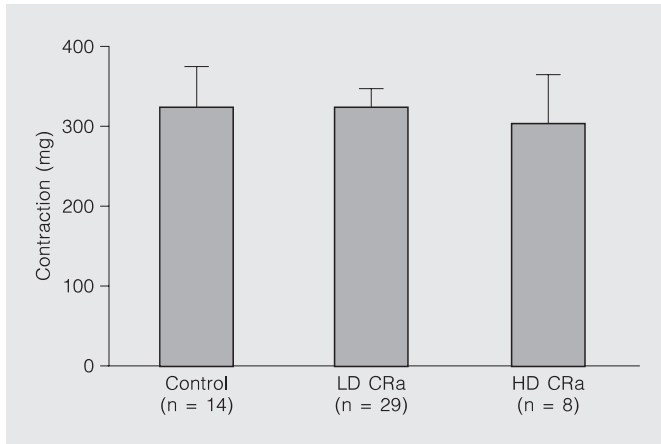


Fig. 1. Contractile response of TSM to EFS. The TSM isolated from saline (control), LD CRa-, or HD CRa-immunized guinea pigs at 24 h post-CRa inhalation challenge were stimulated with EFS. Data are expressed as isometric tension and presented as mean $\pm$ SE.

Antigen-Specific Contractile Responses

The airway smooth muscle rings of the CRa-immunized guinea pigs showed contractions to the CRa challenges in a dose-dependent manner. There was no contraction noted upon CRa challenge in the TSM and BSM isolated from control guinea pigs. The threshold dose of CRa required to contract airway muscles varied by the individual tissues as well as the individual immunized animals. Yet, generally, there were lower threshold doses inducing contractile responses in the rings from the HD group than in those from the LD group. Figure 3 shows the responses to CRa in the TSM isolated from HD or LD CRa-immunized and control guinea pigs. The dose-response curve of the tissues from HD guinea pigs shifted leftward and upward compared to that of the tissues from LD animals. A significantly stronger contraction was observed in the rings of the HD group than in that of the LD animals ($p < 0.0001$).

Similar dose-dependent contractile responses of the airway smooth muscles to CRa were also seen in the TSM of the nonimmunized guinea pigs when the tissues were passively sensitized by incubation in the sera from the actively CRa-immunized guinea pigs (data not shown). The addition of OA did not induce any contraction in either CRa-immunized or control tissues.

Effects of LY-171883 and Pyrilamine on the CRa-Induced Contraction

We studied further the contractilities of the actively immunized and passively sensitized tissues after the tissues

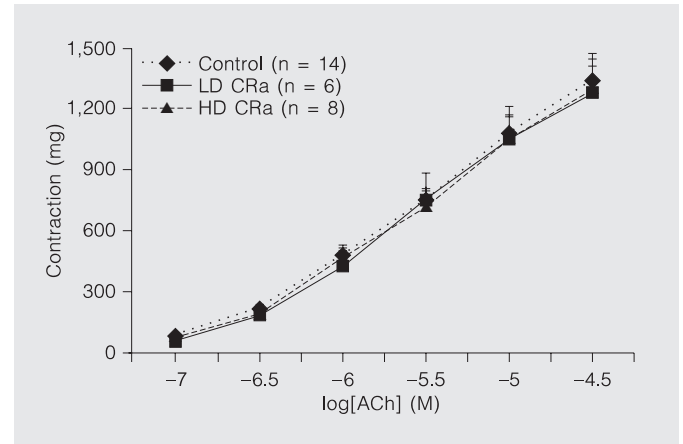


Fig. 2. Cumulative dose-response curves to ACh in BSM. The tissues isolated from saline (control)-, LD CRa-, or HD CRa-immunized guinea pigs at 24 h post-CRa inhalation challenge were stimulated with cumulative doses of ACh. Data are expressed as isometric tension and presented as mean $\pm$ SE.

