Pulmonary C-fiber Activation Enhances Respiratory-Related Activities of the Recurrent Laryngeal Nerve in Rats

I-Jung Lu, Li-Chi Ku, Jin-Tun Lin, Kun-Ze Lee and Ji-Chuu Hwang

Department of Biology
National Taiwan Normal University
Taipei, Taiwan, ROC

Abstract

The purpose of the current study was to characterize the response of the recurrent laryngeal nerve (RLN) to pulmonary C-fiber activation. Male rats of Wistar strain were anesthetized by urethane (1.2 g/kg, i.p.). Tracheostomy was performed. Catheter was inserted into the femoral artery and vein. Additional catheter was placed near the entrance of the right atrium via the right jugular vein. The animal was then paralyzed with gallamine triethiodide, ventilated and maintained at normocapnia in hyperoxia. Activities of the phrenic (PNA) and recurrent laryngeal nerves (RLNA) were monitored simultaneously. Two experimental protocols were completed. In the first experiment, various doses of capsaicin were delivered into the right atrium to activate pulmonary C-fibers with vagal intact. Low dose of capsaicin (1.25 μg/kg) produced apnea, a decrease in amplitude of PNA, an enhancement of RLNA during apnea and recovery from apnea, hypotension, and bradycardia. High dose of capsaicin (5 and 20 μg/kg) evoked the same tendency of response for both nerves and biphasic changes in blood pressure. Dose dependency was only seen in the period of apnea but not observable in nerve amplitudes. After bilateral vagotomy, low dose of capsaicin produced an increase in PNA without apnea, no significant change in RLNA, and hypertension. These results suggest that activation of vagal and nonvagal C-fibers could produce different reflex effects on cardiopulmonary functions. The reflex responses evoked by these two types of afferents might play defensive and protective roles in the airways and lungs.

Key Words: pulmonary C-fiber, recurrent laryngeal nerve, phrenic nerve, vagus nerve, apnea, hypotension, hypertension, bradycardia, capsaicin, rat

Introduction

The lung is one of the organs of animals and human beings to contact directly with the environment. Air inhalation containing irritant gases, e.g. tobacco smoke or wood smoke, would irritate the airway and produce a reflex change in cardiopulmonary functions such as apnea, hypotension, and bradycardia (18, 21). Usually, this triad is described as the pulmonary chemoreflex and mainly mediated by the activation of pulmonary C-fibers (5). In the laboratory, direct stimulation of pulmonary C-fibers with capsaicin could initiate the same pulmonary chemoreflex as those of airway irritation (11, 28). In addition, activation of pulmonary C-fibers may also induce local axon reflex to initiate the so-called neurogenic inflammation, such as an increase in vascular permeability, bronchoconstriction, and augmented mucus secretion. All of these reflex responses, locally and centrally, are considered as a reflex defense mechanism to prevent the airways from the access of irritant gas.

The larynx displays activities of abduction and adduction during respiratory cycle. Contraction of the laryngeal adductor muscles, which is innervated by the recurrent laryngeal nerve (RLN), would narrow the diameter of vocal cord and was considered as the mechanism responsible for braking air flow during early expiration (38). Narrowing of the laryngeal diameter or laryngeal closure could prevent aspiration

Corresponding author: Dr. Ji-Chuu Hwang, Department of Biology, National Taiwan Normal University, Tel: 886-2-29332904 ext. 362, Fax: 886-2-29312904, E-mail: jchwang@cc.ntnu.edu.tw
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of foreign materials into the respiratory tract. In this regard, Stransky et al. (31) reported that laryngeal constriction could be caused by activation of pulmonary C-fiber. Diaz et al. (7) recently observed that laryngeal adductor muscle such as thyroarytenoid discharged continuously during the period of apnea in pulmonary edema caused by halothane inhalation. This continuous activity of thyroarytenoid muscle was dependent on the presence of pulmonary C-fibers (8). Thus, we hypothesized that excitation of pulmonary C-fibers may cause a reflex to enhance the expiratory activity of the RLN and, in turn, to increase adductor muscle activity and laryngeal resistance. This reflex response may benefit the airways and lungs to prevent from being access of foreign substance. However, reflex response of the RLN to pulmonary C-fiber activation has not been completely defined in rats. The aim of this study was to examine this hypothesis. The results showed that respiratory-related activities of the RLN during the period of phrenic apnea and recovery from apnea were enhanced in response to pulmonary C-fiber activation. This enhancement of RLN activity was abolished after bilateral vagotomy.

Materials and Methods

Animal Preparation

Male rats of Wistar strain (478 ± 11 g) were used. The animal was purchased from the Animal Center of Medical School of National Taiwan University and housed in animal room, keeping in 25 °C environment with access of water and food. During experiment, the animal was treated with atropine (0.5 mg/kg, i.m.), anesthetized with urethane (1.2 g/kg, i.p.), and then placed upon a supine position. The right femoral artery and vein were catheterized for blood pressure measurement and drug administration, respectively. Tracheotomy was performed. Polyethylene tubing was inserted into the jugular vein and was further advanced until the tip was close to the right atrium. The rat was then paralyzed with gallamine triethiodide (Sigma, 5 mg/kg, i.v.) and ventilated by ventilator with a constant tidal volume of 4-5 ml and frequency of 60-70 breaths per minute. The expiratory outlet of the ventilator was placed under a pressure of 3 cm H_2O to keep a near normal functional residual capacity. End-tidal fractional concentration of carbon dioxide ($F_{ET}CO_2$) was monitored with a CO_2 analyzer (Electrochemistry, CD3A) and maintained at normocapnia in hyperoxia by the administration of oxygen mixture with CO_2 and adjustment of the frequency of ventilator. Body temperature was maintained at 37-38 °C by an electric blanket.

Nerve Recording

After cutting clavicle and removing part of the sternohyoid as well as the surrounding tissues on the left side, the phrenic nerve could be observed from the base of the 4th spinal nerve and was then cut peripherally. Activity of the phrenic nerve (PNA) was amplified, filtered (0.3~10 kHz) and integrated (R-C circuit) (14). PNA was displayed on an oscilloscope (Tektronix 5111), recorded on the tape via a PCM (Neuro-Corder 890), and also on the chart recorder (Grass 7D). In some experiments, PNA was recorded simultaneously on the tape and hard disc of a computer via the PowerLab system (ADIntrument) simultaneously.

