Sensitizing effects of chronic exposure and acute inhalation of ovalbumin aerosol on pulmonary C fibers in rats

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Zhang G, Lin R-L, Wiggers ME, Lee L-Y. Sensitizing effects of chronic exposure and acute inhalation of ovalbumin aerosol on pulmonary C fibers in rats. J Appl Physiol 105: 128–138, 2008. First published April 24, 2008; doi:10.1152/japplphysiol.01367.2007.—The effect of ovalbumin (Ova) sensitization on pulmonary C-fiber sensitivity was investigated. Brown-Norway rats were sensitized by intraperitoneal injection of Ova followed by aerosolized Ova three times per week for 3 wk. Control rats received the vehicle. At the end of the third week, single-unit fiber activities (FA) of pulmonary C fibers were recorded in anesthetized, artificially ventilated rats. Our results showed the following: 1) Ova sensitization induced airway inflammation (infiltration of eosinophils and neutrophils) and airway hyperresponsiveness in rats; 2) baseline FA in sensitized rats was significantly higher than that in control ones; 3) similarly, the pulmonary C-fiber response to right atrial injection of capsaicin was markedly higher in sensitized rats, which were significantly amplified after the acute Ova inhalation challenge; and 4) similar patterns, but smaller magnitudes of the differences in C-fiber responses to adenosine and lung inflation, were also found between sensitized and control rats. In conclusion, Ova sensitization elevated the baseline FA and excitability of pulmonary C fibers, and the hypersensitivity was further potentiated after the acute Ova inhalation challenge in sensitized rats. Chronic allergic inflammatory reactions in the airway probably contributed to the sensitizing effect on these lung afferents.

Airway hyperresponsiveness; inflammatory mediators; allergen; vasoconstriction; asthma

Asthma is a disease characterized by episodic and reversible airway obstruction and inflammation, accompanied by airway hyperresponsiveness (AHR) (42). AHR is defined as exaggerated bronchomotor responses to various nonallergic and non-specific stimuli, and its pathogenic mechanism is not fully understood, but is known to involve an interaction among multiple types of cells in the airways during allergic inflammatory reaction.

Nonmyelinated (C fiber) vagal afferents represent a major type of sensory nerves innervating the airways and lung. Activation of these afferents by inhaled irritants or by endogenous mediators is known to induce pronounced cardiorespiratory reflex responses, via both centrally mediated reflex pathways and local axon-reflex mechanism (9, 38). These responses include, but are not limited to, bronchoconstriction, airway hypersecretion, cough, tachypnea, etc. Increasing evidence has suggested that hypersensitivity of these afferents plays an important role in the manifestation of various symptoms associated with airway inflammatory diseases (9, 38). However, the changes in bronchopulmonary C-fiber sensitivity during allergic airway inflammatory reaction have not been fully characterized.

Several animal models of asthma have been developed and reported in the literature for studying the pathogenic mechanisms of AHR. One of the extensively used models is Brown-Norway (BN) rats actively sensitized with ovalbumin (Ova); it has been demonstrated that chronic allergic inflammation develops in the airways of sensitized rats, and many of their pathophysiological features closely resemble those observed in patients suffering from allergic asthma (13, 14). For example, in sensitized animals, acute inhalation challenge with aerosol Ova triggers early- and late-phase airway responses in a pattern similar to those found in asthmatic patients after acute exposure to allergens (11, 12, 14). Therefore, the present study was carried out in this animal model of allergen-induced asthma to investigate the following: 1) whether the excitability of pulmonary C fibers in the responses to mechanical and chemical stimulants was altered in the rats that were sensitized with Ova; and 2) if so, whether the responses were altered by acute Ova inhalation challenge in sensitized rats.

METHODS

The experimental procedures were performed in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health, and were also approved by the University of Kentucky Institutional Animal Care and Use Committee.

Animal sensitization and allergen exposure. Pathogen-free male BN rats weighing 250–350 g were divided into two groups (control and sensitized groups). Sensitized rats were actively sensitized by an intraperitoneal injection of a suspension containing 2 mg Ova in 1 ml Imject Alum as adjuvant. Three days after the initial intraperitoneal injection, sensitized rats were exposed to Ova aerosol for 15 min each time, three times per week for 3 wk, following the protocol established by previous investigators (14). During exposure, the unsensitized rat was placed in a Plexiglas restrainer (University of Kentucky, Center for Manufacturing), and it breathed spontaneously and continuously through a nose cone connected to a free stream of air-aerosol mixture under a negative-pressure exhaust hood. Ova solution (wt/vol concentration: 1.25% in saline) was nebulized and delivered by an ultrasonic nebulizer (model 099HD, Devilbiss, Somerset, PA) at a droplet size ranging from 0.5 to 5 μm. Control rats received the intraperitoneal injection and aerosol inhalation of the vehicle (isotonic saline) following the identical procedures.

