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# Ovalbumin sensitization alters the ventilatory responses to chemical challenges in guinea pigs

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**Xu, Fadi, Jianguo Zhuang, Tongrong Zhou, and Lu-Yuan Lee.** Ovalbumin sensitization alters the ventilatory responses to chemical challenges in guinea pigs. *J Appl Physiol* 99: 1782–1788, 2005. First published July 14, 2005; doi:10.1152/jappphysiol.00613.2005.—Patients with chronic bronchial asthma show a depressed ventilatory response to hypoxia (DVH), but the underlying mechanism remains unclear. We tested whether DVH existed in ovalbumin (Ova)-treated guinea pigs, an established animal model of asthma. Twelve guinea pigs were exposed to Ova (1% in saline) or saline aerosol (control) for 5 min, 5 days/wk, for 2 wk. After completing aerosol exposure, the animals were anesthetized and exposed to systemic hypoxia. Ova treatment had no effects on animal body weight, baseline cardiorespiratory variables, or arterial blood O<sub>2</sub> and CO<sub>2</sub> tensions, but it attenuated the ventilatory response to hypoxia (10 breaths of pure N<sub>2</sub>) by 65% ( $P < 0.05$ ). When the animals were subjected to intracarotid injections of sodium cyanide (20 μg) and doxapram (2 mg) to selectively stimulate carotid chemoreceptors, the ventilatory responses were reduced by 50% ( $P < 0.05$ ) and 74% ( $P < 0.05$ ), respectively. In contrast, Ova exposure failed to affect the ventilatory response to CO<sub>2</sub> (7% CO<sub>2</sub>–21% O<sub>2</sub>–balance N<sub>2</sub> for 5 min;  $P > 0.05$ ). Furthermore, the apneic response evoked by stimulating bronchopulmonary C fibers (PCFs) with right atrial injection of capsaicin (5 μg) was markedly increased in the Ova-sensitized group ( $5.02 \pm 1.56$  s), compared with the control group ( $1.82 \pm 0.45$  s;  $P < 0.05$ ). These results suggest that Ova sensitization induces a DVH in guinea pigs, which probably results from an attenuation of the carotid chemoreceptor-mediated ventilatory excitation and an enhancement of the PCF-mediated ventilatory inhibition.

carotid body; bronchopulmonary C fibers; hypoxia; capsaicin; inflammation

RESPIRATORY FAILURE FROM ASTHMA is a common clinical symptom and a primary cause of high mortality. A study through a period of 7 yr indicated that 68% of asthmatic patients had two or more episodes of respiratory failure that led to an ~10% death rate (30). Ventilatory arrest in the near-fatal nature of the exacerbations is dominant compared with cardiac arrest (26). It is generally recognized that patients with chronic bronchial asthma have a depressed ventilatory response to hypoxia (DVH) that may contribute to the respiratory failure (4, 11, 14–16, 21, 33, 38), although an absence (27) of DVH or an increase of ventilatory response to hypoxia (20) was also reported. Hudgel and Weil (14, 15) first reported a severe DVH in asthmatic individuals in 1974. Subsequent studies confirmed this observation and further indicated that the amplitude of minute ventilation ( $\dot{V}_E$ ) in response to progressive isocapnic hypoxia was significantly attenuated in both young and adult

patients compared with age-matched normal subjects (4, 11, 16, 21, 33, 38). A severely diminished hypoxic perception was also found in asthmatic patients; a typical example is that the patients who had asthma displayed cyanosis but had no dyspnea at all (21). This blunted hypoxic perception could impair the behavioral control of breathing and worsen hypoxemia by reducing ventilatory augmentation. To date, the pathophysiological mechanisms underlying DVH is not fully understood.

Multiple factors were thought to contribute to respiratory impairments in asthmatic individuals, which complicated and hindered the investigation of the possible causes of DVH. For example, long-term hypoxemia-induced DVH has been observed in residents living at high altitude (34, 35) and in patients with congenital cyanotic heart disease (8). However, available data showed that some asthmatic patients with DVH exhibited hypoxemia (14, 15, 26, 31), whereas others did not (21, 32). Although DVH is generally observed in asthmatic individuals, the results concerning the ventilatory response to hypercapnia were controversial. A lack of significant change (10, 32, 38), decrease (14, 15, 27), or increase in the ventilatory response to hypercapnia (20, 28, 37) has been observed in asthmatic patients. This controversy coupled with other possible factors involved with DVH, such as the heredity (14, 19), severity of sickness (21), and the different number of previous respiratory failures (38), complicated the study of mechanisms underlying DVH.

