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Separation of bronchoconstriction from increased ventilatory drive in a nonhuman primate model of chronic allergic asthma

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Van Scott, Michael R., Dale Aycock, Emily Cozzi, Kenneth Salleng, and Howard W. Stallings III. Separation of bronchoconstriction from increased ventilatory drive in a nonhuman primate model of chronic allergic asthma. *J Appl Physiol* 99: 2080–2086, 2005. First published August 18, 2005; doi:10.1152/jappphysiol.00537.2005.—The relationship between allergen-induced ventilatory drive and bronchoconstriction was investigated in dust mite-sensitive cynomolgus macaques periodically exposed to low doses of aerosolized antigen for up to 5.5 yr. Initially, the animals responded to aerosolized dust mite allergen at a concentration of 350 arbitrary units (AU)/ml with simultaneous increases in lung resistance (RL) and respiratory rate (RR). With time, RL and RR became differentially sensitive to allergen provocation. At the end of the study period, aerosolized allergen at a concentration of 15 AU/ml doubled RR without increasing RL. When mechanically ventilated to maintain tidal volume, higher concentrations of allergen could be delivered, and RL increased. Inhaled disodium cromoglycate and intravenous diphenhydramine attenuated the increase in RR, indicating that allergen-induced release of histamine and activation of H₁ receptors mediated the response. Inhaled β -adrenergic agonists attenuated the RR response to dust mite and to direct histamine provocation. These results demonstrate that chronic periodic allergen challenge increases the allergic sensitivity of histamine-dependent reflexes controlling ventilatory drive. Activation of these reflexes is independent of overt bronchoconstriction, but can be inhibited by β -adrenergic agonists, indicating that β -adrenergic agonists exert their effect independent of bronchodilation.

C-fiber endings; rapidly adapting receptors; histamine; β -adrenergic agonist

ASTHMA IS RECOGNIZED AS A progressive, chronic disorder resulting from exposure to environmental factors in susceptible individuals. Bronchoconstriction and airway inflammation are hallmark features of asthma, and they are often accompanied by lung hyperinflation, cough, dyspnea, and sensation of chest tightness (12). The latter manifestations are commonly used in diagnosis of the disease, yet the processes leading to altered afferent and efferent activity in the asthmatic lung, their long-term ramifications, and their relationship to bronchoconstriction and inflammation are not understood.

Observations in humans and animals indicate that enhanced neural reflexes in asthma can be dissociated from bronchoconstriction. Alteration in breathing patterns, including increased respiratory rate (RR), is commonly observed in otherwise asymptomatic asthma patients (17). Aeroallergen exposure in allergic dogs increases RR in parallel with bronchoconstriction (1), and the effect is observed in the presence of terbutaline, indicating that airway constriction is not responsible for the increased ventilatory drive. Antigen challenge in *Ascaris suum*

-sensitive rhesus monkeys increases both RR and pulmonary resistance (RL), and the peak RR precedes the peak RL, providing evidence that the two processes are not tightly linked (13). The current evidence therefore indicates that ventilatory drive after allergen challenge can be dissociated from bronchoconstriction, but definitive proof has not been reported.

The increase in ventilatory drive after allergen challenge is inhibited by disodium cromoglycate, indicating that mast cell release of histamine underlies the response (22). Furthermore, β -adrenergic agonists inhibit increases in RR after both allergen challenge (22) and direct histamine provocation (4, 6). However, histamine and β -adrenergic receptors are expressed on both smooth muscle cells and neurons within the airways, making it unclear whether the stimulation of ventilatory drive by histamine is due to a direct effect on sensory receptors or a secondary effect of bronchoconstriction.

In the present study, a chronic model of dust mite-sensitive asthma in nonhuman primates was used to definitively dissociate the ventilatory and bronchoconstriction responses to allergen and to define the role of histamine in the ventilatory response to aerosolized dust mite. Dust mite-sensitive cynomolgus macaques (21) were exposed to aerosolized dust mite every 6–8 wk for up to 5.5 yr. Over time, allergic sensitivity increased, and a clear separation of allergen-induced ventilation and bronchoconstriction developed, with the former being observed at a log-fold lower concentration of allergen than the latter. The increase in ventilatory drive at low levels of allergen was attenuated by histamine H₁ receptor blockade and β -adrenergic agonists. The results support the hypothesis that allergen-induced increase in ventilatory drive is a direct response to histamine released during allergen exposure and that β -adrenergic agonists are able to inhibit the process at a point distal to histamine release and independent of a bronchodilator effect.