Animal preparation. After 3 wk of either Ova or saline exposure, rats were initially anesthetized with an intraperitoneal injection of α-chloralose (100 mg/kg) and urethane (500 mg/kg) dissolved in a 2% borax solution; whenever necessary, supplemental doses (one-tenth of the initial dose) of the same anesthetics were injected intravenously to maintain anesthesia. After the baseline FA was established by previous investigations (14), during exposure, the anesthetized rat was placed in a Plexiglas restrainer (University of Kentucky, Center for Manufacturing), and it breathed spontaneously and continuously through a nose cone connected to a free stream of air-aerosol mixture under a negative-pressure exhaust hood. Ova solution (wt/vol concentration: 1.25% in saline) was nebulized and delivered by an ultrasonic nebulizer (model 099HD, Devilbiss, Somerset, PA) at a droplet size ranging from 0.5 to 5 μm. Control rats received the intraperitoneal injection and aerosol inhalation of the vehicle (isotonic saline) following the identical procedures.

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maintain abolition of pain reflexes elicited by paw pinch. The right femoral artery and the left jugular vein were cannulated for recording arterial blood pressure (ABP) and administration of pharmacological agents, respectively; the latter was advanced until its tip was positioned just above the right atrium. A short tracheal cannula was inserted just below the larynx via a tracheotomy. Tracheal pressure (Ptr) was measured (MP45-28; Validyne, Northridge, CA) via a side port of the tracheal cannula. After a midline thoracotomy was performed, the lung was artificially ventilated with a respirator (model 7025, UGO Basile, Comerio-Varese, Italy), and the expiratory outlet of the respirator was placed under 3 cmH2O pressure to maintain a near-normal functional residual capacity. tidal volume (VT) and respiratory frequency were set at ~8 ml/kg and 48–50 breaths/min, respectively, to mimic those of unilaterally vagotomized rats. Body temperature was maintained at ~36°C by means of a heating pad placed under the animal lying in a supine position.

**Electrophysiological recording of pulmonary C-fiber activity.** Single-unit pulmonary C-fiber activity was recorded as previously described (25). Briefly, the right cervical vagus nerve was separated from right carotid artery. The caudal end of the cut vagus nerve was placed on a small dissecting platform and immersed in a pool of mineral oil. A thin filament was teased away from the desheathed nerve trunk and placed on a platinum-iridium hook electrode. Action potentials were amplified (model PS111K, Grass Instruments, Quincy, MA), monitored by an audio monitor (model AMRRS, Grass Instruments), and displayed on an oscilloscope (model 2211, Tektronix, Wilsonville, OR). The thin filament was further split until the afferent activity arising from a single unit was electrically isolated. The nerve trunk was ligated just above the diaphragm to eliminate afferent signals arising from lower visceral organs. The afferent activity of a single unit was first searched by hyperinflation (3–4 × VT) and then identified by the immediate (delay <1 s) response to a bolus injection of capsaicin (1.0 μg/kg) into the right atrium. Finally, the general locations of pulmonary C fibers were identified by their responses to the gentle pressing of the lungs with a blunt-ended glass rod. The signals of the afferent activity, Ptr and ABP were recorded on a thermal writer (model TW11, Gould Instrument Systems, Valley View, OH) and on a videocassette recorder format data recorder (model SLY-N900, Sony Electronics, Park Ridge, NJ). Fiber activities (FA) in impulses per second (imp/s) were analyzed by an online data acquisition system (model TS-100, Bioceybernetics, Taipei, Taiwan) in 0.5-s intervals.

