Capsaicin-induced activation of pulmonary vagal C fibers produces reflex laryngeal closure in the rat

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Lu, I-Jung, Kun-Ze Lee, and Ji-Chuu Hwang. Capsaicin-induced activation of pulmonary vagal C fibers produces reflex laryngeal closure in the rat. J Appl Physiol 101: 1104–1112, 2006.—To gain further insights into the coordination between laryngeal abduction and adduction, we recently reported that intravenous administration of capsaicin to induce activation of pulmonary C fibers (PCFs) initiates the pulmonary chemoreflex, characterized by apnea, hypotension, and bradycardia, and a concomitant enhancement of activities of the entire RLN during the apneic period and the period of inspiration and expiration when recovering from the apnea (22). The increase in RLN activity is mostly due to excitation of intralaryngeal TA branch activity of the RLN and is totally abolished after a bilateral vagotomy, suggesting mediation by vagal afferents (22, 23). This excitation reflects an augmentation of the amplitude as well as an advance in the onset of intralaryngeal TA branch activity from the expiratory period to the inspiratory stage (see Fig. 3 of Ref. 23). In a coordinated manner with this excitation of the adductor, the activity of the Abd RLN decreases in amplitude, and its onset is delayed. These results strongly suggest that the activity of the TA muscle, one of the adductors, may increase the likelihood of causing adduction of the vocal folds or even closing of the glottis. Increased activities of the TA during apnea have been shown in pulmonary edema caused by halothane inhalation (8, 9), which is vagal C-fiber dependent, in lambs. Thus our first aim was to confirm whether TA electromyographic (EMG) activity is increased by intravenous administration of capsaicin to induce activation of PCFs. Specifically, we focused on whether the TA EMG might advance such that it discharges during inspiration.

Laryngeal adduction is a component of the pulmonary chemoreflex and laryngeal chemoreflex. The laryngeal chemoreflex can be induced by local stimulation of the laryngeal mucosa or intravenous infusion of substance P (2, 3, 4, 30). It can also be triggered by laryngeal exposure to wood smoke (21) or a femoral injection of capsaicin (16). Laryngeal adduction caused by increases in TA EMG activities result in increases in laryngeal resistance (27, 28) and/or increases in subglottal pressure (SGP) (10, 29). Hence, our second aim was to confirm whether SGP increases under a situation of laryngeal adduction evoked by intravenous administration of capsaicin to induce activation of PCFs. To gain further insights into the coordination between laryngeal abductor and adductor muscles, Therefore, the best way to determine how reflexive laryngeal constriction depends on PCF activation caused by intravenous administration of capsaicin would be to directly observe the movement of the vocal folds or the glottis. To examine whether the glottis is tightly closed by intravenous administration of capsaicin, we focused on the TA activity, which is one of the abductors, may increase the likelihood of causing adduction of the vocal folds or even closing of the glottis. Increased activities of the TA during apnea have been shown in pulmonary edema caused by halothane inhalation (8, 9), which is vagal C-fiber dependent, in lambs. Thus our first aim was to confirm whether TA electromyographic (EMG) activity is increased by intravenous administration of capsaicin to induce activation of PCFs. Specifically, we focused on whether the TA EMG might advance such that it discharges during inspiration. Laryngeal adduction is a component of the pulmonary chemoreflex and laryngeal chemoreflex. The laryngeal chemoreflex can be induced by local stimulation of the laryngeal mucosa or intravenous infusion of substance P (2, 3, 4, 30). It can also be triggered by laryngeal exposure to wood smoke (21) or a femoral injection of capsaicin (16). Laryngeal adduction caused by increases in TA EMG activities result in increases in laryngeal resistance (27, 28) and/or increases in subglottal pressure (SGP) (10, 29). Hence, our second aim was to confirm whether SGP increases under a situation of laryngeal adduction evoked by intravenous administration of capsaicin to induce activation of PCFs. Specifically, we focused on whether the TA EMG might advance such that it discharges during inspiration.

