Capsaicin administration inhibits the abducent branch but excites the thyroarytenoid branch of the recurrent laryngeal nerves in the rat

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Lu, I-Jung, Kun-Ze Lee, Jin-Tun Lin, and Ji-Chuu Hwang. Capsaicin administration inhibits the abducent branch but excites the thyroarytenoid branch of the recurrent laryngeal nerves in the rat. J Appl Physiol 98: 1646–1652, 2005. First published January 13, 2005; doi:10.1152/japplphysiol.01133.2004.—Our recent study showed that both inspiratory and expiratory activities of the recurrent laryngeal nerve (RLN) were enhanced by capsaicin administration in the rat (Lu IJ, Ku LC, Lin JT, Lee KZ, and Hwang JC. Chin J Physiol 45: 143–154, 2002). There are two intralaryngeal branches of the RLN: one innervates the thyroarytenoid (TA) muscle and the other innervates the abductor (Abd) muscles. To examine whether these two intralaryngeal branches respond similarly to capsaicin administration, their discharges as well as activities of the phrenic nerve (PNA) and the superior laryngeal nerve (SLNA) were monitored in anesthetized and ventilated rats at normocapnia in hyperoxia. The low dose of capsaicin (0.625 μg/kg) produced a cardio-pulmonary chemoreflex, showing apnea, a decrease in PNA, hypotension, and bradycardia, and significant decreases in SLNA and the activity of the Abd branch. Concurrently, there was an increase in the intralaryngeal TA activity during both apnea and recovery from apnea. The high dose of capsaicin (1.25 μg/kg) evoked larger chemoreflexive responses and laryngeal nerve activities. In addition, both doses of capsaicin initiated a similar delay in the onset of Abd activity and SLNA but an earlier onset for the TA branch to commence during inspiration. A bilateral vagotomy abolished the laryngeal responses to capsaicin administration. However, PNA and blood pressure were enhanced with capsaicin administration after the vagotomy. These results suggest that laryngeal adduction in response to capsaicin administration is vagal afferent dependent and that it may also represent reflexive protection for the airway and lungs.

Activation of pulmonary C fibers (PCFs) has been reported to constrict the larynx (26, 27). Pulmonary edema caused by inhalation of halothane reportedly produced apnea and excited activity of the TA muscles during apneic periods in lambs (5). The excited TA muscle activity manifested itself in a continuous-discharge and PCF-dependent manner (6). Our laboratory’s recent study in rats (18) established that capsaicin administration produces excitation of RLN discharge during apnea and increases the inspiratory and expiratory activities of the RLN during recovery from apnea. RLN excitation during apnea and the expiratory period after recovery from apnea narrows the glottis, producing a protective mechanism that prevents gaseous irritants from entering the lower airways. However, augmentation of inspiratory RLN activity during recovery from apnea may widen the glottis and result in aspiration of foreign substances. A reasonable “defensive” response of the RLN to capsaicin administration would be to decrease its activity during inspiration but increase its activity during expiration when recovering from apnea. To address this issue, we first tested the hypothesis of whether capsaicin administration produces a decrease in RLN activity during inspiration. If so, activities of the SLN innervating other laryngeal abductor muscles should also decrease. This would agree with our laboratory’s recent observation that the phasic hypoglossal discharge decreases with capsaicin administration (17). What, then, is the mechanism responsible for RLN enhancement during inspiration after recovery from apnea? One of the possibilities is that expiratory discharges of the RLN might commence earlier, as pointed out by Dutschmann and Paton (7). Thus our second objective was to test the hypothesis that expiratory discharges of the RLN might advance to fire during inspiration with capsaicin administration. This earlier onset might produce inspiratory RLN activity that is higher than the control during recovery from apnea. To test these two hypotheses, we recorded the activities of the intralaryngeal branches of the RLN and SLN in response to capsaicin administration in anesthetized rats. The results obtained supported our hypotheses. Part of the data was presented as abstract at the Nineteenth Joint Annual Conference of Biomedical Sciences in 2004 (19).
Twenty-eight rats were studied. Nine were used to study the intralaryngeal RLN, and nine were used to study the SLN. Among these 19 animals, 10 rats were examined in the bilateral vagotomy study. An animal was first treated with atropine (0.5 mg/kg im), anesthetized with urethane (1.2 g/kg ip), and then placed in a supine position during the experiment. Two catheters were inserted into the right femoral artery and vein for the measurement of blood pressure (BP) and administration of drugs, respectively. A tracheotomy was performed. Polyethylene (PE) tubing was placed close to the right atrium via the jugular vein. The rat was then paralyzed with gallamine triethiodide (5 mg/kg iv) and artificially ventilated with a constant tidal volume of ~4–5 ml at a frequency of ~60–70 breaths/min. A nearly normal functional residual capacity was maintained by placing the expiratory outlet of the ventilator under a pressure of 3 cmH₂O. The end-tidal fractional concentration of carbon dioxide (\(F_{\text{ET}} \text{CO}_2\)) was continuously monitored with a CO₂ analyzer (Electrochemistry, CD3A) via PE50 tubing connected to a 27-gauge needle to sample gas for the tracheal catheter, and normocapnia (\(F_{\text{ET}} \text{CO}_2 = -0.04–0.05\)) was maintained in hyperoxia by adjusting the frequency and volume of the ventilator. The body temperature was maintained at ~37–38°C with an electric lamp.

