

## High-frequency electrical stimulation of cervical vagi reduces airway response to methacholine

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### Abstract

**AIM:** To test whether high-frequency electrical stimulation (HES) of the bilateral cervical vagus nerves reduces the airway responses to methacholine (MCh).

**METHODS:** Guinea pigs were pretreated with saline (Sal,  $n = 9$ ) or ovalbumin (Ova,  $n = 10$ ) aerosol for two weeks (5 min/d, 5 d/wk) and subsequently anesthetized, paralyzed, tracheotomized and artificially ventilated. Both total lung resistance ( $R_L$ ) and dynamic pulmonary compliance ( $C_{dyn}$ ) were recorded. In addition, the effects of vagal low-frequency electrical stimulation (LES, monophasic, 50 Hz) and HES (monophasic and biphasic, 1 and 2.5 kHz) for about 10 s or 2 min on the responses of  $R_L$  and  $C_{dyn}$  to MCh aerosol-induced bronchoconstriction were compared in both groups of guinea pigs. In a few guinea pigs, the impact of biva-gotomy on the  $R_L$  responses to MCh was assessed.

**RESULTS:** Before MCh challenge, LES, but not HES, significantly increased  $R_L$  by about 30% ( $P < 0.01$ ) and decreased  $C_{dyn}$  by about 20% ( $P < 0.01$ ) similarly in both groups. MCh aerosol for 2 min elevated  $R_L$  and diminished  $C_{dyn}$  more in Ova- than Sal-treated animals ( $R_L$ :  $313\% \pm 52\%$  vs  $113\% \pm 17\%$ ,  $P < 0.01$ ;  $C_{dyn}$ :  $-56\% \pm 7\%$  vs  $-21\% \pm 3\%$ ,  $P < 0.01$ ). During MCh-induced airway constriction, LES further enhanced, but HES decreased  $R_L$  and this decrease was greater in Ova- (about 45%) than Sal-treated animals (about 34%,  $P < 0.01$ ) with little change in cardiovascular activity. On the other hand, LES further reduced whereas HES increased  $C_{dyn}$  more in Ova- (about 20%) than Sal-treated animals (about 13%,  $P < 0.01$ ). In addition, biva-gotomy almost eliminated the  $R_L$  and  $C_{dyn}$  responses to MCh.

**CONCLUSION:** We conclude that vagal HES is able to alleviate the bronchoconstriction induced by MCh in anesthetized guinea pigs, likely *via* reversible inhibition/blockade of vagal conduction.

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**Key words:** Bronchodilation; Acetylcholine; Airway hyperreactivity; Ovalbumin; Asthma

**Core tip:** In summary, our study revealed that vagal high-frequency electrical stimulation (HES) significantly suppressed the airway response to methacholine (MCh) more greatly in the ovalbumin- than saline-treated animals, while vagal low-frequency electrical stimulation always increased airway resistance. Importantly, the HES-evoked bronchodilation during MCh challenge is concomitant with the on-and-off electrical stimulation and with no effect on cardiovascular activity. These, along with the greatly blunted airway resistance response to MCh after biva-gotomy, suggest that vagal HES may be a potentially useful approach in alleviating asthmatic bronchoconstriction (likely *via* reversible inhibition or blockade of the vagal nerve conduction).

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## INTRODUCTION

Airway hyperreactivity results predominantly from an increased acetylcholine release from parasympathetic nerves innervating the airways, leading to local smooth muscle contraction in asthmatic patients<sup>[1]</sup> and in animal models of asthma<sup>[2,3]</sup>. It was assumed that cholinergic agonists, such as methacholine (MCh), produced bronchoconstriction only *via* their direct effect on airway smooth muscle. However, this opinion has been challenged by several recent studies. Vagal activation by conventional electrical stimulation at low frequency (< 20 Hz) augments the airway responsiveness to MCh in rats<sup>[4]</sup>. Conversely, bivotomy or vagal blockade by cooling profoundly decreases and even eliminates bronchoconstriction induced by application of cholinergic agonists into the respiratory tract in mice, rats, sheep, and dogs<sup>[5-8]</sup>. These results strongly demonstrate the critical role basic vagal tone *per se* plays in generating the airway smooth muscle responses to acetylcholine.

Accumulating evidence has shown a blocking effect of electrical stimulation at high frequencies [high-frequency electrical stimulation (HES)  $\geq 1$  kHz] on mammal peripheral nerve fibers' conduction, thereby diminishing or abolishing the nerve's functions<sup>[9-11]</sup>. For example, HES applied to the sciatic nerve of anesthetized cats substantially decreases the alpha motor neural discharges as shown by recording of the antidromic potentials of single fibers of L7 ventral root<sup>[11]</sup>. Therefore, we hypothesized that vagal HES (similar to bivotomy) would reduce the airway responses to MCh challenge in guinea pigs, especially those with ovalbumin (Ova)-sensitization as an established animal model of asthma<sup>[12,13]</sup>.

## MATERIALS AND METHODS

### Animals

Pathogen-free Hartley Duncan guinea pigs were purchased from Charles River Laboratories, Inc. (Wilmington, MA) and housed in the animal facility in filter top cages with *ad libitum* water and food access. The room was constantly ventilated and the temperature was kept at 23 °C. The animals were quarantined for 2 wk before the experiments. The experimental protocols were approved by the Institutional Animal Care and Use Committee at Lovelace Respiratory Research Institute, Albuquerque, NM, which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International, United States.

