Inferior pharyngeal constrictor electromyographic activity during permeability pulmonary edema in lambs

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Diaz, Véronique, Irenej Kianicka, Patrick Letourneau, and Jean-Paul Praud. Inferior pharyngeal constrictor electromyographic activity during permeability pulmonary edema in lambs. J. Appl. Physiol. 81(4): 1598–1604, 1996.—Newborn mammals exhibit an active expiratory upper airway closure during the first hours of extrauterine life. We have recently shown that permeability pulmonary edema led to active expiratory glottic closure in awake newborn lambs while hypoxia (inspired O2 fraction 8%; 15 min) did not. In the present study, we tested the hypothesis that expiratory glottic closure was accompanied by an increase in pharyngeal constrictor muscle expiratory electromyographic (EMG) activity. We studied seven awake nonsedated lambs aged 8–20 days. Airflow (facial mask + pneumotachograph), blood gases (arterial catheter), and EMG activity of both the thyroarytenoid muscle (a glottic adductor) and the inferior pharyngeal constrictor muscle were recorded before and after intravenous injection of halothane (0.05 ml/kg) to induce a permeability pulmonary edema. A central apnea (duration 15 s to 5 min) with continuous thyroarytenoid and inferior pharyngeal constrictor activity was observed within seconds after halothane injection. One lamb died despite rescuing maneuvers. An expiratory phasic thyroarytenoid and inferior pharyngeal constrictor muscle activity with simultaneous zero airflow gradually took place and, by 30 min after halothane injection, was present at each expiration in the six remaining lambs. Expiratory glottic and pharyngeal constrictor muscle EMG activity was subsequently present during the whole study period (1.5–5 h), even after correction of the initial hypoxia. Permeability lung edema was present at postmortem examination in all seven lambs. We conclude that a permeability pulmonary edema induced by intravenous halothane in nonsedated lambs enhances both glottic and pharyngeal constrictor muscle expiratory EMG. We hypothesize that expiratory constriction of the inferior pharyngeal constrictor muscle could participate in the active expiratory upper airway closure; this, in turn, might improve alveolo-capillary gas exchange by increasing the end-expiratory lung volume.

expiratory airflow braking; pharyngeal constrictor muscle

ACTIVE EXPIRATORY AIRFLOW BRAKING is an essential ventilatory strategy in newborn mammals (19). It can be recorded in normal-term human neonates in the first minutes after birth, during initial adaptation to extrauterine lung ventilation (6). In pathological situations, the expiratory airflow braking becomes audible as an expiratory grunting. This classic alarm signal was recognized long ago in hyalin membrane disease, transient tachypnea of the newborn, meconium aspiration, and infectious alveolitis. Its importance as a defense mechanism in these situations has been highlighted by the observation that endotracheal intubation (i.e., by-passing the upper airways) worsened hypoxia and led to severe acidosis (8).

The mechanisms underlying expiratory grunting are still under debate. Although it is well accepted that an active glottic closure brought about by the contraction of glottic adductor muscles (i.e., the thyroarytenoid muscle) accompanies expiratory airflow braking (12), the electrical activity (electromyographic (EMG) activity) of the other upper airway muscles has not been systematically studied in such situations. Moreover, the factors triggering active expiratory glottic closure are still unresolved. Hypoxia, usually present in neonates with respiratory problems, has been considered by some researchers to be responsible for expiratory grunting (2, 8). However, in a recent series of experiments conducted in term lambs from the first day of life, Praud et al. (22, 23, 25) have shown that hypoxia does not lead to expiratory airflow braking or to enhancement of thyroarytenoid muscle EMG activity.

From the premise that all neonates with expiratory grunting have excess water in their lungs, we have shown that pulmonary edema in lambs consistently triggered an expiratory thyroarytenoid muscle contraction with airflow braking (24). These results led us to hypothesize that vagally mediated afferent activity could be responsible for expiratory grunting in neonates, in agreement with previous studies in lambs showing that volume receptive information from the lungs is of overriding importance for controlling glottic adductor muscle expiratory activity (7, 19).