Nerve recording. The phrenic nerve (PN) was dissected as described in our laboratory’s recent study (18). Briefly, the PN was dissected by cutting the clavicle and removing part of the sternohyoid as well as the surrounding tissues; it was identified at the base of the fourth spinal nerve and then was cut peripherally. The PN was placed on a bipolar electrode connected to the inputs of an amplifier (Grass AC preamplifier, P5111F). Phrenic nerve activity (PNA) was amplified, filtered (~0.3–10 kHz), integrated (time constant = 0.05 s) (11, 18), and displayed on an oscilloscope (Tektronix 5111). Integrated PNA was then recorded on the hard disc of a laboratory computer by using the PowerLab system (ADInstrument).

The RLN was carefully dissected along the right side of the trachea and was further traced into the larynx by opening the laryngectomy cartilage. With the aid of a surgical microscope, one of the intralaryngeal branches of the RLN, which runs along the lateral side of the larynx to innervate the TA muscles, was identified by its conspicuous inspiratory discharges and was named the TA branch. Another intralaryngeal branch, which innervates the laryngeal abducent muscles, was identified by its inspiratory activities and was named the abducent branch. Both branches were cut as distally as possible. Activities of both the TA and the abducent branches were recorded the same way as that of the PN. However, a monopolar electrode was normally used for the abducent branch because of its relatively short length.

The SLN runs along the vagus and enters the larynx to innervate the CT muscle. It was dissected and traced along the vagus and identified at the level of the larynx. The SLN was cut peripherally. SLNA was recorded the same way as that of PNA.

Experimental protocol. Two protocols were completed. In the first protocol, we simultaneously recorded activities of the PN, TA branch, abducent branch, and SLN in response to capsaicin administration. Two doses of capsaicin, 0.625 and 1.25 µg/kg, which had previously been used in our laboratory (17, 18), were administered into the right atrium via a Hamilton microsyringe, which was connected to PE tubing inserted into the jugular vein. In this protocol, the onset of activities of both the abducent and TA branches was compared with that of the PN after capsaicin administration. In the second protocol, responses of the intralaryngeal branches of the RLN and PN after capsaicin administration were examined before and after a bilateral vagotomy. The objective of this protocol was to demonstrate whether differential responses of these two intralaryngeal branches are vagal dependent. In these two protocols, a low or high dose of capsaicin was randomly delivered. A time interval of 40 min was allowed between the two doses of capsaicin to avoid possible tachyphylaxis.