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venous administration of capsaicin to induce activation of PCF, we took motion pictures with a digital camera to trace the movement of the vocal folds before, during, and after intravenous administration of capsaicin. Our data showed that in spontaneously breathing rats, increases in the TA EMG as a consequence of intravenous administration of capsaicin caused the vocal folds to move adductively, resulting in the full closure of the glottis as indexed by an increase in the SGP.

MATERIALS AND METHODS

Animal preparation. Thirty-five male Wistar rats (495 ± 17 g) were used. They were purchased from the Animal Center of the Medical School of National Taiwan University. They were housed in a room kept at 25°C with access of water and food ad libitum. Experiments were performed under experimental protocols approved by the Animal Care and Use Committee of National Taiwan Normal University.

Five of these animals were used in the study of EMG activity, and in 2 of the 15, the RLN was bilaterally sectioned. Thirteen rats were used for the study of the SGP, and the RLN of three of these animals were bilaterally sectioned. Of the remaining seven rats used for the observation of vocal fold movements, five were used for image analysis of the motion picture of the vocal fold movement.

Rats were weighed and treated with atropine (0.5 mg/kg IM) on the day of the experiment and were anesthetized with urethane (1.2 g/kg ip). A tracheotomy was performed with the animal in a supine position. Catheters were placed in the right femoral artery and vein for blood pressure (BP) measurement and drug administration, respectively. A third catheter was positioned close to the right atrium via the jugular vein. Spontaneous breathing was maintained through tracheal tubing. The end-tidal fractional concentration of carbon dioxide was continuously monitored with a CO₂ analyzer (Electrochemistry, CD3A). The body temperature was maintained at 37~38°C with a heating blanket or lamp.

EMG monitoring. The diaphragm was dissected via a ventrolateral approach by making an incision at the level of the transverse abdo-

minis muscle. A bipolar electrode was then inserted until it made contact with the diaphragm. A good contact was indicated by the display of the diaphragmatic EMG. Thereafter, the shaft of the electrode was secured using a small hemostat. In some studies, the diaphragmatic EMG was monitored using a pair of stainless steel wires (A-M system, #7935), which was sutured onto the diaphragm. Diaphragmatic EMG activity was amplified (Grass AC preamplifier P5111F, Quincy, MA), filtered (0.3~3 kHz), and recorded (see below). The initial incision was then sutured.

By removing most of the tongue and surrounding tissues, the larynx could be observed under a surgical dissecting microscope (Wild). With the aid of the microscope, a pair of electrodes made up of two could be observed under a surgical dissecting microscope (Wild). The initial incision was then sutured.

Monitoring of the movement of the vocal folds. The movement of the vocal folds was observed and monitored with a digital camera (Sony, W1) under a surgical microscope (Wild) before, during, and after intravenous administration of capsaicin. After the microscope was focused to observe the vocal folds, the digital camera was mounted on a tripod, and the lens was adjusted next to the eyepiece of the surgical microscope. The movement of the vocal folds was continuously recorded and stored in a memory stick (Sony memory stick PRO 512 MB) during the experiment for offline image analysis.

Experimental protocol. Three protocols were conducted in the present study. In the first protocol (n = 15), the TA EMG was examined in response to intravenous administration of capsaicin. Two doses of capsaicin, 0.625 and 1.25 μg/kg, were randomly adminis-

tered via an integrator (with a time constant of 0.05 s), monitored, recorded, and TTOT (the sum of TI and TE) were computed from tracings of the diaphragmatic EMG before and after intravenous administration of capsaicin.