Several animal models of asthma have been developed for studying the pathogenic mechanisms and therapeutic treatments of bronchial hyperreactivity, a basic characteristic of asthma. The most common antigen employed is ovalbumin (Ova) (12). Guinea pigs actively sensitized with Ova provide a well-established animal model because many of their pathophysiological features closely resemble those observed in asthmatic patients (2, 6, 17). Because using this model could greatly minimize and/or eliminate the differences of heredity and severity of sickness, the present study was carried out to determine whether Ova sensitization produced depressed ventilatory responses to stimulations of carotid chemoreceptors in guinea pigs. Furthermore, we investigated whether the ventilatory response to hypercapnia was also altered in the same animals.

Stimulation of bronchopulmonary unmyelinated (C) fibers (PCFs) substantially inhibits inspiration. Right atrial injection of capsaicin, a selective stimulant of PCFs, induced an apnea in different species (1, 42). Recent evidence showed that acute

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hypoxia profoundly increased capsaicin-induced inspiratory inhibition (42), implying an interaction of simultaneous activation of PCFs and carotid chemoreceptors in control of breathing. Interestingly, pulmonary inflammatory mediators, such as histamine, bradykinin, and prostaglandins, are known to exert potent stimulatory effects on PCFs (23), and pulmonary inflammation is known to exist in Ova-exposed guinea pigs (17, 41). Indeed, airway mucosal inflammation resulted in hypersensitivity of PCFs in rats (22). Ova exposure has been demonstrated to cause a depolarization of membrane potentials of nodose ganglion neurons in guinea pigs (39), implying an Ova-induced sensitization of PCFs. Therefore, in the present study, we also investigated whether Ova exposure altered PCF-mediated ventilatory inhibition.

## METHODS

The procedures described below were conducted according to the recommendations of the *Guide for the Care and Use of Laboratory Animals* [DHHS Publication No. (NIH) 85-23, revised 1985, Office of Science and Health Reports, Bethesda, MD 20892], and they were also approved by the University of Kentucky Institutional Animal Care and Use Committee.

**Ova exposure.** Twelve male, pathogen-free Hartley guinea pigs were evenly divided into two groups with matched litter and body weight (initial weight ~200 g). Each animal was placed in a whole body plethysmograph (model SN 117829, Buxco Electronic, Sharon, CT) for 1 h/day for 3 days. After the habituation, the animals were either exposed to aerosolized Ova daily for 5 min, 5 days/wk, for 2 wk (Ova treatment) or to normal saline aerosol (Sal treatment) in an identical manner to serve as the control. During the exposure, guinea pigs were placed in the whole body plethysmograph under a negative-pressure exhaust hood. The chamber was connected to an ultrasonic nebulizer (model 100, Devilbiss, Somerset, PA) by which Ova solution (1% wt/vol) was introduced at the same output rate and droplet size as described in a previous report (41). Diphenhydramine (8 mg) was injected intraperitoneally in both groups 1 h before each exposure in the second week to alleviate the bronchospasm caused by release of histamine during the Ova exposure. Before the first and the last aerosol exposure, the animal body weight was obtained and ventilation was measured by using the whole body plethysmograph.

**General animal procedure.** One day after the last exposure with Ova or Sal, the animal was anesthetized by chloralose (100 mg/kg ip) and urethane (500 mg/kg ip). Appropriate supplemental anesthesia, as needed, was administered intravenously to suppress corneal and withdrawal reflexes. The core temperature was monitored with a rectal probe and maintained at ~36.5°C by a heating pad and radiant heat. The trachea below the larynx was tracheotomized by blunt dissection and cannulated with a tracheal cannula connected to a pneumotachograph. The tracheal pressure (Ptr) was recorded via a pressure transducer that was connected to a side port of the tracheal cannula. The left femoral vein and artery were cannulated; the former was used for administration of anesthetics and the latter for monitoring arterial blood pressure (ABP) and heart rate (HR). A catheter (PE 50) was inserted from the right common carotid artery with the tip ~3 mm below the bifurcation for delivering the agents to stimulate carotid chemoreceptors. The right jugular vein was isolated, and a catheter (PE 50, ~10 cm long) was advanced close to the right atrium for injection of capsaicin. The inserted depth of the catheter was determined by measuring the distance from the heart (felt from the heartbeat) to the cannulating site. Respiratory flow was measured with the pneumotachograph and a differential pressure transducer. The pneumotachograph was made of stainless steel and had a linear flow-pressure relationship in the range of 0–20 ml/s and a flow resistance of 0.046 cmH<sub>2</sub>O·s·ml<sup>-1</sup>, with a dead space of ~0.2 ml. The flow signal was integrated by PowerLab/8SP (ADInstruments,

Castle Hill, Australia) to generate tidal volume (V<sub>T</sub>). A three-way switch was attached to the inspiratory inlet of the one-way breathing valve attached to the pneumotachograph and used to select the inhaled gas mixture, i.e., room air, hypoxia, or hypercapnia. End-tidal pressure of PO<sub>2</sub> and PCO<sub>2</sub> were monitored via an infrared O<sub>2</sub>-CO<sub>2</sub> analyzer (model 78356A, Hewlett-Packard, Louisville, KY). After stabilization of the baseline cardiorespiratory variables for at least 10 min, the animals received the following stimuli sequentially.