The aim of the present study was to gain further insight into the activity of the upper airway muscles, namely the inferior pharyngeal constrictor, during active expiratory airflow braking in lambs. The inferior pharyngeal constrictor has not been previously studied in neonates, and its role in normal and pathological situations, at a period when upper airway function is so crucial, is unknown. We have thus recorded inferior pharyngeal constrictor EMG during baseline room air breathing and during a prolonged period of expiratory airflow braking triggered by permeability pulmonary edema. The results show that expiratory airflow braking is accompanied by enhanced expiratory activity, not only of the thyroarytenoid muscle but also of the inferior pharyngeal constrictor.

MATERIALS AND METHODS

Animals

Seven lambs born at term by spontaneous vaginal delivery were used in the study. One lamb had also been included in a previous study on laryngeal muscle activity during pulmo-
nary edema (24). The animals were aged 8–20 days and weighed between 4.7 and 10.1 kg. All lambs were housed with their mother in our animal quarters for several hours to a few days before surgery. Animals received penicillin and gentamicin daily after surgery. The protocol of this study was approved by our institution's ethics committee for animal research.

Surgical Preparation

Surgery was performed under general anesthesia (2% Fluothane and 30% N₂O). Under direct vision, we inserted intramuscular bipolar electrodes (enameled chrome wire, 0.7-mm diam; Chromel, Groupe Technique et Scientifique Médicale, Castelnaudary, France) into both the thyroarytenoid and inferior pharyngeal constrictor muscles. Details with regard to electrode insertion into the thyroarytenoid muscle have been described previously (24). For the inferior pharyngeal constrictor, we carefully turned the larynx and inserted the electrode perpendicularly to the muscular fibers attached to the lateral lamina of the thyroid cartilage, near its posterior margin (18). The leads were subcutaneously tunneled to exit on the back of the animals. Electrode placement was confirmed by a systematic autopsy after completion of the experiment.

A catheter was inserted in the brachial artery of all lambs for blood gas sampling. Blood gas values were corrected for rectal temperature of each lamb (Mon-a-Therm 6500).

Measurement Apparatus

The measurement apparatus has been described in previous studies (23, 24). Briefly, a face mask, specifically designed for each lamb, was attached to a size 0 pneumotachograph (model 21070B plus model 8815A respiratory integrator, Hewlett-Packard, Waltham, MA). The thyroarytenoid muscle and the inferior pharyngeal constrictor muscle EMG signals were amplified and band-pass filtered at 30–1,000 Hz (model PS11 AC preamplifier and model 7DA DC driver amplifier, Grass Quincy, MA) before undergoing a 100-ms moving time averaging (Dept. of Electronics, Faculty of Medicine, University of Sherbrooke, Sherbrooke, PQ, Canada). The integrator used had parallel outputs to a chart recorder and a computer. An eight-channel polygraph (model 7D, Grass) recorded instantaneous airflow, tidal volume (VT), and the raw and integrated EMG signals of both muscles. An IBM-compatible microcomputer (Televideo TeleCAT-286) analyzed the airflow, integrated EMG signals, and inspired O₂ fraction at a 40-Hz sampling rate. Collected data were stored on disk for further analysis.

Study Design

The design of the study allowed us to infer the role of increased lung water on the expiratory glottic and pharyngeal dynamics in nonsedated lambs.

For the study, each unsedated lamb was positioned in a sling. A venous catheter was first inserted under local anesthesia (2% lidocaine) in the superior vena cava through the superficial jugular vein. The face mask was then applied and the head carefully positioned. Each lamb was given at least 10 min to adjust to the mask. After 3 min of room air baseline recording, the lambs were given an intravenous injection of 0.05–0.075 ml/kg halothane over 1–2 min (14). The ambient temperature was kept between 20 and 22°C and the humidity between 50 and 70% throughout the experiments.