The recurrent laryngeal nerve (RLN) was dissected along the trachea on the right side and was cut distally. Activities of the RLN was recorded the same way as that of the phrenic nerve. For recording vagal afferent activities in some experiments, vagotomy was performed at cervical level on the left side. After removal of nerve sheath, a clear rhythmic activity of vagal afferents following the rhythm of the ventilator could be obtained and was undiscernibly when the ventilator was ceased. Therefore, these vagal activities represented the activities of the pulmonary stretch receptors (PSR) activated by the ventilator. From the integrated neurogram, activities of the C-fibers evoked by capsaicin administration would shift the PSR activities upward. With the observation of vagal afferent discharge, we were able to examine whether lung C-fibers were activated by capsaicin treatment.

Experimental Protocol

Two experimental protocols were performed in the present study. In the first protocol, twenty animals were used. Each rat received three doses, which were 1.25, 5, and 20 μg/kg of capsaicin. Various doses of capsaicin ranging from 10 μg/kg in dogs to 5 or 1 μg/kg or even lower doses in rats were used for activation of lung C-fibers (2, 6, 13, 23, 24, 26). Aim of this protocol was to find a suitable dose of capsaicin to activate pulmonary C-fibers based on the apnea observed. A time interval of 40 minutes apart between any two doses of capsaicin challenges was allowed to avoid the possible tachyphylaxis.

The second experimental protocol was completed to examine whether response of the RLN to C-fiber activation was vagal- or nonvagal-dependent. Based on the first protocol, 1.25 μg/kg of capsaicin was enough to elicit both apnea and enhancement of RLN activity. Thus, each animal received a low dose of capsaicin (1.25 μg/kg) before vagotomy. After bilateral sectioning of the vagus nerves, the same

dose of capsaicin was given again to evaluate if the response of RLNA was still observable. If low dose of capsaicin could not elicit reflex apnea and an increase in RLNA after vagotomy, a higher dose of capsaicin (5 μg/kg) was then given 40 minutes later. Dose of 20 μg/kg of capsaicin was not used in the second protocol because it elicited almost the same amplitude of the RLN response as that initiated by 5 μg/kg. To observe RLNA, vagotomy was performed on the right side at a level just caudal to the branch of the RLN by intrathoracic approach and the left vagus nerve was interrupted at the cervical level. With this protocol, reflex response of the RLN to C-fiber activation could be determined whether it depended on vagal or nonvagal components.

Data Analysis and Statistical Examinations

The results on the tape were played back and digitized into a personal computer via the PowerLab system and analyzed with software written by visual C^{++}. Data stored in the hard disc were directly retrieved by the software for analysis. Neural amplitudes of twenty consecutive respiratory cycles before capsaicin treatment was determined and averaged as the control. Neural activities following capsaicin treatment were taken as experimental values and were further transformed into % of the control. For the RLN, both inspiratory and expiratory discharges were determined following the timing of phrenic nerve discharge. During the apneic period, peak activity of the RLN was also determined. T_I (time for phrenic inspiration), T_E (time between phrenic inspiration) and T_{TOT} (sum of T_I and T_E) were computed from the tracing of phrenic nerve discharge before and after capsaicin treatment. Multiple comparisons of data were performed by one-way ANOVA followed by a Dunnett's modified *t*-test (36). Comparison of data of blood pressure and heart rate was evaluated by paired *t*-test. P values less than 0.05 were considered as statistical significance. Data are expressed as mean ± S.E.M.

Results

Activation of lung C-fibers by low dose of capsaicin (1.25 μg/kg) produced apnea and a reduction of PNA. High doses of capsaicin (5 or 20 μg/kg) initiated apnea and an increase in PNA. During the period of apnea, activities of the RLN increased and displayed a rhythmic profile similar to inspiratory and expiratory discharges. Resumption from apneic response, amplitude of PNA showed a gradual recovery toward the control with low dose of capsaicin, whereas it displayed an increase to higher than that of the control with high dose. However, respiratory-related discharge of the RLN was returned to the control gradually. Concomitantly, hypotension and bradycardia were observed. After bilateral vagotomy, low dose of capsaicin produced neither immediate phrenic apnea nor changes in RLN activities. With high dose of capsaicin, apnea was initiated and PNA was increased. Enhancement of respiratory-related discharge of the RLN during apnea was not observed. Blood pressure was hypertensive. Thus, it appears that activation of vagal and nonvagal C-fibers by capsaicin displays different influences upon cardiopulmonary functions.

Response of the Phrenic Nerve to C-fiber Activation by Capsaicin

Low dose of capsaicin injection (1.25 μg/kg; Fig. 1, upward arrowhead) into the right atrium activated vagal C-fibers (Int. VAA) and simultaneously caused a short period of cease of PNA (apnea). During the recovery from apnea, PNA gradually recovered to the control. In response to high dose of capsaicin (5 μg/kg), PNA showed a longer period of apnea (Fig. 1 B). After recovery from apnea, PNA increased for a period of time, and then returned to control. No changes in PNA could be discerned in response to the same volume of vehicle (Fig. 1C) and saline injection (data not shown).

A consistent response to lung C-fiber activation by capsaicin was observed in all animals. We therefore pooled the data into groups. In response to 1.25 μg/kg of capsaicin, mean PNA of the first breath was reduced to 78.2 ± 4.8 % of the control (Fig. 2, $P < 0.05$) and then returned to the control. Higher doses of capsaicin produced increases of PNA instead of a reduction. With 5 μg/kg of capsaicin injection, mean PNA increased gradually and reached to a significant level between 9th (122.3 ± 6.3 % of the control; Fig. 2, $P < 0.05$) and 15th (135 ± 6.3 % of the control, Fig. 2, $P < 0.05$) breaths after capsaicin treatment, and then returned to the control. In response to the highest dose of capsaicin (20 μg/kg), significant increase in PNA was seen at the second breath (113.8 ± 5.0 % of control, Fig. 2, $P < 0.05$) after treatment. Increases of PNA displayed stepwise and reached to a peak at 15th breath (140.0 ± 8.4 % of the control, Fig. 2, $P < 0.05$) after capsaicin. The significant increase sustained for one minute (129.6 ± 5.0 % of the control, Fig. 2, $P < 0.05$) and then decreased slowly to the control at the end of third minute following capsaicin injection (Fig. 2).

