Effect of inhaled carbon dioxide on laryngeal abduction

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Cheetham J, Jones A, Martin-Flores M. Effect of inhaled carbon dioxide on laryngeal abduction. *J Appl Physiol* 118: 489–494, 2015. First published December 18, 2014; doi:10.1152/japplphysiol.00469.2014.—Hypercapnia produces a profound effect on respiratory drive and upper airway function to maintain airway patency. Previous work has evaluated the effects of hypercapnia on the sole arytenoid abductor, the posterior cricoarytenoid (PCA), using indirect measures of function, such as electromyography and direct nerve recording. Here we describe a novel method to evaluate PCA function in anesthetized animals and use this method to determine the effects of hypercapnia on PCA function. Eight dogs were anesthetized, and a laryngeal mask airway was used, in combination with high-speed videendoscopy, to evaluate laryngeal function. A stepwise increase in inspired partial pressure of CO₂ produced marked arytenoid abduction above 70-mmHg end-tidal CO₂ (ETCO₂) (P < 0.001). Glottic length increased above 80-mmHg ETCO₂ (P < 0.02), and this lead to underrepresentation of changes in glottic area, if standard measures of glottic area (normalized glottic gap area) were used. Use of a known scale to determine absolute glottic area demonstrated no plateau with increasing ETCO₂ up to 120 mmHg. Ventilatory parameters also continued to increase with no evidence of a maximal response. In a second anesthetic episode, repeated bursts of transient hypercapnia for 60 s with an ETCO₂ of 90 mmHg produced a 43–55% increase in glottic area (P < 0.001) at or shortly after the end of the hypercapnic burst. A laryngeal mask airway can be used in combination with videendoscopy to precisely determine changes in laryngeal dimensions with high temporal resolution. Absolute glottic area more precisely represents PCA function than normalized glottic gap area at moderate levels of hypercapnia.

CARBON DIOXIDE IS THE MAIN STIMULANT OF VENTILATORY DRIVE IN MAMMALS. Central chemoreceptors detect increases in hydrogen ion concentration in the cerebrospinal fluid and produce an elevation in alveolar ventilation (5, 17). Hypercapnia also stimulates a vagal reflex, increasing respiratory rate (f) and tidal volume (VT) (17). These changes produce stimulation of upper airway muscles to increase airway patency (4, 11, 31) and reduce resistance to airflow (3, 8). The only upper airway muscle to produce dilation of the rima glottidis is the posterior cricoarytenoid (PCA) muscle (3, 4, 7, 25, 31), which abducts the arytenoid cartilages during inspiration.

Prior investigations into the relationship between hypercapnia and laryngeal function have demonstrated an increase in inspiratory PCA abductor activity with progressive hypercapnia (31). This increase is synchronous with increases in diaphragmatic activity and VT (11, 31). Under these hypercapnic conditions, a steady-state maximal response is reached more rapidly in the PCA than in other airway dilating muscles, such as the genioglossus (10, 11, 31).

In addition to these central mechanisms, direct stimulation of luminal laryngeal chemoreceptors by CO₂ produces an excitatory response similar to that produced by negative upper airway pressure (31). The majority of this work has used electromyography (EMG) to determine PCA activity (4, 7, 10, 11, 21). In this study, we describe a novel technique that avoids invasive instrumentation, so reducing the possibility of instrumentation affecting response.

We compare the effects of increasing hypercapnia on laryngeal abduction using a novel endoscopic method in anesthetized dogs. We use a canine model, as prior studies have elucidated the relationship between hypercapnia and laryngeal function in this species (1, 5, 18, 26, 31), and the canine larynx is a widely accepted preclinical animal model for human laryngeal disease (16, 25). This approach could also be used to assess laryngeal function longitudinally after experimental nerve injury or following reinnervation of the abductor muscles using nerve graft (2, 30).