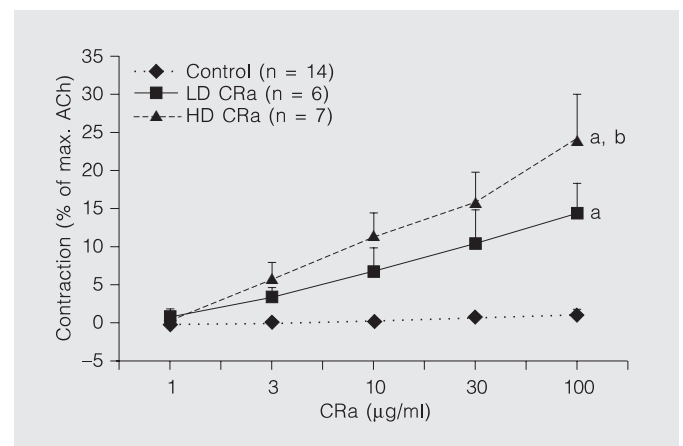


Fig. 3. Cumulative dose-response curves to CRa in TSM. The tissues isolated from saline (control)-, LD CRa-, or HD CRa-immunized guinea pigs at 24 h post-CRa inhalation challenge were stimulated with cumulative doses of CRa. Data are expressed as a percentage of maximal contraction of ACh (3×10^{-5} M) and presented as mean $\pm$ SE. ^a $p < 0.0001$ vs. control; ^b $p < 0.0001$ vs. LD CRa.

were preincubated with LY-171883 and pyrilamine, in comparison with those of the control incubation (preincubated with vehicle). It was found that the CRa-induced contractions of the tissues were markedly attenuated by preincubation with pyrilamine and LY-171883 (both $p < 0.001$, fig. 4). LY-171883 produced a greater inhibitory effect on the CRa-induced contractions of TSM ($p < 0.001$) than did pyri-

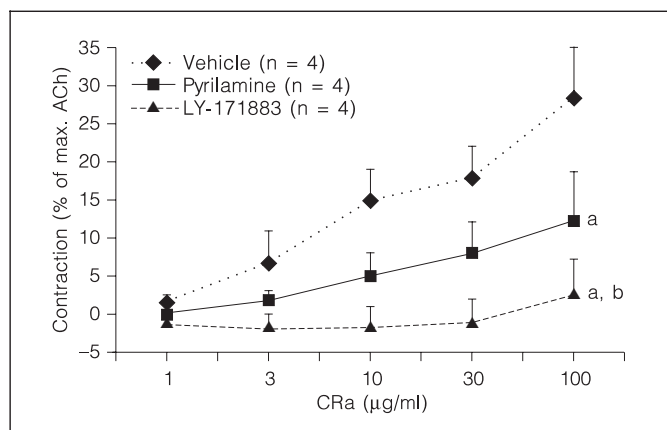


Fig. 4. Effects of pyrilamine or LY-171883 on CRA-stimulated contraction of TSM. The tissues isolated from HD CRA-immunized guinea pigs at 24 h post-CRA inhalation challenge were preincubated with vehicle, pyrilamine (10^{-6} M), or LY-171883 (10^{-5} M) for 20 min and then stimulated with cumulative doses of CRA. Data are expressed as a percentage of maximum contraction of ACh (3×10^{-5} M) and presented as mean $\pm$ SE. Numbers of tissues were from 4 animals for each group. ^a $p < 0.001$ vs. vehicle; ^b $p < 0.001$ vs. pyrilamine.

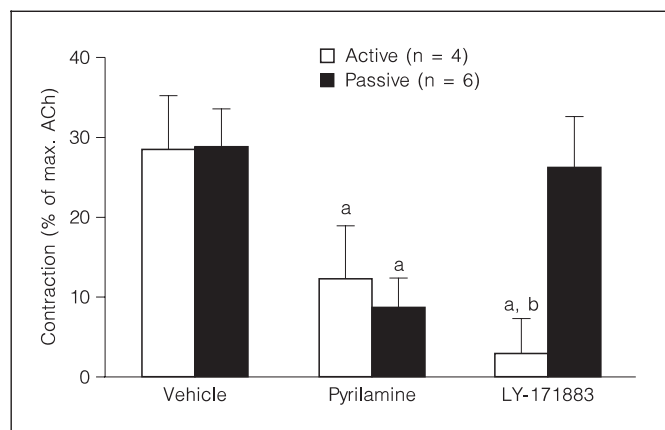


Fig. 5. Effects of pyrilamine or LY-171883 on CRA-induced contraction of the actively immunized or passively sensitized TSM. The TSM were preincubated with vehicle, pyrilamine (10^{-6} M), or LY-171883 (10^{-5} M) for 20 min and then stimulated with 100 µg/ml CRA. Data are expressed as a percentage of maximum contraction (3×10^{-5} M) of ACh and presented as mean $\pm$ SE of numbers of tissues. 'Active' refers to TSM isolated from 4 actively CRA-immunized guinea pigs, 'passive' to TSM isolated from 6 nonimmunized guinea pigs and passively sensitized by incubation with the sera of CRA-immunized guinea pigs. ^a $p < 0.001$ vs. vehicle; ^b $p < 0.01$ vs. the tissue passively sensitized and incubated with LY-171883.

mine. Moreover, both antagonists showed less inhibitory effects on contractions of BSM than those noted in the TSM. However, the difference between TSM and BSM did not reach statistical significance ($p > 0.05$).