### Ova-exposure

Nineteen male guinea pigs were used and divided into

two groups with matched-litter and body weight (initial weight about 250 g). Each animal was placed in a whole-body plethysmograph (SN 117829; Buxco Electronic Inc., Sharon, CT) for 1 h per day for 3 d. Following the habituation, the animals as previously reported<sup>[14,15]</sup> were either exposed to aerosolized Ova ( $n = 10$ ) daily for 5 min, 5 d per week, for 2 wk (Ova-treatment) or to saline (Sal) aerosol ( $n = 9$ ) in an identical manner to serve as a control. During the exposure, the individual guinea pig was placed in the plethysmograph under a negative-pressure exhaust hood. The plethysmograph was connected to an Aeroneb Pro nebulizer (Mountain View, CA) by which Ova (1% wt/vol) or its vehicle solution was introduced at 0.7 mL/min with a mass median aerodynamic diameter of 3.7  $\mu\text{m}$  similar to those reported previously<sup>[14,15]</sup>. Diphenhydramine (8 mg) was injected intraperitoneally (*ip*) in both groups 1 h before each exposure in the second week to alleviate the bronchospasm caused by release of histamine during Ova-exposure<sup>[15]</sup>. Before the first and the last aerosol exposure, the animal's body weight was measured.

### General animal preparation

One day after the last exposure with Ova or Sal, anesthesia was induced using a mixture of ketamine + xylazine (40 + 5 mg/kg; *im*). Supplementary anesthetics (4.0 + 0.5 mg/kg; *im*) were provided as needed to suppress corneal and withdrawal reflexes. The trachea was cannulated below the larynx and connected to a pneumotachograph (Frank's Mfg. Co., Albuquerque, NM) to measure the respiratory flow via a differential pressure transducer (ML141, AD Instruments, Castle Hill, Australia). The pneumotachograph had a linear flow-pressure relationship in the range of 0-20 mL/s with a flow resistance of 0.046 cmH<sub>2</sub>O s/mL and a dead space of about 0.2 mL. The animals were exposed to a gas mixture of 30% oxygen in nitrogen throughout the experiment. The right femoral artery was cannulated for monitoring and recording arterial blood pressure (ABP) and heart rate (HR) through a blood pressure transducer (MLT0380, AD Instruments). The right femoral vein was cannulated for administration of agents. The cervical vagi were carefully isolated and placed on bipolar stimulating electrodes respectively. The guinea pigs were then paralyzed with pancuronium (0.1-0.3 mg/kg for induction and 0.1 mg/kg per hour for maintenance, *iv*) and ventilated at a constant frequency ( $f_R$ ) of 70 breaths/min with tidal volume ( $V_T$ ) adjusted to keep end tidal pressure of CO<sub>2</sub> ( $P_{ETCO_2}$ ) at about 40 torr<sup>[4,16]</sup>. Sudden spontaneous increases in arterial blood pressure (> 10 mmHg) and/or any irregular rhythm or increase in heart rate (> 15%) were indicators for supplementary anesthesia after paralysis.

### Measurement of airway resistance

As we reported previously<sup>[17]</sup>, total lung resistance ( $R_L$ ) and pulmonary compliance ( $C_{dyn}$ ) were measured and recorded on a breath-by-breath basis. Briefly, a catheter was inserted into the intrapleural cavity between the 5<sup>th</sup> and 6<sup>th</sup> ribs and the incision subsequently sutured and sealed

**Table 1** Baseline parameters in the Sal- and Ova-treated guinea pigs

Animals	R <sub>L</sub> (cmH <sub>2</sub> O s/mL)	C <sub>dyn</sub> (mL/cmH <sub>2</sub> O)	MABP (mmHg)	HR (bpm)
Sal (n = 9)	0.10 ± 0.02	0.35 ± 0.02	51.6 ± 1.6	327 ± 13
Ova (n = 8)	0.12 ± 0.02	0.34 ± 0.03	55.2 ± 3.7	311 ± 9

No significant differences of total lung resistance (R<sub>L</sub>), dynamic pulmonary compliance (C<sub>dyn</sub>), mean arterial blood pressure (MABP), or heart rate (HR) were found between the two groups.

airtight with silicone jelly in order to measure intrapleural pressure. The pneumothorax was corrected by briefly opening the intrapleural catheter to ambient air while holding hyperinflation ( $3 \times V_T$ ). Transpulmonary pressure was measured as the difference between the tracheal and intrapleural pressure.