We hypothesized that the response of the PCA muscle to increasing end-tidal carbon dioxide (ETCO₂) would reach a plateau, and that the response to a transient burst of hypercapnia at a specified level would be highly repeatable. The goal of this study was to determine the laryngeal and ventilatory responses to increasing levels of ETCO₂, and to determine the repeatability of the response to repeated short bursts of inhaled CO₂.

MATERIALS AND METHODS

Ethics Statement

This study was performed in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals, the National Institutes of Health guide for Care and Use of Laboratory Animals, federal and state regulations, and was approved by the Cornell University Institutional Animal Care and Use Committee. Animals were brought into the research unit and given a 7-day acclimatization period before any procedure. Daily record logs of medical procedures were maintained. Cages with elevated floors were cleaned daily and disinfected biweekly. The animals were fed twice a day to maintain proper body condition and allowed water ad libitum. Group housing provided socialization and ample space for exercise. At the conclusion of the study, dogs were adopted out through local Center for Animal Resources and Education procedures. ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines for reporting in vivo experiments were used throughout (15).

Animals and Instrumentation

Eight female Beagle dogs (age 7–9 yr, body weight 9.9 ± 0.6 kg, range 8.4–11.8 kg), with no history of upper airway disease and normal laryngeal function, determined endoscopically, were used. Dogs were fasted for at least 6 h before anesthesia. At the conclusion of each procedure, dogs were monitored for 1 h before returning to their group housing.

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Dogs were anesthetized in two discrete episodes separated by at least 7 days. After fasting, each dog was sedated with dexmedetomidine (2 μg/kg iv). Anesthesia was induced with isoflurane in oxygen via face mask and maintained at a constant end-tidal isoflurane concentration (~1 minimum alveolar concentration [MAC]; 1.3%), with a constant infusion rate of dexmedetomidine (2 μg·kg⁻¹·h⁻¹ iv) maintained thereafter. These anesthetic agents were selected based on data showing their minimal effect on suppressing spontaneous ventilation and laryngeal function (14, 27). Ondansetron, an antiemetic, was administered 0.2 mg/kg iv 5–10 min before the induction of anesthesia.

Following induction of anesthesia, animals were instrumented with a laryngeal mask airway (LMA; size 3, AES, Black Diamond, WA), placed orally to seal the rima glottis and connected via a three-way adapter to an anesthetic breathing circuit (32). The cuff of the LMA was inflated to ensure a secure seal around the rima glottidis during ventilation.

Laryngeal function was observed with a high-definition videendoscope (3 mm outer diameter, FLEX-XC, Karl Storz, Tuttinglen, Germany) placed through the free port of the three-way adapter into the lumen of the LMA and advanced just anterior to the larynx.

A respiratory profile monitor (Novametrix NICO Non-Invasive Cardiac Output Monitor Model 7300) was interfaced between the three-way adapter and the anesthetic breathing circuit for measurement of ventilatory variables: the f, \( ETCO_2 \), inspiratory \( V_T \), peak inspiratory \( (PFI) \) and expiratory flow \( (PF E) \). Inspired \( (V_I) \) and expired minute ventilation \( (V_E) \) were calculated from inspiratory and expiratory \( V_T \) \( (V = f \cdot V_T) \), where \( V \) is volume.

Animals were allowed to ventilate spontaneously and received lacted Ringer solution (~5 mL·kg⁻¹·h⁻¹ iv) during anesthesia. Routine anesthetic monitoring (ECG, oscillometric arterial blood pressure, pulse oximetry, capnography, temperature, and inspired and expired alveolar concentrations) was used in all animals. Following induction of anesthesia, the level of expired anesthetic agent (isoflurane) was measured in all dogs and maintained at 1.3% (1 MAC). The vaporizer was adjusted to maintain this anesthetic plane. Animals were allowed to breathe spontaneously for at least 15 min before perturbation.

Response to increasing levels of \( ETCO_2 \). After a stable plane of anesthesia was reached, a 1-min videendoscopic recording of the larynx during spontaneous ventilation was obtained with the videendoscope; ventilatory variables were recorded concurrently.

