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Ovalbumin sensitizes vagal pulmonary C-fiber afferents in Brown Norway rats

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Kuo YL, Lai CJ. Ovalbumin sensitizes vagal pulmonary C-fiber afferents in Brown Norway rats. *J Appl Physiol* 105: 611–620, 2008. First published June 5, 2008; doi:10.1152/jappphysiol.01099.2007.—Sensitization of vagal lung C fibers has been postulated to contribute to the development of asthma, but support for this notion is still lacking. We investigated the characteristics and function of pulmonary C fibers (PCFs) in ovalbumin (OVA)-sensitized Brown Norway rats, an established animal model of asthma. Rats were sensitized with intraperitoneal injection of OVA or were treated with saline (control). In *study 1*, with the use of open-chest and artificially ventilated rats, inhalation of 5% OVA aerosol evoked an augmented increase in total lung resistance in the OVA-sensitized rats, compared with the control rats. Bilateral vagotomy or subcutaneous pretreatment with a high-dose of capsaicin for blocking of C-fiber function equally attenuated this augmented total lung resistance response, suggesting the involvement of PCFs. In *study 2*, with the use of anesthetized, spontaneously breathing rats, right atrial injection of capsaicin (1 $\mu\text{g/kg}$; a PCF stimulant) evoked an augmented apneic response in the OVA-sensitized rats, compared with the control rats. In *study 3*, with the use of open-chest, paralyzed, and artificially ventilated rats, the afferent PCF responses to right atrial injection of capsaicin (0.5 and 1.0 $\mu\text{g/kg}$), phenylbiguanide (8 $\mu\text{g/kg}$; a PCF stimulant), or adenosine (0.2 mg/kg; a PCF stimulant) were enhanced in the OVA-sensitized rats, compared with the control rats. However, the baseline activities of PCFs and their afferent responses to mechanical stimulation by lung hyperinflation in the OVA-sensitized and control rats were comparable. Our results suggested that OVA-sensitized Brown Norway rats possess sensitized vagal PCFs, which may participate in the development of the airway hyperactivity observed in these animals.

lung vagal afferents; pulmonary chemoreflex; capsaicin; inflammation; asthma

ASTHMA IS AN AIRWAY DISEASE characterized by variable airway obstruction and airway inflammation. Although the pathogenesis of this disease has not been fully elucidated, infiltration of inflammatory cells followed by subsequent activation and release of various chemical mediators has been suggested. The lung vagal afferents play a critical role in the regulation of respiratory functions and airway defense reflexes under a variety of pathophysiological conditions. Among these vagal afferents, the bronchopulmonary C fibers, which constitute ~75% of the afferent fibers in the vagal branches innervating the entire respiratory tract, function as a primary sensor for detecting various chemical stimuli in the airways or pulmonary circulation (15, 32). In general, these C fibers are very sensitive to various endogenous inflammatory mediators or inhaled irritants but relatively insensitive to mechanical stimuli (e.g.,

bronchoconstriction; Refs. 15, 32). Stimulation of the vagal pulmonary C fibers (PCFs) immediately elicits pulmonary chemoreflexes (e.g., apnea, bradycardia, and hypotension; Refs. 15, 32) accompanied by a number of other reflex responses mediated through the cholinergic pathway, such as bronchoconstriction, hypersecretion of mucus, and bronchial vasodilatation (15, 32). In fact, activation of PCFs produces cough, airway irritation, and chest tightness, all of which are clinical signs of asthmatic patients (4, 15, 44). Furthermore, after perineural capsaicin treatment of both cervical vagi to block the conduction of capsaicin-sensitive C fibers, antigen-induced bronchomotor responses were largely prevented (35, 38). Taken together, these observations indicate that activation of PCFs may be involved, at least partially, in the development of antigen-induced respiratory responses.

Ovalbumin (OVA), the most common antigen, has been used to establish an animal model of asthma. The actively sensitized Brown Norway (BN) rats when challenged with OVA exhibit many of the key features of allergic asthma. This model has been used extensively for studying the pathophysiological mechanisms and therapeutic treatments of asthma (1, 43). More importantly, airway inflammation is known to exist in OVA-exposed animals or asthmatic patients; the inflammatory mediators, either formed endogenously or administered exogenously, are known to exert potent stimulatory and sensitizing effects on PCFs (15, 32). Udem et al. (54) reported that OVA sensitization increased the excitability of nodose ganglion neurons in guinea pig, implying an OVA-induced sensitization of PCFs. Bergren (6) showed that OVA sensitization enhanced PCF responses to capsaicin and bradykinin at high doses in guinea pigs. However, it is not known whether OVA induced the sensitizing effects of PCFs to mechanical or other chemical stimulants. To perform this study, we used the OVA-sensitized BN rat model and selected capsaicin, phenylbiguanide (PBG), and adenosine as chemical stimulants that are thought to selectively activate PCFs on the nerve terminals via activation of the vanilloid type 1 (VR1) receptor (11, 30), 5-HT₃ receptor (21), and adenosine A₁ receptor (23), respectively. Additionally, we also used lung hyperinflation as the challenge to test the PCF response to mechanical stimulation. Therefore, the present study was carried out to evaluate whether OVA sensitization enhances the sensitivity of the pulmonary chemoreflexes, which is known to be elicited by stimulation of PCFs (32), and, if so, to investigate whether the OVA sensitization augments the stimulatory effects of chemical stimulants and lung hyperinflation on PCF using the single-fiber recording technique.

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METHODS

All surgical and experimental procedures were carried out using protocols approved by the Institutional Animal Care and Use Committee of Tzu Chi University.

Sensitization procedures. A total of 100 inbred male BN rats (weight = 296 ± 4 g) were divided into the OVA-sensitized and control groups; the former group received an intraperitoneal injection of OVA (1 ml, 1 mg/ml) in 0.9% NaCl containing 100 mg/ml $\text{Al}(\text{OH})_3$ on 3 consecutive days, whereas the latter received an intraperitoneal injection of 0.9% NaCl. Eleven days after the last OVA or saline treatment, animals were used for the subsequent studies of bronchomotor, reflex, and PCF responses. This sensitization protocol was adopted from that reported by Coleman et al. (14), who demonstrated that the level of circulating IgE antibodies increases rapidly 7–14 days after the intraperitoneal injection of OVA in rats.

Capsaicin pretreatment. Some rats received capsaicin pretreatment before OVA sensitization to block C-fiber function with a protocol suggested by Jancso et al. (27). For this purpose, each rat was subcutaneously injected with increasing dosages of 50, 100, and 150 mg/kg capsaicin for 3 consecutive days (58). One day after the last dose of capsaicin pretreatment, these rats were subjected to OVA sensitization.