Drug preparation. Capsaicin (Tocris, Bristol, UK) was freshly prepared during each experiment by dissolving 5 mg in a mixture of 1 ml of 95% ethanol and 1 ml of Tween 80 (23). This solution was then diluted with saline (pH 7.4) to make a volume of 10 ml. Thus a stock capsaicin solution with a concentration of 500 μg/ml was obtained. This stock capsaicin solution was further diluted with saline to make a solution of 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 and statistical analysis. Data were directly retrieved from the hard disk and analyzed with software written in visual C++. EMG amplitudes or the SGP of 20 consecutive respiratory cycles before intravenous administration of capsaicin were determined and aver-

aged as the control. Experimental data of the EMG amplitudes or SGP after intravenous administration of capsaicin were analyzed breath-by-breath for 15 respiratory cycles. Additional analysis of the EMG amplitudes and SGP was also completed by averaging 10 breaths at the end of the first, second, and third minutes after capsaicin treatment to determine if the responses had fully recovered from the effect of capsaicin. Data of the diaphragmatic and TA EMGs and SGP were further transformed into a percent of the control. T₁ (the period for diaphragmatic inspiration), T₉ (the period between diaphragmatic EMG readings), and T RouteServiceProvider (the sum of T₁ and T₉) were computed from tracings of the diaphragmatic EMG before and after intravenous

J Appl Physiol • VOL 101 • OCTOBER 2006 • www.jap.org
administration of capsaicin. The mean BP and HR before and after intravenous administration of capsaicin were analyzed using the Data pad module of the PowerLab system.

Data for the motion picture taken with the digital camera were stored in the hard disk of a personal computer. The motion pictures were retrieved with Flash MX 2004 (Macromedia, San Francisco, CA) and analyzed by clicking the frame on the timeline to read the image of individual static pictures displayed on the worksheet. Each static image picture displayed on the worksheet, analyzed frame by frame, represents the instantaneous position of the vocal folds as well as the extent of glottal widening or narrowing during the respiratory cycle. Hence, we were able to correlate the image picture with the events such as changes in the diaphragmatic EMG and SGP. The widest aperture of the glottis represents the image at the end of inspiration, whereas the narrowest opening represents the image at the end of expiration. Thus images of glottal aperture during inspiration and expiration and before and after intravenous administration of capsaicin could be captured by PhotolImpact (Ulead, Taipei, Taiwan) and then correlated with the recorded tracings of the diaphragmatic EMG and fluctuations of the SGP.

To quantify the glottal area, the glottal images with full abduction and adduction captured by PhotolImpact were dragged into the worksheet of the PhotoShop (Adobe, San Jose, CA). After the free edges of the vocal folds in each image were traced (24), the number of pixels encompassed by the vocal fold edges was then displayed in the histogram dialog box. Changes in the glottal area during respiratory cycle and in response to intravenous administration of capsaicin could be calculated by comparing the number of pixels encompassed by a reference area taken simultaneously with the images.

Multiple comparisons test was performed by two-way ANOVA (32) for repeated measures. Bonferroni test was then performed to examine the significant differences of responses to capsaicin treatment from the control by SPSS version 13.0. A level of 0.0025 (0.05/20) was determined as significant criterion. Data are expressed as the means ± SE.

RESULTS

Cardiopulmonary responses to intravenous administration of capsaicin. Following intravenous administration of capsaicin, most of the animals immediately exhibited apnea (n = 23; Fig. 1A), whereas some rats displayed shallow breathing (n = 5). Regardless of whether the animal displayed apnea or shallow breathing, the diaphragmatic EMG was always decreased for the first respiratory cycle. Thus data on the diaphragmatic EMG was 84% of the control (Fig. 2A, \( P = 5.83 \times 10^{-4} \)) with low-dose capsaicin. However, the decrease in the first diaphragmatic EMG caused by high-dose capsaicin was not statistically significant (Fig. 2A, \( P = 0.105 \)).