### Experimental procedures

In our pilot studies, the conventional stimulations (5, 10, 25, 50 and 100 Hz; 0.5 V; 0.2 ms; monophasic and biphasic square-waveform) were delivered from a biphasic stimulus isolator (BSI-950, Dagan Corporation Minneapolis, MN) and applied to stimulate the vagi. We found that among these stimulations, a stimulation at 50 Hz with monophasic square-waveform resulted in the reproducible and greatest R<sub>L</sub> response, which was applied as the low-frequency stimulation (LES). In addition, HES at 1 or 2.5 kHz with monophasic waveform (HESm1 and HESm2) or with biphasic waveform (HESb1 and HESb2) were also applied. Both types of HES are reportedly able to block nerve conduction in previous studies<sup>[11,18-21]</sup>. These stimulations were randomly applied with the ratio of LES *vs* HES equal to 1:3 in each MCh exposure, and each stimulus lasted for a 10-s with an approximately 30 s interval or occasionally for 2-min period (only HES). Thirty minutes after completion of these electrical stimulations, the animals were subsequently exposed to MCh aerosol (100 µg/mL per minute for 2 min<sup>[4]</sup>) delivered by the nebulizer into the intake port of the ventilator by using an airflow 20 mL/s with a nebulizer output of 0.7 mL/min. The same patterns of electrical stimulations applied before MCh exposure were repeated with the first electrical stimulation applied at about 30 s of the plateau R<sub>L</sub> response to MCh. After recovery from the MCh exposure, the animal received the same MCh exposure and electrical stimulations again once or twice to ensure the reproducibility of the R<sub>L</sub> responses. The above procedures were performed in 9 Sal- and 8 Ova-treated animals. In the remaining two other Ova-treated guinea pigs, the R<sub>L</sub> response to the same MCh challenge was measured before and after bivatotomy to determine whether bivatotomy would cause a similar inhibition of the R<sub>L</sub> response to MCh.

### Statistical analysis

Raw data of the airflow, tracheal and intrapleural pressures, P<sub>ET</sub>CO<sub>2</sub>, arterial blood pressure, and rectal tem-

perature were digitized, monitored, and recorded by using a PowerLab/8sp (model ML 785; ADInstruments Inc., Colorado Springs, CO) connected to a computer employing the PowerLab Chart 5 software. Derived parameters, including the R<sub>L</sub>, C<sub>dyn</sub>, V<sub>E</sub>, V<sub>T</sub>, f<sub>R</sub>, MABP, and HR, were calculated online using the functions of the software. After stabilization of these variables, the R<sub>L</sub> baseline was determined by averaging R<sub>L</sub> values for 30 s immediately before vagal electrical stimulation or MCh exposure. The electrical stimulation-evoked R<sub>L</sub> responses before and after MCh aerosol were expressed as delta percent ( $\Delta\%$ ) change from the baseline R<sub>L</sub> values. The R<sub>L</sub> responses to the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> MCh exposure were compared to determine the reproducibility. In our study, multiple trials of a given electrical stimulation in the individual animal were averaged to calculate our group data unless mentioned otherwise. All data were presented as means  $\pm$  SE. Student *t*-test was applied to detect the significant differences in baseline values between the two groups (Ova- and Sal-treated). Two-way ANOVA was used to differentiate all responses evoked by the stimulations (chemical or electrical) between the two groups or among the R<sub>L</sub> responses to different trials of MCh exposure. When an overall test was found to be significant, Fisher's post-hoc tests were followed for specific comparisons between individual groups. Differences were considered significant at a *P* value < 0.05.