General preparations. For all experiments, animals were anesthetized with an intraperitoneal injection of α -chloralose (100 mg/kg; Sigma Chemical, St. Louis, MO) and urethane (500 mg/kg; Sigma Chemical) dissolved in a 2% borax solution. The rats were tethered in a supine position, and the trachea was cannulated below the larynx with a short tracheal tube via a tracheotomy. A polyethylene catheter was inserted into the jugular vein and advanced until the tip was close to the right atrium for intravenous administration of pharmacological agents. The volume of each bolus injection was 0.1 ml, which was first injected into the catheter (dead space of ~ 0.2 ml) and then flushed into the circulation by an injection of 0.3 ml saline. The right femoral artery was cannulated for measurement of arterial blood pressure and heart rate. During the course of the experiments, supplemental doses of α -chloralose ($20 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) and urethane ($100 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) were administered to maintain abolition of pain reflexes induced by pinching the tail of the animal. In the study of reflex responses, rats breathed spontaneously. In the bronchomotor and electrophysiological studies, rats were ventilated by a respirator (Harvard 683; South Natick, MA) at a constant volume ~ 2.0 – 2.5 ml. The frequency of the respirator was set at 60 breaths/min and was kept constant in each experiment. A midline thoracotomy was then performed, and the edges of the rib cage were retracted. The expiratory outlet of the respirator was placed under 3 to 4 cm of water to maintain a near normal functional residual capacity. These rats were paralyzed with pancuronium bromide (0.05 mg/kg iv; Orgnon Teknika, Bostel, Holland). Periodically, the effect of pancuronium was allowed to wear off so that the depth of anesthesia could be checked. The body temperature of all animals was maintained at $\sim 36^\circ\text{C}$ throughout the experiment by means of a servo-controlled heating blanket.

Measurements of bronchomotor and reflex responses. To measure bronchomotor responses, tracheal pressure (transpulmonary pressure in open-chest preparation; Ptr) was monitored with a pressure transducer (Validyne MP45-28; Northridge, CA) via a side tap of the tracheal cannula. Total lung resistance (RL) was determined by using the subtraction method (40). To measure reflex responses, respiratory flow was measured with a pneumotachograph (Fleisch 4/O; Richmond, VA) coupled with a differential pressure transducer (Validyne MP45-12), and integrated to give tidal volume. Respiratory frequency, expiratory duration, and tidal volume were measured on a breath-by-breath basis.

Recording of PCF afferent activity. Afferent activities arising from PCFs were identified and recorded by methods described previously (29). Briefly, the right vagus nerve was sectioned, and a fine afferent

filament was split from the desheathed nerve trunk and placed on a platinum-iridium recording electrode. Action potentials were amplified (Grass P511K; Quincy, MA), monitored with an audio monitor (Grass AM8), and displayed on an oscilloscope (Gould 420; Cleveland, OH). The fine nerve filament was subdivided until activity from only 1 or 2 units was obtained. Both vagi were ligated just above the diaphragm to eliminate the electrical signals arising from the abdominal viscera. The afferent activity of a single unit was first searched for by hyperinflation (3–4 times tidal volume) and then identified by the immediate (delay < 1 s) response to a bolus injection of capsaicin (0.5 and $1.0 \mu\text{g/kg}$) into the right atrium. Finally, the conduction velocity of the afferent fiber was measured as described previously (7). Fibers with conduction velocities of < 2 m/s were considered as PCFs. Before the end of each experiment, the general locations of C fibers studied were identified within the lung structure by gently probing the tissues with a polyethylene rod (diameter = 2 mm).

Pharmacological agents. A mixture of α -chloralose (100 mg/ml) and urethane (500 mg/ml) was dissolved in a 2% borax solution. The stock solution of capsaicin (5 mg/ml) was prepared by dissolving capsaicin in 10% Tween 80, 10% ethanol, and 80% isotonic saline. The stock solutions of PBG (1 mg/ml) and adenosine (10 mg/ml) were prepared by dissolving PBG and adenosine in distilled water and saline, respectively. The desired concentrations of these PCF stimulants were made daily by diluting their stock solutions with saline on the basis of the body weight of the animal. All these pharmacological agents were obtained from Sigma Chemical.

Experimental procedures. Three series of experiments were carried out. Rats were grouped according to following treatments: nonsensitization by saline (control), OVA sensitization (OVA), capsaicin pretreatment before OVA sensitization (capsaicin + OVA), and bilateral vagotomy before OVA aerosol inhalation (vagotomy + OVA). *Study series 1* and *2* were designed to determine the effect of OVA sensitization on PCF-mediated responses and to determine whether the effect of OVA sensitization can be abolished by blocking the C-fiber function using capsaicin pretreatment or by bilateral vagotomy. *Study series 3* was designed to investigate whether the sensitivity of a single PCF to stimulants may be altered in OVA-sensitized rats. Therefore, in *study series 1*, all four groups (each $n = 10$) were used to measure bronchomotor responses to OVA aerosol. OVA aerosol (5%) was generated by an ultrasonic nebulizer (model 2511; PulmoSonic, DeVilbiss, PA) and was delivered to the airways to induce bronchoconstriction via a delivery circuit (48) when the pneumotachograph was removed. RL responses to OVA aerosol were measured and compared among the study groups. In *study series 2*, the control, OVA, and capsaicin + OVA groups (each $n = 10$) were used to measure PCF-mediated pulmonary chemoreflex responses. The cardiopulmonary reflex responses to right atrial injection of capsaicin ($1 \mu\text{g/kg}$) were measured and compared among the study groups. In *study series 3*, only control and OVA groups (each $n = 15$) were used because afferent responses of PCFs to stimulants may be impaired in the capsaicin + OVA group. The afferent responses of PCFs to right atrial injections of capsaicin (0.5 and $1.0 \mu\text{g/kg}$), PBG ($8 \mu\text{g/kg}$), or adenosine (0.2 mg/kg) and to lung hyperinflation were measured and compared between two study groups. These PCF stimulants were tested in each fiber, and one receptor was recorded from each animal. To allow the baseline fiber activity to return to control levels and to avoid any accumulated effect, an elapsed time of ~ 15 min was allowed between any two injections. Lung hyperinflation was achieved by inflating the lung with a constant air flow until tracheal pressure reached 15 and 30 cmH_2O , respectively, and was maintained at that pressure for 10 s after the respirator was turned off. Results were discarded for those receptors that had become inactive during the test and/or were unresponsive to capsaicin injection at the end of the test period.