METHODS

Animals. Fifteen dust mite-sensitive cynomolgus macaques were obtained from LABS of Virginia (Yemassee, SC). The animals had been sensitized as described previously (21), by subcutaneous injections of allergen adsorbed to Alum [312 arbitrary units (AU) of *Dermatophagoides pteronyssinus* (Dp) and *Dermatophagoides farinae* (Df) extract, Greer Laboratories, Lenoir, NC; Imject Alum, Pierce, Rockford, IL]. The animals were obtained in two cohorts (Fig. 1). One cohort of two animals was sensitized when the animals were 2–2.5 yr of age. The other cohort of 13 animals was sensitized from birth. The animals ranged in age from 2.5 to 7.5 yr and weighed 1.5–6.8 kg at the time of this study. The dust mite-sensitive animals were group housed at East Carolina University in rooms ventilated

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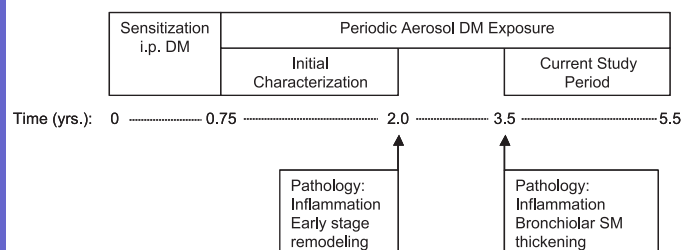


Fig. 1. Timeline of the longitudinal study. The colony represents 2 cohorts, 1 sensitized from birth and the other sensitized at 2–2.5 yr of age. Following sensitization, the animals were challenged with aerosolized allergen, on average, once every 40 days. The early functional characteristics of the dust mite (DM)-sensitized animals were published previously (21). Pathology results demonstrated airway wall remodeling characteristic of asthma and thickening of bronchiolar smooth muscle (SM).

with high-efficiency particulate-filtered air. Animal husbandry was conducted under US Department of Agriculture guidelines. All protocols were approved by the Institutional Animal Care and Use Committee of East Carolina University. Eight control animals that had been sham sensitized from birth were also studied.

Allergen exposure. The animals were challenged with aerosolized dust mite (Dp/Df mix, 1–2,500 AU/ml for 4 min) every 4–8 wk. Allergen was nebulized using a Devilbiss ultrasonic nebulizer and delivered through a pediatric face mask or endotracheal tube depending on whether pulmonary function testing was required. Sensitivity was determined by delivering ascending concentrations of allergen (1, 10, 100, 500, and 2,500 AU/ml) until either a $>100\%$ increase in RL , $>50\%$ decrease in dynamic lung compliance (C_{dyn}), or $>100\%$ increase in RR was observed. Pulmonary function was monitored for 15 min between each dose. Routine periodic exposures involved provocation with a single, minimal dose required to observe a pulmonary response.

Pulmonary function testing. Animals were anesthetized with 2.0 mg/kg telazol by intramuscular injection and maintained on propofol delivered by continuous intravenous infusion at $10\text{--}15\text{ mg}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$. An endotracheal tube and esophageal balloon were inserted. C_{dyn} and RL were measured by standard computer analysis of the flow and pressure signals, digitalized by a MuMed PR800 physiological recorder (MuMed, London, UK). Flow was measured using a heated Fleisch pneumotachograph (size 00, Fleisch, Lausanne, Switzerland) connected to a Validyne pressure transducer (model DP 45-14, Validyne Engineering, Northridge, CA). Intrathoracic pressure was measured using a model DP 45-24 Validyne pressure transducer. Saline was delivered via a Devilbiss ultrasonic nebulizer for 4 min (2 ml/min delivered), and pulmonary function was monitored for 2 min to establish baseline parameters. Animals were exposed to aerosolized dust mite for 4 min, and pulmonary function was monitored for 15 min. When indicated, vecuronium (0.035 mg/kg) was delivered intravenously, and the animals were mechanically ventilated at a RR and tidal volume (V_T) equivalent to the parameters measured during the baseline period. Adjustments were made to maintain end-tidal CO_2 at 40 Torr. Animals were then exposed to dust mite, and pulmonary function was monitored for up to 15 min. Arterial oxygen saturation was monitored by pulse oximetry (SurgiVet model V3304, Harvard Apparatus, Holliston, MA) throughout the protocol, and supplemental O_2 was delivered if readings fell below 70%.

Pharmacological agents. The following agents were delivered as indicated: disodium cromoglycate (DSCG; EI-121, Biomol, Plymouth Meeting, PA), 98 mM in saline, nebulized for 10 min immediately before allergen exposure; isoproterenol (I5627, Sigma, St. Louis, MO), 40 mM in saline nebulized 40 s immediately before allergen exposure; salbutamol (S8260, Sigma), 8.5 mM in saline nebulized for 10 min immediately before allergen challenge; diphenhydramine hydrochloride (Baxter, Deerfield, IL), 2.5 mg/kg delivered intrave-

nously in 3 ml of saline over 1 min; and histamine diphosphate (H7375, Sigma) nebulized in saline at concentrations of 0.0066 mg/ml (21 μM) to 1 mg/ml (3.3 mM) for 2 min. All experimental drug treatments were performed as part of the normal periodic allergen challenge, and treated animals were crossed over to nontreatment groups in subsequent challenges to ensure that treatment did not affect the basal response to allergen.

Statistics. Changes due to drug treatment and allergen challenge were expressed as a percent change from the saline control period for untreated animals, while allergen challenge was expressed as percent change from the drug treatment period for treated animals. Statistical significance was determined using Student's two-tailed t -test to compare two groups and using ANOVA with Fisher's least significant difference post hoc test to evaluate three or more groups (level of significance: $P \leq 0.05$). All values are reported as means $\pm$ SE.