**Inflammatory cell analysis in bronchoalveolar lavage fluid.** Bronchoalveolar lavage fluid (BALF) was collected from another two groups (control and Ova sensitized) of rats after 3 wk of vehicle and Ova exposures, respectively. BALF was obtained by injecting 10 ml of sterile saline (5 ml, twice) into the lungs of the anesthetized rat via opening the intrapleural catheter to ambient air during a held hyperinflation (3 × VT). During the experiment, the animals were paralyzed with pancuronium bromide (50 μg/kg intravenously). Transpulmonary pressure was measured as the difference between Ptr and Ppl with a differential pressure transducer (model MP 45-28, Validyne, Northridge, CA). Respiratory flow was measured with a heated pneumotachograph and a differential pressure transducer (model MP 45-14, Validyne). All signals were recorded on a chart recorder (model 7, Grass, Quincy, MA); total pulmonary resistance (Rt) and dynamic lung compliance (Cdyn) were analyzed continuously by an on-line computer on a breath-by-breath basis (TS-100 series, Bioceybernetics, Taipei, Taiwan).

**Acute Ova inhalation challenge.** Ova aerosol (3%) was delivered directly into the lungs for 5 min; aerosol was generated by a ultrasonic nebulizer (Lumiscope 6610, Lumiscope, East Brunswick, NJ) that was connected between the outlet of the respirator and the tracheal cannula to prevent contamination of the breathing circuit by the residual Ova. The mass mean diameter of aerosol under our experimental setting was estimated to be 3–4 μm. To shorten the protocol to maintain the signal stability of single-fiber recording, acute inhalation Ova challenge was administered for a shorter duration (5 min) at a higher concentration of Ova solution (3%).

**Methacholine dose-response curve.** To verify airway hyperresponsiveness, responses of Rt and Cdyn to inhalation challenge with increasing concentrations of methacholine (MCh) aerosol (0.1, 0.3, 1, 3 mg/ml solutions) were determined in each animal. MCh aerosol was generated and delivered in the same manner as described above for 1 min. There was a 15-min interval between two consecutive MCh aerosol challenges. The lungs were hyperinflated (3 × VT) at 2 min before each MCh aerosol challenge.

**Experimental protocol.** Three series of experiments were carried out. Study series 1 was carried out to determine the presence and severity of airway inflammation by differential leukocyte counts of the BALF on day 21 at 4–6 h after the last inhalation exposure to Ova aerosol. Study series 2 was designed to investigate whether the bronchomotor responses to inhaled MCh aerosol and acute Ova inhalation challenge were altered by Ova sensitization in anesthetized rats. On day 24, the dose-response curve to MCh aerosol was obtained in each of the animals. Subsequently, responses of Rt and Cdyn were measured continuously in each animal before and for 30 min immediately after acute Ova inhalation challenge. Study series 3 was carried out to determine whether the excitability of pulmonary C fibers was altered by chronic Ova sensitization and to determine whether the changes were further augmented by acute Ova inhalation challenge. The baseline activities and the responses to lung inflation and chemical stimulation were determined in anesthetized, open-chest, and artificially ventilated rats before and within 45 min after acute Ova challenge in both control and sensitized rats on day 24. The baseline FA was recorded first, followed by the response to lung inflation, and 5 min later the response to chemical stimulation. There was always a 10-min interval between two chemical stimulation trials. Baseline FA was averaged over 60 s in each fiber before and 5–15 min after acute Ova challenge (3%, 5 min). Constant-pressure lung inflation was applied by inflating the lung with a constant airflow (~12 ml/s) until Ptr reached 15 or 30 cmH2O, and it was maintained at that pressure for 10 s after the respirator was turned off. Two chemical agents (capsaicin 0.25 and 0.5 μg/kg; adenosine 75 and 150 μg/kg) were selected as stimulants of pulmonary C fibers for the following reasons: capsaicin is a potent stimulant of pulmonary C fibers and a selective agonist of the transient receptor potential vanilloid type 1 receptor (TRPV1) (6); adenosine is a metabolite of ATP and another pulmonary C-fiber stimulant, but its action is mediated by activating adenosine A1 receptors (24). The volume of each bolus injection was 0.15 ml, which was first slowly injected into the catheter (dead space ~0.2 ml) and then flushed into the right atrium by an injection of 0.4 ml saline before and within 45 min after the acute Ova challenge (3%, 5 min). Mean ABP (MABP) and heart rate (HR) were measured as 30-s averages before and after acute Ova challenge in both control and sensitized rats.
that of control ones (n/H11005 detectable change in RL was found in control rats. The decrease 30 min after challenge in sensitized rats; in contrast, no decrease in Cdyn in a dose-dependent manner in both control and sensitized rats (Fig. 2). The bronchomotor responses to bronchosalveolar lavage fluid. *P < 0.05 and †P < 0.01, significant difference between control and sensitized rats (paired t-test).