**Systemic hypoxia.** Both groups of animals were exposed to 10 consecutive breaths of 100% N<sub>2</sub> to test the cardiorespiratory responses to systemic hypoxia. The protocols for N<sub>2</sub> were repeated and arterial blood samples taken immediately before hypoxic inhalation and termination.

**Activation of peripheral and central chemoreceptors.** To test the effect of Ova exposure on the peripheral chemoreceptors-mediated ventilatory responses, both groups of animals were randomly exposed to intracarotid injection of sodium cyanide (20 µg) and doxapram (2 mg) 5 min after pure N<sub>2</sub> exposure. These stimuli have been demonstrated to augment ventilation predominately via activating peripheral chemoreceptors (25, 36, 43) without systemic hypoxia. Inhalation of hypercapnic gas mixtures (7% CO<sub>2</sub>-21% O<sub>2</sub>-balance N<sub>2</sub>) for 5 min was subsequently applied to stimulate central chemoreceptors. The protocols for CO<sub>2</sub> were repeated and arterial blood samples taken immediately before hypercapnic inhalation and termination. A 10-min interval was allowed for animals' recovery from each chemical challenge.

**Activation of PCFs.** A stock solution of capsaicin (400 µg/ml, Sigma, St. Louis, MO) was made in a vehicle of 10% Tween 80, 10% ethanol, and 80% isotonic saline. During each injection, solutions of the desired concentration were prepared with saline dilution, placed in the catheter (0.1 ml), and then flushed into the right atrium by bolus injection of saline (0.2 ml). The bolus injection duration was <1.5 s. Capsaicin (5 µg) was injected 10 min after the cardiorespiratory responses to hypercapnia returned to or were close to the control level.

**Data acquisition and analysis.** ABP, HR, Ptr, V<sub>T</sub>, respiratory frequency (f), expiratory duration (T<sub>E</sub>), and  $\dot{V}_E$  were monitored and recorded on an eight-channel chart recorder, and analyzed by an online computer. The baseline cardiorespiratory variables were expressed as absolute values, whereas their responses to chemical stimulations were presented as percent changes from control (without stimulation). The cardiorespiratory baseline values (arterial blood gases) and their responses to various chemical challenges were recorded and compared between Ova and Sal animals. Student's *t*-test and one-way analysis of variance with the Newman-Keuls post hoc test were utilized to determine the differences of baseline cardiorespiratory variables and the responses to chemical challenges obtained in the Ova and Sal guinea pigs, respectively. *P* < 0.05 was used to identify the significant difference.

## RESULTS

**Hypoxia-induced DVH in anesthetized Ova guinea pigs.** Ova exposure decreased the ventilatory responses to acute systemic hypoxia with little effect on cardiovascular responses. Figure 1A displays typical experimental recordings of cardiorespiratory responses to 10 breaths of pure N<sub>2</sub> in a Sal (*left*) and Ova guinea pig (*right*), respectively. Hypoxia apparently increased ventilation in the Sal guinea pig, but this response was strikingly attenuated in the Ova animal. Group data (Fig. 1B) showed that hypoxia significantly elevated  $\dot{V}_E$ , V<sub>T</sub>, and f in both Sal and Ova guinea pigs with an accompanied hypotension response. However, the responses of  $\dot{V}_E$  in Ova guinea pigs were significantly smaller than those observed in the Sal animals mainly because of a reduction of V<sub>T</sub> response (*left*). The hypotension in response to N<sub>2</sub> was not markedly affected by Ova (*right*).