RESULTS

Changes in responses to allergen over time. This study was conducted as part of a larger longitudinal study of a small colony of dust mite-sensitive cynomolgus macaques. The timeline of the longitudinal study is shown in Fig. 1. The characteristics of the colony immediately after sensitization have been described previously (21). A subset of the original colony was retained, and it has been followed for a total of 5.5 yr to date. The subset, which was used for this study, included 2 animals sensitized as adults and 13 animals sensitized as neonates. In 2002, at the end of the initial characterization period, the animals in this subset responded to aerosolized dust mite at concentrations between 50 and 2,500 AU/ml (mean = 350 ± 175 AU dust mite/ml). The response included simultaneous increases in RR and RL , and decreases in V_T and C_{dyn} (Fig. 2). Compared with sham-sensitized control animals challenged with dust mite at 2,500 AU/ml, all of the changes in the dust mite-sensitive group except the increase in RL were significantly greater than the sham-sensitized group. These responses were observed in spontaneously breathing animals without evidence of apnea or respiratory failure.

After the initial characterization period, the animals were challenged with aerosolized allergen once every 4–8 wk. The allergen dose was titrated to elicit a 50% decrease in C_{dyn} or 100% increase in RL . The average time between challenges was 40 ± 1 days. With time, sensitivity to the allergen increased. Within 2 yr of inducing sensitization, spontaneously breathing animals routinely exhibited apnea if challenged with aerosolized dust mite at concentrations >100 AU/ml. In addition, a separation of functional responses became apparent, with low levels of allergen provocation inducing changes in breathing patterns and high levels of allergen provocation inducing bronchoconstriction. This separation in responses was demonstrated by challenging spontaneously breathing dust mite-sensitive animals with increasing doses of aerosolized allergen until a 100% increase in RR was observed (Fig. 3). The animals were then mechanically ventilated, and the allergen concentration was increased 10-fold. For the dataset presented in Fig. 3, the provocative allergen concentration that induced a 100% increase (PC_{100}) in RR was 15 ± 7 AU/ml. V_T and C_{dyn} changed in parallel with the RR during this early response to low-level allergen provocation. In contrast, this allergen concentration had no detectable effect on RL . After the initial response was observed, the animals were mechanically ventilated and challenged with higher concentrations of aerosolized allergen. C_{dyn} decreased further, and RL increased.

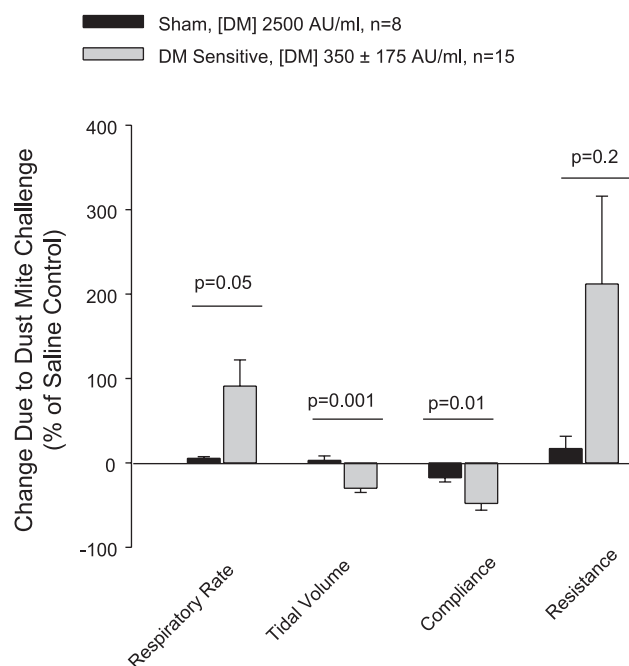


Fig. 2. Response of sham and DM-sensitized animals to aerosolized DM during the initial characterization period. Spontaneously breathing animals were challenged with saline followed by increasing concentrations of aerosolized DM until a $\geq 50\%$ drop in dynamic compliance (C_{dyn}) or $\geq 100\%$ increase in lung resistance (R_L) was observed. The response to DM was normalized to the saline control. Values are means $\pm$ SE recorded at the highest concentration of DM delivered; n , no. of animals. The DM-sensitive animals responded at a mean DM concentration of 350 ± 175 arbitrary units (AU)/ml, whereas all sham animals received 2,500 AU/ml without achieving the target responses. Note that at this time in the study no DM-induced respiratory failure was observed before the target responses were achieved.

The PC_{100} for R_L was 409 ± 127 AU/ml. These data provided evidence that periodic exposure to dust mite at levels that induced functional responses increased the sensitivity of the respiratory tract to airborne allergen, resulting in differential sensitivities of processes controlling bronchoconstriction and breathing patterns.

Prior observations revealed that animals sensitized to dust mite as adults exhibited less allergen-induced bronchoconstriction than animals sensitized as neonates (21). The dataset used to generate Fig. 3 was therefore separated on the basis of the age at which they were sensitized. As observed previously, the two animals sensitized as adults required larger concentrations of allergen to increase R_L compared with the neonatally sensitized animals. However, both subsets exhibited dissociation in breathing patterns and bronchoconstriction in response to allergen (Fig. 4). The PC_{100} values for RR and R_L increases in the neonatally sensitized animals were 15 ± 8 AU dust mite/ml and 310 ± 126 AU dust mite/ml under spontaneously breathing and ventilated conditions, respectively. In contrast, the animals sensitized as adults required dust mite concentrations of 10 and 1,000 AU dust mite/ml to elicit equivalent changes in RR and R_L . Thus the time at which the animal was sensitized affected the degree of allergic sensitivity, but not the pattern of responsiveness after chronic periodic provocation.