A vagotomy was performed at the midcervical level on the left side and was performed below the branch of the RLN on the right side by an intrathoracic approach with the aid of a microscope.

Chemical preparation. Capsaicin (Tocris, Bristol, UK) was freshly prepared by dissolving 5 mg in 1 ml of 95% ethanol, and then 1 ml of Tween 80 was added. This solution was diluted with saline (pH 7.4) to make up a volume of 10 ml so that a concentration of 500 µg/ml of stock capsaicin was achieved. This stock capsaicin solution was further diluted with saline to 1.25 µg/kg according to each animal’s body weight. The vehicle was a solution containing 1 ml of 95% ethanol, 1 ml of Tween 80, and 8 ml of saline.

Data analysis and statistical examinations. Data on the hard disk were retrieved by the software and analyzed using software written by Visual C++. Neural activities of 20 consecutive respiratory cycles before capsaicin administration were determined and averaged as the control. Neural activities after capsaicin administration were analyzed and taken as the experimental values and then were further transformed into a percentage of the control. The onset of the activities of these four nerves was also determined and compared with that of PNA. The TI (duration of phrenic inspiration), TE (duration between phrenic inspirations), and duration of the respiratory cycle (sum of TI and TE) were computed from the tracing of the PNA before and after capsaicin administration. Multiple comparisons of data were performed by one-way ANOVA followed by a Dunnett’s modified t-test (28). Student’s t-test was used to compare the onset of nerve activity with PNA and the change in BP and respiratory pattern before and after vagotomy. A P value of <0.05 was considered statistically significant. Data are expressed as means ± SE.

RESULTS

Inhibition of PNA by capsaicin administration. The low dose (0.625 µg/kg) of capsaicin administration into the right atrium caused apnea for 2.13 ± 0.16 s as well as decreases in PNA (Fig. 1A). The high dose of capsaicin (1.25 µg/kg) evoked a longer apneic response (3.06 ± 0.21 s) as well as decreases in PNA (Fig. 1B), which gradually returned to control values. Inhibition of PNA occurred after an average latency of 1.05 ± 0.21 s after the administration of capsaicin. No change in PNA was observed after administration of the same volume of saline (Fig. 1C) or vehicle (Fig. 1F). In general, capsaicin induced significant decreases in mean PNA for several breaths (Fig. 2A, P < 0.05); then PNA gradually returned to control values.

Inhibition of the abducent branch of the RLN after capsaicin administration. Capsaicin administration inhibited activities of the abducent branch, which were significantly reduced (Fig. 1, A and B) to 81 and 76% of the control, respectively. These reductions remained for 15 breaths (Fig. 2C, P < 0.05).

The activity of the abducent branch always occurred earlier than PNA (lines L1 and L2 in Fig. 3) by 81.20 ms on average (Fig. 4A, P < 0.05). However, the activity of the abducent branch was delayed after PNA by −109.10 ± 18.13 ms with the low dose of capsaicin and 70.30 ± 16.10 ms with the high dose of capsaicin (Fig. 4A, P < 0.05). This delayed onset remained for 15 breaths before returning to the control level.

Inhibition of SLNA after capsaicin administration. Capsaicin administration inhibited SLNA similar to that of PNA and of the abducent branch of the RLN. The mean SLNA was significantly reduced by capsaicin administration (Fig. 2B, P < 0.05). The onset of SLNA preceded that of the PNA by an average of 69.70 ± 28.10 ms (Fig. 4B, P < 0.05) before capsaicin administration and was delayed (Figs. 3 and 4B, P < 0.05) to a level similar to that for the PNA after capsaicin administration.
Fig. 1. Activities of the phrenic nerve (PNA), abducent branch of the recurrent laryngeal nerve (Abd RLNA), and thyroarytenoid (TA) branch of the recurrent laryngeal nerve (TA RLNA) in response to capsaicin administration in a rat. Before the vagotomy, PNA and Abd RLNA decreased, whereas TA RLNA increased, in response to capsaicin administration (A and B). Concomitantly, apnea, hypotension, and bradycardia were observed (A and B). After a bilateral vagotomy, inhibitions of cardiopulmonary responses disappeared with the low dose (0.625 μg/kg) of capsaicin administration (D). However, the high dose of capsaicin (1.25 μg/kg) produced moderate increases in PNA, blood pressure, and respiratory frequency (E). Saline and vehicle evoked no changes in cardiopulmonary functions (C and F). The horizontal line represents 10 s. BP, blood pressure.