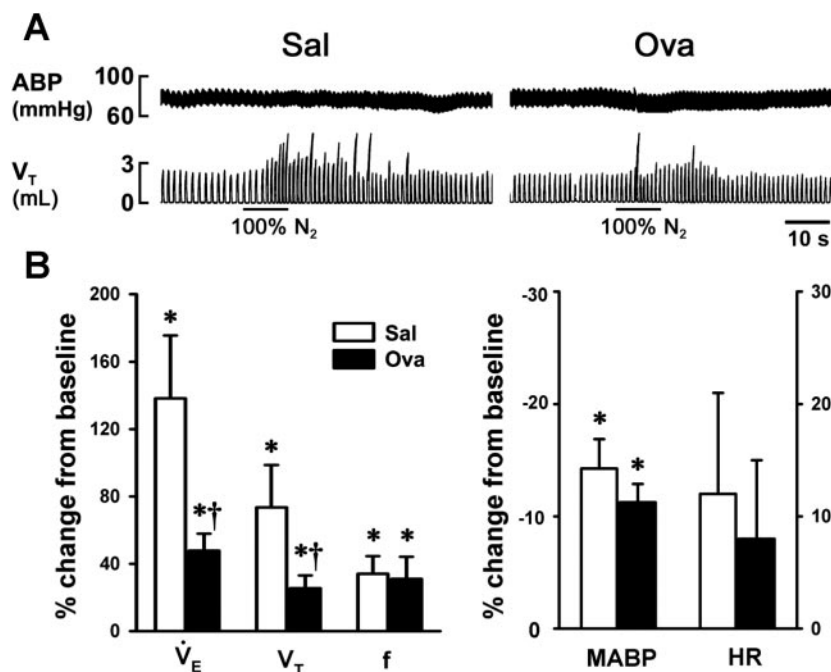


Fig. 1. Cardiorespiratory responses to systemic hypoxia. *A*: experimental records showing the cardiorespiratory responses to inhalation of 10 breaths of pure  $N_2$  in a saline control (Sal; left) and ovalbumin-sensitized (Ova; right) guinea pig. Traces from top to bottom are arterial blood pressure (ABP), and tidal volume ( $V_T$ ). *B*: group data of the ventilatory and cardiovascular responses. Values are means  $\pm$  SE.  $\dot{V}_E$ , minute ventilation;  $f$ , respiratory frequency; MABP, mean ABP; HR, heart rate. \*Significant differences between the data collected before and during hypoxic stimulation,  $P < 0.05$ . †Significant differences between Sal and Ova guinea pigs,  $P < 0.05$ .

**Effect of Ova-exposure on baseline cardiorespiratory variables.** No significant differences were found in  $\dot{V}_E$ ,  $V_T$ ,  $f$ ,  $P_{tr}$ , ABP, and HR between Sal and Ova guinea pigs during eupneic breathing (Table 1). Considering the increase in animal weight after 12 days of aerosol exposure, we compared changes in animals' weight and ventilation between the two groups. As exhibited in Fig. 2A, the animals' weight was significantly increased in both groups, but the changes were not significantly different between Ova and Sal guinea pigs. The ventilation (Fig. 2B) in Sal animals appeared slightly higher than that in Ova guinea pigs. However, the difference was not significant ( $P > 0.05$ ). As a result, the ratio of the elevation in  $\dot{V}_E$  and weight ( $\Delta\dot{V}_E/\Delta W$ , Fig. 2C) were not markedly changed by Ova exposure.

**Cardiorespiratory responses to stimulation of peripheral chemoreceptors.** Pure  $N_2$  inhalation used in our experiment led to systemic hypoxia, i.e., arterial  $O_2$  tension ( $P_{aO_2}$ ) decreased from 91 to 44 Torr (detailed in *Baseline arterial blood gases and their responses to hypoxia and hypercapnia*). To selectively stimulate peripheral chemoreceptors without systemic hypoxia, intracarotid injections of cyanide and doxapram were used. As illustrated in Fig. 3, cyanide injection augmented  $\dot{V}_E$  in Sal animals predominantly via enhancing  $V_T$ , and this response was significantly attenuated by Ova exposure (Fig. 3A). In comparison, mean ABP (MABP) and HR were not markedly affected by cyanide in either group of animals (Fig. 3B). Similar results were obtained during application of doxapram (Fig. 4). The respiratory response was significantly di-

minished in the Ova animals by reduction in both  $V_T$  and  $f$  (Fig. 4A) compared with the Sal group. In contrast, the increases in MABP and HR induced by doxapram were not significantly different between Ova and Sal groups (Fig. 4B).

**Cardiorespiratory responses to activation of central chemoreceptors.** To determine the effects of Ova exposure on the cardiorespiratory response to stimulation of central chemoreceptors, we compared the responses to steady-state hypercapnia (7%  $CO_2$ -21%  $O_2$ -balance  $N_2$ ) for  $\sim 5$  min in both groups. Surprisingly, different from the ventilatory response to hypoxia, the respiratory response to hypercapnia was not significantly different between these two groups. As shown in Fig. 5A, hypercapnia significantly increased the ventilatory response by elevating  $V_T$  and  $f$  in both Sal and Ova guinea pigs with no significant difference between the two groups. Furthermore, hypercapnia did not significantly alter MABP and HR in either group (Fig. 5B).

**Baseline arterial blood gases and their responses to hypoxia and hypercapnia.** We compared  $P_{aO_2}$  and arterial  $CO_2$  tension ( $P_{aCO_2}$ ) during eupneic breathing, hypoxia, and hypercapnia in both groups to verify whether Ova exposure alters  $P_{aO_2}$  and  $P_{aCO_2}$ . We found that the levels of  $P_{aO_2}$  were not significantly different between these two groups during either eupneic breathing (room air) or hypoxia (Fig. 6A). Similarly, the levels of  $P_{aCO_2}$  were not significantly different between these two groups during eupneic or hypercapnic breathing (Fig. 6B).