The sensitivity to dust mite remained high for prolonged periods. All protocols conducted on these animals incorporate a washout period of 4–8 wk between allergen challenges.

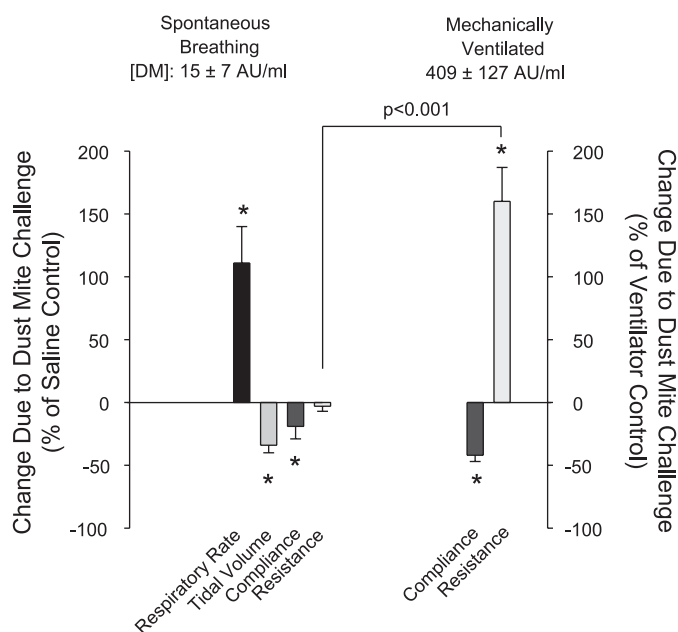


Fig. 3. Differential sensitivity of pathways controlling breathing patterns and bronchoconstriction observed during the present study period. Spontaneously breathing animals were challenged with saline followed by increasing concentrations of aerosolized DM until a $\geq 100\%$ increase in respiratory rate (RR) was observed. The response to DM was normalized to the saline control. The animals were then placed on a mechanical ventilator, a new baseline was established, and the DM concentration was increased until a $\geq 50\%$ decrease in C_{dyn} or $\geq 100\%$ increase in R_L was observed. The response to DM provocation during mechanical ventilation was normalized to the baseline obtained on the ventilator. Values are means $\pm$ SE for 14 animals (1 of the neonatally sensitized animal represented in Fig. 2 could not be studied at the time of this protocol). *Significantly different from zero, $P < 0.05$.

Recently, a dust mite-sensitive animal that had not been exposed to dust mite for 10 mo due to chronic therapy and monitoring for recurrent rectal prolapse was challenged with aerosolized allergen. After 10 mo, the animal responded to 10

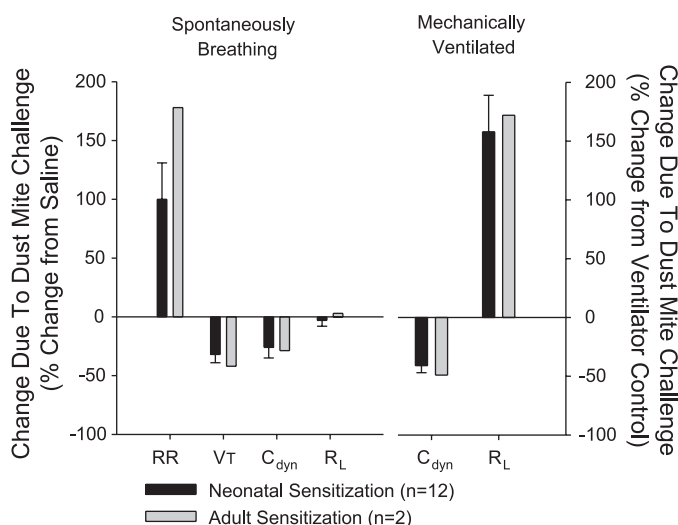


Fig. 4. Comparison of pulmonary function responses in animals sensitized to DM as adults and neonates. The animals used to generate the dataset for Fig. 3 were separated on the basis of the time of sensitization. Twelve neonatally sensitized animals were challenged with 15 ± 8 AU DM/ml and 310 ± 126 AU DM/ml under spontaneously breathing and ventilated conditions, respectively. In contrast, 2 adult sensitized animals were challenged with 10 and 1,000 AU DM/ml. Values are means $\pm$ SE.

AU/ml of aerosolized dust mite with a 150% increase in RR and 42% decrease in C_{dyn}. When mechanically ventilated and challenged with 100 AU/ml of aerosolized dust mite, he exhibited a 120% increase in R_L. Thus heightened sensitivity to dust mite appeared to be maintained for prolonged periods.