**Materials.** Solution of Ova was prepared daily at the concentration described earlier. Imject Alum as adjuvant was purchased from Pierce Biotechnology (Rockford, IL). Stock solution of capsaicin (250 μg/ml) was prepared in 1% Tween 80, 1% ethanol, and 98% saline and stored at −20°C. Stock solutions of adenosine (10 mg/ml) and MCh (250 mg/ml) were prepared in saline and stored at −20°C. Solutions of these chemical agents at desired concentrations for injection or aerosolization were then prepared daily by dilution with isotonic saline based on the animal’s body weight. All chemical agents were purchased from Sigma Chemical (St. Louis, MO).

**Statistical analysis.** Data were analyzed with a two-way repeated-measures ANOVA, unless mentioned otherwise. One factor was the interaction, pairwise comparisons were made with a post hoc analysis (Fisher’s least significant difference). A P value <0.05 was considered significant. Data are reported as means ± SE.

**RESULTS**

A total of 52 rats (control, n = 26; sensitized, n = 26) were used in this study. The average body weight of control rats (300.8 ± 3.7 g) was significantly higher than sensitized rats (279.6 ± 4.4 g).

**Study series 1: effect of chronic Ova sensitization on airway inflammatory cells infiltration.** The total number of leukocytes in the BALF collected from sensitized rats (n = 7) exceeded that of control ones (n = 7) by 73%. In particular, the percentage of eosinophils [change (Δ) = 1,633%; P < 0.01; paired t-test], neutrophils (Δ = 1,159%; P < 0.01), and lymphocytes (Δ = 183%; P < 0.05) in the BALF of sensitized rats was distinctly higher than that in control rats (Table 1).

**Study series 2: effect of chronic Ova sensitization on bronchial reactivity.** There was no significant difference in the average baseline Rt or Cdyn between control and sensitized rats, but the bronchomotor response to acute Ova aerosol challenge was significantly higher in sensitized rats (Fig. 1). The same Ova inhalation challenge evoked an intense increase of the Rt, which sustained and remained above the baseline for 30 min after challenge in sensitized rats; in contrast, no detectable change in Rt was found in control rats. The decrease in Cdyn after the Ova inhalation challenge was also significantly greater in sensitized rats (Fig. 1). Even in control rats, Cdyn decrease significantly below the baseline after acute Ova challenge, which was presumably caused by the aerosol (liquid) deposition in the lung because the decrease in Cdyn could be readily reversed by hyperinflation of the lung (data not shown).

Inhalation of MCh aerosol induced an increase in Rt and a decrease in Cdyn in a dose-dependent manner in both control and sensitized rats (Fig. 2). The bronchomotor responses to MCh aerosol were significantly higher in sensitized rats at the higher concentrations of MCh (1.0 and 3.0 mg/ml; Fig. 2), verifying the presence of non-specific AHR in the sensitized group.

**Study series 3: effect of Ova sensitization on the excitability of pulmonary C fibers.** A total of 35 pulmonary C fibers were studied in 28 rats (control rats: 311.9 ± 5.7 g, n = 14; sensitized rats: 294.4 ± 4.7 g, n = 14). The distribution of these receptors was as follows: 2 in upper lobe; 7 in middle lobe; 12 in lower lobe, and 6 in accessory lobe; all were located in the right lung. The locations of remaining eight pulmonary C fibers were not identified, but all of them were activated by нар

**Table 1. Effect of Ova sensitization on leukocyte counts in BALF collected at 4 – 6 h after the acute Ova inhalation challenge**