Following this baseline period, inspired \( CO_2 \) partial pressure was increased in a stepwise manner by ~20 Torr per step, in 5-min intervals. At the end of each interval, a 1-min video of laryngeal arytenoid movement was recorded. Within this 1-min period, three observations of \( ETCO_2 \), inspiratory \( V_T \), \( PFI \), \( PFE \), and \( f \) were made. \( CO_2 \) administration was discontinued when \( ETCO_2 \) reached 120 mmHg or when no further increase in \( V_T \) was observed with increasing \( ETCO_2 \). Arterial blood samples for blood-gas analysis were drawn at baseline, at maximum arytenoid abduction, and at a range of intermediate abduction levels from the right metatarsal artery and were analyzed using a point-of-care device (i-STAT system, Abbott Point of Care, Princeton, NJ). Dogs recovered from anesthesia following data collection and received mannitol (0.5 g/kg iv).

Repeatability of response to a short burst of \( ETCO_2 \). To determine the repeatability of the change in cross section of the rima glottidis with repeated \( CO_2 \) administration on laryngeal abduction, we exposed each dog to three transient 1-min bursts of \( CO_2 \) during a separate experimental protocol performed under anesthesia. Animals were anesthetized and instrumented as for the first experiment. Following 15 min of a stable end-tidal isoflurane level, animals were exposed to a 1-min period of inhaled \( CO_2 \) to produce to \( ETCO_2 \) of ~90 mmHg. This level of hypercapnia was selected to ensure that \( ETCO_2 \) was greater than the beginning of the plateau phase of rima glottidis response (80 mmHg) and so avoid potential variability between animals. The burst was repeated three times with 15 min of washout between bursts. This level of inspired \( CO_2 \) was determined from the previous experiment to be the minimal level necessary to produce maximal laryngeal abduction. Arterial blood-gas analysis was performed at the conclusion of the first 1-min burst of \( CO_2 \) (i.e., at maximal \( ETCO_2 \)). Glottic opening during each burst was recorded in a 210-s video, with the first 30-s capturing baseline function without \( CO_2 \) exposure, the subsequent 60-s capturing response during the increase of inspired \( CO_2 \) levels, and the concluding 120-s capturing laryngeal function after \( CO_2 \) administration ceased. Ventilatory response \( (f, \text{ inspiratory } V_T, \text{ expiratory } V_T, V_I, \text{ and } V_E) \) was determined before and at the end of \( CO_2 \) administration for each burst.

Observation of laryngeal function and quantification of glottic area. Laryngeal function was recorded using a high-definition videendoscope placed into the lumen of the LMA. A sterile rod (2-mm diameter) was also placed through the LMA and positioned in the field of view with the tip just posterior to the corniculate processes of the arytenoid cartilages to provide a known and constant standard scale within the same plane as the rima glottidis and within the view of the endoscope so that absolute cross section of the rima glottidis could be determined. Care was taken not to touch the epiglottis or arytenoids to avoid laryngospasm. The endoscope was then fixed in place to avoid any movement within the LMA. Still-frame images were obtained from the digital videos using MPEG Video Wizard DVD 5.0 software (Video Wizard, Womble Multimedia, Cupertino, CA). All images were selected at maximal abduction during inspiration.

Framed images were calibrated using the known width of the sterile rod and measured using image analysis software (Able Image, Mu Laboratories). The length of the rima glottis was measured from the posterior to the anterior commissure. The left and right glottic area were measured by tracing the glottic gap area, identified as the medial surfaces of the vocal cords and corniculate processes (Fig. 1).

Data Analysis

Response to increasing levels of \( ETCO_2 \). Measurements of arytenoid abduction during inspiration in the \( CO_2 \) interval trial were obtained from three respiratory cycles selected at random and averaged during each 1-min video. Arytenoid abduction immediately before \( CO_2 \) administration was classified as baseline. Both absolute glottic area and glottic area normalized by length [normalized glottal gap area (NGGA) = glottic area/length³] were calculated (14, 22, 29). Previous reports using NGGA have assumed that glottic length is unchanged by experimental perturbation. Here, both measures of glottic area (NGGA and absolute measurement) are reported to allow comparison between the two approaches.