Data analysis and statistics. All physiological signals were analyzed (BioCybernetics 1.0; Tapipei, Taiwan) and recorded by a thermal array recorder (Gould TA11) and also recorded on tape (Neurocorder

DR-890; New York, NY) for later analysis. These physiological parameters were analyzed using a computer equipped with an analog/digital converter (Gould DASA 4600) and software (BioCybernetics 1.0). Neural activity of PCFs and cardiorespiratory responses were measured at 1-s intervals or on a breath-by-breath basis, respectively. In *study series 1*, because changes in RL induced by the OVA aerosol inhalation exhibited a biphasic pattern as reported by Baraniuk (3), responses between 0 to 240 min and from 241 to 600 min after the termination of OVA aerosol inhalation were defined as the early and late phases, respectively. RL, mean arterial blood pressure, and heart rate were calculated for a 1-min period every 5 min during 0–30 min and every 30 min during 31–600 min after OVA aerosol inhalation. In *study series 3*, averaged baseline fiber activity was calculated over the 10-s intervals; peak responses were defined as the maximal value averaged over 10-s and 2-s intervals for lung hyperinflation and injection of various chemical stimulants, respectively. Results were evaluated by a two-way mixed factorial ANOVA followed by Duncan's test when appropriate. $P < 0.05$ was considered statistically significant. All data were presented as means $\pm$ SE.

RESULTS

Effects of OVA sensitization on bronchomotor response. At baseline, RL was not significantly affected by OVA sensitization. On average, the baseline RLs in control ($n = 10$), OVA ($n = 10$), capsaicin + OVA ($n = 10$), and vagotomy + OVA ($n = 10$) groups were 0.20 ± 0.02 , 0.20 ± 0.01 , 0.18 ± 0.01 , and 0.20 ± 0.01 cmH₂O/ml/s, respectively. In the control group, inhalation of 5% OVA aerosol for 10 min caused a slight increase in RL. In contrast, inhalation of 5% OVA aerosol markedly elicited an increase of RL in the OVA group. The analysis of response over time revealed that RL after OVA aerosol inhalation exhibited a bimodal pattern (Fig. 1A). The early phase exhibited an initial rapid increase in RL, which reached a peak response at ~5–60 min after OVA inhalation and declined gradually to a lower plateau level at ~150–270 min; the late phase maintained at this plateau level and slightly increased in RL between 330–510 min used OVA provocation. As a group, the RL increased from baseline of 0.20 ± 0.01 cmH₂O·ml⁻¹·s⁻¹ to a peak of 0.33 ± 0.03 cmH₂O·ml⁻¹·s⁻¹ during the early phase and to a peak of 0.30 ± 0.02 cmH₂O·ml⁻¹·s⁻¹ during the late phase after OVA inhalation. The late phase was significantly higher than the plateau (0.25 ± 0.02 cmH₂O·ml⁻¹·s⁻¹) after the early phase. Furthermore, pretreatment with high dose of capsaicin markedly abolished the increase in RL induced by OVA inhalation (Fig. 1); the baseline RL and early and late peak responses were 0.18 ± 0.01 , 0.19 ± 0.01 , and 0.20 ± 0.02 cmH₂O·ml⁻¹·s⁻¹, respectively. Similarly, the bimodal pattern in bronchomotor response by OVA sensitization was also largely eliminated by vagotomy; the baseline RL and early and late peak responses were 0.20 ± 0.01 , 0.21 ± 0.01 , and 0.21 ± 0.01 cmH₂O·ml⁻¹·s⁻¹, respectively (Fig. 1).

OVA sensitization enhanced pulmonary chemoreflex. At baseline, no significant differences were found in respiratory frequency, tidal volume, minute ventilation, heart rate, and mean arterial blood pressure among the control, OVA, and capsaicin + OVA groups. A bolus injection of capsaicin (1 μ g/kg) suppressed the respiratory rate, heart rate, and arterial blood pressure in control groups. The inhibition of the respiratory rate resulted in apnea leading to a prolongation of expiratory duration, whereas the inhibition of heart rate and arterial blood pressure was characterized by transient but

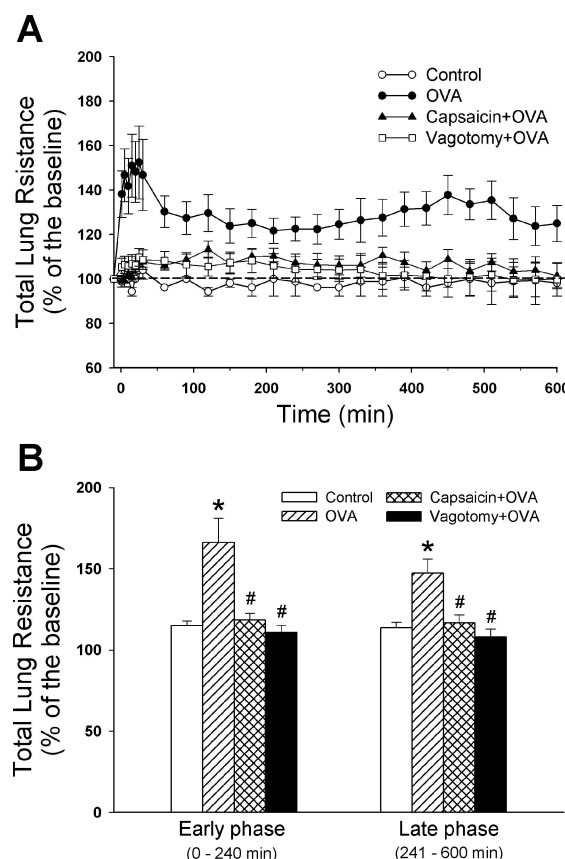
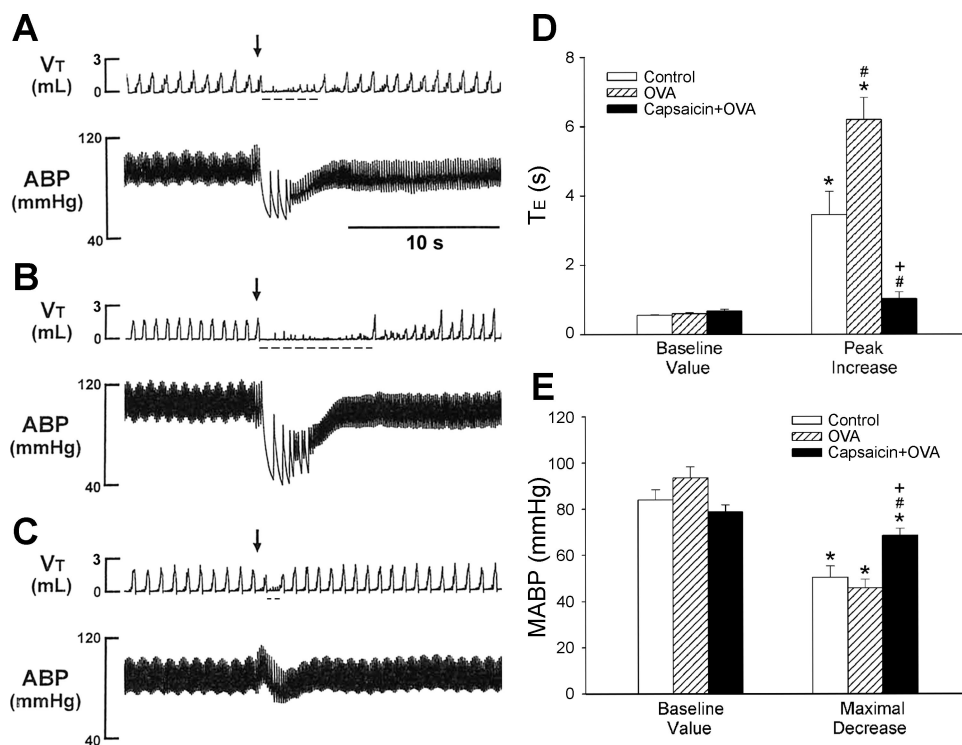