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Cells, ×10⁶</th>
<th>Basophils, %</th>
<th>Eosinophils, %</th>
<th>Lymphocytes, %</th>
<th>Monocytes, %</th>
<th>Neutrophils, %</th>
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<tr>
<td>Control (n = 7)</td>
<td>12.32 ± 2.89</td>
<td>0.00 ± 0.00</td>
<td>0.26 ± 0.07</td>
<td>4.63 ± 1.03</td>
<td>92.97 ± 1.06</td>
<td>2.20 ± 0.71</td>
</tr>
<tr>
<td>Sensitized (n = 7)</td>
<td>21.46 ± 3.95</td>
<td>0.00 ± 0.00</td>
<td>4.46 ± 0.41†</td>
<td>13.14 ± 2.54*</td>
<td>54.71 ± 8.13*</td>
<td>27.71 ± 8.68†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals. Ova, ovalbumin; BALF, bronchoalveolar lavage fluid. *P < 0.05 and †P < 0.01, significant difference between control and sensitized rats (paired t-test).
effects greater in sensitized rats. The change in FA (ΔFA) in response to lung inflation was calculated as the difference between the average FA over the 10-s interval during lung inflation and the baseline FA (60-s average) in each fiber. Before acute Ova challenge, the response to lung inflation (Ptr = 30 cmH₂O) in sensitized rats (ΔFA = 1.43 ± 0.42 imp/s) was significantly higher than that in control rats (ΔFA = 0.46 ± 0.1 imp/s; P < 0.05); after acute Ova challenge, the same lung inflation evoked a greater response in both groups, but the response (ΔFA) was greater in sensitized rats (2.83 ± 0.64 imp/s) than in control rats (0.85 ± 0.19 imp/s; P < 0.05). The FA response to lung inflation was markedly augmented by acute Ova challenge in sensitized rats (P < 0.01) but not in control rats (Figs. 4 and 5). The response to lower intensity of lung inflation (Ptr = 15 cmH₂O) in sensitized rats was significantly higher than that in control rats after acute Ova challenge but not before the Ova challenge (Fig. 5).

Responses of pulmonary C fibers to chemical stimulants (capsaicin and adenosine) were also clearly elevated in sensitized rats. The ΔFA in response to a stimulus was calculated as the difference between the peak FA and the baseline FA (ΔFA = 0.19 ± 0.05 imp/s, Impulses per second).

Table 2. Effects of acute Ova inhalation challenge on average baseline MABP and HR in both control and sensitized rats

<table>
<thead>
<tr>
<th></th>
<th>MABP, mmHg</th>
<th>HR, beats/min</th>
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<tbody>
<tr>
<td></td>
<td>Before Ova</td>
<td>After Ova</td>
</tr>
<tr>
<td>Control (n = 14)</td>
<td>86.1 ± 3.3</td>
<td>96.9 ± 3.4</td>
</tr>
<tr>
<td>Sensitized (n = 14)</td>
<td>91.3 ± 3.1</td>
<td>102.5 ± 4.5</td>
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</table>

Values are means ± SE; n, no. of animals. Mean arterial blood pressure (MABP) and heart rate (HR) were measured before and within 45 min after acute Ova challenge (3%, 5 min).
nerve (e.g., Fig. 6). The responses of pulmonary C fibers to the high dose (0.5 μg/kg) of capsaicin (ΔFA = 12.2 ± 2.3 and 17.5 ± 2.6 imp/s, before and after acute Ova challenge, respectively) in sensitized rats were distinctly higher than those in control rats (ΔFA = 4.15 ± 0.86 imp/s and 3.81 ± 0.93 imp/s, before and after acute Ova challenge, respectively; P < 0.05 and P < 0.01) (Figs. 6, 7, and 8). In sensitized rats, the duration of discharge in response to capsaicin (0.5 μg/kg) was also significantly increased after acute Ova challenge (P < 0.01; Fig. 8B). Interestingly, the decrease in HR and MABP induced by capsaicin appeared to last a longer duration after acute Ova challenge in both control and sensitized rats (e.g., Figs. 6 and 7). The pulmonary C-fiber response to the lower dose of capsaicin (0.25 μg/kg) was also distinctly different between control and sensitized rats, both before and after acute Ova challenge. These results further showed that the FA responses to capsaicin were significantly augmented by acute Ova challenge in sensitized rats (P < 0.05; Fig. 8) but not in control rats. The differences in response to right atrial injection of adenosine between control and sensitized rats were similar, but less pronounced, compared with the difference in the response to capsaicin between the two groups. The response to the high dose of adenosine (150 μg/kg) in sensitized rats was higher than that in control rats both before and after acute Ova challenge, whereas the response to the low dose (75 μg/kg) was significantly higher in the sensitized rats only after acute Ova inhalation challenge (P < 0.05; Fig. 9). There was no significant difference in the duration of fiber discharge after the adenosine injection either between control and sensitized rats, or before and after acute challenge (Fig. 9).