Response of the RLN to C-fiber Activation by Capsaicin

Pulmonary C-fiber activation by capsaicin excited respiratory-related activities of the RLN (Fig. 1A). Activity of the RLN (RLNA) was excited,

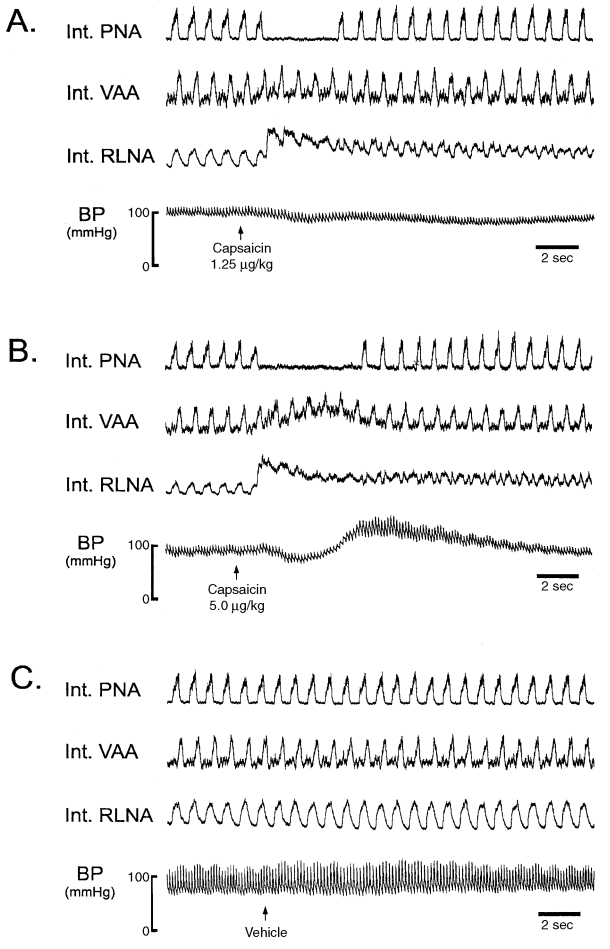

Fig. 1. Pulmonary C-fiber activation by capsaicin produced inhibition of phrenic nerve activity (Int. PNA) and excitation of discharge of the recurrent laryngeal nerve (Int. RLNA) in one rat. Int. VAA represents integrated activity of the vagal afferents, which showed rhythmic discharge caused by ventilator and an upward baseline shift following capsaicin treatment. Panels A and B represent low (1.25 μg/kg) and high (5 μg/kg) doses of capsaicin treatment. Panel C represents vehicle treatment. The horizontal line represents 2 second. BP, blood pressure.

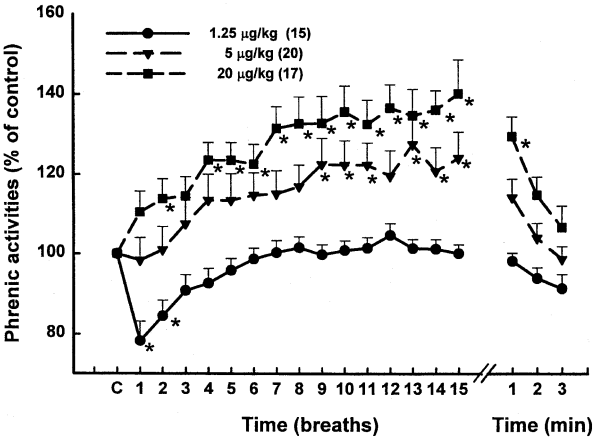

Fig. 2. Time courses of phrenic nerve activities in response to capsaicin treatment. Low dose of capsaicin (1.25 μg/kg) produced a significant decrease in PNA and then returned to the control. High dose of capsaicin (5 and 20 μg/kg) evoked increases in PNA. These increases of PNA maintained at significant level for several breaths even for one minute before returning to the control. Data were expressed as mean ± S.E. of mean. *, $P < 0.05$ compared with the control, which represents an average of consecutive twenty respiratory cycles before capsaicin. Number in the parenthesis is observation numbers.

increased to a peak, remained rhythmic during the period of apnea, and then gradually returned to the control following the resumption from apnea. This rhythmic discharge of RLNA during apnea appeared to be respiratory-related based on the observation of residual activities of PNA. In ten animals observed, low dose of capsaicin did not completely inhibit PNA so that two or three residual discharges seen in each neural breath as shown in Fig. 5A. We therefore divided RLNA into two parts, one for that during apneic period and the other for those during the recovery from the inhibition of PNA. The profile of the RLNA response to capsaicin treatment was very different from those of the control. Increase in expiratory activities of the RLN after capsaicin could be divided into two components, one preceding PNA and the other during the postinspiratory phase. It appeared like an "M" profile with lower amplitude of inspiratory discharge in between. It is interesting to note that profile of RLNA during apnea also showed an "M" shape. For convenience, we did not specify these two components of expiratory discharge and considered as a whole expiratory response in quantitative analysis.

Enhancements of RLNA during apneic period and resumption from apnea were all significant compared with the control. As shown in Fig. 3B, mean expiratory RLNA was significantly increased beginning from the first breath with low dose of capsaicin (1.25 μg/kg) (165.6 ± 18.3% of the control, $P < 0.05$). This substantial augmentation of RLNA was gradually returned to the control. High dose of 5 and 20 μg/kg capsaicin produced increases of expiratory RLNA to 201.7 ± 16.2% and 193.0 ± 16.3% of the control (Fig. 3B, $P < 0.05$), respectively. These significant augmentations sustained for at least fifteen breaths before returning to the control. Enhancement of inspiratory RLNA in response to capsaicin treatment showed a similar tendency as that of expiratory RLNA. Hence, capsaicin of 1.25, 5, and 20 μg/kg evoked increases of inspiratory RLNA to 144.0 ± 16.9%, 152.3 ± 11.6%, and 132.2 ± 6.6% of the control, respectively (Fig. 3A, $P < 0.05$). With pulmonary C-fiber activation by 1.25, 5, and 20 μg/kg of capsaicin, RLNA during apneic period were enhanced to 212.9 ± 27.3%, 270.5 ± 21.7%, and 271.9 ± 23.4% of the control, respectively (Fig. 4, *P*

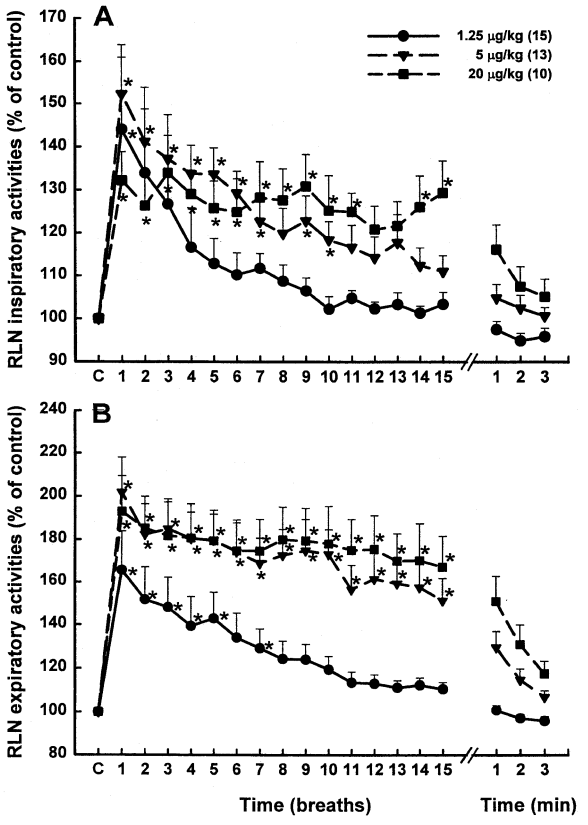

Fig. 3. Time courses of recurrent laryngeal nerve activities (RLNA) in response to capsaicin treatment. Enhancements of RLNA reached the peak beginning from the first breath after recovery from apnea with all doses of capsaicin treatment. Panels A (upper) and B (lower) represent inspiratory and expiratory discharges of the RLN, respectively. Data are expressed as mean ± S.E. of mean. *, $P < 0.05$ compared with the control, which represents an average of consecutive twenty respiratory cycles before capsaicin. Number in the parenthesis is observation numbers.