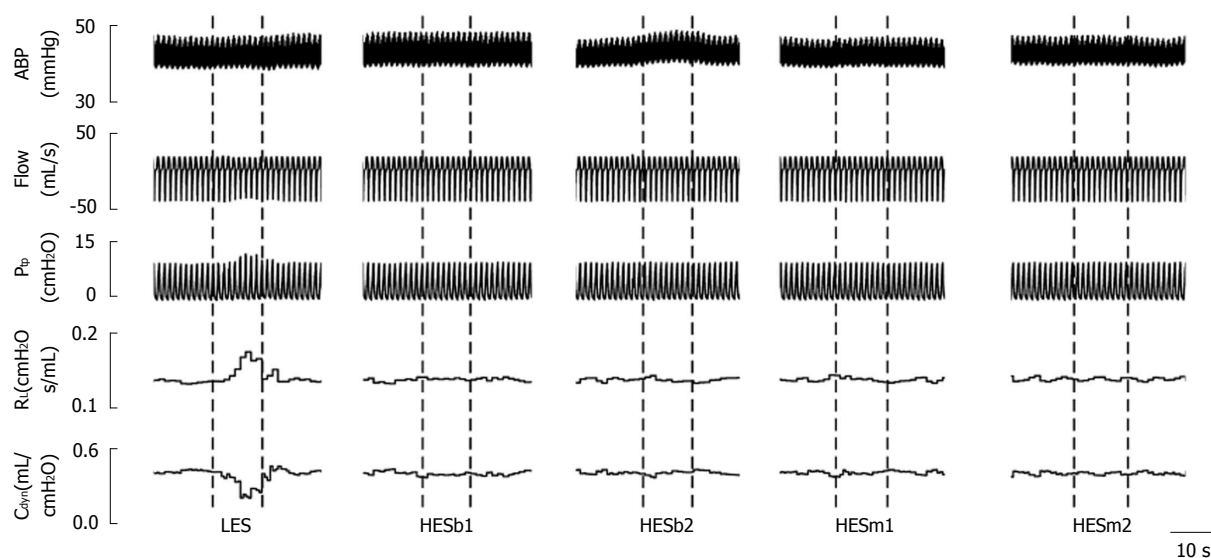
## RESULTS

### Airway responses to vagal electrical stimulations before MCh challenge

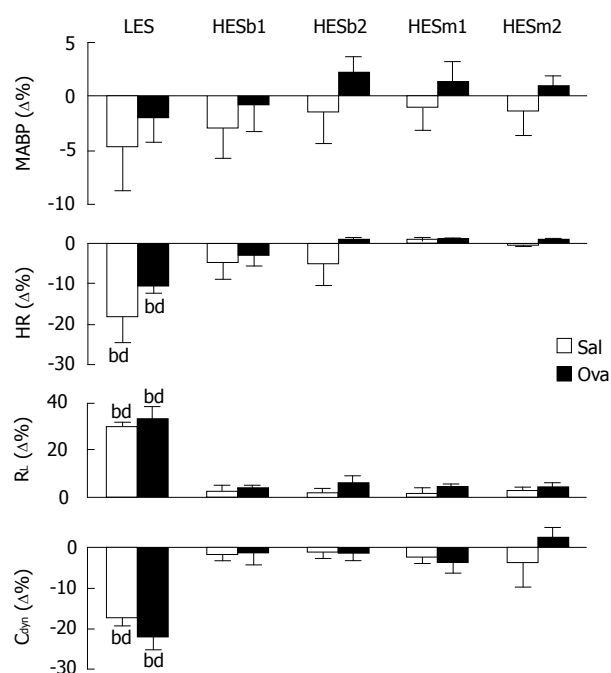
After completion of the Sal- or Ova-pretreatment, animal body weights were not significantly different between the two groups ( $358 \pm 12$  g *vs*  $363 \pm 15$  g). Following anesthesia and paralysis, animals' baseline airway and cardiovascular activities and their responses to the electrical stimulations were compared. As listed in Table 1, all of these baseline values were not significantly different between the two groups, which are consistent with the previously reported data from guinea pigs<sup>[5,14]</sup>. LES immediately increased the R<sub>L</sub> by about 30% and decreased the C<sub>dyn</sub> by approximately 18%; this is similarly associated with bradycardia in the Sal- and Ova-treated guinea pigs. The evoked responses disappeared rapidly after withdrawing the electrical stimulation. In sharp contrast, all HESs (HESm1, HESm2, HESb1, and HESb2) failed to significantly alter R<sub>L</sub>, C<sub>dyn</sub>, MABP, and HR. The typical experimental recordings and the corresponding group data are illustrated in Figures 1 and 2, respectively.

### Airway responses to aerosol MCh

In both groups of guinea pigs, exposure to MCh aerosol for 2 min evoked significant bronchoconstrictive response as evidenced by an increase in R<sub>L</sub> and decrease in C<sub>dyn</sub> (Figure 3). The R<sub>L</sub> response reached a plateau about 2 min after applying MCh, and the plateau lasted for  $2.8 \pm 0.2$  min followed by a relatively rapid recov-



**Figure 1** Typical total lung resistance and dynamic pulmonary compliance responses to electrical stimulations in an Ova-sensitized guinea pig. The traces from the top to bottom are arterial blood pressure (ABP), airflow (Flow), Trans-pulmonary pressure ( $P_{tp}$ ), total lung resistance ( $R_L$ ) and dynamic pulmonary compliance ( $C_{dyn}$ ). The dashed lines in each column reflect the on- and off-set of a given electrical stimulation. Vagal electrical stimulation included low-frequency electrical stimulation (LES, monophasic stimulation at 50 Hz); HESb1 and HESb2 (biphasic waveform at 1 and 2.5 kHz); and HESm1 and HESm2 (monophasic waveform at 1 kHz and 2.5 kHz), which are the same for the following figures.



**Figure 2** Group data showing the responses to vagal electrical stimulations. MABP: Mean arterial blood pressure; HR: Heart rate;  $R_L$ : Total lung resistance;  $C_{dyn}$ : Dynamic pulmonary compliance.  $n = 9$  and  $8$  in the Sal- and Ova-pretreated guinea pigs; Mean  $\pm$  SE. <sup>b</sup> $P < 0.01$ , vs the data immediately before electrical stimulation presented as "0"; <sup>d</sup> $P < 0.01$ , low-frequency electrical stimulation (LES) vs high-frequency electrical stimulations (HESs).

ery to their pre-exposure baselines. The amplitudes and durations of evoked  $R_L$  responses to the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> MCh exposure in Sal-treated and Ova-treated animals are listed in Table 2. The lack of significant difference among the  $R_L$  amplitude and duration in response to the three MCh exposures strongly indicates that these evoked

**Table 2** The reproducibility of total lung resistance responses to methacholine exposure controls

Group	Amplitude (%)			Duration (min)		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Sal	127 $\pm$ 25	136 $\pm$ 28	128 $\pm$ 30	7.9 $\pm$ 0.4	8.0 $\pm$ 0.5	7.5 $\pm$ 0.5
Ova	355 $\pm$ 58	370 $\pm$ 68	351 $\pm$ 63	8.2 $\pm$ 0.5	8.4 $\pm$ 0.5	7.7 $\pm$ 0.6

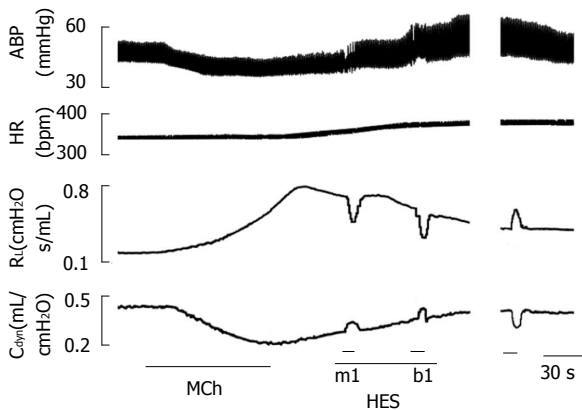
$n = 9$  for Sal- and Ova-treated guinea pigs for trial 1 and 2, and  $n = 5$  for trial 3 for both groups; mean  $\pm$  SE; no significant difference was found among the  $R_L$  amplitude and duration in response to the three methacholine exposures in both groups of animals. Sal: Saline; Ova: Ovalbumin.