**Right atrial injection of capsaicin-induced apnea.** To clarify the effect of Ova exposure on PCF-mediated cardiorespiratory

Table 1. Comparisons of baseline cardiorespiratory variables in anesthetized Sal and Ova guinea pigs

| <i>n</i> | $\dot{V}_E$ , ml/min | $V_T$ , ml    | $f$ , breaths/min | $P_{tr}$ , cmH <sub>2</sub> O | MABP, mmHg     | HR, beats/min    |
|----------|----------------------|---------------|-------------------|-------------------------------|----------------|------------------|
| Sal, 6   | 170.6 $\pm$ 26.6     | 2.5 $\pm$ 0.3 | 67.8 $\pm$ 5.7    | 6.9 $\pm$ 0.2                 | 73.2 $\pm$ 2.9 | 323.0 $\pm$ 43.1 |
| Ova, 6   | 153.6 $\pm$ 10.1     | 2.7 $\pm$ 0.2 | 64.8 $\pm$ 3.1    | 7.1 $\pm$ 0.5                 | 72.3 $\pm$ 2.2 | 342.0 $\pm$ 21.3 |

Values are means  $\pm$  SE; *n*, no. of animals. Values of minute ventilation ( $\dot{V}_E$ ), tidal volume ( $V_T$ ), respiratory frequency ( $f$ ), tracheal pressure ( $P_{tr}$ ), mean arterial blood pressure (MABP), and heart rate (HR) observed in saline control (Sal)- and ovalbumin-sensitized (Ova) guinea pigs were not significantly different.

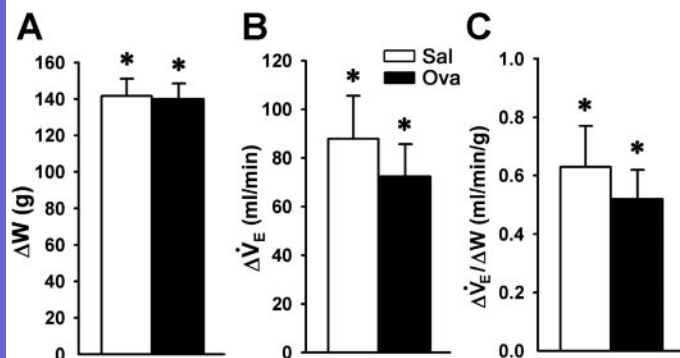


Fig. 2. Effect of Ova exposure on guinea pigs' weight and eupneic ventilation. Changes in animals' weight ( $\Delta W$ ; A),  $\dot{V}_E$  ( $\Delta \dot{V}_E$ ; B), and ratio of  $\Delta \dot{V}_E$  to  $\Delta W$  ( $\dot{V}_E / \Delta W$ ; C) after 12-day aerosol inhalation are compared between the Sal and Ova guinea pigs. Values are means  $\pm$  SE. \* $P < 0.05$  for day 1 vs. day 13.

responses, we compared the responses to capsaicin in Sal and Ova guinea pigs. An example of experimental recordings is depicted in Fig. 7A. Apnea occurred when capsaicin (5  $\mu$ g) was administered into the right atrium in a Sal guinea pig (left). In sharp contrast, the same dose of capsaicin caused a much longer apnea in an Ova animal (right). Group data (Fig. 7B) indicated that there was no significant difference of baseline  $T_E$  between these two groups, but the capsaicin-induced apnea was augmented by Ova exposure (left). Additionally, capsaicin injection led to hypotension that was not different between the Sal and Ova animals (right).

## DISCUSSION

Presence of DVH in Ova guinea pigs provides a useful animal model for further investigating the relevant mechanisms. Our major finding in this study is that DVH occurs in Ova-sensitized guinea pigs, which is a well-established animal model with many of their pathological features closely resembling those observed in asthmatic patients (2, 6, 17). This DVH is not associated with changes in cardiovascular activity during hypoxia, which is in agreement with the clinical assessment that the ventilatory rather than cardiac dysfunction is the major cause of the death in the asthmatic individuals (26). Patients

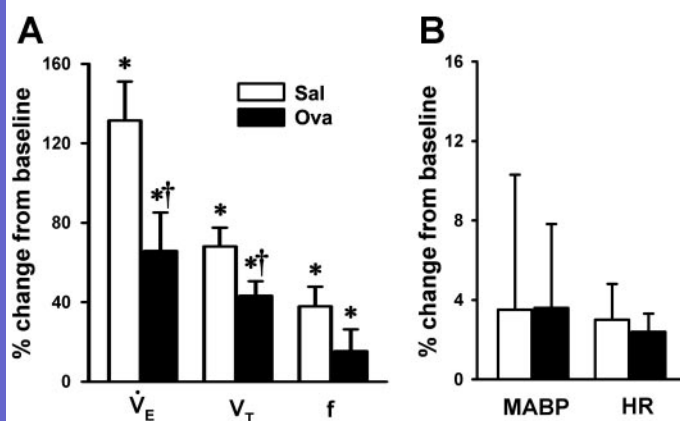


Fig. 3. Group data of the respiratory (A) and cardiovascular responses (B) to intracarotid injection of sodium cyanide in Sal and Ova guinea pigs. Values are means  $\pm$  SE. \*Significant differences of the data collected before and during stimulation,  $P < 0.05$ . †Significant differences between Sal and Ova guinea pigs,  $P < 0.05$ .