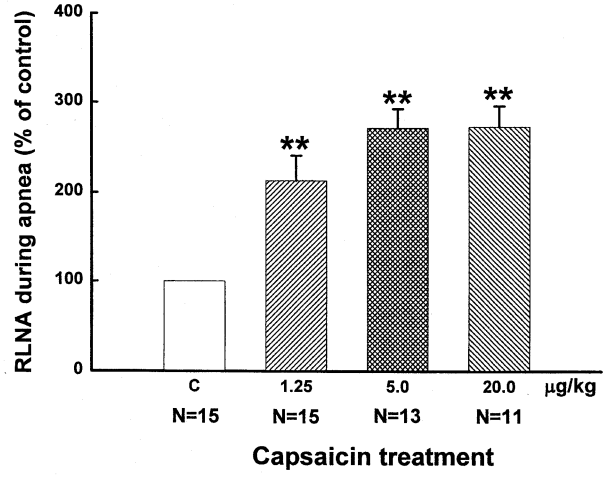

Fig. 4. Mean value (± S.E.M.) of RLNA during apnea with pulmonary C-fiber activation by various doses of capsaicin. Bar represented S. E.M. **, $P < 0.01$ compared with the control (C). N, observation numbers.

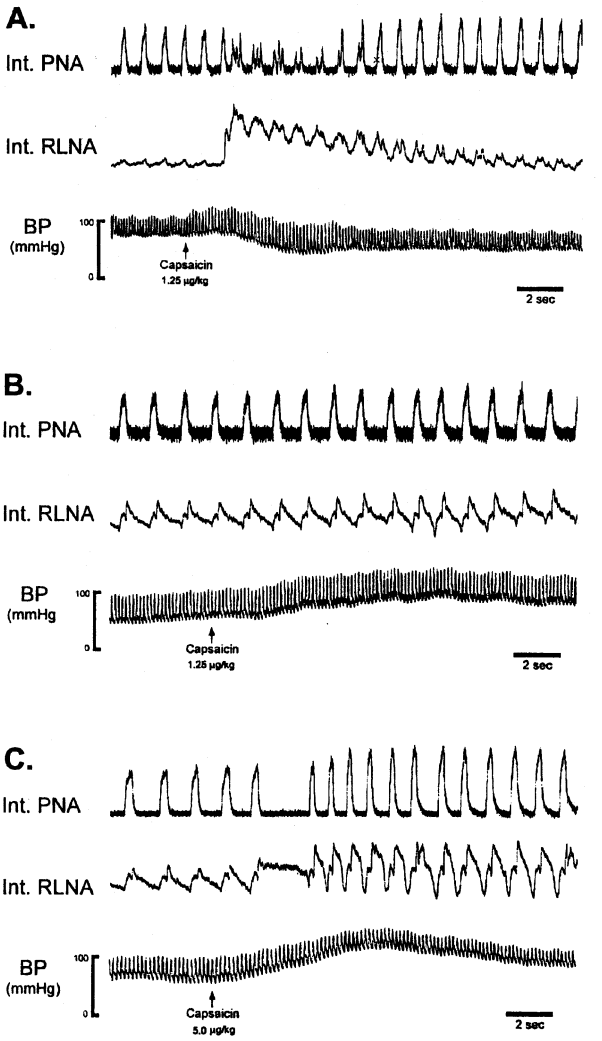

Fig. 5. Example of cardiopulmonary responses to capsaicin treatment before and after bilateral vagotomy was observed from one animal. Decrease in PNA and increase in RLNA by a dose of 1.25 μg/kg capsaicin (panel A) were abolished after bilateral sectioning of the vagus nerves (panel B). With high dose of capsaicin (5 μg/kg), apnea was induced but increase in RLNA during apnea was unobservable (panel C). When resumption from apnea, PNA and RLNA both showed increase in amplitude. Hypotension with capsaicin treatment (panel A) was reversed to be hypertensive after vagotomy (panels B and C). In this animal, capsaicin could only cause decrease in PNA instead of apnea. The horizontal line represents 2 second.

< 0.01). High doses of capsaicin may produce a slightly larger effect on RLNA than low dose but the comparison was not significant (Figs. 3 and 4).

Responses of PNA and RLNA to C-fiber Activation after Vagotomy

Before bilateral vagotomy, low dose of capsaicin (1.25 μg/kg) produced an inhibition upon PNA, and an excitatory effect on RLNA (Fig. 5A) similar to those observed in the first experiment. Mean PNA

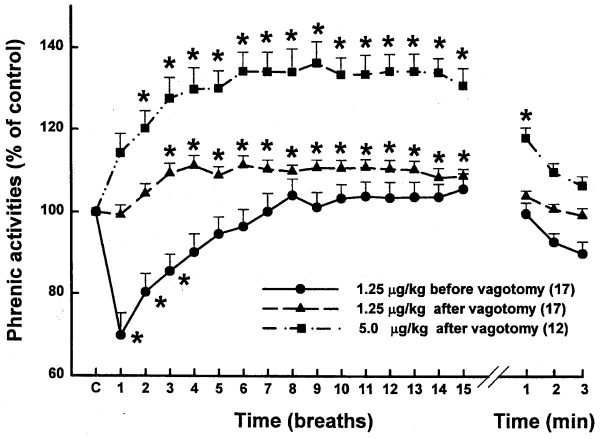

Fig. 6. Time courses of phrenic nerve activities in response to capsaicin treatment before and after vagotomy. Low dose of capsaicin (1.25 μg/kg) produced a significant decrease in PNA before vagotomy and then returned to control. Following bilateral sectioning of the vagus nerves, capsaicin treatment with both low and high doses significantly evoked increases in PNA. Data are expressed as mean ± S.E.M. *, $P < 0.05$ compared with the control. Number in the parenthesis is observation numbers.