responses are reproducible. The MCh exposure caused a remarkable hypotension (phase I) and a hypertension subsequently (phase II) that gradually returned to its pre-exposure baseline level. In parallel to phase II, there was a tachycardia evoked by MCh aerosol without HR change during phase I. The group data (Figure 4) showed that the MCh-induced  $R_L$  increase and  $C_{dyn}$  decrease were significantly greater in the Ova- than Sal-treated animals (313% vs 113% and -57% vs -20% respectively;  $P < 0.01$ ). The changes of MABP and HR, however, were not significantly different between the two animal groups. These data are consistent with previously reported airway and cardiovascular responses to MCh<sup>[22-25]</sup>.

#### Airway responses to vagal electrical stimulations after exposure to aerosolized MCh

When the  $R_L$  response reached the plateau after MCh inhalation, the different electrical stimulations were randomly applied. Typical experimental recordings are exhibited in Figure 3, in which LES still enhanced  $R_L$  but HESs





**Figure 3** Typical examples exhibiting cardiovascular and airway responses to methacholine aerosol (100  $\mu\text{g/mL}$  per minute for 2 min) and to vagal electrical stimulation in an anesthetized and paralyzed Ova-pretreated guinea pig. The traces from the top to bottom are arterial blood pressure (ABP), heart rate (HR), total lung resistance ( $R_i$ ) and dynamic pulmonary compliance ( $C_{\text{dyn}}$ ). The duration of the break is about 1 min. MCh: Methacholine. HES: High-frequency electrical stimulation.

suppressed the  $R_i$  response to MCh challenge. Statistically, the amplitude of the suppression was greater in the Ova- (49%) than the Sal-treated animals (35%). Similar results were also observed in  $C_{\text{dyn}}$ . The breakdown of the responses to individual HESs is detailed in Figure 5. The inhibitory effects on the  $R_i$  responses to MCh aerosol were not markedly different between the stimulations at 1 and 2.5 kHz and between the stimulations with different waveforms. In addition, the HES-induced alteration in  $R_i$  was not associated with statistical changes in ABP and HR, although ABP fluctuated a bit (Figures 3 and 5). In this study, 4 electrical stimulations were applied during each  $R_i$  plateau response to MCh aerosol. The bronchodilation responses ( $R_i$ ) evoked by the first and last HES (usually with about 1.5 min apart) were not significantly different in amplitude (in Sal-treated GPs:  $-37\% \pm 5\%$  vs  $-33\% \pm 6\%$  and in Ova-treated GPs:  $-51\% \pm 6\%$  vs  $-48\% \pm 7\%$ ;  $P > 0.05$ ). In addition, no remarkable fatigue of the bronchodilation in response HES was observed during continuously stimulating vagi for 2 min (Figure 6). These data support the notion that detectable fatigue and/or nerve damage is minimal in the experimental timeframe of this study.

#### Airway responses to prolonged electrical stimulations after MCh challenge

We also tested whether prolongation of the vagal stimulation duration would produce similar bronchodilation after MCh exposure and whether the vagal nerve remained viable. As exhibited in Figure 6, the prolonged 2-min HES stimulation still produced a bronchodilation with a limited effect on both MABP and HR. Moreover, this response was reversible and repeatable.

#### Airway responses to MCh aerosol before and after bivagotomy

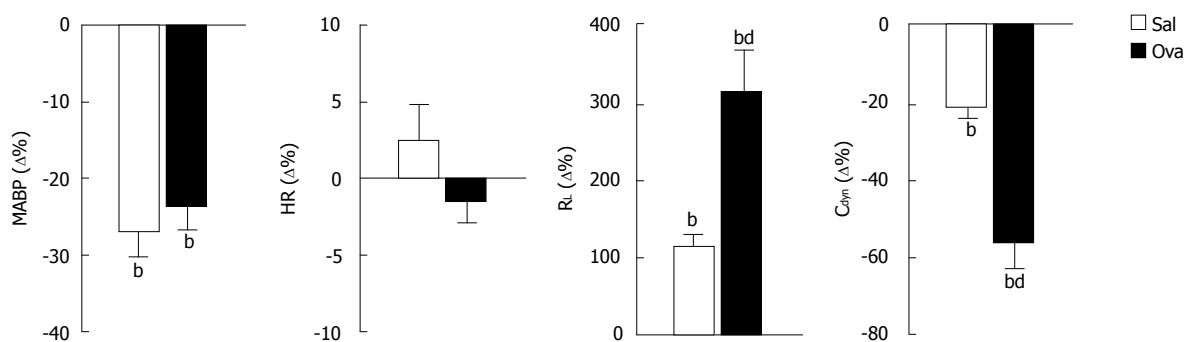
The objective of this study was to clarify whether, similar to vagal HES, bivagotomy would substantially diminish

the airway responses to MCh aerosol in our experimental preparation. We found that as compared to the responses measured with intact vagi, bivagotomy did not significantly change the baseline  $R_i$  and  $C_{\text{dyn}}$  or the BP and HR in the Sal- and Ova-treated animals, which is similar to the previous reports in anesthetized rats<sup>[5,26]</sup>. Most importantly, the  $R_i$  and  $C_{\text{dyn}}$  responses to MCh aerosol were dramatically reduced by the bivagotomy ( $55\% \pm 11\%$ ) and the MCh-induced hypotension tended to be smaller (Figure 7).