A potential role for mast cell-derived histamine release. Mast cell activation and histamine release were thought to play a major role in the early response to allergen, and a previous study in *Ascaris*-sensitive rhesus monkeys demonstrated that mast cell stabilization by DSCG attenuated the allergen-induced increase in RR and decrease in V_T (22). The ability of aerosolized DSCG to inhibit the changes in breathing pattern after low-level allergen provocation was therefore investigated. DSCG pretreatment had no effect on baseline resistance ($\Delta R_L = -1 \pm 4\%$, where Δ is change), C_{dyn} ($\Delta C_{dyn} = 6 \pm 11\%$), V_T ($\Delta V_T = 0 \pm 2\%$), or RR ($\Delta RR = -6 \pm 3\%$). In contrast, DSCG attenuated the response to low-level allergen provocation as indicated by decreased changes in RR, C_{dyn}, and V_T (Fig. 5). The difference in responsiveness could not be accounted for by differences in the allergen concentration. The results indicated that the ventilatory response to low-level allergen challenge was mediated at least in part by mast cell release of histamine.

Further evidence for a role of histamine in the response to low-level allergen provocation was obtained by pretreating animals with diphenhydramine, an H₁-receptor antagonist, before allergen challenge. Diphenhydramine hydrochloride alone (2.5 mg/kg, administered intravenously) caused a transient increase in RR and decrease in V_T in some animals ($\Delta RR = 67 \pm 42\%$, $\Delta V_T = -11 \pm 7\%$) that resolved within 3 min. Diphenhydramine attenuated the changes in RR and V_T induced by subsequent provocation with dust mite (Fig. 6). Intravenous injection of vehicle had no effect. These data

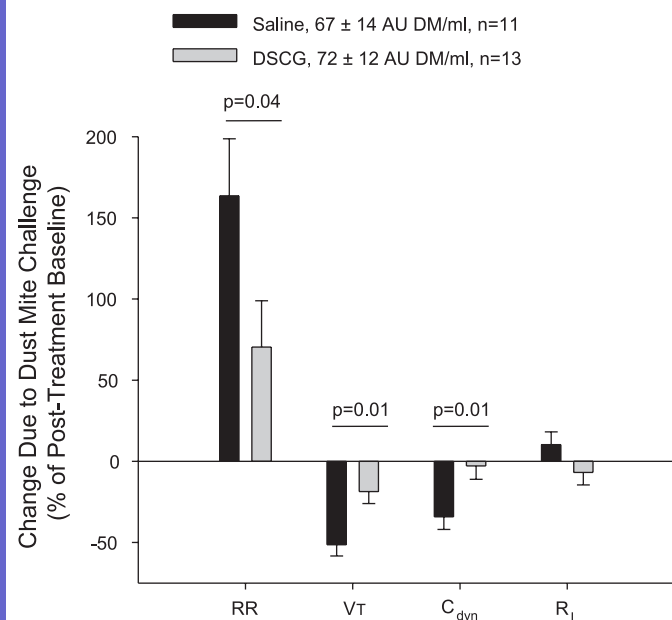


Fig. 5. Effect of disodium cromoglycate (DSCG) on responses to low-dose allergen provocation. Animals were treated with nebulized DSCG or saline, and then they were challenged with a dose of allergen known to increase ventilatory drive. The response to DM was normalized to the baseline readings after treatment. Values are means $\pm$ SE; *n*, no. of animals. *P* values indicate the level of significance between the saline and DSCG treatment groups.

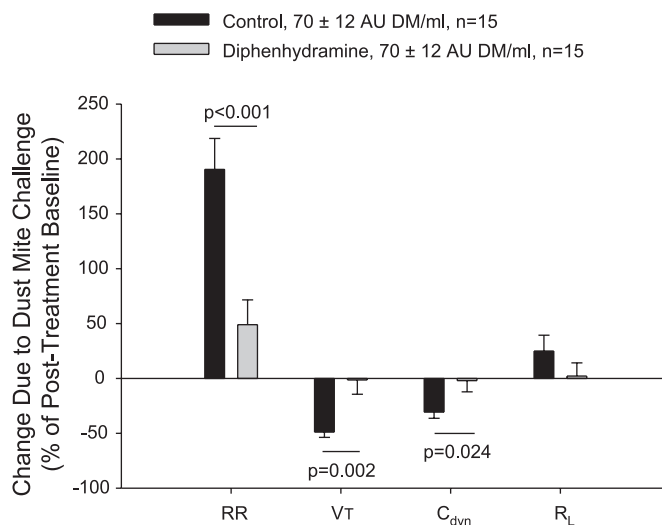


Fig. 6. Effect of diphenhydramine on responses to low-dose allergen provocation. The response to inhaled DM was determined in all animals and normalized to inhaled saline (solid bars). Four weeks later, the animals were challenged with saline to determine the baseline pulmonary function, and treated with diphenhydramine (2.5 mg/kg iv). When pulmonary function returned to baseline, the animals were challenged with the same dose of dust mite used 4 wk earlier (shaded bars). The response to DM was normalized to the postdiphenhydramine baseline. Values are means $\pm$ SE; *n*, no. of animals. *P* values indicate the statistical difference in the response to DM with and without diphenhydramine treatment.

indicated that histamine acting through H₁ receptors mediated the effect of dust mite on respiratory drive.