Fig. 1. Mean total lung resistance used OVA aerosol inhalation in saline (control), ovalbumin-sensitized (OVA), high dose of capsaicin pretreatment + OVA-sensitized (capsaicin + OVA), and vagotomy + OVA-sensitized (vagotomy + OVA) groups of rats. A: OVA aerosol was inhaled at time 0; total lung resistance was measured on a breath-by-breath basis, and was averaged over 1-min periods in each rat. B: early and late phases were defined as peak responses between 0–240 and 241–600 min after OVA aerosol inhalation, respectively. *Significantly different from responses to control group. Data in each group are means $\pm$ SE of 10 animals and are expressed as percentage of baseline response; #significantly different from responses to OVA group.

prominent bradycardia and hypotension. The capsaicin-induced apnea was greatly prolonged in OVA-sensitized rats (Fig. 2, B and D). The apneic durations (a prolonged expiratory duration) induced by capsaicin injection in control and OVA groups were 3.45 ± 0.68 ($n = 10$) and 6.20 ± 0.64 s ($n = 10$), respectively. In contrast, bradycardia and hypotension caused by bolus injection of capsaicin were not significantly different between the control and OVA groups (Fig. 2E). In addition, the OVA-enhanced apneic duration to right atrial injection of capsaicin was almost completely abolished by capsaicin pretreatment (1.03 ± 0.19 s; Fig. 2D).

OVA sensitization induces hypersensitivity of PCFs. A total of 36 PCFs were studied in 30 rats. The conduction velocities were 0.85–1.96 m/s (1.16 ± 0.06 m/s; $n = 26$). The baseline activity of the PCFs studied was irregular and sparse (0.05 ± 0.03 impulses/s; $n = 36$), displaying a distinct sensitivity to capsaicin and a weak or no response to lung hyperinflation. The response to capsaicin was always tested at an initial dose of 0.5 μ g/kg. In 3 of 36 C fibers, the dose was increased to 1.0 μ g/kg when the initial dose failed to produce a detectable and consistent stimulatory effect on these fibers. At the control level, the right atrial bolus injection of a low dose of capsaicin

Fig. 2. Cardiorespiratory responses to right atrial injection of capsaicin in control (A), OVA (B), and capsaicin + OVA (C) rats. A, B, and C: arrows indicate onset time of capsaicin injection (1 μ g/kg); the dashed lines indicate the apneic duration to capsaicin. D and E: group data of apneic response and hypotension to capsaicin injection. VT, tidal volume; ABP, arterial blood pressure; TE, expiratory duration; MABP, mean ABP. *Significantly different from corresponding baseline; #significantly different from responses to control group; +significantly different from responses to OVA group. Data in each group are means $\pm$ SE of 10 animals.



(0.5 μ g/kg; $n = 18$) immediately evoked a mild and short burst of discharge (Fig. 3A); the peak response was 1.10 ± 0.23 impulses/s. However, the peak activity of the low dose of capsaicin in the OVA group of C fibers was markedly enhanced (5.33 ± 1.28 impulses/s; $n = 18$). Similar enhancement in the response to capsaicin was also elicited in a high dose of capsaicin (1.0 μ g/kg; $n = 18$). In the control and OVA groups of PCFs, the peak activity of the high dose of capsaicin was 5.82 ± 0.59 ($n = 18$) and 10.97 ± 1.60 impulses/s ($n = 18$), respectively. Additionally, the baseline activity of PCFs was not significantly different between OVA and control groups (Fig. 3).

The potentiating effect of OVA sensitization was not only limited to the response to capsaicin. The responses of these PCFs to injection of a low dose of PBG (8 μ g/kg; Fig. 4) and adenosine (0.2 mg/kg; Fig. 5) were also enhanced in OVA-sensitized rats. On average, these peak activities of PCFs induced by PBG were 4.30 ± 0.73 impulses/s in the control group and 9.05 ± 1.83 impulses/s in the OVA group; the peak responses caused by adenosine were 1.60 ± 0.55 impulses/s in the control group and 6.15 ± 1.21 impulses/s in the OVA group. Similarly, the peak activities to PBG and adenosine were enhanced in OVA-sensitized animals (Figs. 4 and 5).

OVA sensitization failed to alter the responses of PCFs to lung inflation at low pressure ($P_{tr} = 15$ cmH₂O) or high pressure (30 cmH₂O; Fig. 6). Lung inflation at high pressure significantly increased the activity of PCFs in both the control and OVA groups; however, PCF activity was not significantly different between the control and OVA groups (Fig. 6B). The peak activities of PCFs in control and OVA groups were 0.12 ± 0.03 and 0.16 ± 0.03 imp/s, respectively, during lung inflation at $P_{tr} = 30$ cmH₂O (10-s average).