Data Analysis

Minute ventilation (VE/kg), VT (VT/kg), breathing frequency (f), and duty cycle (Ti/TT) were computed for each breath in all lambs. Averages were calculated over 15 s (i.e., 15–23 breathing cycles) during baseline recordings and every 30 min postinjection. We looked for expiratory airflow braking on the flow-volume curves computed breath by breath during the above-defined 15-s epochs in each lamb. The thyroarytenoid and the inferior pharyngeal constrictor EMGs were observed throughout the experimental procedure. The percentage of breaths accompanied by thyroarytenoid muscle expiratory EMG activity was calculated during the above-defined 15-s epochs in each lamb. The inferior pharyngeal constrictor expiratory EMG activity was quantified by averaging the maximal voltage of the integrated signal over the same 15-s epochs and expressing the average values as a percentage of the value during baseline room air breathing (taken as 100%). Finally, average values for all lambs as a whole were calculated for statistical study.

At the end of the experiment, the lambs were killed, and pulmonary edema was evaluated by macroscopic observation and then by the measurement of lung-to-body weight and wet-to-dry lung weight ratios (33).

Statistical Analysis

Fifteen-second-averaged values for breathing pattern parameters, percentage of cycles with expiratory airflow braking or with thyroarytenoid muscle expiratory EMG activity, and inferior pharyngeal constrictor expiratory EMG activity were compared by analysis of variance for repeated measures, completed by contrast comparison (SuperANOVA 1989, Abacus Concepts, Berkeley, CA).

Lung-to-body weight ratio and wet-to-dry lung weight ratio of five of seven lambs with a control group [n = 8; age 15.6 ± 9.5 (SD) days, weight 6.9 ± 2.3 kg] were also compared by a Mann-Whitney test.

RESULTS

The injection of 0.05–0.075 ml/kg halothane induced a response in all lambs but two, which required two and four injections, respectively (total amount 0.125 and 0.2 ml/kg, respectively), before a response was observed. These two lambs behaved like the others thereafter.

Baseline Room Air Breathing

Ventilatory parameters, VE, VT, f, and Ti/TT during baseline room air breathing are presented in Fig. 1.

Thyroarytenoid muscle EMG activity and flow-volume curves. As previously reported in awake nonsedated lambs during baseline room air breathing (22), we observed no expiratory thyroarytenoid muscle EMG activity in the seven lambs studied (Fig. 2). Non-respiratory-related bursts of thyroarytenoid muscle EMG were recorded only during swallowing movements. As in the previous studies by Praud et al. (22–25), no expiratory airflow braking was observed except in one lamb that had 10% of respiratory cycles with expiratory airflow braking in baseline conditions.

Inferior pharyngeal constrictor. An expiratory phasic EMG of the inferior pharyngeal constrictor muscle was observed in six of the seven lambs during baseline room air breathing (22–25) but was not Used by 10.22.2017 from http://jap.physiology.org/Downloaded from
air breathing. Its characteristic shape (somewhat variable from 1 breath to another in the same lamb) consisted of an expiratory plateau preceded by an early peak and ending with a second, late expiratory peak (Fig. 2). The seventh lamb had a tonic expiratory EMG of the inferior pharyngeal constrictor muscle. During inspiration, we observed a tonic activity of the inferior pharyngeal constrictor muscle, with an average amplitude of 42 ± 23% of the reference value (baseline expiratory activity) for the seven lambs as a whole.

Response to Halothane Injection

The intravenous injection of halothane induced a permeability pulmonary edema in all lambs, as observed by gross anatomic abnormalities during systematic postmortem examination. Areas of focal hemorrhaging and atelectasis predominant in the dependent portions of the lungs were present with endoluminal foam and bloody pleural liquid. The lung-to-body weight and wet-to-dry lung weight ratios were significantly increased in the halothane group compared with the control group (P < 0.01; Fig. 3). Moreover, a histological analysis performed after hematein, eosin, and safran staining pointed out heterogeneous abnormalities, varying from one area to another: vascular congestion and interstitial and/or alveolar edematous hemorrhagic infiltrates. Inflammatory cell accumulation was rarely observed in the pulmonary interstitium.