Effect of β -adrenergic agonists on induced respiratory drive. β -Adrenergic agonists had been shown to reduce allergen-induced changes in RR and V_T in *Ascaris*-sensitive rhesus macaques (22). The effect of β -adrenergic agonists on the ventilatory responses to allergen and histamine was therefore investigated. Effects on the ventilatory response to dust mite was investigated using nebulized isoproterenol. Isoproterenol treatment by itself increased baseline heart rate (HR) ($\Delta HR = 35 \pm 6\%$; $P < 0.001$), C_{dyn} ($\Delta C_{dyn} = 17 \pm 5\%$; $P = 0.005$), V_T ($\Delta V_T = 11 \pm 5\%$; $P = 0.05$), and RR ($\Delta RR = 18 \pm 6\%$; $P = 0.01$). Baseline R_L was decreased by isoproterenol ($\Delta R_L = -8 \pm 3\%$; $P = 0.03$). As shown in Fig. 7, pretreatment with isoproterenol abolished the subsequent changes in RR, V_T, and C_{dyn} induced by low-level allergen provocation. To distinguish between a β -adrenergic agonist effect on histamine release and histamine targets, animals were treated with salbutamol and then challenged directly with aerosolized histamine. Fifteen animals were challenged with aerosolized histamine to determine the provocative dose that induced a 50% increase (PC₅₀) in RR. Two weeks later, eight animals were pretreated with nebulized salbutamol, the remaining animals were pretreated with saline, and the response to histamine provocation was reevaluated. Direct histamine provocation increased RR (PC₅₀ = 0.29 ± 0.03 mM) and decreased V_T [provocative dose that induced a 30% increase (PC₃₀) = 0.46 ± 0.07 mM; Fig. 8]. Salbutamol by itself increased baseline HR and RR ($\Delta HR = 19 \pm 4\%$, $P < 0.001$; $\Delta RR = 12 \pm 5\%$, $P < 0.001$; *n* = 8), but had no significant effect on baseline resistance ($\Delta R_L = -6 \pm 19\%$), C_{dyn} ($\Delta C_{dyn} = 5 \pm 10\%$), and V_T ($\Delta V_T = 5 \pm 3\%$). Salbutamol pretreatment shifted the dose-response curve to histamine to the left, in-

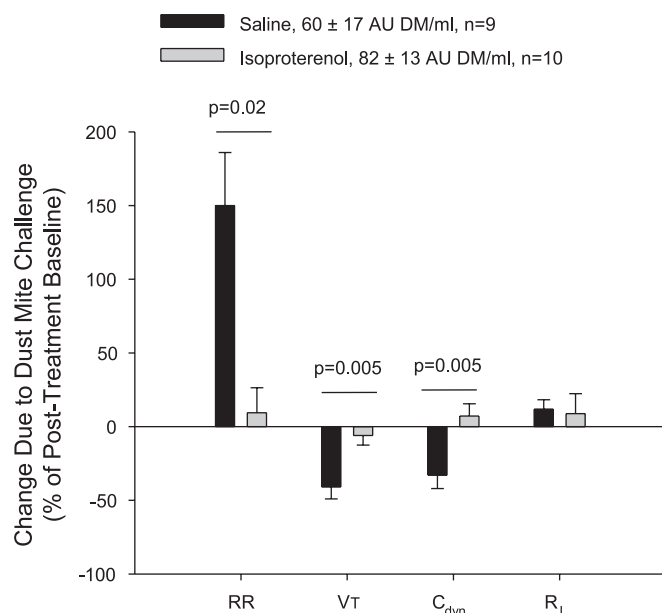


Fig. 7. Effect of isoproterenol on responses to low-dose allergen provocation. Animals were treated with nebulized isoproterenol or vehicle, a posttreatment baseline was established, and the animals were challenged with DM. The response to DM was normalized to the posttreatment baseline. Values are means $\pm$ SE; *n*, no. of animals. *P* values indicate the statistical difference in the response to DM between the saline and isoproterenol treatment groups.

creasing the PC_{50} for RR and abolishing the change in V_T at the doses of histamine that were tested (open symbols, Fig. 8). In contrast, pretreatment with saline had no effect on the dose response to histamine (PC_{50} for RR = 0.42 ± 0.1 mM; PC_{30} for V_T = 0.65 ± 0.20 mM; *n* = 6, one animal was noncompliant). The effect of salbutamol on the PC_{50} was statistically