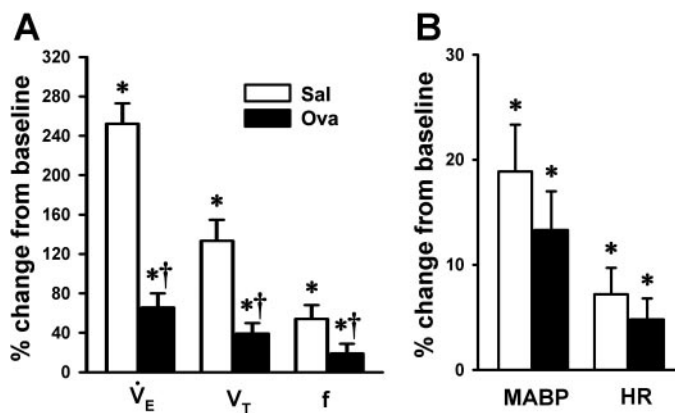


Fig. 4. Group data for the respiratory (A) and cardiovascular responses (B) to intracarotid injection of doxapram in Sal and Ova guinea pigs. Values are means  $\pm$  SE. \*Significant differences of the data collected before and during stimulation,  $P < 0.05$ . †Significant differences between Sal and Ova guinea pigs,  $P < 0.05$ .

with chronic bronchial asthma are known to have DVH (4, 11, 14–16, 21, 33, 38) that worsens the ventilatory compensation during hypoxia, and these patients are apt to lapse into a critical condition. However, the pathophysiology involved in the DVH is not fully understood because of the limitations on experimental study in humans. For example, the multiple factors, such as differences in heredity (14, 19), severity of sickness (21), and arterial blood gases (10, 32, 38), have complicated and hindered the study of the pathogenic mechanisms of DVH. Long-term hypoxia-induced DVH has been observed in high-altitude residents (34, 35) and in patients with congenital cyanotic heart disease (8). Therefore, it has been hypothesized that severe hypoxemia, which might occur during an asthma attack, can decrease ventilation (13). However, our data do not support this assumption. We found that Ova-exposure caused DVH in guinea pigs without significant changes in  $P_{aO_2}$  and  $P_{aCO_2}$  during eupneic breathing, demonstrating that DVH is not the result of preexisting hypoxemia. In fact, hypoxemia is not uniformly observed in asthmatic individuals (3, 14, 15, 21, 26, 31, 32) and not necessarily related to the degree of asthmatic severity (21). In addition, Ova treatment in guinea pigs failed to change body weight, baseline cardiorespiratory activities, baseline total pulmonary resistance, and dynamic lung compli-

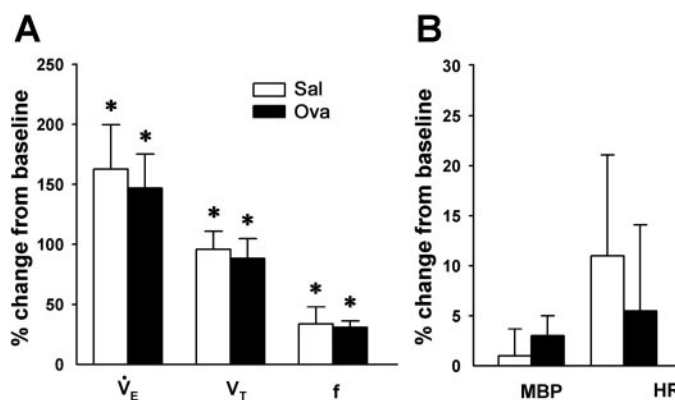


Fig. 5. Ventilatory (A) and cardiovascular responses (B) to hypercapnia obtained from the Sal and Ova guinea pigs. Values are means  $\pm$  SE. \* $P < 0.05$  between the variables derived from Sal and Ova animals.