showed significant reduction in the first breath (69.7 ± 5.5% of the control, Fig. 6, $P<0.05$) following apnea and then returned to the control (Fig. 6). After vagotomy, low dose of capsaicin (1.25 μg/kg) produced no immediate apneic inhibition, but a slightly enhancement on PNA (Fig. 5B). In some animals, mild apnea still occurred. Averaged increase in PNA remained at significant level ($P<0.05$) for several breaths (Fig. 6). In response to 5 μg/kg of capsaicin, a period of apnea was evoked (Fig. 5C). Increase in PNA was observed in the very beginning recovery from apnea and was kept at several breaths before returning slowly to the control level (Fig. 5C). Substantial enhancement of mean PNA was seen at the second breath (120.2 ± 4.3% of the control, Fig. 6, $P < 0.05$), remained at significant level until one minute, and then returned to the control (Fig. 6). This respon-sive pattern was very different from that produced by vagal C-fiber. Activation of vagal C-fibers initiated apnea and then an inhibition on PNA whereas nonvagal C-fibers evoked an early inhibition to produce apnea and then a delayed excitation for a period of time.

After bilateral vagotomy, enhancement of rhythmic RLNA caused by nonvagal C-fiber activation was not observed during apneic period, but still remained at the end of expiration (Fig. 5C). Thus, in response to 1.25 μg/kg of capsaicin, peak of mean expiratory RLNA was increased to 234.9 ± 21.7% of the control before vagotomy (Fig. 7B, $P < 0.05$) and then returned to the control. After bilateral vagotomy, the same dose of capsaicin did not produce significant change in expiratory RLNA (Fig. 7B, $P > 0.05$). With

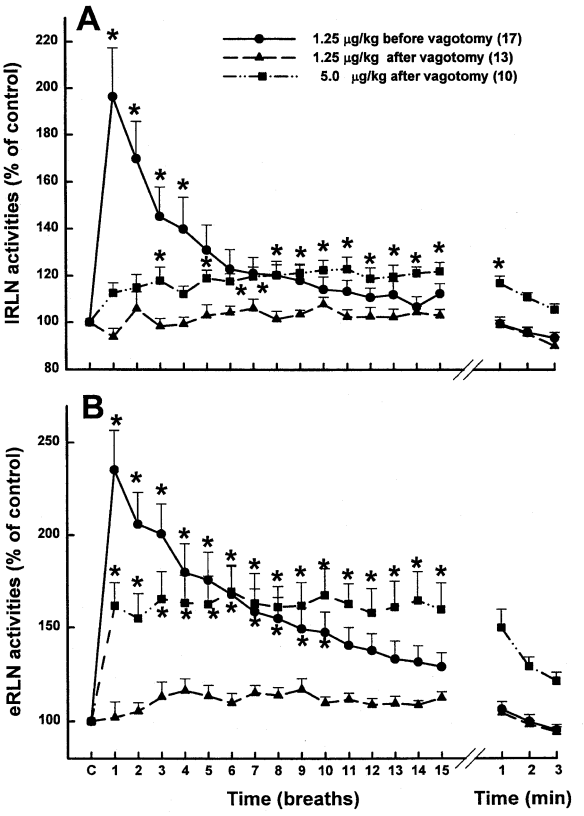

Fig. 7. Time courses of RLNA in response to capsaicin treatment before and after vagotomy. Low dose of capsaicin (1.25 μg/kg) produced significant enhancements of both inspiratory (panel A) and expiratory (panel B) RLNA before vagotomy and no changes after vagotomy. High dose of capsaicin (5 μg/kg) evoked increases of both inspiratory and expiratory RLNA ($P < 0.05$). Data are expressed as mean ± S.E.M. *, $P < 0.05$ compared with the control. Number in the parenthesis is observation numbers.

5 μg/kg of capsaicin treatment, expiratory RLNA was substantially enhanced to 161.9 ± 12.5% of the control (Fig. 7B, $P < 0.05$) and remained at a plateau profile for more than ten breaths before returning to the control. Peak average of inspiratory RLNA in response to low dose of capsaicin was increased to 196.3 ± 20.9% of the control (Fig. 7A, $P < 0.05$) and then gradually returned to the control before vagotomy. This increase was not observable after bilateral vagotomy. High dose of capsaicin (5 μg/kg) initiated significant increases in inspiratory RLNA (Fig. 7A, $P < 0.05$). During apnea, enhancement of average RLNA with 1.25 μg/kg capsaicin was 297.8 ± 25.6% of the control before vagotomy (Fig. 8, $P < 0.05$) and was not observed after vagotomy regardless the doses of capsaicin injected.

Changes in Respiratory Pattern in Response to Pulmonary C-fiber Excitation

In response to pulmonary C-fiber activation,

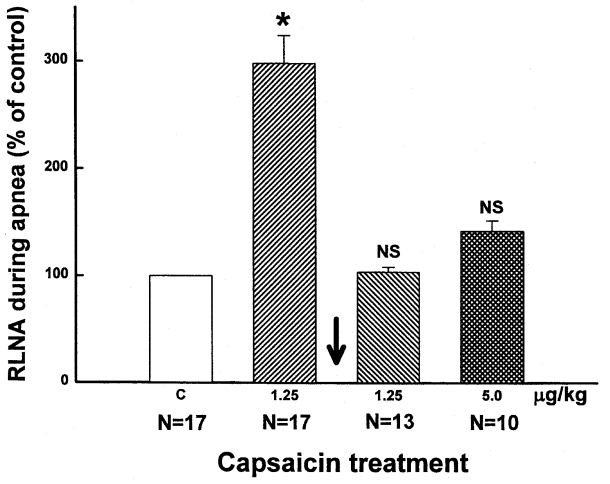

Fig. 8. Mean value (± S.E.M.) of RLNA during apnea induced by capsaicin treatment before and after vagotomy. Vertical bar represented S.E.M. **, $P < 0.01$; NS, no significant compared with the control (C). N, observation numbers. Down-arrowhead represents vagotomy.