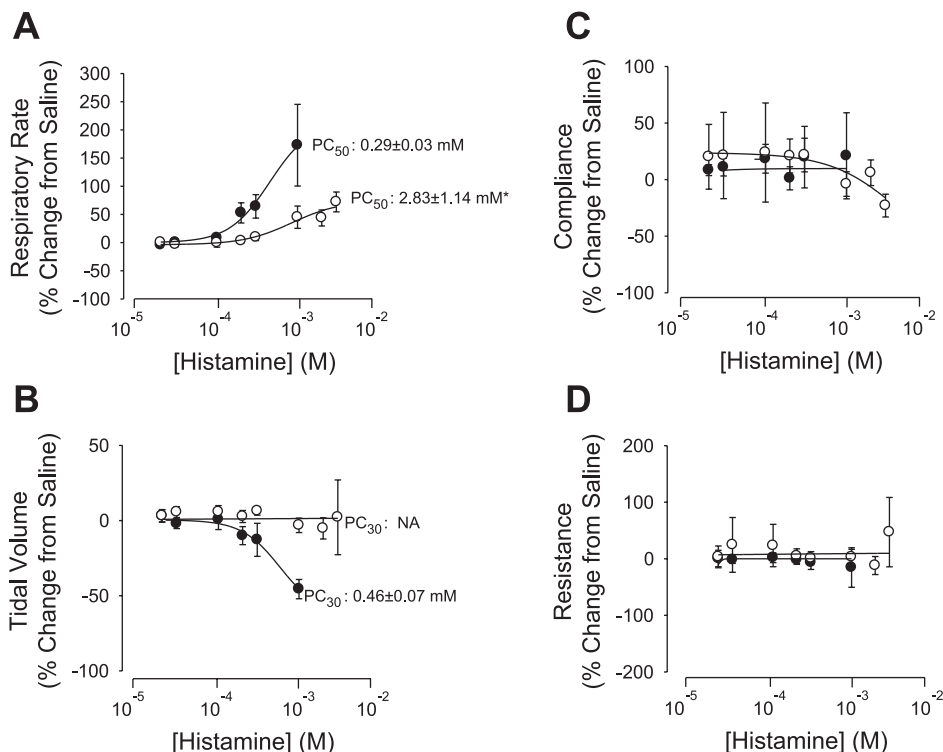
significant compared with both the aggregate PC_{50} values for all untreated animals and the PC_{50} values for the vehicle control group (ANOVA, $P < 0.008$), and when analyzed using paired *t*-test of the responses of the same animals with and without salbutamol pretreatment ($P = 0.05$). These findings indicated that β -adrenergic agonists inhibited histamine-induced respiratory drive, and therefore could act at a site distal to mast cells and basophils to inhibit allergen-induced respiratory drive.

DISCUSSION

Periodic exposure of dust mite-sensitive cynomolgus macaques to antigen increases the allergic sensitivity of the respiratory tract and induces differential sensitivity in breathing patterns and bronchoconstriction. In the present study, low-level allergen provocation was used to study the ventilatory response to allergen independent of the bronchoconstriction response. The results demonstrate that dust mite-induced changes in breathing patterns are mediated by histamine through H_1 receptors and are independent of allergen-induced bronchoconstriction. Yet, β -adrenergic agonists are highly effective in attenuating both allergen- and histamine-induced changes in breathing patterns. These findings may be important in understanding clinical observations in some asthmatic individuals and how asthma exacerbations reoccur after periods without overt asthma symptoms.

Few studies have investigated the progressive changes in pulmonary function that result from repetitive exposure of the respiratory tract to environmental stimuli. Patterson and Harris (14) followed individual *Ascaris suum*-sensitive rhesus monkeys for up to 13 yr, and they found that only a few animals retained asthma-like responses to inhaled antigen for >1 yr after the initial induction of IgE by *Ascaris* infection. Focusing

Fig. 8. Effect of salbutamol on the response to aerosolized histamine. A: respiratory rate. B: tidal volume. C: compliance. D: resistance. Baseline histamine responsiveness was determined in all animals by challenging with saline followed by increasing doses of histamine and normalizing the responses to the saline control (solid symbols; *n* = 15). Two weeks later, a subset of the animals were pretreated with salbutamol and rechallenged with histamine. The responses to histamine were normalized to the posttreatment baseline (open symbols; *n* = 8). The remaining animals were pretreated with vehicle. The vehicle had no effect on the dose-response curve, and the data are presented in the text. Values are means $\pm$ SE. PC_{30} , provocative allergen concentration that induced a 30% increase; PC_{50} , provocative allergen concentration that induced a 50% increase; NA, not achieved within the doses tested. *Different from the histamine response in the absence of salbutamol, $P < 0.008$.



on this subpopulation of animals with prolonged *Ascaris* sensitivity, Weissberg and Garay (22) documented early-phase changes in RR and V_T to *Ascaris* antigen for up to 1.5 yr after the initial sensitization. Plopper and colleagues demonstrated that sensitization of neonatal rhesus monkeys to dust mite and subsequent chronic exposure to dust mite allergen and ozone results in expression of asthma features for up to 6 mo (3, 7, 11, 18, 20). The present study extends these observations by demonstrating that neonatal induction of allergic sensitivity followed by limited periodic challenge with low levels of aerosolized allergen (once every 6–8 wk) results in hypersensitivity of the airways that is retained for over 5 yr after the initial induction. Furthermore, with time, the processes controlling bronchoconstriction and breathing patterns become differentially sensitive to allergen.