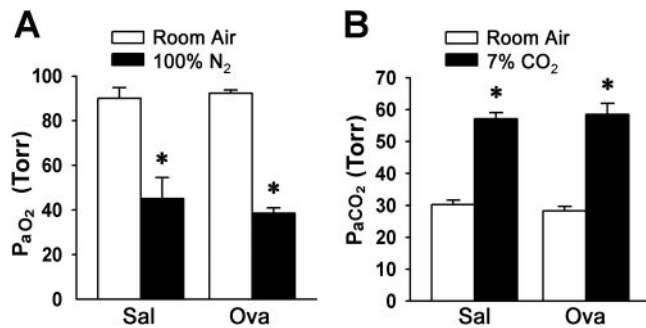


Fig. 6. Effects of Ova exposure on arterial blood gases during hypoxia and steady-state hypercapnia. Changes of arterial  $O_2$  tension ( $P_{aO_2}$ ; A) and arterial  $CO_2$  tension ( $P_{aCO_2}$ ; B) from breathing room air, hypoxia, and hypercapnia were compared between the Sal and Ova guinea pigs. Values are means  $\pm$  SE. \* $P < 0.05$  between the variables derived before and during stimulation.

ance (41). These results, together with the fact that our study was carried out on the guinea pigs with matched litter and similar severity of sickness, strongly demonstrate that Ova treatment-induced DVH does not result from those multiple factors presumably existing in asthmatic individuals. Therefore, the Ova guinea pig is a very unique animal model for elucidating the mechanisms underlying DVH.

*DVH in Ova guinea pigs involves, at least partially, the suppression of the carotid chemoreceptor-mediated respiratory responses.* It is well known that systemic hypoxia-induced ventilatory responses contain two components in both anesthetized and conscious species, including rats, i.e., peripheral chemoreceptor-mediated excitation and central hypoxic depression (29). Therefore, DVH could be the results of diminishing the carotid chemoreceptor-mediated excitatory response and/or strengthening hypoxic central depression. Intracarotid injection of cyanide or doxapram has been demonstrated to elevate ventilation mainly via activating peripheral chemoreceptors (25, 36, 43). Our data that the ventilatory responses to these selective stimuli were inhibited by Ova exposure convincingly demonstrated that DVH was generated, at least partially, by attenuating the carotid chemoreceptor-mediated respiratory response.

*Ova treatment uniquely causes DVH without effect on the cardiorespiratory responses to hypercapnia.* An interesting finding in our study is that, different from the effect on hypoxic ventilatory responses, Ova exposure failed to affect the ventilatory response to steady-state hypercapnia. In clinical studies, the data of the ventilatory responses to hypercapnia in asthmatic individuals are controversial. Some results from the asthmatic individuals showed a lack of significant change in the ventilatory response to hypercapnia (10, 32, 38), whereas a decreased (14, 15, 27) or increased hypercapnic ventilation (20, 28, 37) was also documented. These clinically observed discrepancies in the hypercapnic ventilation may be the results of varied baseline blood gases in these patients. Either a presence or absence of hypercapnia and acidosis has been reported in asthmatic individuals (3, 14, 15, 21, 26, 31, 32). The major difference of reflex arc involved in hypoxia- and hypercapnia-induced ventilatory responses is suggested to be the chemoreceptor location; the former is dominantly localized at carotid body and the latter at the central nervous system (9). The observation that DVH occurred in the Ova guinea pigs without changes in the ventilatory responses to hypercapnia

may be due to dysfunction of carotid chemoreceptor chemoreception. It is known that carotid chemoreceptors are also sensitive to  $CO_2$ , but their contribution to the ventilatory responses to hypercapnia, compared with hypoxia, is much smaller. This may have contributed, at least partially, to the lack of effect on Ova on the ventilatory response to hypercapnia.

*Ova treatment augments PCF response to capsaicin, which may contribute to depression of the carotid chemoreceptor-mediated respiratory responses.* We found that Ova exposure significantly increased the apneic response to stimulation of PCFs, clearly demonstrating an augmented PCF-mediated inspiratory inhibition. This augmentation may be because of a sensitization/stimulation of PCFs induced by Ova exposure because stimulation of PCFs could bring about airway hyper-reactivity and inflammatory aggravation, and mucus hypersecretion that are the major characteristics of Ova guinea pigs (2, 6, 12, 17). In agreement, Ova exposure led to a depolarization of membrane potentials of nodose ganglion neurons in guinea pigs (39). Repeated exposure of house dust mite allergen aerosols in adult monkeys significantly enhanced the resting membrane potential and the number of action potential of neurons within the nucleus tractus solitarius (5). Because both nodose ganglion and the nucleus tractus solitarius are known to contain the pulmonary sensory neurons and their second-order neurons, respectively, these data provide indirect experimental evidence to support the assumption that chronic allergen exposure induces peripheral and central neuroplasticity, and facilitates PCF afferent inputs. In the present study, we did not attempt to address the issue how Ova exposure sensitized PCFs. However, it is well established that Ova exposure in guinea pigs produces substantial pulmonary inflammation (17, 41) and increased the release of inflammatory mediators, such as histamine (40), bradykinin (24), and prostaglandins (18, 40). Because all of these mediators are known to sensitize/stimulate