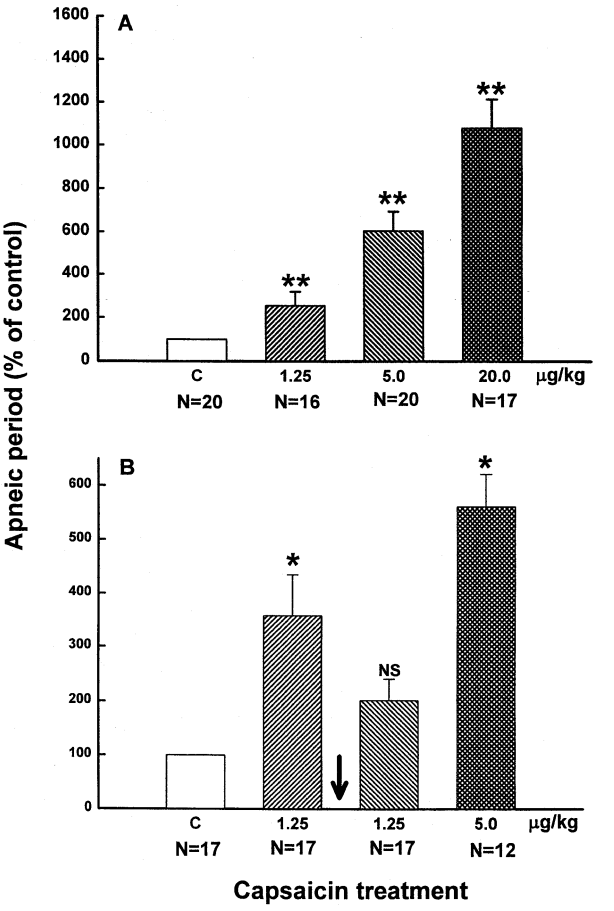

Fig. 9. Comparisons of apneic period induced by capsaicin treatment. A dose-dependent prolongation of apnea in response to various doses of capsaicin was occurred before bilateral vagotomy (A). After bilateral vagotomy, low dose of capsaicin (1.25 µg/kg) did not evoke a prolongation of apnea (panel B), whereas significant prolongation of apnea was also observable with high dose of capsaicin treatment (B). *, $P < 0.05$; **, $P < 0.01$; NS, no significant compared with the control. N, observation number.

respiratory frequency had a tendency to decrease before bilateral vagotomy and to increase after vagotomy. Yet, these changes in respiratory pattern were insignificant. Substantial changes were only observed in the period of apnea. Before bilateral vagotomy, average period of apnea was prolonged to 253.6 ± 67.7%, 602.6 ± 90.8%, and 1074.3 ± 136.8% of the control in response to 1.25, 5, and 20 µg/kg of capsaicin, respectively (Fig. 9 A, $P < 0.01$ compared with the control). In the second experiment, 1.25 µg/kg of capsaicin treatment prolonged the mean period of apnea to 358.0 ± 77.5% of the control before bilateral vagotomy (Fig. 9B, $P < 0.05$) and was 202.3 ± 39.2% of the control after vagotomy (Fig. 9B, $P > 0.05$). High dose of capsaicin (5 µg/kg) prolonged T_E to 561.6 ± 60.4% of the control (Fig. 9B, $P < 0.05$). Respiratory frequency was significantly prolonged after vagotomy (average of T_{TOT} was 0.85 sec before vagotomy and was 1.21 sec after vagotomy). This prolongation was mainly due to the increase of T_E from 0.63 ± 0.05 sec to 0.96 ± 0.27 sec ($P < 0.05$). In response to nonvagal C-fiber activation, T_E had a tendency to be shortened, which reached its statistical significance with 5 µg/kg of capsaicin (Fig. 10, $P < 0.05$). Period of T_I was 0.22 ± 0.01 sec before vagotomy and was 0.25 ± 0.01 after vagotomy. Significant change in T_I with capsaicin treatment was not observed.

Cardiovascular Responses to Pulmonary C-fiber Activation

In the first experiment, decrease in blood pressure (BP) and heart rate (HR) were recorded when pulmonary C-fibers were activated by capsaicin. Low dose of capsaicin (1.25 µg/kg) caused decreases in both BP and HR as shown in Fig. 1. High dose of capsaicin (5 µg/kg) produced biphasic effect upon BP, showing an early decrease and a delay increase. In the second protocol, the same cardiovascular responses as the first experiment were seen. Thus, averages of BP and HR were significantly reduced in response to low dose capsaicin (Fig. 11). Mean BP and HR were 80.3 ± 3.4 mmHg and 498.0 ± 9.0 beat per minute (BPM) respectively. In response to low dose of capsaicin treatment, decreases in BP and HR were 11.4 ± 2.0 mm Hg and 83.9 ± 9.2 BPM (Fig. 11, $P < 0.001$). After bilateral vagotomy, low dose of capsaicin caused substantial hypertension (increasing 22.8 ± 3.3 mm Hg from the control; Fig. 11, $P < 0.001$) and a significant bradycardia (reducing 30.1 ± 7.5

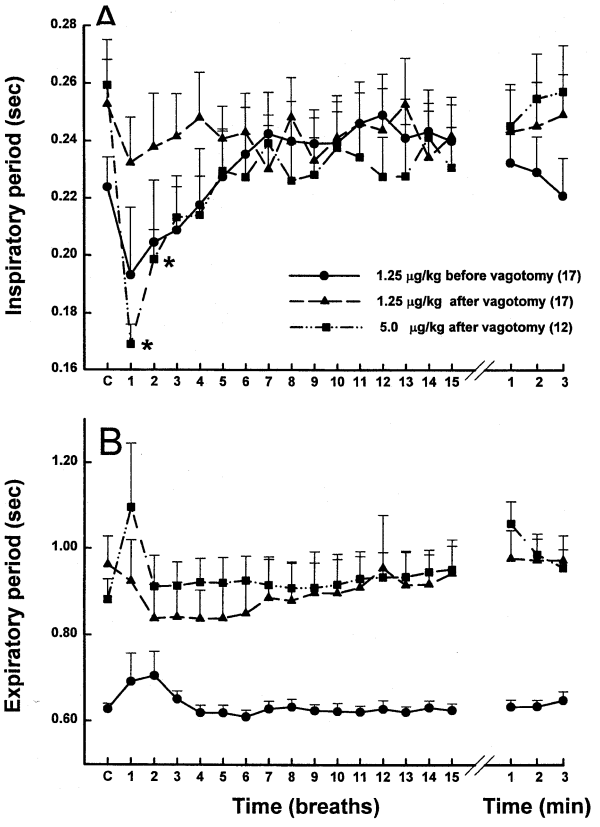

Fig. 10. Changes in inspiratory (panel A) and expiratory (panel B) periods induced by capsaicin treatment. Bilateral vagotomy significantly prolonged the period of expiration. Only inspiratory period was shortened in response to high dose of capsaicin treatment. *, $P < 0.05$ compared with the control (panel A).