Previous studies in *Ascaris*-sensitive dogs and rhesus macaques provided evidence that ventilatory response to allergen was not a direct outcome of bronchoconstriction. Using allergic dogs, Cotton and colleagues (1) observed that *Ascaris* antigen increased RR in parallel with bronchoconstriction but that pretreatment with terbutaline inhibited the bronchoconstriction without altering the ventilatory response. While this observation provided evidence that alteration in breathing patterns could be induced without bronchoconstriction in dogs, the lack of effect of terbutaline on the ventilatory response to allergen provocation conflicted with the effects of isoproterenol observed in monkeys. The discrepancy has been discussed previously and attributed to the dose of terbutaline that was used in the dogs (22). Using *Ascaris*-sensitive rhesus macaques, Patterson and Harris (13) demonstrated that the magnitudes and temporal aspects of the ventilation and bronchoconstriction responses to airborne antigen were different. These investigators also observed that at least one monkey exhibited a change in RR at a lower dose of antigen than required to induce bronchoconstriction (13). Our data extend the observations by Patterson and Harris and demonstrate that chronic periodic allergen challenge accentuates the dissociation between the bronchoconstriction and ventilatory responses to allergen due primarily to an increase in the sensitivity of processes controlling breathing patterns.

Previous observations in rhesus macaques indicated that histamine mediated the ventilatory response to aerosolized allergen. Weissberg and Garay (22) reported that DSCG inhibited allergen-induced ventilatory drive, leading to the hypothesis that mast cell release of histamine was responsible for the allergen-induced ventilatory drive. These investigators did not measure R_L during the allergen challenge, and therefore they could not distinguish between a direct affect of allergen on ventilation and a secondary effect resulting from airflow limitation. Our findings confirm that histamine mediates the effect of low-level allergen provocation on ventilation, provides evidence that histamine acts through H_1 receptors to increase ventilation, and establishes that the effect is not secondary to airflow limitation resulting from bronchoconstriction.

Weissberg and Garay (22) further noted that isoproterenol inhibited the allergen-induced increase in ventilation. Coupled with the observation that DSCG inhibited the allergen-induced ventilation, this observation led to the hypothesis that β -adrenergic agonists inhibited histamine release from mast cells and thereby attenuate the ventilation response to allergen. Extensive evidence has accumulated over the past decade to support

the idea that β -adrenergic agonists modulate mast cell function. However, this may not be the primary inhibitory action of β -adrenergic agonists. β -Adrenergic agonists are more effective than DSCG in inhibiting allergen-induced increases in ventilation (compare Figs. 5 and 7), and they are at least equally effective as H_1 -receptor blockade (compare Figs. 6 and 7). Furthermore, salbutamol inhibits the ventilatory changes associated with direct administration of histamine (Fig. 8) indicating that β -adrenergic agonists can act at a point in the pathway that is downstream of histamine release from mast cells and basophils. Further work is required to define the relative importance of the β -agonist inhibitory effects on histamine release and the histamine target.

Rapid shallow breathing after allergen provocation, and apnea at higher allergen doses, are consistent with activation of C-fiber endings (9, 24). These nerve endings are located throughout the airways and lung parenchyma; are known to be responsive to inflammatory mediators, including histamine; have been shown to be present in macaques; and can become hypersensitive in response to inflammation (9, 15). Tachypnea and cough are also associated with rapidly adapting pulmonary stretch receptor (RAR) activation, depending on their location within the airways (23); and discharge of RARs is increased by histamine (16). Interestingly, blockade of vagal C fibers has been shown to reduce histamine-stimulated discharge of RARs in dogs (19), indicative of interactions between afferent pathways. In addition, there is evidence that different aspects of the response to allergen provocation are mediated by different afferent pathways. In dogs, vagal C-fiber blockade increases RR, but it has a relatively small effect on histamine-induced changes in V_T and C_{dyn} (19). Terbutaline treatment in combination with vagal C-fiber blockade inhibits the changes in V_T and C_{dyn} . Thus allergen-induced release of histamine is likely to increase ventilatory drive through a complex mechanism involving multiple sensory pathways.

In summary, the results of this study demonstrate that periodic exposure to low levels of allergen differentially increases the sensitivity of a histamine-dependent pathway controlling ventilatory drive. The mechanisms by which the afferent pathways have become hypersensitive to provocation have not been elucidated. While it is known that sensory receptors can be sensitized by inflammatory mediators (10), the hypersensitivity in the cynomolgus macaques is observed for long periods (weeks to months) after a low-dose allergen challenge when the neutrophilic and eosinophilic inflammation from the previous exposure has largely resolved. Plopper and colleagues (8) have demonstrated that allergen and ozone exposure in macaques leads to neural remodeling, which could lead to prolonged hypersensitivity of afferent pathways. Alternatively, the number of mast cells resident in the airway mucosa may be increased. There is evidence of mast cell proliferation in the large and small airways from subjects with fatal asthma (2). However, if proliferation of mast cells is responsible, it is unclear why reflex pathways controlling breathing patterns are affected to a greater extent than pathways involved in bronchoconstriction. Further investigation is required to address these questions. Our results further demonstrate that periodic exposure to allergen at levels too low to induce significant bronchoconstriction and airflow limitation are able to maintain allergic sensitivity over long periods as indicated by stimulation of histamine-mediated processes in the airways. This

observation may have direct relevance to recent clinical trials with omalizumab, which demonstrate that IgE pathways play a central role in maintaining the asthma phenotype (5). Finally, the ability to definitively dissociate RL and RR responses to allergen provides definitive evidence that the ventilatory response to release of endogenous histamine subsequent to allergen challenge is independent of bronchoconstriction.

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