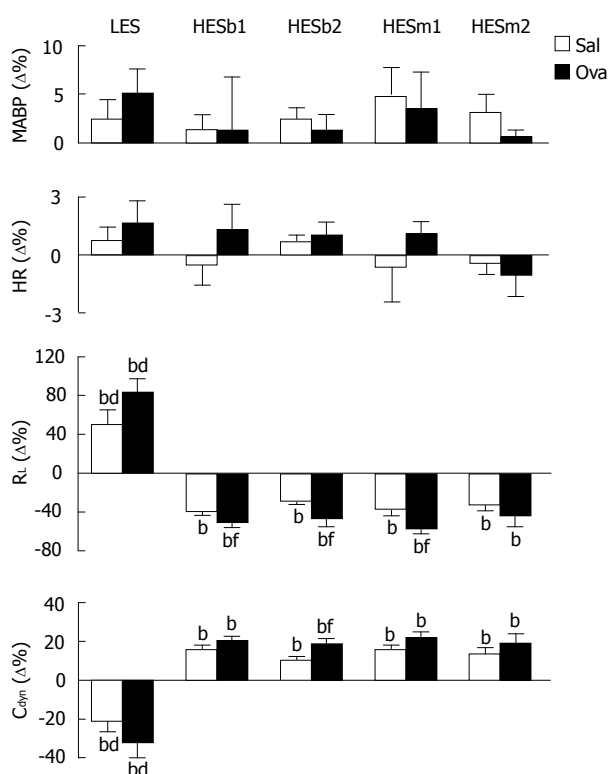
## DISCUSSION

Multiple mechanisms (parasympathetic nerve-dependent or -independent) are involved in generating airway hyper-reactivity. In the present study, we addressed an essential issue as to whether HES reversibly impairs vagal conduction *via* blocking acetylcholine release from parasympathetic fibers, and thereby alleviates the MCh-produced bronchoconstriction. We found that, differing from an excitatory effect of LES, vagal HES greatly inhibited the airway constrictive responses to inhalation of MCh aerosol in anesthetized guinea pigs, especially in those sensitized by Ova. Actually, vagal LES-induced bronchoconstriction<sup>[22-24]</sup> and reduce  $C_{\text{dyn}}$ <sup>[27]</sup> have been observed in rats, rabbits, and guinea pigs. This excitatory response is achievable mainly *via* promoting release of acetylcholine from parasympathetic fibers innervating airway smooth muscle. Although vagal LES provoked a relatively greater  $R_i$  increase and lower  $C_{\text{dyn}}$  in Ova- than Sal-treated guinea pigs, these differences were not significant. There are four unique features of our HES-induced bronchodilation. (1) vagal HES during the bronchoconstrictive response to MCh challenge evokes a rapid bronchodilation that is consistent with the rapid tracheal relaxation by electrical stimulation *in vitro*<sup>[28]</sup>; (2) the electrical stimulation-evoked bronchodilation was reversible and reproducible, implying a minimal damage of vagal HES on the nerve in our study. This finding is consistent with the reported effects of HES characterized by its repeated and reversible blockade of nerve conduction regardless of the electrical parameters<sup>[20,29]</sup>; (3) the vagal HES-evoked bronchodilation was not associated with remarkable changes in HR and ABP; and (4) the HES-evoked bronchodilation after MCh challenge was significantly greater in Ova- than Sal-treated animals. Collectively, our data clearly suggest that vagal HES could substantially inhibit the bronchoconstrictive responses to cholinergic agonists. Asthma is a chronic disease; however, severe asthmatic attack is acute. In our study, the airway responses to MCh mimic the asthmatic attack, while HES-induced bronchodilation during the acute bronchoconstriction may provide a clue of alleviating the asthmatic attack.

A large body of evidence has shown multiple neural pathways responsible for regulating the airway smooth muscle tone<sup>[30]</sup>. Of them, two neural pathways are excitatory. Acetylcholine released from parasympathetic postganglionic nerves innervating airway smooth muscles produces airway contraction *via* acting on muscarinic 3



**Figure 4** Peak airway responses to methacholine aerosol (100  $\mu\text{g/mL}$  per minute for 2 min) and the associated cardiovascular changes.  $n = 9$  and  $8$  in Sal- and Ova-treated guinea pigs respectively; mean  $\pm$  SE. <sup>b</sup> $P < 0.01$ , vs the data of pre-methacholine (MCh) challenge presented as "0"; <sup>d</sup> $P < 0.01$  between Sal- and Ova-treated groups. MABP: Mean arterial blood pressure; HR: Heart rate; RL: Total lung resistance; C<sub>dyn</sub>: Dynamic pulmonary compliance.