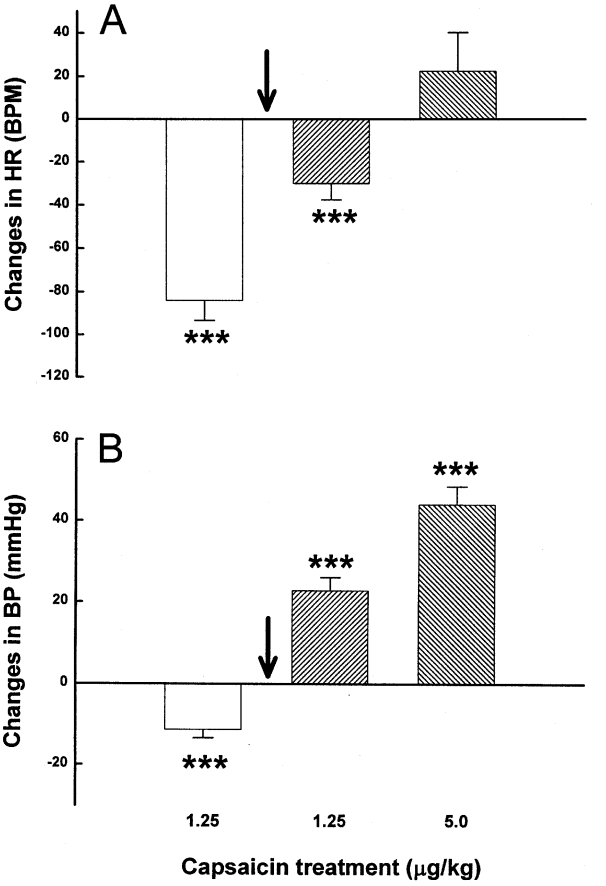

Fig. 11. Changes in blood pressure (BP) and heart rate (HR) in response to capsaicin treatment. Hypotension and bradycardia induced by low dose of capsaicin were significant before vagotomy. Following bilateral sectioning of the vagus nerves, the same dose of capsaicin caused an increase in BP and a substantial reduction of HR. With high dose of capsaicin treatment, hypertension with a mild tachycardia was discerned. ***, $P < 0.001$ compared with value before vagotomy. Down-arrowhead represents vagotomy.

BPM from the control; Fig. 11, $P < 0.001$). High dose of capsaicin produced a larger hypertensive effect (Figs. 5 and 11, $P < 0.001$) and a tendency of tachycardia. These results indicate that vagal C-fiber activation produced hypotension but nonvagal C-fiber activation caused hypertension.

Discussion

There are three main findings of the present study. First, activation of pulmonary C-fibers could elicit an inhibition upon PNA but simultaneously an excitatory effect on respiratory-related discharge of the RLN. This excitation of the RLN was totally abolished after bilateral vagotomy, suggesting that excitation of the RLN is vagal dependent. This has never been observed previously in rats. Second, activation of nonvagal C-fibers produced an early inhibition of PNA, and a delayed excitation upon PNA and RLNA. Third, excitation of vagal C-fibers produced decreases in BP and HR, whereas activation of nonvagal C-fibers produced an increase in BP.

Cardiopulmonary Response to Activation of Vagal C-Fibers

It has been documented that there is a species difference for capsaicin activation of lung C-fibers. The dose used in different species of animals was quite dissimilar, ranging from 10 μg/kg in dogs (6, 23) to 5 μg/kg in rats (26). It also varied even in the same species of animal, ranging from 5 to 1 μg/kg or even lower in rats (2, 13, 24). Rats are the animals getting popular to be used in investigating pulmonary chemoreflex (21). Thus, defining a suitable dose for activating vagal C-fibers to initiate pulmonary chemoreflex is important. Since capsaicin was injected into the right atrium to activate pulmonary C-fibers by circulation, the immediate response of this chemoreflex could be considered as activation of C-

fibers located in the lung. This immediate pulmonary chemoreflex is correlated well in timing with vagal afferent activity (VAA in Fig. 1). In our preparation, averaged time for initiating cardiopulmonary reflex by low dose of capsaicin was 2.3 sec before vagotomy and 5 sec after vagotomy. Specifically, apnea, reduction in PNA, excitation of RLNA, and hypotension were not discernable after vagotomy. We therefore believed that these immediate responses should be evoked by vagal C-fiber activation.

With this experimental protocol, activation of vagal C-fibers elicited an inhibition on respiration, resulting in apnea followed by a reduction in amplitude of PNA and then returned to the control (Figs. 1 and 2). These results are comparable well with other reports (5, 10, 26, 29). Apnea is due to a complete inhibition of PNA instead of a switch from inspiration to expiration caused by the pulmonary stretch receptors or a prolongation of T_E. This is based on two lines of evidence in the present study. First, ten animals observed remained residual PNA without conspicuous apnea when capsaicin was administrated (Fig. 5A). Second, inspiratory activity of the superior laryngeal nerve in two animals showed incomplete inhibition during apnea (data not shown). This point of view was also suggested by other report (30). In the present study, we did not observe a rapid small neural discharge of the phrenic nerve as that in other reports showing shallow breathing following pulmonary C-fiber activation. Yet, we did find that incomplete inhibition of PNA gave rise to a rapid resumption of the phrenic activity so that PNA displayed 2 or 3 even 4 small activities in each neural breath. Whether the incomplete inhibition of PNA by pulmonary C-fiber activation represented shallow breath remains to be determined.

Response of the RLN to Activation of Vagal C-fibers

The RLN displayed conspicuous inspiratory and expiratory discharges during respiratory cycle. It innervates the muscles of the larynx, mainly the posterior cricoarytenoid (PCA) muscle. EMG of PCA muscle showed respiratory-related activities. Inspiratory activity of the laryngeal muscles would dilate the glottis to reduce the laryngeal resistance, whereas expiratory activity abducted the glottis to increase the resistance. The present data showed that enhancement of RLNA in response to vagal C-fiber activation was seen not only during apnea but also during recovery from apnea (Figs. 3 and 4). The enhancement of RLNA during apnea was not discerned after bilateral vagotomy (Figs. 5C and 8). These results strongly implied that enhancement of RLNA during apnea is mediated by vagal C-fibers. The same conclusion was also reached in lamb by other studies (8, 27). Profile of this enhancement had a peak in the very beginning of capsaicin injection. It displayed respiratory rhythm like "M" shape containing two expiratory components with inspiratory activity in between. The enhancement of RLNA may suggest that a tightly complete laryngeal closure had occurred during apnea when vagal C-fibers were activated. This may benefit to the airways and lungs to avoid further insult by irritant gas. In the point of view of defense mechanism, this protective function of RLN response to vagal C-fiber activation has somewhat similarity to the diving reflex although responsive pattern is different. In the diving reflex, a continuous adduction without interruption of glottis was observed (9). In fact, RLNA response remaining at the end of expiration after vagotomy was considerably similar to that of diving response. Increases in RLNA during apnea may also indicate that laryngeal resistance was increased with vagal C-fiber activation. In this regard, Stransky et al. (31) had observed that apnea and a complete laryngeal closure were discerned in rabbits in response to intravenous injection of phenyl diguanide. Palecek et al. (26) also reported a complete closure of the upper airway and an increase in laryngeal resistance in rats after capsaicin treatment. The high dose of capsaicin used in Palecek's study makes it difficult to explain the results as to whether they are from vagal or nonvagal origin. Lara et al. (19) believed that glottal constriction could maintain a larger lung volume to benefit gas exchange. Taking together, enhancement of RLNA would either benefit gas exchange during temporary cessation of breathing or prevent from being further insult of irritant gas. Thus, our results would have physiological implications in terms of defense mechanism when vagal C-fibers are stimulated.