**DISCUSSION**

Results of the present study showed that baseline activity of pulmonary C fibers was significantly elevated in the rats sensitized by chronic inhalation of aerosolized Ova, compared with that in the control rats. Chronic Ova exposure also produced a distinctly higher sensitivity of these afferents to chemical stimulants and lung inflation. The increase in fiber sensitivity to capsaicin was demonstrated by pronounced increases in both the peak activity and discharge duration in response to the same chemical challenge (Figs. 6–8). In addition, after an acute Ova inhalation challenge, the increase in baseline activity and the excitability were further augmented in sensitized rats. In sharp contrast, no significant change in the fiber activity or sensitivity was detected in control rats following the acute Ova inhalation challenge.
BN rats have been established as an animal model of allergic asthma by previous investigators (13, 21, 52). Many features induced by Ova sensitization are very similar to those observed in human allergic asthma, including production of an antigen-specific immunoglobulin (Ig) E antibody; upregulation of T helper 2 cytokines such as interleukin (IL)-4 and IL-5; early- and late-phase airway reaction to the inhaled antigen; inflammatory cell (e.g., eosinophils, neutrophils) infiltration; and AHR to nonspecific bronchoactive challenge. Our study showed a significant and sustained increase of airway resistance to acute Ova challenge in sensitized rats, which demonstrated the AHR to the antigen challenge (Fig. 1). At the same time, differential cell counts of the BALF revealed inflammatory cell (eosinophils, neutrophils) infiltration in the airways of sensitized animals (Table 1). These results are therefore in agreement with those reported by previous investigators in the BALF of sensitized rats (13) and in the sputum of asthmatic patients during asthmatic attack (49). Furthermore, elevated bronchomotor response and sensitivity to MCh challenge indicated AHR in sensitized rats (Fig. 2), which are consistent with those reported by previous investigators using the same animal model (13, 15).

In Ova-sensitized animals, acute inhalation challenge with aerosolized Ova triggers early- and late-phase airway responses in the pattern and time course similar to those found in asthmatic patients after acute exposure to allergens (11, 12, 14). The early-phase response, which usually subsides within an hour, is exemplified by severe bronchoconstriction, mediated primarily by mast cell-derived mediators, including histamine and leukotrienes (LTs). Four to six hours later, a more sustained late-phase reaction, characterized by airway obstruction and inflammatory reaction, develops as a result of the action of various endogenous inflammatory mediators, cytokines and chemokines generated by the resident inflammatory cells (e.g., mast cells, macrophages, and epithelial cells) as well as the recruited inflammatory cells (lymphocytes, neutrophils, and eosinophils) (5).

Extensive evidence suggests a close link of the inflammatory mediators released from eosinophils and neutrophils to the airway hypersensitivity developed during allergic airway inflammatory reaction. The eosinophil is one of the primary effector cells in airway inflammation, and it produces a wide range of inflammatory mediators, including granule-derived cationic proteins such as eosinophil major basic protein (MBP), lipid mediators such as prostaglandins and LTC4, and cytokines (32). Eosinophils may also localize to airway nerves by recognizing neuronal adhesion molecules (34), which allows the mediators released from eosinophils to act directly on the airway nerves. Previous studies demonstrated that activated eosinophils acted directly on dorsal root ganglion neurons to stimulate their neuuropeptides release and implicated MBP as a key mediator of this direct effect (17). Recent studies in our laboratory showed that intratracheal instillation of cationic proteins consistently induced intense stimulation and sensitization of pulmonary C fibers, and the cationic charge carried by these proteins is primarily responsible for generating the stimulatory and sensitizing effects on pulmonary C-fiber afferents (18, 20, 36).

Activated eosinophils can also synthesize and secret newly formed lipid mediators, and some of these mediators are known to exert potent sensitizing effects on pulmonary C fibers. For example, prostaglandin (PG) E2 has been reported to enhance the cough sensitivity to capsaicin in healthy human volunteers (7). Indeed, it has been clearly demonstrated that PGE2 can sensitize pulmonary C fibers in anesthetized rats (22), as well as isolated vagal pulmonary sensory neurons (35). Besides prostaglandins, LTC4, a potent bronchoconstrictor, can also stimulate the release of tachykinins from airway afferent nerve fibers in isolated trachea/bronchus of guinea pigs (39).