![Fig. 1. Cardiopulmonary responses to capsaicin administration in one of the observed rats.](http://jap.physiology.org/)
Concomitant with the respiratory responses, intravenous administration of capsaicin also resulted in immediate decreases in the blood pressure and heart rate (Fig. 1A). The blood pressure was 94.38 ± 2.46 mmHg before capsaicin and was reduced by 17.94 ± 2.15 (P = 6.94 × 10⁻⁵) and 23.6 ± 2.77 mmHg (P = 1.21 × 10⁻⁷ compared with control by 2-way ANOVA), respectively, with the low and high doses of capsaicin. The heart rate was 402.19 ± 10.49 beats/min before capsaicin and was decreased by 257 ± 20.62 (P = 1.73 × 10⁻⁷) and 309.25 ± 14.37 beats/min (P = 1.21 × 10⁻⁷ compared with control by 2-way ANOVA), respectively, in response to low- and high-dose capsaicin.

In response to the low and high doses of capsaicin, the periods for shallow breathing seen in five animals were 0.86 ± 0.02 and 1.82 ± 0.58 s, respectively. After recovery from the shallow breathing, diaphragmatic EMG readings for the first respiratory cycle were 43.39% (P = 0.001) and 39.21% (P = 0.001) compared with control; values of T_I were 0.17 and 0.15 s and those of T_E were 0.13 and 0.85 s, respectively, with the low and high doses of capsaicin.

Administration of vehicle or saline (data not shown) produced no effect on the diaphragmatic EMG or respiratory patterns.

Increases in TA EMG with intravenous administration of capsaicin. Baseline TA EMG activity occurred during T_E and commenced immediately following the decrease in the diaphragmatic EMG and then decreased gradually to the baseline (Fig. 1Ba). In response to intravenous administration of capsaicin, the TA EMG immediately advanced at the onset and rose to a peak during apnea (Fig. 1, A and Bb). This increase in the TA EMG remained at an elevated level during T_I and T_E even after having recovered from the apnea (Fig. 1, A and Bb), and thus the phasic activity of the TA EMG was transformed into a continuously discharging pattern (Fig. 1A). This continuous TA EMG was characterized by notchlike activity during diaphragmatic bursts (Fig. 1Bc), which meant that the TA EMG did not fully return to the baseline (Fig. 1Bd). However, this notchlike activity totally disappeared whenever an augmented breath occurred (Fig. 3). The percentage increase in the TA EMG during T_I after capsaicin treatment was not quantified due to lack of the TA EMG during T_I before intravenous administration of capsaicin.

In grouped data, average increases in the TA EMG were 286% and 332% (Fig. 4A, P = 9.54 × 10⁻⁵ and 3.28 × 10⁻⁵) of the control during apnea, and 230% and 276% of the control (Fig. 4B, P = 1.75 × 10⁻⁴ and 1.76 × 10⁻³) for the first respiratory cycle following apnea with the low and high doses of capsaicin, respectively. The mean TA EMG increase after recovery from apnea remained significant for at least nine respiratory cycles before returning to the control level (Fig. 4B, P = 1.38 × 10⁻³ at the 9th breath with low-dose capsaicin and P = 1.3 × 10⁻⁴ at 10th breath with high-dose capsaicin). This capsaicin-induced increase in the TA EMG was totally abolished after bilateral sectioning of the RLN, demonstrating an RLN-specific response, which disappeared after a bilateral vagotomy, indicating that this response is mediated vagally (data not shown). Regardless of whether RLN was intact or sectioned, intravenous administration of the vehicle evoked no effect on the TA EMG (n = 2).

Increases in the SGP with intravenous administration of capsaicin. SGP usually fluctuates with the respiratory cycle. As seen in Fig. 1, A and B, the SGP decreased during periods with diaphragmatic EMG and increased during periods without diaphragmatic EMG activity (Fig. 1Ba). The difference in this fluctuation was 0.82 ± 0.41 cmH₂O in the present study. Following intravenous administration of capsaicin, this fluctuation in the SGP peaked during the beginning of apnea (Fig. 1,
A and Bb) and remained high after recovery from apnea for several respiratory cycles and then gradually returned to the control level (Figs. 1, A and Bd, and 5B). Average increases in the SGP were 3.850% and 3.800% of the control during the apneic period (Fig. 5A, P = 2.73 × 10^{-4} and 2.100% and 2.350% of the control for the first respiratory cycle (Fig. 5B, P = 6.68 × 10^{-4}) in response to the low and high doses of capsaicin, respectively. This rise in the SGP following intravenous administration of capsaicin remained at a significant level for 5 breaths (Fig. 5B, P = 1.72 × 10^{-3} for low-dose capsaicin and P = 4.11 × 10^{-4} for high-dose capsaicin); the SGP then slowly returned to the control level. The capsaicin-induced increase in the SGP was abolished after bilateral sectioning of the RLN (data not shown) or a bilateral vagotomy (data not shown). Intravenous administration of vehicle produced no changes in the SGP.