Changes relative to baseline for glottal area were calculated as a percentage of both absolute glottal area (calibrated images) and NGGA during each incremental level of inspired \( CO_2 \).

Fig. 1. Representative appearance of canine glottis during resting breathing under general anesthesia [end-tidal \( CO_2 \) \( (ETCO_2) = 40 \text{ mmHg}; A \) and hypercapnia \( (ETCO_2 = 120 \text{ mmHg}; B \) from a fixed endoscopic position via a laryngeal mask airway. Solid lines denote glottic length, and dotted lines outline glottic area.
Three values for each ventilatory variable were obtained and averaged during each CO₂ increment. Changes relative to baseline for glottic area and length, NGGA, and each ventilatory variable (f, inspiratory VT, PFI, and PFE) were analyzed using a mixed-effect model, with animal identity as a random effect. ETCO₂ level was treated as a categorical variable to allow for potential nonlinear relationships. Linear contrasts were used to determine the differences in rima glottidis dimensions between each level of elevated ETCO₂ and baseline values. Statistical analysis was performed using JMP (SAS Institute, Cary, NC). Significance was set at P < 0.05 throughout.

Repeatability of response to a short burst of inhaled CO₂. For the second trial, glottic cross section was measured for every inspired breath during each respiratory cycle of each burst (three bursts), and data were averaged over each 10-s interval for analysis. Baseline images included all breaths within the 30-s period before CO₂ administration. Data were expressed as NGGA, as data from the first experiment did not identify any changes in glottis length at the ETCO₂ levels used to produce each burst. To explore any sensitization effects of repeated transient hypercapnia, the repeatability of the response to a 30-s burst of inspired CO₂ was determined using standard guidelines (6).

RESULTS

The LMA was placed without complication in all animals. LMA placement allowed complete endoscopic visualization of the rima glottidis with concurrent arytenoid abduction. The inferior and posterior commissures of the rima glottidis were clearly visualized in each trial. Placement of the known length scale in the same superior-inferior plane as the rima glottidis was achieved without complication. All dogs recovered uneventfully from both anesthetic procedures, and no deleterious effects from CO₂ administration were observed.

Response to Increasing Inhaled CO₂ Levels

Glottic length and area during inspiration at baseline were 5.6 ± 0.8 mm (mean ± SE) and 6.3 ± 1.5 mm², respectively. During exposure to stepwise increases in inspired CO₂ levels, NGGA steadily increased, with a response significantly greater than baseline when ETCO₂ reached 70 mmHg (P < 0.0001, linear contrast). A plateau was reached at this point, and NGGA at ETCO₂ levels ≥75 mmHg was not significantly greater than NGGA at 70 mmHg (linear contrast, P = 0.52). When using absolute glottic area as a measure of laryngeal response, area reached a level significantly greater than baseline at 65 mmHg (linear contrast, P = 0.03) and continued to further increase until 80 mmHg (linear contrast, P < 0.01) when a plateau was reached with no further increase in absolute glottic area (linear contrast, P = 0.15).

This disparity in measurement of the response using absolute area or NGGA was explained by the variation in glottic length with increasing ETCO₂, which remained constant with ETCO₂ less than 80 mmHg (linear contrast, P = 0.35, compared with baseline). Above this level of hypercapnia, glottic length increased by 25% from baseline (Fig. 2, linear contrast, P = 0.02). Within the range of ETCO₂ used in these experiments, maximal response observed in absolute glottic area increased by 172 ± 27.4% from baseline (least squares mean ± SE), NGGA increased by 69.9 ± 11.6%, and glottic length increased by 30 ± 7.9% of baseline.