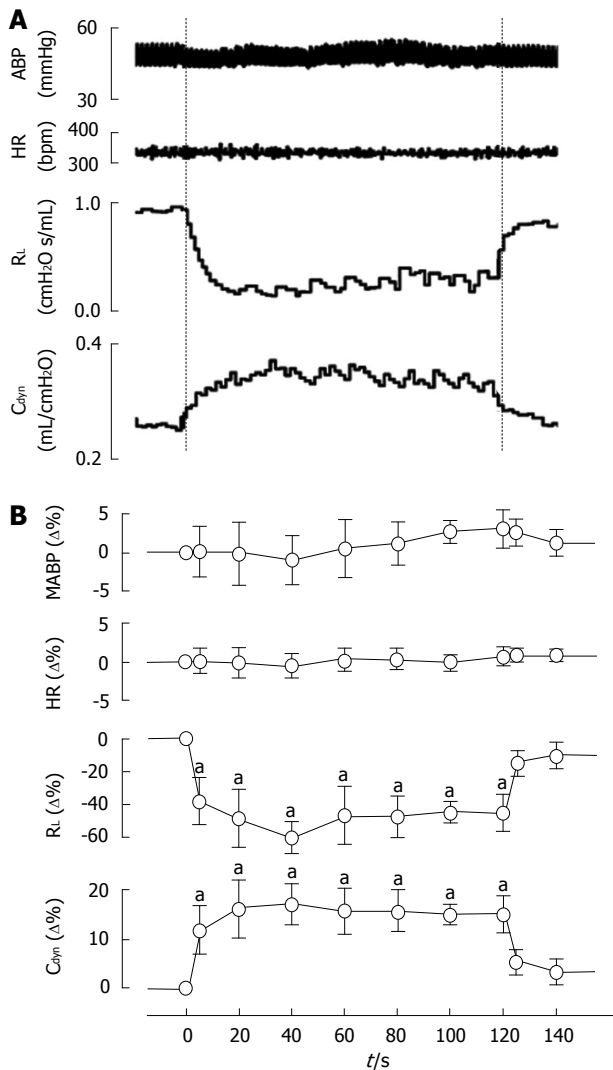


**Figure 5** Airway and cardiovascular responses to the vagal electrical stimulations after exposure to methacholine aerosol (100  $\mu\text{g/mL}$  per minute for 2 min).  $n = 8$  and  $9$  in Sal- and Ova-treated guinea pigs; Mean  $\pm$  SE. <sup>b</sup> $P < 0.01$ , compared to the data before methacholine (MCh) challenge; <sup>d</sup> $P < 0.01$  LES vs HESs; <sup>f</sup> $P < 0.01$  between Sal- and Ova-treated groups. MABP: Mean arterial blood pressure; HR: Heart rate; RL: Total lung resistance; C<sub>dyn</sub>: Dynamic pulmonary compliance.

cholinoceptors of smooth muscle. In addition, activation of vagal pulmonary afferent endings releases tachykinins, such as substance P (SP) and neurokinin A (NKA), causes smooth muscle contraction. The two others are inhibitory. Parasympathetic postganglionic fibers can release inhibitory nonadrenergic, noncholinergic (iNANC) neurotransmitters, such as nitric oxide (NO) and vasoactive intestinal peptide (VIP); and  $\beta_2$  adrenoceptors mediate the muscle relaxation in response to circulating adrenaline. We assume that vagal HES leads to bronchodilation during MCh exposure *via* inhibiting or blocking vagal

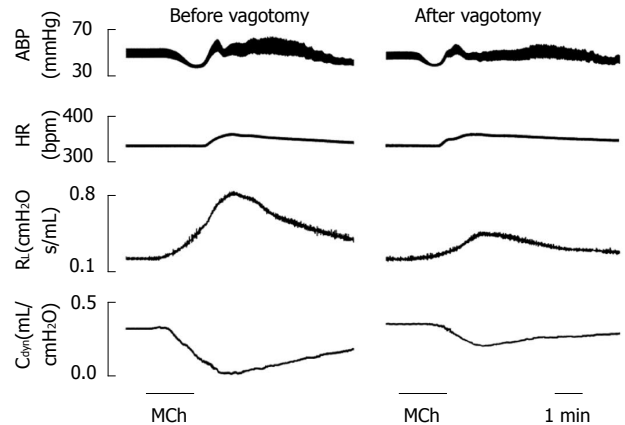
conduction to attenuate release of acetylcholine from parasympathetic fibers innervating airway smooth muscle for several reasons. Vagal HES-induced bronchodilation during MCh is similar to the blunted airway response to MCh after bivagotomy that was previously reported<sup>[5-8]</sup> and confirmed in this study. The similarity of vagal HES and bivagotomy was further confirmed by the evidence that vagal HES (Figures 1 and 2) and bivagotomy<sup>[5,6]</sup> failed to alter baseline RL and C<sub>dyn</sub>. These data suggest that RL and C<sub>dyn</sub> at rest state are largely determined by the mechanics of the airway and lung tissue independent of the basal cholinergic tone. In agreement with our data, the ability of HES to block nerve conduction was first found by Reboul<sup>[18]</sup> and subsequently confirmed by other investigators<sup>[11,19-21]</sup>. Particularly, a square-waveform HES, similar to that used in this study, has been shown to block the sciatic nerve in anesthetized cats<sup>[11]</sup>. The possibility of the involvement of SP and NKA release from pulmonary afferents in the HES-induced bronchodilation is discussed later. The HES-induced bronchodilation unlikely results from activation of the two inhibitory neural pathways. The iNANC pathway-mediated relaxation was evoked by low stimulating frequency ( $< 35$  Hz) and often required several minutes to reach the plateau<sup>[31]</sup>. Different from these, we used HES that induced a rapid bronchodilation. Electrical stimulation of airway sympathetic nerves could also evoke bronchodilation in the guinea pig<sup>[32,33]</sup>. Thus, one may ask whether the HES current spread could stimulate the adjacent airway sympathetic nerves, leading to the bronchodilation observed in our study. This opinion is strongly argued by lacking any significant cardiovascular response to vagal HES in this study. Taken together, our results demonstrate that vagal LES causes bronchoconstriction and bradycardia *via* activating the vagus nerve, while vagal HES leads to bronchodilation during MCh aerosol independent of stimulating waveforms, likely through inhibiting or blocking vagal conduction.