DISCUSSION

The results of this study show that aerosolized OVA markedly increased RL with a bimodal pattern in OVA-sensitized rats. The early phase displayed an initial rapid increase in RL that reached a peak response at ~ 5 –60 min after OVA inhalation and 240 min later followed by the late-phase asthmatic reaction. These responses in OVA-sensitized BN rats have provided an asthmatic animal model that has been used for studying the pathogenic mechanisms, because the bronchoconstrictive features closely resemble those seen in asthmatic patients (8). Bilateral vagotomy or subcutaneous pretreatment with a high-dose of capsaicin for blocking of C-fiber function equally attenuated this augmented RL response, suggesting the involvement of PCFs. Additionally, OVA sensitization enhanced the apneic response to right atrial injection of capsaicin in anesthetized, spontaneously breathing BN rats, compared with the control rats. The prolonged apneic response was also markedly attenuated by pretreatment with capsaicin. Electrophysiological recording of the afferent activity of a single PCF further demonstrated that the C-fiber responses to right arterial injection of capsaicin, PBG, and adenosine were markedly enhanced in the OVA-sensitized rats. In contrast, OVA sensitization failed to alter the afferent response of PCFs to hyperinflation of the lung. Hence, our results suggest that the sensitizing effects on PCFs existed in OVA-sensitized BN rats, which may participate in the development of the airway hyperreactivity observed in these animals.

Allergen provocation of asthmatic patients elicits a biphasic response of bronchoconstriction in $\sim 60\%$ of subjects, consisting of an early phase reaction and a late phase reaction (13). The early phase reaction occurs ~ 15 min after allergen provocation and is thought to be mediated by allergen binding to

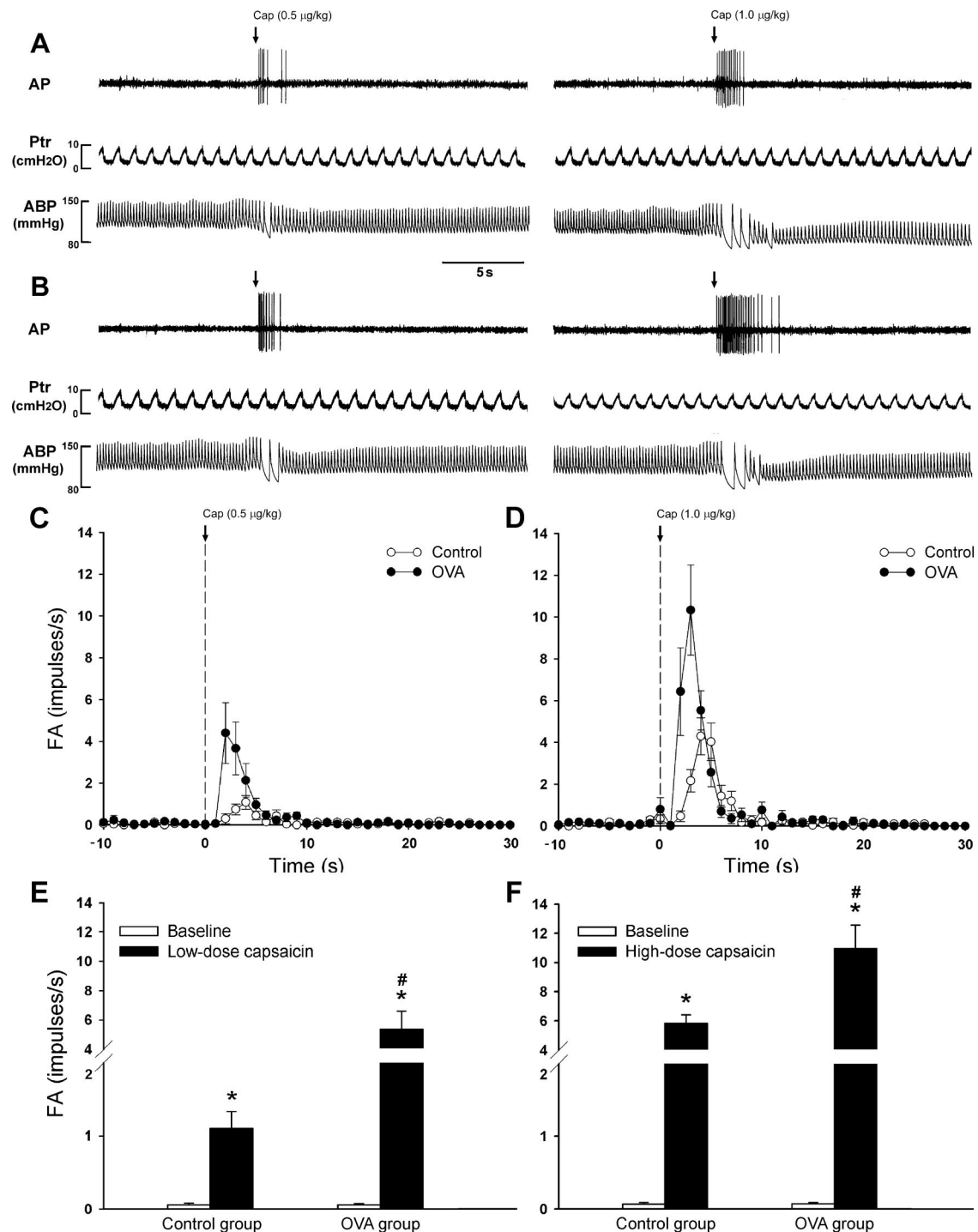


Fig. 3. Effect of OVA sensitization on pulmonary C-fiber responses to low (0.5 $\mu\text{g/kg}$; A and B, left; C and E) and high (1 $\mu\text{g/kg}$; A and B, right; D and F) doses of capsaicin in anesthetized, open-chest rats. A: responses of a control rat; B: responses of an OVA rat. Capsaicin solution (0.1 ml) was first slowly injected into the catheter (dead space = 0.2 ml) and then flushed into the right atrium (at the arrow) as a bolus with saline. Approximately 15 min elapsed between injections. In C and D, vertical dashed lines indicate onset time of low-dose and high-dose of capsaicin injections, respectively. In E and F, fiber activity (FA) represents the peak FA (average over 2-s intervals) and the baseline FA (average over 10-s intervals). AP, action potential; Ptr, tracheal pressure. *Significantly different from corresponding baseline; #significantly different from responses to control group. Data in each group are means $\pm$ SE of 18 fibers from 15 rats.