LY-171883 and pyrilamine also exerted inhibitory effects on the CRA-induced contractions of the passively sensitized TSM. However, LY-171883 showed significantly different inhibition of the CRA-induced contractions in the passively sensitized TSM from those noted in the actively immunized ones ($p < 0.01$). As shown in figure 5, the contraction of passively sensitized TSM was barely inhibited by the preincubation with LY-171883. In contrast, pyrilamine showed similar or slightly stronger inhibitory effects in the passively sensitized contractions (70%) than those of actively immunized TSM (57%), although there was no statistical significance ($p > 0.05$).

Discussion

The present study demonstrates that the airway smooth muscles, both those isolated from the CRA-sensitive guinea pigs immunized by chronic aerosol exposures and those passively sensitized by incubation with the sera from CRA-immunized guinea pigs, showed anaphylactic contractile

responses to antigen while they showed normal responses to cholinergic stimulations (EFS and ACh). Increased contractile response to cholinergic stimulations is often considered as one of the mechanisms of the airway hyperreactivity to physical, chemical and pharmacological stimuli, a hallmark of asthma. The hyperresponsiveness of airway smooth muscles to cholinergic stimulation in vitro is frequently observed in allergen-sensitized animals, particularly in association with late-phase asthmatic response [6–8]. Scheerens et al. [7] reported an increased responsiveness of airway smooth muscles to carbachol 24 h after antigen provocation in the toluene diisocyanate-sensitized mice. Using OA-sensitized guinea pigs, Ishida et al. [8] and Wang et al. [9] documented that there was an increased responsiveness of the trachea to ACh. Recently, Kageyama et al. [20] demonstrated that chronic allergen (OA) exposure enhanced guinea pig TSM constriction to EFS, but not to the exogenous addition of ACh. This could indicate that chronic allergen sensitization and subsequent airway inflammation cause cholinergic airway hyperresponsiveness, presumably due to the facilitation of cholinergic neurotransmitter. However, there have been reports showing unchanged, even decreased, responsiveness of airway smooth muscles to cholinergic stimulations in other allergic asthma models [10–13]. Wagner et al. [10] observed that there was no alteration in the contractile

responses to methacholine, histamine, prostaglandin $F_{2\alpha}$, and thromboxane A_2 analogue challenges between TSM isolated from control and *Ascaris suum*-sensitized sheep, although increased responses to histamine and a thromboxane A_2 analogue were found in the lung parenchyma strips. Wills-Karp and Gilmour [11] reported that the constrictor response of isolated tracheas to ACh was not significantly altered after multiple allergen challenges in vivo in which inflammatory cells were increased. Cheng and Townley [12] reported a decreased sensitivity and response to methacholine and histamine in the TSM of OA-sensitized guinea pigs. Compton et al. [13] also noted that the trachea of OA-immunized guinea pigs displayed a decreased contractile response to a muscarinic agonist carbachol. Interestingly, different responses (increased, decreased, or unchanged) to nonspecific stimulations were also found in airway smooth muscles obtained from asthmatic subjects in vitro [2–5]. Schellenberg and Foster [3] reported that BSM obtained from a human asthmatic who was allergic to grass responded to methacholine and LTs with equivalent contractions to those seen in nonasthmatic tissues.

The reason(s) for the normal contractile responses to cholinergic stimuli seen in the allergic airways in our study is not clear. It may be related to variations in the experimental methodology, such as the methods of allergic sensitization and challenge, the type and the amount of allergen used, the use of an adjuvant, the route of allergen administration, and the duration of allergen sensitization and challenge. Unlike our CRa aerosol sensitization a great majority of other animal models were immunized with an allergen given intraperitoneally and often with an adjuvant [9–11, 14]. In our study the state of immunization of guinea pigs was proven by the presence of anti-CRa IgG1 [16–18] as well as by the cellularity in BAL fluid (table 1). Yet, our findings, normal contractile response to ACh and leukocytosis in BAL, closely resemble the report by Wills-Karp et al. [12], whose guinea pigs were intraperitoneally immunized with OA. The guinea pigs showed significant leukocytosis in BAL fluid at 24 h after antigen challenge, although they did not measure asthmatic responses as we did. Our study confirms that aerosol CRa-immunized airway smooth muscles also show normal constrictor response to cholinergic stimuli. These airway muscles were isolated from asthmatic inflammation [18, 19]. Further studies are needed to clarify such conflicting reports seen in allergic animals. It is possible, however, that the CRa-immunized guinea pig may show cholinergic hyperreactivity in vivo despite normal contractility in vitro; several studies reported demonstrating a distinct disassociation of airway responsiveness to nonspecific stimulation between in vivo and in

vitro [14, 22]. Santing et al. [14] reported that tracheal muscle from OA-immunized guinea pigs responded normally to histamine and methacholine in vitro while the airway reactivity to histamine in vivo was increased 3- to 4-fold of control. Stewart and Fennessy [22] also had similar findings.