Glottal closure in response to intravenous administration of capsaicin. With the aid of a microscope and a digital camera, we observed and recorded the movement of the vocal folds during the respiratory cycle. Data from one of these three animals studied, from which the vocal fold movement was simultaneously monitored with the diaphragmatic EMG and SGP, were presented in Fig. 6. It appears that the glottis widens (or abduction of the vocal folds) during inspiration (Insp) and narrows (or adduction) during expiration (Exp) (Fig. 6Ca). Concomitant with the widening of the glottis (or the abduction of the vocal folds) were decreases in the SGP and increases in diaphragmatic EMG activity (Fig. 6Ba). Likewise, narrowing of the glottis (or adduction of the vocal folds; Exp in Fig. 6Ca) was associated with an increase in the SGP (Fig. 6Ba) during expiration as indexed by an absence of diaphragmatic EMG activity (Fig. 6Ba). Intravenous administration of capsaicin (the high dose in this example) resulted in marked adduction of the vocal folds during the respiratory cycle, leading to tight closure of the glottis during apnea (Fig. 6Cb, left), which correlated with enhanced fluctuation of the SGP (Fig. 6Bb, also Fig. 6Ba). In the particular animal shown in Fig. 6Ba, the fluctuation of the SGP during apnea caused by capsaicin was so high that its peak response could not be observed with the original setting of the amplifier (Fig. 6Ba). To display this peak response, the gain of the amplifier was reduced (Fig. 6Bb). However, this adjustment in the gain of the amplifier had one pitfall—the fluctuation of the SGP before capsaicin could not be observed (Fig. 6Ba). This tight glottal closure caused by capsaicin was observed...
under the surgical microscope in the first two animals and was later recorded from five animals by taking motion pictures with a digital camera. The widening and narrowing of the glottis were recorded on the disk, and the images captured from the motion pictures were analyzed. The glottal widening and narrowing was transient and remained for a very short period of time. This behavior of repeated opening (widening) and closing (narrowing) of the glottis (Fig. 6C) as reflected by fluctuations in the SGP (Fig. 6Cb) was maintained for at least 1 min before completely returning to the control level as shown Fig. 6A.

The glottal area before and after intravenous administration of capsaicin was quantified from these five animals to examine how many changes in the glottal aperture were in response to PCF activation by capsaicin. Due to variation in the glottal area before intravenous administration of capsaicin was well documented (7, 13, 17–21, 28). Apnea and reflex laryngeal adduction during recovery from apnea for the first and the subsequent breaths before completed recovery. It seems that the glottal area is immediately recovered from tight closure during apnea because of the insignificance.

**DISCUSSION**

The main finding of the present study is that the glottis tightly closes in response to intravenous administration of capsaicin. This tight glottal closure was due to the large increase in TA EMG activity resulting in adduction of the vocal folds as indexed by the marked increase in the SGP. Direct evidence of glottal closure following intravenous administration of capsaicin was provided by images taken with a digital camera. This sequence of respiratory events may be critical for protecting the airways and lungs when an animal is exposed to environmental gaseous irritants.

Cardiopulmonary responses to intravenous administration of capsaicin. The cardiopulmonary chemoreflex (apnea and decreases in the blood pressure and heart rate) induced by intravenous administration of capsaicin has been well documented (7, 13, 17–21, 28). Apnea and reflex laryngeal adduction can also be induced by stimulations applied to the larynx (3, 4, 28) or by inhaled wood smoke (17, 21).