Enhancement of RLNA during apnea in response to vagal C-fiber activation may infer some other physiological implications. We would discuss some of our opinion here. Decreases in BP and HR simultaneously occurred with respiratory responses to vagal C-fiber activation. Mechanism for these responses has not yet elucidated. It is obvious for us to speculate that hypotention and bradycardia may be due to an increase in outflow of the parasympathetic nerves. Hence, cardiovascular response would change after interruption of the vagus nerves. In this regard, Wang et al. (37) recently reported that a group of neurons with their nerve fibers along the cardiac vagal branches were localized within or near the nucleus ambiguus (NA). Activity of these neurons showed respiratory-modulated pattern and commenced during early expiration. More interesting is that these neurons were excited by injection of phenyl biguanide into the right atrium. Two of these neurons were still excited by phenyl biguanide even without phrenic nerve

activity. The NA is one of the areas from where the cardiac vagal neurons originate. It is also the location of motor neurons that innervate the PCA muscles in rats (3, 33). Neurons of cardiovascular and respiratory control system are localized so close or even coupled together. They may interact each other, such as postinspiratory modulation of cardiac vagal neurons. Paton and Nolan (25) recently reported that laryngeal motor neurons had similar reflex pattern in timing to that of cardiac vagus in response to various types of stimulation, such as upper airway negative pressure, pharyngoesophageal mechanical and arterial chemical. Hence, enhancement of RLNA probably implied an increase in cardiac vagal activity with vagal C-fibers activation although direct measurement was not performed. If that is the case, bradycardia evoked by vagal C-fiber activation could be explained by the increased activity of cardiac vagal output.

Cardiopulmonary Response to Activation of Nonvagal C-fibers

Comparing the data, cardiopulmonary responses to capsaicin treatment were different before and after bilateral vagotomy. Activation of vagal C-fibers caused apnea and/or reduction of amplitude of PNA, hypotension, bradycardia, and excitation on the RLN. In contrast, excitation of nonvagal C-fibers initiated apnea, increase in PNA, no excitation of the RLN during apnea, and pressor effect. Besides apneic response, these two afferent systems seem to initiate opposite cardiopulmonary responses. These results manifested that these two afferent systems probably impinged differential influence upon cardiopulmonary functions. This differential influence was also observed in other report (22). The problem is what are the physiological function(s) and significance with activation of nonvagal C-fibers? Is it still related to the defense mechanism? Before answering these questions, it would be better to consider what is nonvagal C-fiber system. The nonvagal C-fibers are rather complicated. It was thought that afferents passing through the sympathetic stellate ganglions are part of it (16, 17). In this regard, Kostreva et al. (16) reported that stimulation of sympathetic afferent could inhibit phrenic nerve activity. Coon et al. (4) observed that stimulation of lung receptors by KCl would produce excitatory effect upon EMG of the trianguralis sterni muscle. Tseng et al. (32) reported that plasma extravasation of neurogenic inflammation evoked by capsaicin was totally eliminated after bilateral sectioning of the vagus nerves and stellate ganglions. Cardiac afferent fibers also pass through the stellate ganglion. Activation of cardiac afferent fibers by capsaicin has been reported to augment PNA (34). In addition, extrathoracic C-fibers may also be excited by high dose of capsaicin through circulation. Activation of nociceptors in skeletal muscle was reported to increase phrenic nerve discharge (35) and BP (20). Activation of nociceptors in forelimb was demonstrated to produce hypopnea, tachycardia, and hypertension (1). Cardiopulmonary reflex caused by activation of muscle nociceptors in the present study is probably not occurred, which is due to a stable tracing of BP and PNA in response to a mechanical stimulation pinching on the paw. The superior laryngeal nerve may also be involved in apneic response to femoral injection of capsaicin (15). The possibility of the trigeminal nerve to cause apnea should not be excluded (9). Therefore, data showing apnea, increase in PNA, and hypertension after vagotomy in the present study would represent a result of processing those nonvagal afferents mentioned above in the central nervous system. Different components of these nonvagal C-fibers may produce various influences upon cardiopulmonary responses. Thus, more studies are necessary to define their role in modulation of cardiopulmonary functions. Nevertheless, we would like to take more considerations on our data. After vagotomy, the "M" shape pattern of the RLN was no observable during apnea. Increases in PNA and respiratory-related RLNA including both inspriatory and expiratory discharges during recovering period from apnea were all significant when nonvagal C-fibers were excited by capsaicin. The enhancement of RLNA during postinspiration indicates probably that there may be an increase in laryngeal abduction to augment the resistance of the airways. It is interesting that T_I was concomitantly decreased during this period (Fig. 10). Hence, the animal may take a short deep breath under this condition to generate a higher airway pressure to expel the irritant gas, which had already been breathed in. These reflex responses seen in our study may play a role in protecting the airways and lungs. However, it is not clear whether the response of the expiratory muscle such as abdominal muscles to nonvagal C-fiber activation is increased. In this regard, activities of the transversus abdominalis and triangularis sterni muscles were augmented by application of substance P or neurokinin B on the ventral medullary surface (12). Moreover, excitation of the triangularis sterni muscle by tachykinin acting on the ventral medulla is compatible with that by cardiac afferent activation reported from Coon et al. (4).

Considering the discussion above, opposite effect of vagal and nonvagal C-fiber activation on cardiopulmonary reflex seems to be contradictory at a first glance. In contrast, it is actually a consistency in the point of view for defense protection. Both afferent systems may contribute to defense mechanism, in which vagal C-fibers for the immediate reflex and nonvagal C for the delayed response. One of our

observations showed significant reduction in HR with low dose of capsaicin after vagotomy (Fig. 11). Decrease in sympathetic outflow may not be possible because significant hypertension is occurred. Residual vagal efferent to the heart was not observed after vagotomy. We have so far no adequate explanation for this phenomenon.

In conclusions, discharge of the RLN was enhanced, whereas PNA was inhibited in response to vagal C-fiber activation. The enhancement of RLN response may be beneficial to the respiratory system in term of defending the insult of irritant gas. In addition, activation of vagal C-fibers and nonvagal C-fibers may play different roles in cardiopulmonary functions.

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