The neutrophil is one of the first inflammatory cells to be recruited into the airway after allergen exposure (48, 50). The neutrophil contains an array of products, including lipids, cytokines, proteases, microbicidal products, reactive oxygen species (ROS), and nitric oxide (47). Many of these endogenous substances are possible contributors to the AHR. For example, it has been reported that airway challenge of aerosolized H2O2, one of ROS known to be released in the airways from eosinophils, neutrophils, and epithelial cells (27), stimulated capsaicin-sensitive vagal pulmonary afferents in a dose-
dependent manner, and that its sensory transduction was mediated through both TRPV1 and P2X purinergic receptors (45). In addition, several cytokines have also been suggested to be involved in the sensitization of nociceptors (46). For example, IL-1β, IL-6, and tumor necrosis factor-α can be produced by many cell types, including mast cells, eosinophils, neutrophils, and macrophages in the lung and airways. More recently, Yu et al. (53) demonstrated that IL-1β stimulated airway nociceptors in anesthetized rabbits.

After acute Ova inhalation challenge, the enhanced sensitivity of pulmonary C fibers was further augmented in sensitized rats. The increased sensitivity occurred coinciding with the period of the early-phase response reported previously in sensitized rats (11, 41, 54), and mast cell degranulation presumably played a major part of the reaction; on exposure to antigen, IgE binds to the high-affinity IgE receptor FcεRI expressed on the mast cells, and triggers the release of mediators such as histamine, PGs LTs, and cytokines (4). A previous study in our laboratory has shown that inhalation of a low dose of aerosolized histamine augmented the afferent responses of vagal pulmonary C fibers to both lung inflation and right-atrial injection of capsaicin in anesthetized, open-chest dogs (37).

Tryptase is frequently considered as a marker of mast cell degranulation (43). Increased concentration of this protease has been found in BALF of asthmatic patients (1). A recent study by Gu and Lee (19) showed that intratracheal instillation of trypsin significantly amplified the capsaicin-induced pulmonary chemoreflex responses mediated by pulmonary C-fiber afferents. In isolated vagal pulmonary sensory neurons, pretreatment with trypsin potentiated the capsaicin-induced whole cell inward current (19), and this effect by trypsin is probably mediated though an activation of protease-activated receptor-2 and the protein kinase C-dependent transduction pathway.

Bradykinin, a nonapeptide released from mast cells and found in the BALF of asthmatic patients (2), has been shown to stimulate bronchial C-fiber afferents in anesthetized dogs (33). Similarly, in an isolated preparation of guinea pig trachea and bronchus, bradykinin activated the C-fiber sensory terminals innervating these airways, particularly those originating from the jugular ganglia (31). Bradykinin is also known to increase the sensitivity of C fibers in isolated guinea pig airways and in Ova-sensitized guinea pigs (3, 16). In addition, 5-hydroxytryptamine, another autacoid derived from mast cells, stimulated vagal bronchopulmonary C fibers in guinea pig lungs (8). Together, these studies have provided strong evidence suggesting that these mediators derived from mast cell degranulation are probably responsible for the stimulation and sensitization of pulmonary C fibers after acute inhalation challenge with Ova in sensitized rats.

Our results illustrating the Ova-induced increase in excitability of these C-fiber endings to lung inflation have important
physiological implications because these endings are relatively insensitive to changes in lung volume under normal conditions (23). Furthermore, expansion of the lung is a natural stimulus that occurs commonly in physiological conditions; for example, an increase in VT can occur on taking a deep inspiration (sigh) at rest or during hyperventilation in heavy exercise. Activation of bronchopulmonary C fibers is known to play a part in evoking the sensation of airway irritation, cough, and reflex bronchoconstriction (9, 38). Therefore, the hypersensitivity of pulmonary C fibers to lung inflation may be involved, at least in part, in generating these symptoms and AHR found in patients with allergic airway inflammation.