All animals displayed an excitatory ventilatory response to increasing levels of ETCO₂ (Fig. 2). A 2.5- to 3-fold increase in inspiratory flow and 3- to 4-fold increase in Vt were observed over the range of ETCO₂. No plateau effect was observed for f, ETCO₂ (mmHg)

![Fig. 2. Effect of increasing ETCO₂ on glottic area and glottic length, respiratory rate, inspiratory flow, and minute volume. All data are expressed as a percentage of baseline area and as least squares means ± SE. NGGA, normalized glottal gap area.](http://jap.physiology.org/doi/10.1152/japplphysiol.00469.2014)
Inspiratory flow, or $\dot{V}_I$, which all gradually increased with increasing hypercapnia, suggesting that exposure to $\text{ETCO}_2$ up to 120 mmHg did not produce a maximal ventilatory response.

Baseline $\text{ETCO}_2$, before the administration of additional CO$_2$, was 42.7 ± 1.2 mmHg. Hypoxemia did not develop in any animal during anesthesia. Arterial $P_O_2$ was 541 ± 29 Torr (mean ± SD) at baseline and 465 ± 61 Torr at peak $\text{ETCO}_2$ levels. In addition, hemoglobin desaturation (measured continuously with a pulse oximeter) was not observed, and arterial O$_2$ saturation from pulse oximetry remained at >95% in all experiments in all animals. There were no significant differences in bicarbonate levels between peak $\text{ETCO}_2$ of 26.7 ± 1.8 mmol/l and baseline $\text{ETCO}_2$ of 24.1 ± 1.6 mmol/l ($P = 0.06$).

As anticipated, $\text{ETCO}_2$ demonstrated an inverse and linear relationship with arterial blood pH. For every 10-mmHg increase in $\text{ETCO}_2$, pH decreased by 0.033 ± 0.0023 units ($R^2 = 0.92$). Based on the laryngeal response, the plateau identified in NGGA in this first trial and the relationship with arterial pH, an $\text{ETCO}_2$ of 90 mmHg was selected for the second trial in NGGA in this first trial and the relationship with arterial blood pH. For every 10-mmHg increase in $\text{ETCO}_2$, pH decreased by 0.033 ± 0.0023 units ($R^2 = 0.92$). Based on the laryngeal response, the plateau identified in NGGA in this first trial and the relationship with arterial pH, an $\text{ETCO}_2$ of 90 mmHg was selected for the second trial (repeatability). This level of hypercapnia ($\text{ETCO}_2 = 90$ mmHg) was associated with an arterial blood pH of 7.07. As anticipated, $\text{ETCO}_2$ correlated very closely with arterial $P_{CO_2}$ ($R^2 = 0.90$, $P < 0.0001$).

**Response to Repeated Transient Elevation in $\text{ETCO}_2$**

In the second trial, a rapid increase in inspiratory normalized glottis area (NGGA) was observed within 20–30 s after the administration of CO$_2$, with a peak response at or up to 10 s after the end of CO$_2$ administration (Fig. 3). Peak NGGA response to a 60-s burst of CO$_2$ for bursts 1-3 was 139.5 ± 6.4, 147.2 ± 4.4, and 143.7 ± 4.6% of baseline, respectively (least squared mean, all $P < 0.001$ compared with baseline, linear contrast) (Fig. 3). Model fits were all good at $R^2 = 0.72, 0.72$, and 0.75, respectively. Absolute areas at peak response were (mean ± SE) 7.7 ± 0.92, 8.3 ± 1.1, and 9.3 ± 1.2 mm$^2$, respectively, for bursts 1-3. There was no significant change in length of the rima glottis during the 60-s burst compared with baseline ($P = 0.19$, mixed-effect model). There were no significant differences between bursts across the range of $\text{ETCO}_2$ applied ($P = 0.35$) or when evaluating peak response only ($P = 0.55$). Recovery after each burst was slower than the rise in glottic area after the onset of the burst, and glottic cross-sectional area returned to within 10% of baseline at the conclusion of a 2-min recovery period in all trials. Minimal changes in expiratory area were observed (Fig. 3).