Fig. 2. Time courses of PNA, SLNA, Abd RLNA, and TA RLNA in response to capsaicin administration. Low and high doses of capsaicin (0.625 and 1.25 μg/kg) produced significant decreases in PNA (A), SLNA (B), and Abd RLNA (C). The decrease in Abd RLNA remained for several breaths before returning to the control level (C). The same doses of capsaicin administration evoked significant increases in TA RLNA (D). After a bilateral vagotomy, decreases in PNA and Abd RLNA and increases in TA RLNA evoked by capsaicin administration were not observed (A, C, and D). However, PNA significantly increased with the high dose of capsaicin after the vagotomy (A). Values are means ± SE. *P < 0.05 compared with control values, which represent the average of 20 consecutive respiratory cycles before capsaicin administration, by multiple comparisons test. The number in parentheses represents the number of animals observed.
Excitation of the TA branch of the RLN after capsaicin administration. Capsaicin administration produced excitation of expiratory discharges of the TA branch, including the amplitude and onset time (Figs. 1, A and B, and 2D). During apnea, the activity of the TA branch reached its highest level and then slightly decreased (Fig. 1, A and B). This increase in TA branch activities remained at this high level during recovery from apnea. In general, discharges of the TA branch commenced at the very beginning of stage I expiration (line L3 in Fig. 3). However, after capsaicin administration, these discharges began earlier during inspiration (line L4 in Fig. 3). This earlier onset of the TA branch caused its activity in stage I expiration to overlap with the activity of stage II expiration of the previous respiratory cycle. This joining together resulted in a continuity of TA branch activity with a transient decrease during duration of phrenic inspiration (Ti), (L5). I and II respectively represent stage I and II expiration. The short horizontal bar indicates 1 s.

No changes in the abducent or TA branches with capsaicin after the vagotomy. Ten animals were used for the vagotomy study. A bilateral vagotomy abolished the inhibitory effect of capsaicin administration on PNA and abducent branch activity (Fig. 1, D and E). Furthermore, augmentation of TA branch activities with capsaicin administration was also eliminated (Fig. 1D). After the bilateral vagotomy, PNA was not reduced with the low dose of capsaicin but was significantly increased with the high dose of capsaicin (Fig. 2A, P < 0.05). Decreases in abducent branch activity and increases in that of the TA branch were not observed with either the low or high dose of capsaicin (Fig. 2, C and D, P > 0.05). Earlier onset of TA branch activity was not discerned. SLNA was not evaluated after the vagotomy.

Changes in respiratory patterns in response to capsaicin administration. Before the bilateral vagotomy, capsaicin administration increased the respiratory frequency (Rf), which was mainly due to shortening of Ti (Fig. 6A, P < 0.05). The apneic period was substantially prolonged by capsaicin, and...
DISCUSSION

There are three main conclusions of the present study. First, capsaicin administration decreased inspiratory activities of the abducent branch and SLN but increased TA branch activities. Second, capsaicin administration evoked a delayed onset of the abducent branch and of the SLN, while eliciting earlier onset of TA branch activities. Third, these responses of the intralaryngeal branches of the RLN were abolished after the bilateral vagotomy, suggesting that laryngeal responses are vagally mediated.