Capsaicin is a potent stimulant of the pulmonary C fibers, and a selective activator of the TRPV1 receptor; the sensitivity to capsaicin is a characteristic feature of pulmonary C-fiber afferents in various animal species (9, 23, 38). Previous investigators have reported increases in the levels of airway acidity, airway temperature, and lipooxygenase metabolites of arachidonic acid in asthmatic patients (26, 44, 51), all of which are known to activate the TRPV1 (29). Other inflammatory mediators released in asthmatic airways (e.g., PGs, bradykinin, etc.) do not activate the TRPV1 directly, but they can lower its activation threshold (18, 28). It has been reported that patients with asthma are more sensitive to the tussive effect of TRPV1 agonists (10). In addition, selective TRPV1 antagonists can inhibit cough elicited by Ova inhalation challenge in sensitized guinea pigs (40). The present study further demonstrated a distinctly elevated sensitivity of pulmonary C fibers to capsaicin in Ova-sensitized rats (Figs. 7 and 8). Because capsaicin is a selective activator of TRPV1, these results seem to suggest a possible involvement of an increase in the response of TRPV1 channels, although direct evidence has not been established in this study. We speculate that the enhanced response may have resulted from an increase in the expression or excitability of TRPV1, or both, in the sensitized rats; the precise mechanisms were not determined in the present study. Furthermore, our results suggested that other ion channels (e.g., adenosine A1 receptors) were also involved in the Ova-induced sensitizing effect on pulmonary C fibers because there were significant increases in the C-fiber responses to adenosine and lung inflation in sensitized rats (24), and stimulation of C fibers by neither of these stimuli is known to involve TRPV1 activation.

It is well documented that stimulation of pulmonary C fibers elicits a number of reflex responses mediated through the cholinergic pathway, such as bronchoconstriction, airway hypersecretion, and bronchial vasodilation. In addition, certain neuropeptides, such as tachykinins and calcitonin gene-related peptide, released locally from these nerve endings can produce airway smooth muscle contraction, protein extravasation, and
chemotactic effects on inflammatory cells (e.g., mast cells, leukocytes, etc) (30); sustained stimulation of these afferents may, therefore, lead to the development of neurogenic inflammation (30). Based on the results obtained in this study, it seems reasonable to suggest that an increase in the sensitivity of pulmonary C-fiber endings may play an important role in the development and manifestation of AHR induced by allergen sensitization.

In conclusion, the present study demonstrated that Ova sensitization induces a pronounced sensitizing effect on vagal pulmonary C-fiber afferents. Potent effects of certain chemical mediators released from inflammatory cells (eosinophils, neutrophils, and mast cells) are possibly involved in enhancing the sensitivity of pulmonary C fibers in sensitized rats. Further studies will be required to evaluate the relative contributions of specific types of inflammatory cells and endogenous mediators in the development of pulmonary C-fiber hypersensitivity, and to identify the signaling pathways possibly involved in the interaction between inflammatory cells and pulmonary C-fiber endings in allergen-sensitized airways.

Fig. 8. Effect of Ova sensitization on the average peak response and the duration of discharge of pulmonary C fibers in response to right atrial bolus injection of capsaicin in anesthetized, open-chest, and artificially ventilated rats. A and B: average peak responses (top) and durations of discharge (bottom) of pulmonary C fibers in response to right atrial injections of capsaicin at 0.25 and 0.5 μg/kg, respectively. Open bars, control rats (n = 11 in A, n = 13 in B); filled bars, sensitized rats (n = 10 in A, n = 15 in B). Values are means ± SE. ∆FA, difference between the peak FA (average over 2-s interval) within 5 s after the injection and the baseline FA (average over 60-s interval) in each fiber. Duration of discharge was calculated as the time when FA exceeded the average baseline FA by 1 imp/s. Responses were tested before and 15–30 min after the acute Ova challenge (3%, 5 min) in both control and sensitized rats. *P < 0.05 and **P < 0.01, significant difference in the response or the duration of discharge between control and sensitized rats. †P < 0.05 and ††P < 0.01, significant difference in the response or the duration of the discharge between before and after acute Ova challenge.

Fig. 9. Effect of Ova sensitization on the average peak response and the duration of discharge of pulmonary C fibers in response to right atrial bolus injection of adenosine in anesthetized, open-chest, and artificially ventilated rats. A and B: average peak responses (top) and durations of discharge (bottom) of pulmonary C fibers in response to right atrial injections of adenosine at 75 and 150 μg/kg, respectively. Values are means ± SE. Open bars, control rats (n = 12); filled bars, sensitized rats, n = 12. ∆FA, difference between the peak FA (average over 10-s interval) and the baseline FA (average over 60-s interval) in each fiber. Duration of discharge was calculated as the time when FA exceeded the average baseline FA by 0.5 imp/s. Responses were tested before and 25–45 min after acute Ova challenge (3%, 5 min) in both control and sensitized rats. *P < 0.05, significant difference in the response between control and sensitized rats.
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