Bolus administration of capsaicin to the right atrium via the right jugular vein has been demonstrated to activate pulmonary vagal C and Aδ-fibers (6, 14). Activation of pulmonary Aδ-fibers has been reported to evoke an increase in respiration, whereas activation of vagal C fibers has been reported to produce a decrease in respiration (6, 14, 26). Thus the cardiopulmonary chemoreflex following intravenous administration of capsaicin may be due largely to activation of pulmonary vagal C fibers. However, the excitatory effect caused by activation of Aδ-fibers may have dampened the inhibitory effect caused by activation of C fibers and might partly explain why decreases in the PNA induced by the high dose of capsaicin (Fig. 2A) were smaller than those induced by the low dose of capsaicin. The small decrease in the diaphragmatic EMG activity caused by the high dose of capsaicin may partly have been due to an excitatory influence derived from activation of nonvagal C fibers (22).

TA EMG excitation by intravenous administration of capsaicin. Our present data showing continuous activity of the TA EMG following intravenous administration of capsaicin were
very similar to previous reports (10, 15, 12–27) and also to the laryngeal chemoreflex (3, 4). However, differences exist in the response between our present findings and these previous reports. First, the TA EMG activity during apnea following intravenous administration of capsaicin was highly augmented and then slightly reduced to remain at a sustained activity level with notchlike activity during TI before gradually returning to the control level, and second, the TA EMG activity advanced to commence earlier during inspiration. This earlier onset of the TA EMG might have caused the existence of TA activity during TI and the transformation of the phasic TA EMG into a continuous discharge pattern. This response profile was well matched with our recent observation of the intralaryngeal TA branch of the RLN to intravenous administration of capsaicin (23). This pattern of augmented TA EMG indicated that tight closure of the glottis during apnea was probably evoked by intravenous administration of capsaicin. This notion was supported by the current observation of vocal fold movements as shown in Fig. 6Bb.

The mechanism for glottal closure in response to PCF activation caused by capsaicin is still unknown. Signals of PCF activation must be processed within the central nervous system. In this regard, Bauman and Wang (5) recently reported that microinjection of a vasoactive intestinal peptide and neurokinin B into the nucleus tractus solitarius, an area that receives signals from vagal C fibers, can produce apnea and a sustained or phasic increase in the TA EMG. Dutschmann and Paton (10) found that administration of strychnine, which blocks glycine receptors, produces a shift in glottal adduction from early expiration to inspiration. Our data were well compatible with the report regarding the sustained increase in the TA EMG (5) and the shift in glottal adduction during inspira-