Early transient response. In six of seven lambs, a prolonged central apnea lasting from 5 to 230 s was observed during the first minutes after injection. Continuous thyroarytenoid and inferior pharyngeal constrictor muscle EMG activity was observed during apneas. Amplitude of both continuous EMGspresented simultaneous variations. Six lambs required rescuing maneuvers, and one lamb died.

Prolonged thyroarytenoid and inferior pharyngeal constrictor muscle response. Expiratory airflow braking (usually to zero flow in early expiration) with thyroarytenoid muscle phasic expiratory EMG activity appeared 16 ± 10 min after halothane injection. At the same time, the phasic expiratory EMG of the inferior pharyngeal constrictor muscle significantly increased (P < 0.02; Fig. 4). Thereafter, expiratory flow limitation with both thyroarytenoid and increased inferior pharyngeal constrictor muscle phasic expiratory EMG activities was recorded (for at least 75% of the breaths) throughout the 1.5- to 5-h recording period (Figs. 5 and 6). No increase in inspiratory inferior pharyngeal constrictor EMG activity was observed in any lamb.

The time course of $V_E$, $V_T$, $f$, and $T_I/T_T$ after halothane injection is illustrated in Fig. 1. We observed a significant and prolonged decrease in $V_T$ (P < 0.001), $f$ (P < 0.001), and $T_I/T_T$ (P < 0.02), whereas the increase in $V_E$ was not significant (P = 0.15). The time course of the arterial blood gases and pH (pH$_a$) recorded in four

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**Fig. 1.** Time course of respiratory parameters during baseline (BL) room air breathing and during pulmonary edema induced by intravenous injection of halothane. Values are means ± SD. A: breathing frequency ($f$). B: duty cycle ($T_I/T_T$). C: tidal volume ($V_T$). D: minute ventilation ($V_E$). Halothane injection (at time 0) was made at end of baseline period. See text for details. *P < 0.05.

**Fig. 2.** Thyroarytenoid and inferior pharyngeal constrictor EMG activity during baseline room air breathing in 1 lamb. TA, raw thyroarytenoid muscle EMG activity; $\int$TA, moving time-averaged TA; IPC, raw inferior pharyngeal constrictor muscle EMG activity; $\int$IPC, moving time-averaged inferior pharyngeal constrictor muscle EMG activity. Inspiration is upward. *, Swallowing movement. Note absence of expiratory TA EMG activity and presence of expiratory IPC EMG activity with a frequent characteristic shape of integrated signal (▲).
lambs (Fig. 7) was variable, with decreased pH appearing to be the more predominant alteration observed.

Effect of blood gas variations on active expiratory airway closure induced by pulmonary edema. Transiently observed hypoxia was corrected spontaneously or with oxygen addition (arterial $\text{PO}_2$ > 70 Torr or transcutaneous arterial $\text{O}_2$ saturation > 92%) in all the lambs. Hypoxia correction did not reverse the increased expiratory upper airway muscle EMG activity or expiratory airflow braking in any of the lambs, as illustrated in the example in Fig. 4. Figure 4 also clearly shows that the increased expiratory upper airway muscle EMG activity and airflow braking were observed despite near-baseline values of arterial $\text{PCO}_2$ ($\text{PaCO}_2$) and pH.

DISCUSSION

The present study demonstrates that induced permeability pulmonary edema in lambs stimulates the expiratory phasic EMG of both pharyngeal and glottic constrictor muscles with consequent expiratory upper airway closure. The key findings are the following: 1) lambs exhibit a spontaneous and consistent expiratory phasic EMG of the inferior pharyngeal constrictor in baseline conditions, whereas thyroarytenoid muscle expiratory activity is absent; 2) permeability pulmonary edema leads to a significant increase in the expiratory phasic EMG activity of the inferior pharyngeal constrictor, simultaneous to the appearance of active expiratory glottic closure with thyroarytenoid contraction; and 3) hypoxia is not the key factor responsible for the increase in upper airway constrictor muscle expiratory EMG activity during pulmonary edema in lambs.