IgE receptors on mast cells (9, 55). Subsequently, this event leads to activation and the release of histamine and leukotrienes, triggering an immediate bronchoconstriction (19, 22). In contrast, the late phase reaction begins 3–4 h after allergen provocation, peaks between 6–12 h, and generally resolves

within 24 h (8, 13, 19). Many studies (16, 20) have suggested that the recruitment of proinflammatory leukocytes into the lung is involved in the development of the late phase reaction used allergen challenge. It is known that stimulation of PCF afferents can elicit bronchoconstriction via the cholinergic

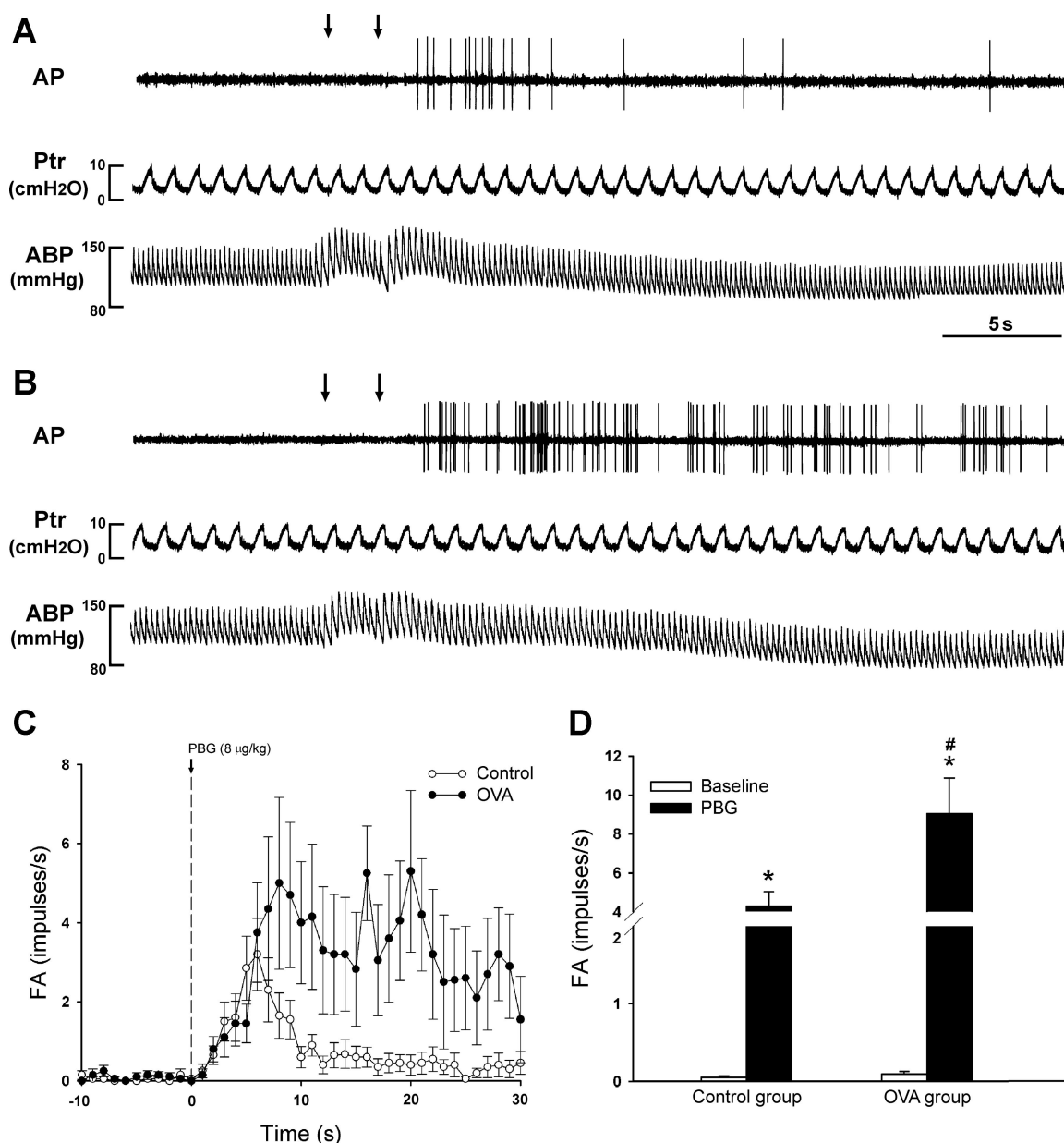


Fig. 4. Effect of OVA sensitization on pulmonary C-fiber responses to right atrial injection of phenylbiguanide (PBG) in control (A) and OVA (B) rats. In A and B, PBG (8 μ g/kg, 0.30–0.35 ml) was injected into catheter at first arrow and flushed into vein at second arrow. In C, vertical dashed line indicates onset time of PBG injection and corresponds to the second arrows in A and B. *Significantly different from corresponding baseline; #significantly different from responses to control group. Data in each group are means $\pm$ SE of 18 fibers from 15 rats. See Fig. 3 legend for further explanation. Note that the significant increase in blood pressure between the 2 arrows due to a volume loading from rapid injection in A and B.

pathway and/or axonal reflex (15). Furthermore, the augmented bronchoconstrictive effect caused by allergen challenge was attenuated by capsaicin pretreatment in guinea pigs (35, 38). Similarly, in the present study, we also found that inhalation with 5% OVA aerosol for 10 min significantly produced an increased RL with a bimodal pattern in OVA-sensitized rats. Our study also suggests that stimulation and/or sensitization of PCFs seem to be involved in the enhanced bronchoconstrictive response to OVA aerosol inhalation induced by OVA sensitization in BN rats because the augmented response was markedly attenuated by either capsaicin pretreatment or bilateral vagotomy. Actually, the inactivation of PCFs by the capsaicin-pretreatment used in this study has not been reported. Previous

studies (27) using this capsaicin-pretreatment mostly focused on its effect to cause depletion of tachykinins. However, in this study, we found that the PCF-mediated apneic responses to right atrial injection of capsaicin were absent in animals with the capsaicin pretreatment. This result suggests that the capsaicin pretreatment can also impair afferent function of PCFs. Accordingly, we speculate that PCFs have a critical role in causing the enhanced bronchoconstriction observed OVA sensitization. Our data, however, cannot rule out the possibility that irritant receptors also have a role in the bronchomotor response by OVA, because capsaicin treatment has been reported to diminish the number of small myelinated A δ fibers (49).