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Fig. 6. Images of vocal fold movement and glottal aperture. Intravenous administration of capsaicin produced a decrease in the BP, apnea, and an increase in the SGP (A; slow-speed recording). After extending the recording to a faster recording speed (5 times that used in A), fluctuations in the SGP following the respiratory cycle were evident (Ba). During normal breathing, the vocal folds were widened or abducted (Ca; Insp) to a mean aperture of 2.89 ± 0.28 mm² (D) that correlated with a decrease in SGP during inspiration (Insp) (Ba); they were then adducted to a small aperture (Ca; Exp) with a mean value of 1.76 ± 0.24 mm² (D) that correlated with an increase in SGP during expiration (Ba; Exp). Vocal folds were completely closed (Cb, left), which was associated with a large increase in the SGP (Bb) during apnea following intravenous administration of capsaicin (high dose of capsaicin in this example). During recovery from apnea, the vocal folds were abducted to an aperture of 1.21 ± 0.20 mm² (D; P = 0.0127 compared with control before capsaicin) and then adducted to 0.26 ± 0.07 mm² in average (D; P = 0.008 compared with control before capsaicin) in the first breath (Cb, middle and right), and then gradually recovered as seen in Cc together with a smaller fluctuation of SGP in Bc.
tion. Moreover, this shift in onset of the TA EMG was temporarily prevented with the occurrence of augmented inspiration and then shifted back to being advanced as shown in Fig. 3. On the basis of our present data, the inhibition probably impinged on the TA motoneurons during inspiration and was released by signal inputs of PCF activation caused by intravascular administration of capsaicin. Whether this inhibitory mechanism during TI is mediated or modulated by glycinergic the nouse administration of capsaicin. Whether this inhibitory released by signal inputs of PCF activation caused by intrave-
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Fig. 3. On the basis of our present data, the inhibition probably 
with the control before intravenous administration of capsaicin (Fig. 5A). This large increase in the SGP strongly suggested 
resulting in the immediate response compared with the control before intravenous administration of capsaicin (Fig. 5A). This large increase in the SGP strongly suggested 
that the glottis was tightly closed when PCFs were activated by intravascular administration of capsaicin. 
The increase in the SGP has been used as one of the parameters to determine whether glottal closure has occurred (11). To examine changes in the glottal aperture during the respiratory cycle following intravascular administration of capsaicin, we inserted a short piece of polyethylene tubing into the trachea at a level below the larynx and another piece on the other side of the trachea through which the animal could spontaneously breathe. We then passed air through the larynx to simulate expiratory airflow. We first used a very small airflow rate and then increased the flow rate in a stepwise manner to a level just strong enough to evoke a conspicuous SGP fluctuation (Fig. 1A, left). Thus the SGP we observed might have been overestimated and much higher than normal. Nevertheless, the relative changes in the SGP before and after intravascular administration of capsaicin should still reflect the relative changes in the glottal aperture. 
Glottal closure during TA EMG excitation by intravenous administration of capsaicin. Vocal fold movements in human subjects have been observed using a laryngoscope connected to the endoscopic lens, and images were recorded on videotape (13). Unfortunately, a laryngoscope small enough for use in a rat is not available. To observe the change of the glottal aperture, we used a digital camera to observe movements of the vocal folds during the respiratory cycle. It looks very clear (Fig. 6) for taking the images with a digital camera. The only precaution is that one needs to make sure that the vocal folds, the shaft of the microscope, and the digital camera are aligned properly. 
From the results of the image analysis, the aperture of the glottis was tightly closed during apnea, which was correlated with the increase in the SGP and TA excitation following intravascular administration of capsaicin. The glottal aperture was partially abducted and showed 40% of the control during first inspiration during recovering from apnea. This may be due to the decrease in activity of the intralaryngeal abducted branch of the RLN (23), which innervates the adductor. The degree of glottal adduction was reduced to a level of <20% of the control during first expiration during recovery from apnea. The large reduction of the glottal aperture during expiration may be ascribed to the great excitation of the TA EMG and of the adductant branch of the RLN (23) evoked reflexively by intravenous administration of capsaicin. This excitation of the TA EMG was reflected in its amplitude and advanced onset from expiratory stage to inspiration (Fig. 1B). The large glottal reduction might contribute to the large fluctuation of the SGP (Fig. 1, A and Bb). Unfortunately, this large reduction of the glottal area during the first breath recovery from apnea was insignificant. It could be due to the small number of animals with data available for analysis (n = 5). Nevertheless, the tight closure of the glottal response to intravenous administration of capsaicin may provide immediately and reflexively defensive mechanism for the airways and lungs on exposure to irritants. This reflexive defensive mechanism would depend on well-coordinated activities between the laryngeal abductor (or adduc tant branch of the RLN) and the adductor (or adductor branch of the RLN) through the central nervous system. 
In conclusion, intravenous administration of capsaicin produces increases in TA EMG activity, resulting in movement of the vocal folds and subsequent closure of the glottis during apnea as well as a small glottal aperture after recovery from apnea. This tight closure of the glottis was indirectly evidenced by the increase in the SGP and directly recorded as images obtained from a digital camera. These responses are vagally mediated and might serve as a protective mechanism when the airway and lungs are exposed to gaseous irritants. 

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REFERENCES