The ventilatory response at the end of the CO$_2$ burst was highly repeatable. No significant differences were observed in $f$ (bursts 1-3, respectively, mean ± SD: 23.0 ± 1.5, 22.9 ± 5.6, and 26 9 ± 9.9 breaths/min, $P = 0.65$), inspiratory $V_T$ (225.2 ± 45.5, 243.8 ± 35.2, and 259.1 ± 49.4 ml/min, $P = 0.39$), or expiratory $V_T$ (223.2 ± 55.3, 244.3 ± 42.0, and 250.8 ± 26.5 ml/min, $P = 0.65$), $V_i$ (5,198 ± 1,510, 5,479 ± 1,154, and 6,825.9 ± 3,080 ml/min, $P = 0.32$), or $V_e$ (5,125.6 ± 1,571, 5,487 ± 1,225.5, and 6,621 ± 3,248 ml/min, $P = 0.43$). Baseline levels for each ventilatory parameter before administration of the CO$_2$ burst were all significantly lower than those at the end of the burst (all $P < 0.001$, mean ± SD at baseline; $f = 14.7 ± 4.4$ breaths/min, inspiratory $V_T = 173.3 ± 8.8$ ml/min, expiratory $V_T = 171.8 ± 11.3$ ml/min, inspiratory minute volume = 2,478 ± 1785 ml/min, expiratory minute volume = 2,435 ± 355 ml/min). Mean arterial pH at the end of the CO$_2$ burst (peak laryngeal response) was 7.1 ± 0.05.

No evidence of sensitization was observed between bursts, and the response to hypercapnia was highly repeatable, with no bias in rima glottis area between the three bursts (mean differences at peak response: burst 1–burst 2, burst 1–burst 3, and burst 2–burst 3 were 0.22, 1.95, and 1.73 mm$^2$, respectively; $P = 0.14, 0.67$, and 0.11, respectively). The coefficients

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**Fig. 3.** Response to repeated transient 1-min burst of 90-mmHg $\text{ETCO}_2$. All data are expressed as a NGGA as a percentage of baseline area and least squares means ± SE. E, expiration; I, inspiration.
of repeatability were all low (2.96, 3.29, and 1.67, respectively), suggesting a highly repeatable response and no evidence of sensitization to repeated CO₂ exposure (6).

**DISCUSSION**

**Summary**

This study demonstrates that hypercapnia produces a marked progressive increase in the cross-sectional area of the rima glottidis, and that these changes can be readily quantified, using a LMA and a known scale in the same plane as the rima glottidis, with high temporal resolution. We also demonstrate that prolonged marked hypercapnia produces an increase in length in the rima glottidis. This change in glottic length suggests that a commonly used method to determine changes in glottic area using length as a standard (NGGA) may not be appropriate under all conditions.

The use of short transient burst of hypercapnia produced a repeatable laryngeal response with an approximate 140% increase in glottic area to 7–9 mm² at or shortly after the end of the burst with no increase in glottic length.

**Novel Observations**

We demonstrate the efficacy of a novel approach to directly observe laryngeal function during perturbation while maintaining a secure airway. We identified a profound elevation in absolute glottic cross section with maximal abduction associated with an increase of over 150% from baseline (a 2.5-fold increase). The magnitude of the response in respiratory parameters (f, inspiratory flow, and minute ventilation) was similar. A short transient burst of hypercapnia produced a marked and significant increase in glottic area of ~50%. This effect was highly repeatable with small differences in peak area between bursts and low coefficients of repeatability.

We also identify a marked increase (20–25%) in the anterior-posterior dimension of the rima glottidis (glottic length) at high levels of ET₇₅₀ (≥80 mmHg), when the applied hypercapnia was prolonged (experiment 1). This is anticipated as this dimension of the rima glottidis is controlled by the cricothyroid muscle, which elongates and tenses the vocal cords by producing anterior displacement of the thyroid cartilage with respect to cricoid cartilage (9, 12, 20, 28, 34) and so lengthens the vocal cord due to its attachment to both the thyroid anteriorly and the cricoid (via the corniculate process of the arytenoid), posteriorly. In this way, contraction of the cricothyroid muscle produces gliding of the thyroid relative to the cricoid cartilage, so lengthening and dilating the rima glottidis during hypercapnia, deep breathing, or brief airway occlusion (13, 19, 20, 33, 34).