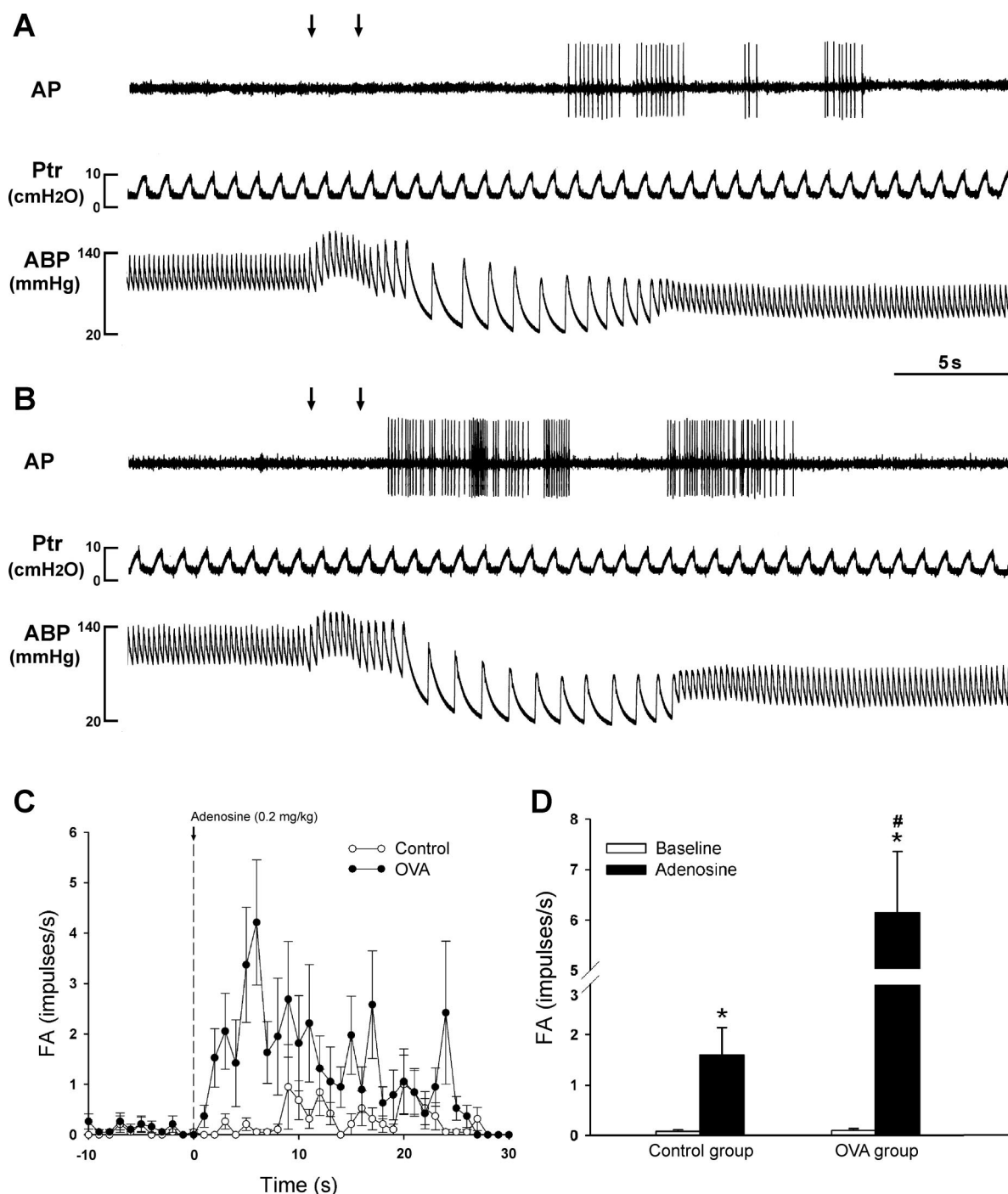


Fig. 5. Effect of OVA sensitization on pulmonary C-fiber responses to right atrial injection of adenosine in control (A) and OVA (B) rats. In A and B, adenosine (0.2 mg/kg, 0.30–0.35 ml) was injected into catheter at first arrow and flushed into vein at second arrow. In C, vertical dashed line indicates onset time of adenosine injection and corresponds to the second arrows in A and B. *Significantly different from corresponding baseline; #significantly different from responses to control group. Data in each group are means $\pm$ SE of 18 fibers from 15 rats. See Fig. 3 legend for further explanation. Note that the significant increase in blood pressure between the two arrows due to a volume loading from rapid injection in A and B.

Our results demonstrated that OVA sensitization enhances the pulmonary chemoreflex sensitivity (Fig. 2) and the sensitivity of PCFs to chemical stimulants (Figs. 3–5). Recent investigations reported that OVA sensitization significantly enhanced PCF activities (6) and the apneic response to capsaicin (57). The results of the present study also provide the direct electrophysiological evidence demonstrating the OVA

sensitization induced PCF hypersensitivity. The importance of PCFs in the regulation of airway functions in both normal and pathophysiological conditions is well recognized (15, 32); therefore, our results substantiate the possibility that PCFs are responsible for producing various pulmonary responses in OVA-sensitized animals. Asthmatic subjects (5, 12) exhibit the sensation of dyspnea, chest tightness, and cough; these respi-

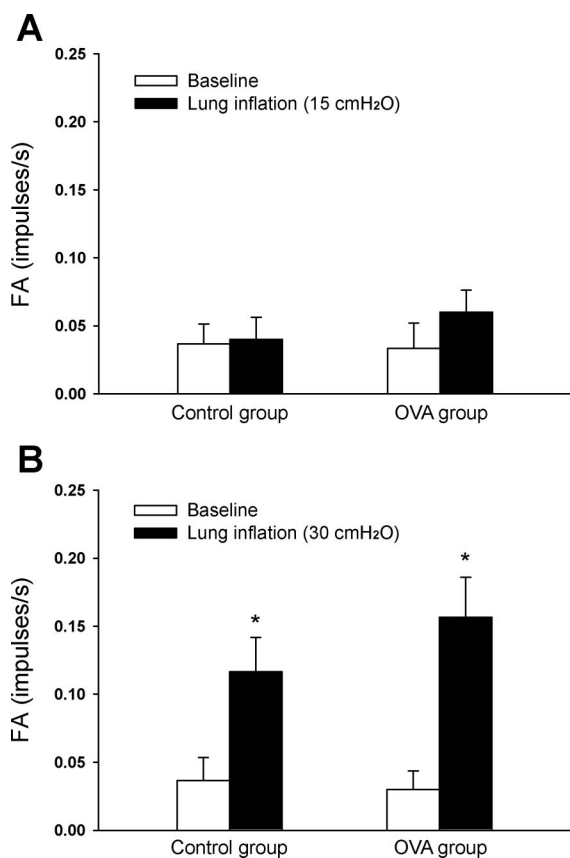


Fig. 6. Effect of OVA sensitization on pulmonary C-fiber responses to lung inflation in control and OVA groups. The responses to constant-pressure lung inflation at $P_{tr} = 15$ cmH₂O and 30 cmH₂O in A and B, respectively. The peak FA and the baseline FA were averaged over 10-s intervals. *Significantly different from corresponding baseline. Data in each group are means $\pm$ SE of 18 fibers from 15 rats.

ratory responses are believed to be signs of activation of PCFs (4, 15, 44). Similarly, previous studies (2, 35, 38) have shown that antigen-induced bronchomotor responses were largely prevented by a high dose of capsaicin in animals. In contrast, pretreatment with capsaicin failed to abolish OVA aerosol-induced airway obstruction in OVA-sensitized guinea pigs (28) and even enhanced irritant-related lung inflammation in polymeric diphenylmethane-diisocyanate-sensitized BN rats (46). The differences may be explained by different animal species, sensitized procedures, and asthmagenic compounds used in these studies and in our study.