Inferior Pharyngeal Constrictor Muscle Activity During Baseline Room Air Breathing

The inferior pharyngeal constrictor EMG activity has previously been studied in adult animals of several species and in humans. Most studies have shown a spontaneous expiratory phasic EMG of the inferior pharyngeal constrictor, such as in anesthetized cats (20, 30), rabbits (28), and monkeys (29) and in unanesthetized dogs (15). On the other hand, one study found that unanesthetized rats exhibited an inspiratory EMG of the inferior pharyngeal constrictor (31). In adult
humans, a phasic expiratory (and sometimes transitional, from midinspiration to midexpiration) EMG of the inferior pharyngeal constrictor was inconsistently observed during wakefulness (32). The reasons for the discrepancies between these previous results are not known and could be explained by different experimental conditions [use of anesthesia (28), posture (29), presence of tracheostomy (20, 30)] and/or by species differences. Furthermore, the precise studied pharyngeal level was not always well defined, and activity patterns among superior, middle, and inferior pharyngeal constrictor muscles seem to differ from species to species. For example, while the three pharyngeal constrictors are active during expiration in awake humans (32), the expiratory EMG of the inferior pharyngeal constrictor contrasts with the inspiratory firing of the superior and middle pharyngeal constrictors in anesthetized rabbits (28).

The present study is the first to describe the inferior pharyngeal constrictor EMG in the neonatal period without anesthetic interference. We observed an expiratory phasic EMG of the inferior pharyngeal constrictor on each respiratory cycle in all but one lamb during baseline conditions. Moreover, we found similar expiratory phasic EMG in 18 other lambs (not involved in the present study) during quiet room air breathing (unpublished observations). Therefore, what would be the purpose of an active expiratory pharyngeal narrowing during quiet breathing in lambs? Contrary to the larynx, the pharynx is a compliant structure submitted to surrounding pressure variations. During expiration, the outflow of expired gas dilates the pharynx and increases physiological dead space. As previously suggested, the inferior pharyngeal constrictor expiratory contraction could stiffen the pharyngeal wall and prevent an increase in dead space (30). The inferior pharyngeal constrictor contraction could also increase inspiratory upper airway resistance, either at the pharyngeal level by decreasing the pharyngeal diameter (17) or at the laryngeal level by approximating the hyoid bone and the thyroid cartilage, a mechanism that closes the glottis (5, 16). The increase in inspiratory upper airway resistance would consequently induce expiratory airflow retardation and delay lung emptying, helping the neonate to preserve a high end-expiratory lung volume.

Inferior Pharyngeal Constrictor Muscle Activity During Permeability Pulmonary Edema

It has long been observed that human newborns exhibit an expiratory airflow braking during the first minutes after birth (19). This phenomenon is prominent in premature newborns, especially in neonatal respiratory distress syndrome, leading to classic expiratory grunting (8). In a recent study, Hutchison et al. (12) demonstrated that, as in human newborns, nonseparated premature lambs exhibited an expiratory airflow braking with expiratory thyroarytenoid muscle phasic EMG during the first hours of life. Grunting newborns, and especially premature babies, all have in common an excess of lung water (1). In a recent study, we showed that increasing lung water content (by inducing a permeability pulmonary edema) led to an expiratory airflow braking with expiratory thyroarytenoid muscle phasic EMG activity in lambs (24). Both studies suggest that the thyroarytenoid muscle participates to the active expiratory airflow braking in newborn lambs.