**Methodological Issues**

Although several prior studies have evaluated the effects of hypercapnia on PCA muscle activity using EMG (4, 7, 10, 11), none have evaluated the effect of PCA contraction on glottic cross section under these conditions. The endoscopic technique we describe, in combination with use of a LMA and a known scale in the plane of the rima glottis, allows precise temporal resolution and determination of absolute changes in glottic dimensions during perturbation. As glottic length increases at higher ET₇₅₀ levels (>80 mmHg), we suggest that NGGA should only be used if any effects of experimental perturbation on glottis length are assessed. If the PCA muscle is experimentally stimulated independently of all other laryngeal muscles (22), then this methodological constraint does not apply.

**Limitations**

One limitation of this approach was that visualization of the canine vocal cords was occasionally difficult, as they could be partially obstructed by the prominent cuneiform processes in the canine larynx. Careful positioning of the animal in sternal recumbency with symmetrical alignment of body, neck, and head and careful positioning of the endoscope to ensure a symmetrical view of the glottis mitigated this limitation in all animals. Visualization of the rima glottidis at profound abduc- using the LMA was straightforward using the techniques we describe. A plateau in absolute glottis area and ventilatory response was not observed within the range of ET₇₅₀ used (up to 120 mmHg).

A further limitation is that animals need to be anesthetized to instrument them for this procedure. We selected anesthetic agents based on their limited effects on spontaneous ventilation and laryngeal movement, but also on their safety margin. Maintenance of a constant end-tidal level of the inhalational anesthetic allows maintenance of a constant depth of anesthesia during data collection within each experiment and between animals. We cannot speculate on the quantitative effects of the anesthetic agents on the laryngeal response to hypercapnia, as that would require obtaining measurements in the absence of anesthetic agents. These effects would be consistent between animals and would not introduce bias.

**Physiological and Clinical Significance**

These data directly identify a profound laryngeal response to both steady-state and transient hypercapnia. We describe the response to repeated transient hypercapnic bursts using NGGA to determine this response. We describe these changes in terms of NGGA as no changes in glottis length were observed at this level of hypercapnia (ET₇₅₀ = 90 mmHg), and previous reports evaluating the canine glottis under sedation have used this approach (14, 29). The magnitude of the changes we identify in glottic cross section under transient or steady-state hypercapnic conditions is comparable to those identified using EMG to determine PCA activity (10, 11, 31).

A videoendoscopic approach, combined with a LMA, allows repeatable assessment of PCA function in a canine preclinical model of laryngeal disease. The canine larynx is a widely used preclinical animal model of human laryngeal disease (16, 25), and the spatial arrangements of cricoid, thyroid, and arytenoid cartilages is similar to that of humans (16), although a number of distinct difference exist, including the anatomy of the hyoepiglotticus muscle/ligament, the size and conformation the epiglottis, and relationship with the soft palate. This approach could also potentially be used in other small (nonrodent) models of laryngeal disease. The use of direct videendoscopy allows detailed observations to be made of the rima glottis with high temporal resolution. By avoiding invasive instrumentation, videendoscopy reduces the possibility of instrumentation affecting response and has also been shown to be a sensitive technique to detect changes in laryngeal function in dogs with naturally occurring laryngeal paralysis (23, 24). This model
system could also be used to assess laryngeal function longitudinally after experimental nerve injury or following reinnervation of the abductor muscles using nerve graft (2, 30).

Conclusion

These data directly demonstrate the effects of repeated transient and steady-state hypercapnia on laryngeal function using a novel method to assess glottic area in a clinically relevant preclinical model.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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