Our electrophysiological evidence indicated that C-fiber responses to right arterial injection of chemical stimulants, such as capsaicin, PBG, and adenosine, were markedly enhanced in the OVA-sensitized rats. Those three chemical stimulants with entirely different chemical structures and pharmacological properties were chosen for testing PCF sensitivity in this study. Capsaicin, the pungent ingredient of the hot pepper, has been used extensively as a tool for identifying C-fiber afferents because of its potency and selectivity in stimulating C-fiber endings and eliciting pulmonary chemoreflex responses (15, 32) by activation of VR1 receptors (11, 30). McLeod et al. (39) showed that activation of VR1 receptors is involved in OVA aerosol-induced cough response in OVA-sensitized guinea pigs. Additionally, OVA-sensitized animals

produced substantial pulmonary inflammation (26, 56), which often occurs concomitantly with tissue acidosis (45). It is known that H^+ by itself does not activate VR1 but modulates the channel properties of the VR1 and enhances its sensitivity to capsaicin (42). However, the OVA-induced hypersensitivity of PCF responses to chemical stimulants cannot be explained by OVA association with tissue acidosis alone since OVA sensitization also elevated the sensitivity of PCFs to PBG and adenosine. These two chemical agents are known to stimulate PCF through completely different mechanisms from those of capsaicin. PBG, a specific 5-HT₃ receptor agonist, is known as another potent stimulatory agent to vagal sensory C neurons in different species (21, 25). Activation of the 5-HT₃ receptor elicited a fast activating and inactivating calcium influx in rat sensory neurons, which are believed to be able to stimulate and sensitize the nerve endings (33). Additionally, activation of the 5-HT₃ receptor is reported to play an important role in the rapid and long-lasting unmasking of functional tachykinin receptors after OVA challenge of nodose ganglia (41). Adenosine, a naturally occurring purine nucleoside generated by the degradation of ATP in all metabolically active cells (10), is elevated under inflammatory conditions and released from mast cells used allergen challenge (34, 36). Clinically, bronchoalveolar lavage fluid from asthmatic patients has also revealed increased adenosine levels compared with normal control patients (18). The possible mechanisms underlying the enhanced excitability of PCFs after OVA sensitization were not studied in the present study. However, it is likely that OVA sensitization may release numerous chemical mediators (17, 51), many of which have been shown to enhance the sensitivity of PCFs. For example, histamine (31), adenosine (23), and prostaglandins (24) have the ability to potentiate the sensitivity of PCFs. Many of these mediators directly bind to specific G-protein-coupled receptors at the nerve terminal, leading to a nonspecific increase in the electrical excitability of the nerve endings (52). Additionally, this can occur indirectly due to phosphorylation of certain voltage-gated sodium channels leading to an increase in membrane conductance (52). Another example of airway inflammation-induced sensitization of these afferents was reported by Undem et al. (54) using an intracellular recording. The excitability of isolated neurons in the guinea pig nodose ganglia, the cell bodies of PCFs, was enhanced by sensitization with OVA. It was suggested that the inflammatory mediators released from degranulation of mast cells located in the ganglion may be involved. However, it should be pointed out that there are noticeable differences in the results between the present in vivo and previous in vitro studies. First, one study (53) found that the majority of C fiber-innervated intrapulmonary tissues presumably are derived from the neurons in nodose ganglion. However, Riccio et al. (47) studied the afferent innervation of guinea pig trachea and found that nodose ganglia contain cell bodies of mostly myelinated A δ afferents but not C fibers. Second, the differences in membrane properties, the expression of channels/receptors, and the signal transduction mechanism between the neuronal soma and the sensory nerve endings may have also contributed to this difference in hypersensitivity of cell soma or sensory terminal. Finally, the ultrafine structures that surround and interact with the sensory nerve endings in living tissue are very different from those used in the isolated neurons.

Although PCFs are usually quiescent during eupneic breathing, they can be activated by lung inflation (25, 32), and an increase in inspired volume occurs commonly during hyper-ventilation caused by hypoxia or exercise. Whether the response of these afferents to lung inflation is augmented by OVA sensitization is not known. In this study, OVA challenge did not alter the sensitivity of PCFs to lung hyperinflation (15 and 30 cmH₂O tracheal pressure) and its baseline activity, whereas OVA markedly increased their responses to chemical stimulants. In fact, PCFs are very sensitive to chemical stimuli but relatively insensitive to mechanical stimuli (50). Therefore, one possibility is that the OVA challenge for 3 consecutive days evoked inflammatory reactions that failed to reach the threshold levels and therefore did not generate a sensitizing effect on the response to lung hyperinflation in PCFs. Second, the wide-range variation of different C-fiber responses to lung hyperinflation reflects the heterogeneity of physiological properties of these afferents (15, 32). The characteristics of the PCF response to lung hyperinflation were also found in this study, because OVA sensitization evoked only a mild potentiation of afferent response to lung hyperinflation and there was no significant difference in control and OVA rats. Third, OVA-induced inflammations did not alter the conductance of specific ion channels that mediate the lung hyperinflation-induced membrane depolarization (37) of the C-fiber sensory terminals. However, we cannot rule out the possibility that prolonged OVA treatment or more severe inflammatory reactions may augment the afferent response to lung hyperinflation and baseline activity of PCFs.

In summary, these results demonstrate that OVA sensitization markedly augmented OVA aerosol-induced elevation of RL and also significantly amplified the apneic response to capsaicin injection. These enhanced effects of OVA may be mediated through the activation of PCFs, because these responses were largely attenuated by pretreatment with a high dose of capsaicin or bilateral vagotomy. Furthermore, OVA sensitization markedly enhanced the sensitivity of PCFs to chemical stimulants. These results suggest that sensitization of PCFs may contribute, at least in part, to the development of exaggerated airway reactions in asthmatic subjects.

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