Cardiopulmonary chemoreflex. The capsaicin-induced cardiopulmonary chemoreflex is well established and has been demonstrated by many investigators (4, 16, 18). This chemoreflex seen in our present study was in agreement with that reported by other studies (4, 9, 12, 15, 27) and similar to that of our laboratory’s recent studies (17, 18). The <2-s latency of this chemoreflex observed in the present study was compatible with results of other reports. This chemoreflex was abolished after a bilateral vagotomy, indicating a vagally dependent pathway as suggested by others (4, 9, 25).

Capsaicin administration to the right atrium has been widely used to activate PCFs (2). However, it has been also reported this effect was dose dependent (Fig. 5B, left, P < 0.05). After the bilateral vagotomy, Te was significantly prolonged (Fig. 6B, P < 0.05). Prolongation of the apneic period evoked by capsaicin administration was not observed with the low dose and was moderate with the high dose of capsaicin (Fig. 5B, right, P > 0.05).

Cardiovascular responses to capsaicin administration. Capsaicin administration decreased both the BP and heart rate (HR) (Fig. 1, A and B). The low and high doses of capsaicin caused the mean BP to drop by 20.64 ± 1.53 and 22.70 ± 1.87 mmHg, respectively (P < 0.05; the BP was 94.99 ± 1.46 mmHg before capsaicin was given). The mean HR was significantly reduced by 56.14 ± 4.32 and 63.33 ± 5.92 beats per minute (bpm) in response to these two doses of capsaicin administration, respectively (P < 0.05; the mean HR was 385.43 ± 10.81 bpm before capsaicin administration). After the bilateral vagotomy, the BP (mean BP was 80.05 ± 2.89 mmHg) was increased by 7.56 ± 0.67 (P > 0.05) and 13.61 ± 1.97 (P < 0.05), whereas the HR (389.01 ± 5.94 bpm) was decreased by 13.27 ± 2.79 and 19.73 ± 3.80 (P < 0.05) with low and high doses of capsaicin administration, respectively.
that capsaicin can excite a small portion of Aδ-fibers or rapidly adapting receptors (2, 10). Hence, inhibition of abducent RLN activity and excitation of TA branch activity that we found may have been caused by excitation of both afferents induced by capsaicin. Activation of Aδ-fibers has been documented to evoke an increase in respiration, whereas activation of vagal C fibers has been confirmed to cause a decrease in respiration (2, 10, 24). Therefore, our findings may largely have been due to a reflex from activation of vagal C fibers. They may also indicate that the excitation caused by Aδ-fibers can be overridden by the inhibition evoked by vagal C fibers.

Our data suggest that the increase in Rf evoked by capsaicin was mainly due to shortening of T1. This increase in Rf and decrease in the phrenic amplitude may correspond to the rapid shallow breathing seen in spontaneously breathing animals (15). Positive-pressure ventilation of the lung may restrain the increase in Rf and laryngeal resistance (20). This was displayed in our present data, showing a significant increase in T1 and minor augmentation of TA activity after the bilateral vagotomy. Because the difference in onset between RLN activity and PNA was computed in the same animal, this restraint might be similar for both nerves and may have produced little effect on our data. However, our data may have been underestimated, as the lung volume produces greater inhibition of the upper airway motor activity than that on the bulbospinal phrenic system as seen in the cat (12).

Reduced glottal dilation with capsaicin administration. The abducent branch and SLN innervate the PCA and CT muscles, respectively. Activity of the PCA and CT muscles abduct the aperture of the glottis (1). Thus capsaicin administration may reduce the glottal aperture to restrict the airflow by decreasing the activities of the laryngeal adductors. Our present findings indicate that capsaicin administration may delay the opening of the glottis, shorten the duration of its opening, and result in a significant decrease in T1. Thus the behavior of the vocal cords and a shortening of T1 after capsaicin administration would minimize entry of gaseous irritants into the airway and lungs.