The airway smooth muscles isolated from CRa-immunized guinea pigs showed dose-dependent contractions upon a series of CRa challenges. Moreover, the data demonstrates the significant fact that the CRa-induced contraction of the airway muscles is related to the amount of the allergen the animals were exposed to during the immunization period. As shown in figure 3, the smooth muscle contractions are stronger in the tissues of HD CRa group than those of LD CRa group. The increment of contractile responses of TSM was associated with the cellular influx in BAL fluid at 24 h following CRa challenge. To our knowledge, this is the first report in the literature confirming that anaphylactic contractile responses of airway muscles are correlated to the amount of allergen to which animals are exposed during sensitization period. Such antigen-specific contractions of asthmatic airways are results primarily from the actions and interactions of released anaphylactic mediators, preformed as well as newly generated, e.g., histamine and LTs, from mast cells via antigen-anaphylactic antibody interaction. The antibody in this model is the CRa-specific IgG1 but not IgE [16–18].

The effects of antagonist, LY-171883 and pyrilamine, on the CRa-induced airway smooth muscle contractions may explain the roles of LTs and histamine in the CRa-induced asthma-like phenomenon. Inhibitors of both anaphylactic mediators partially blocked contractions of TSM. Although obtained data are derived from each inhibitor in one concentration, both inhibitors were proven effective, as reported by others previously [23, 24]. Interestingly, however, the effect of LY-171883 was significantly greater than that of pyrilamine in actively immunized tissues, suggesting that LT C_4/D_4 played a greater role than histamine in the antigen-induced contractions of airway smooth muscles (fig. 4, 5). This is perhaps an anticipated result as LTs are stronger spasmogens than histamine in the allergic asthma. In addition, LY-171883 almost completely inhibited the smooth muscle contraction at lower concentrations of CRa (fig. 4). The reason for such significant inhibition could possibly come from two factors: (1) it might also be due to relaxation of airway smooth muscle during incubation with LY-171883 as we as well as Fleisch et al. noted [25] and (2) low CRa induced a minimal contraction which is blocked further showing no clear difference. The data also points out an interesting fact, the difference noted in the inhibitory effects of LY-171883 and pyrilamine on the antigen-induced con-

tractions between actively immunized and passively sensitized tissues (see fig. 5): pyrilamine was moderately effective in both actively immunized and passively sensitized tissues, while LY-171883 exerted a dramatically stronger inhibitory effect on the contractions in actively immunized TSM (86%) than in passively sensitized TSM (9%). This may suggest that LTs play a more significant role in the actively immunized tissues than in the passively sensitized tissues.

The finding may implicate that an active environmental exposure-induced sensitization, i.e. CRa-allergic asthma, is a more complex phenomenon than merely an anaphylactic antibody-induced phenomenon. Chronic allergen exposure not only induces formation of specific antibody, but also leads to chronic inflammation in the structural organ (lung) along with the emigration and influx of mobile inflammatory cells such as lymphocytes, macrophages and eosinophils. Upon allergen challenge, structural cells and the inflammatory cells produce a wide range of inflammatory mediators released, including LT C₄/D₄ and LT E₄ which have potent bronchospastic actions, being on a molar basis 100–1,000 times as potent as histamine. The interpretation of the present data, along with substantial amounts of LTs noted in the

systemic (postpulmonary) circulation of the CRa-asthmatic guinea pigs in our earlier study [17] could be that LTs are released heavily upon CRa challenge in the CRa-immunized guinea pigs.

The antigen-specific contraction of airway smooth muscles to CRa challenge in vitro is basically similar to the other antigen-specific responses found in other asthma models [24, 26–28]. This fact, together with findings of asthma-like bronchospasms in vivo in the anesthetized [17] as well as in the conscious state [18], is an additional confirmation that the guinea pigs chronically immunized by simple exposure to airborne CRa have characteristics closely resembling those of human cockroach asthma.

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