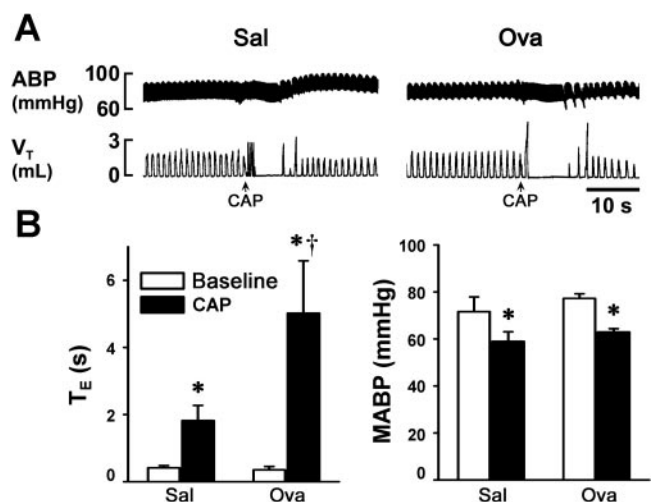


Fig. 7. Cardiorespiratory responses to stimulation of bronchopulmonary C fibers. A: experimental records of the cardiorespiratory responses to right atrial injection of capsaicin (CAP, 5.0  $\mu$ g) in a Sal (right) and Ova guinea pig (left). Traces from top to bottom are ABP and  $V_T$ . Averaged expiratory duration ( $T_E$ ) and MABP are shown on the left and right in B, respectively. Open bars, control variables; solid bars, apneic responses to capsaicin injection. Values are means  $\pm$  SE. \*Significant differences between the data collected before and during stimulation,  $P < 0.05$ . †Significant differences between Sal and Ova guinea pigs,  $P < 0.05$ .

PCFs (23), they are probably involved in Ova sensitization of PCFs. The mechanisms by which Ova exposure induces DVH remain unknown. This depression could be due to diminution of the carotid chemosensitivity and/or central neural excitability responsible for the carotid chemoreceptor-mediated respiratory responses. Recently, our laboratory demonstrated that acute hypoxia profoundly increased PCF-mediated inspiratory inhibition (42), showing a functional interaction of simultaneous activation of PCFs and carotid chemoreceptors in control of breathing. The commissural nucleus of the nucleus tractus solitarius is the first site in the central circuitry where afferent signals from PCFs and carotid chemoreceptors are transmitted and susceptible to modulation (1, 7). Therefore, it is possible that Ova exposure increases PCFs' afferent inputs, resulting from release of the inflammatory mediators, to the nucleus tractus solitarius and thereby inhibits the excitability of local neurons responsible for carotid chemoreceptor-mediated respiratory responses and consequently cause DVH. The information whether Ova-induced inflammatory mediators have directly regulatory effects on carotid chemoreception remains to be established. Undoubtedly, further investigations are required to determine whether the Ova exposure enhances PCF inputs and, if so, whether these changes are produced by Ova-induced pulmonary inflammation. Moreover, it should be interesting to know whether the enhanced PCF inputs can depress the carotid chemoreceptor-mediated respiratory responses, either peripherally and/or centrally.

**Criticism of the present study.** Animals with airway hyperresponsiveness, such as Ova-treated guinea pigs, have been shown to generate greater bronchomotor response to the afferent stimulations (22, 23, 41). Therefore, we cannot completely rule out the possibility that an exaggerated bronchoconstriction may have reduced the  $V_T$  and contributed to the DVH in Ova-treated guinea pigs. We have found that the ventilatory inhibition did not occur during eupneic breathing and in response to hypercapnia in the same animals, indicating that bronchoconstriction alone cannot totally, if at all, account for the depressed ventilation. In support of our findings, previous studies have demonstrated that baseline total pulmonary resistance and dynamic lung compliance are not affected by Ova treatment in guinea pigs (41). Nevertheless, whether hypoxia exaggerates Ova treatment-induced bronchoconstriction remains to be determined. Because anesthesia changes the respiratory responses to a variety of stimuli, further experiments are needed to confirm Ova exposure-induced DVH and exaggeration of PCF-mediated apnea in awake animals.

In summary, we found that DVH existed in anesthetized Ova-guinea pigs, in which the carotid chemoreceptor-mediated ventilatory responses were attenuated but the PCF-mediated apneic response was augmented. These findings suggest that DVH, similar to that observed in asthmatic individuals, exists in the experimental model of asthma and likely results from two primary factors: a blunted carotid chemoreceptor-mediated respiratory excitation and an enhanced PCF-mediated ventilatory inhibition. Further studies are required to define how these two modulations interact to generate DVH. In the present study, the heredity, the baseline cardiorespiratory variables, the ventilatory response to hypercapnia, arterial blood gases, and body weight in the Ova guinea pig were not different from those in the Sal guinea pig. These data demonstrate that the

Ova guinea pig is a unique experimental asthmatic model for further investigating the pathogenesis of DVH.

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