Induction of glottal closure by capsaicin administration. Activation of the laryngeal adductors is known to move the vocal cords closer to the midline of the glottis during expiration. Our present data, showing enhancement of TA branch activity during apnea and recovery from apnea after capsaicin administration, suggest that a strong narrowing of the glottis is brought on by contraction of the laryngeal adductors, thus increasing the laryngeal resistance to expiratory airflow. In this regard, our data are compatible with those of Stransky et al. (26) and Palecek et al. (21). Moreover, laryngeal responses were totally abolished after the bilateral vagotomy (Figs. 1, C and D, and 2, C and D), suggesting that this response was mainly mediated via vagal C fibers. One implication of our results for activation of PCFs by gaseous irritants such as environmental or chemical smoke (10, 13) is that excitation of the TA branch activity may protect the airway and lungs from further insult by gaseous irritants. This protective function of the TA branch in response to capsaicin administration of activating vagal afferents is similar to the diving reflex (7).

Capsaicin-induced commencement of TA branch activity during inspiration has not been previously reported in the rat. This could help interpretation of our laboratory’s recent report (18), in which we found a higher inspiratory activity of the entire RLN compared with the control during recovery from apnea. Increases in the expiratory activity of the TA branch may enhance the activity of the TA muscle resulting in a higher contractile force, which moves the vocal cords closer to the midline of the glottis or even completely closes it. Direct evidence for glottal closure was based on our observation under the microscope of one rat, which showed a tightly closed glottis after capsaicin administration (unpublished data). Recently, Praud and colleagues (8, 22, 23) reported that active closure of the larynx during apnea in lambs was originated centrally. Our present study on reflex-induced laryngeal responses indicates that the regulatory mechanisms for the glottal closure induced by capsaicin administration are likely processed through the central nervous system, although the exact pathway is still not well established.

Glottal closure by cooperation of the abducent and TA branches of the RLN. Glottal aperture is modulated by vocal cord movement, which is determined by rotation of the arytenoids and contraction of the laryngeal muscles (1). Ventrolateral rotation elicited by contractions of the TA muscles moves the vocal cords toward the midline, thus resulting in glottal adduction. In contrast, ventromedial rotation of the arytenoids induced by contractions of the PCA muscles widens the vocal cords, thus initiating glottal abduction. This rotation of the arytenoids, a fulcrumlike action, works in concert with contraction of the laryngeal muscles to move the vocal cords and regulate the aperture of the glottis during the respiratory cycle. Our data showing a decrease in the amplitude and a delay in onset of the abducent branch in combination with an increase in activity and an earlier onset of the TA branch would help regulate glottal closure, suggesting that a coordinated mechanism in the central nervous system is likely activated by inputs of vagal C and Aδ-fibers. Whether this cooperative regulation is mediated by a glycnergic mechanism as shown in trigeminal stimulation (7) is unclear.

Physiological implications. Our data strongly suggest that glottal closure was evoked by capsaicin administration. The pulmonary chemoreflex induced by capsaicin administration has been considered to be beneficial in protecting the airway and lungs (3, 4). Glottal closure, as shown in this study, in combination with apnea provides a highly efficient means for minimizing the effects of gaseous irritants on the airway and lungs. Recently, Lara et al. (14) proposed that adduction of the glottal aperture could maintain a larger lung volume, which may facilitate gaseous exchange. Our present study provides evidence that cooperation of the abducent and TA branches is critical for glottal closure. Taken together, excitation of the intralaryngeal TA branch of the RLN in combination with inhibition of the abducent branch and other abducent motor nerves after capsaicin administration might help gaseous exchange during transient apnea and prevent further insult to the respiratory pathway from gaseous irritants. Thus our data reveal physiological implications of a mechanism for defending the respiratory system when vagal afferents are stimulated.

In conclusion, capsaicin administration provokes a vagally dependent increase in the TA branch of the RLN but decreases in both SLN and abducent branch activities. Harmonic cooperation of an increase in the TA branch activity with reductions in abducent branch and SLN activities in response to capsaicin administration produces a narrowing of the laryngeal aperture.
or even closure of the glottis, which may be beneficial to the respiratory system when confronted with gaseous irritants.

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