There are several concerns and limitations in this study. First, although vagal HES and bivagotomy share a similarity in inhibiting airway contraction in response to MCh aerosol, our data are not conclusive in proving vagal blockade by the HES. Technically, recording vagal afferent fiber signals is doable, but distinguishing these signals



**Figure 6** Cardiovascular and airway responses to 2 min vagal high-frequency electrical stimulation after exposure to methacholine aerosol (100  $\mu$ g/mL per minute for 2 min). A: HESm1-evoked responses in an Ova-treated guinea pig about 2 min after initiation of methacholine (MCh) challenge, in which the baseline values of total lung resistance ( $R_L$ ) and dynamic pulmonary compliance ( $C_{dyn}$ ) were 0.1 cmH<sub>2</sub>O s/mL and 0.4 mL/cmH<sub>2</sub>O respectively; B: The averaged data of MABP, HR,  $R_L$  and  $C_{dyn}$  from three guinea pigs (five trials) are illustrated in panel. In both panels, the traces are arterial blood pressure (ABP/MABP), heart rate (HR),  $R_L$ , and  $C_{dyn}$ .  $n = 5$  trials (3 guinea pigs); Mean  $\pm$  SE;  $^aP < 0.05$ , high-frequency electrical stimulation (HES)-evoked responses vs the data before electrical stimulation presented as "0".

from the artificial signals of vagal HES is very difficult. Second, the cervical vagal trunk consists of both afferent (myelinated and unmyelinated) and efferent (myelinated) fibers<sup>[34]</sup>. Our data are unable to delineate which type(s) of vagal fibers are responsible for the HES-induced change. Previous results in cats have shown that A-fibers of the slow adapting receptors are not critical to the cholinergic-mediated airway responses because lung inflation has limited effects on the airway response to MCh administration<sup>[35]</sup>. On the other hand, bronchopulmonary C-fibers may be involved in modulating the airway response to cholinergic activation *via* the local axonal- and/or the central-mediated reflexes. Nicotinic cholinergic



**Figure 7** Comparison of the cardiovascular and airway responses to methacholine aerosol (100  $\mu$ g/mL per minute for 2 min) before and after vagotomy in an anesthetized and paralyzed Ova-treated guinea pig. The traces are arterial blood pressure (ABP), heart rate (HR), total lung resistance ( $R_L$ ), and dynamic pulmonary compliance ( $C_{dyn}$ ).

receptors exist in bronchopulmonary C-fibers<sup>[36]</sup>. Degeneration of these fibers significantly diminishes the airway responsiveness to MCh aerosol in both rats and guinea pigs<sup>[37,38]</sup>, while stimulation of these fibers causes airway constriction<sup>[34,39]</sup>. Thus, it is possible that vagal HES can induce bronchodilation during MCh challenge, presumably in part *via* inhibiting the conduction of bronchopulmonary C-fibers. Third, though our data failed to show a detectable fatigue and/or nerve damage induced by our HES applied within 2 min, it remains unknown whether HES applied in a prolonged period ( $> 2$  min) would produce such pathophysiological changes. Fourth, our data cannot deny the plausible that pre-exposure to the conventional treatments clinically used may interfere with the effectiveness of HES denoted in the present study. Fifth, our study was performed in anesthetized preparation. Therefore, further experiments in conscious asthmatic animals are needed to rule out the possible interference of anesthetics in the vagal HES-induced bronchodilation.

## COMMENTS

### Background

High-frequency electrical stimulation (HES) of peripheral nerve is reportedly able to inhibit or block the nerve conduction, and thereby to inactivate the nerve-mediated responses. Airway hyperreactivity results predominantly from an increased acetylcholine release from vagal nerves innervating the airways in asthmatic patients and in animal models of asthma. Authors tested whether HES of vagal nerves could inhibit acetylcholine-induced airway hyperreactivity.

### Research frontiers

The results showed that HES of vagus nerves greatly attenuated airway hyperreactivity in response to acetylcholine, suggesting an inhibitory effect of HES on vagus nerves.

### Innovations and breakthroughs

The major innovation of authors' data is to ensure an inhibitory effect of HES on vagus nerves that leads to an attenuation of acetylcholine-induced airway hyperreactivity.

### Applications

An inhibitory effect of HES on vagus nerves, as presented in this study, may provide a therapeutic approach to alleviate airway hyperreactivity in asthmatic patients.

## Terminology

HES: low-frequency electrical stimulation; methacholine; ovalbumin; total lung resistance and dynamic pulmonary compliance.

## Peer review

This is a very good study in which the authors demonstrated that HES of vagus nerves in guinea pigs attenuated the acetylcholine-induced airway hyperreactivity. The results are interesting and potentially translational from data to pre-clinical research.

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