In a more recent study, Hutchison et al. (13) briefly mentioned that asphyxiated premature lambs exhibited an expiratory phasic EMG of the inferior pharyngeal constrictor muscle simultaneous to the expiratory thyroarytenoid muscle EMG and airflow braking in the first minutes after birth. In keeping with this observation, the present study shows that pharyngeal and
laryngeal constrictor muscles can be stimulated simultaneously in experimental conditions mimicking neonatal respiratory problems. While increased inferior pharyngeal constrictor muscle EMG activity could serve to stiffen the pharyngeal wall during increasing airflow, the accompanying upper airway closure with zero flow during expiration recorded in all our lambs is likely related to the enhanced activity of both pharyngeal and laryngeal constrictor muscles. Indeed, it has been previously shown that active laryngeal closure involves intrinsic and extrinsic laryngeal muscles (16) and that the inferior pharyngeal constrictor muscle is considered to be an extrinsic laryngeal muscle: by pulling the thyroid cartilage toward the hyoid bone, it contributes to laryngeal closure (5, 16). Thus we speculate that the increase in expiratory phasic EMG of the inferior pharyngeal constrictor acts together with expiratory thyroarytenoid muscle contraction to produce an expiratory glottic closure with zero flow during high-permeability pulmonary edema in lambs. Furthermore, we speculate that a similar mechanism involving both thyroarytenoid and inferior pharyngeal constrictor muscle operates in premature babies with expiratory grunting.

Origin of the Increase in Pharyngeal Constrictor Muscle Activity

The mechanism(s) linking permeability pulmonary edema to increased pharyngeal constrictor muscle expiratory activity in lambs is unknown. Several possible factors may involve chemo- or mechanoreceptors.

As previously shown for glottic constrictor muscle (12, 22–25), hypoxia in the present study does not appear to be a key factor. Indeed, correction of hypoxia (either spontaneously or with O₂ administration) during pulmonary edema did not decrease the expiratory EMG activity of the inferior pharyngeal constrictor. Likewise, while acidosis and slight hypercapnia were observed in the four studied lambs as a whole, increased inferior pharyngeal constrictor muscle EMG activity was also recorded in the presence of normal arterial pH and PaCO₂ (see Fig. 4), similar to previous findings in preterm lambs for thyroarytenoid muscle EMG (12). Thus, although we cannot rule out that increased PaCO₂ could have participated in the enhancement of expiratory inferior pharyngeal constrictor EMG (30, 32), this does not seem to be a prerequisite in lambs with pulmonary edema.

Reflex mechanisms originating from mechanoreceptors in the lower respiratory apparatus could enhance inferior pharyngeal constrictor muscle activity during pulmonary edema, as for the glottic constrictor muscles. Indeed, previous data have shown that glottic constrictor muscle expiratory activity depends on lung receptive feedback and is important in maintaining end-expiratory lung volume (7, 19). Pulmonary edema decreases lung compliance and hence lung volume. Consequent decrease in afferent messages from slowly adapting receptors could thus enhance both glottic and inferior pharyngeal constrictor muscle expiratory contraction. On the other hand, stimulation of C-fiber endings and rapidly adapting receptors have been shown during pulmonary edema (21, 26, 27), and C-fiber ending stimulation by capsaicin increases glottic constrictor activity (9, 11). Finally, stimulation of pulmonary arterial baroreceptors (by increased pulmonary arterial pressure) may also be involved (3). Thus vagally mediated afferent activity originating from mechanoreceptors in the respiratory apparatus is likely important in enhancing upper airway constrictor muscle activity during pulmonary edema. However, previous data on the effect of vagotomy upon pharyngeal constrictor muscle activity are scarce and contradictory (30) and preclude any firm conclusions.

In summary, the results of the present study, along with previous data from our laboratory, show that the electrical activity of both thyroarytenoid and inferior pharyngeal constrictor muscle in lambs is enhanced in a situation with permanent expiratory airflow braking. We hypothesize that this essential defense strategy in newborn infants involves similar upper airway muscle activity, mainly triggered by the excess of lung water present at birth.

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**Fig. 7.** Time course of arterial blood gases during baseline room air breathing and during pulmonary edema (n = 4). pHa, arterial pH; PaCO₂, arterial P CO₂; PaO₂, arterial P O₂.
REFERENCES


