Vagal afferents and active upper airway closure during pulmonary edema in lambs

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Diaz, Véronique, Dominique Dorion, Irenej Kianicka, Patrick Létourneau, and Jean-Paul Praud. Vagal afferents and active upper airway closure during pulmonary edema in lambs. J. Appl. Physiol. 86(5): 1561–1569, 1999.—The present study was undertaken to gain further insight into the mechanisms responsible for the sustained active expiratory upper airway closure previously observed during high-permeability pulmonary edema in lambs. The experiments were conducted in nonsedated lambs, in which airflow and thyroarytenoid and inferior pharyngeal constrictor muscle electromyographic activity were recorded. We first studied the consequences of hemodynamic pulmonary edema (induced by impeding pulmonary venous return) on upper airway dynamics in five lambs; under this condition, a sustained expiratory upper airway closure consistently appeared. We then tested whether expiratory upper airway closure was related to vagal afferent activity from bronchopulmonary receptors. Five bivagotomized lambs underwent high-permeability pulmonary edema: no sustained expiratory upper airway closure was observed. Finally, we studied whether a sustained decrease in lung volume induced a sustained expiratory upper airway closure. Five lambs underwent a 250-ml pleural infusion: no sustained expiratory upper airway closure was observed. We conclude that 1) the sustained expiratory upper airway closure observed during pulmonary edema in nonsedated lambs is related to stimulation of vagal afferents by an increase in lung water and 2) a decrease in lung volume does not seem to be the causal factor. 

control of breathing; lung volume; newborn; vagus nerve; thyroarytenoid muscle; inferior pharyngeal constrictor muscle

ACTIVE EXPIRATORY UPPER airway closure, often audible as an expiratory grunting, is an essential ventilatory strategy in newborn mammals (8, 19). Its importance as a defense mechanism is highlighted by the clinical observation that bypassing the upper airways in newborns with respiratory distress syndrome worsens hypoxia and leads to severe acidosis (11). The mechanisms underlying active expiratory upper airway closure in newborns are still largely unknown. We have recently shown that induction of a high-permeability pulmonary edema in chronically instrumented, nonsedated lambs consistently enhanced expiratory thyroarytenoid muscle (TA) and inferior pharyngeal constrictor muscle (IPC) electromyographic (EMG) activity to the point of expiratory upper airway closure (5, 22). We hypothesized from these results that increased lung water content, through vagal afferents originating from bronchopulmonary receptors, can cause a sustained, active expiratory upper airway closure in newborns.

The aim of the present study was to further examine the mechanisms linking pulmonary edema and sustained expiratory upper airway closure observed in lambs. More specifically, we tested the following three hypotheses in nonsedated lambs: 1) a sustained active expiratory upper airway closure is also induced during hemodynamic pulmonary edema, 2) vagal afferents arising from bronchopulmonary receptors are responsible for the expiratory upper airway closure observed during pulmonary edema, and 3) a sustained decrease in lung volume leads to a sustained expiratory upper airway closure.

MATERIALS AND METHODS

Fifteen lambs (5 for each series of experiment) aged between 8 and 28 days and weighing between 5 and 13.5 kg were involved in the study. All lambs were born at term by spontaneous vaginal delivery and housed with their mother in our animal quarters for a few days before surgery. The protocol of the study was approved by the ethics committee for animal research of our institution.

Surgical Preparation

Aseptic surgery was performed while the lambs were under general anesthesia (2% halothane-40%-N2O-58% O2) after premedication by an intramuscular injection of ketamine (10 mg/kg) and acepromazine (0.1 mg/kg) and a subcutaneous injection of atropine (0.2 mg/kg). Intramuscular bipolar electrodes (enameled chrome wire, 0.7-mm diameter; Chromel, GTSM, Castelnaudary, France) were inserted under direct vision into both TA and IPC muscles. Details with regard to electrode insertion have been described previously (5, 16). 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. Depending on the type of experiment scheduled, surgery was completed on the lamb as follows.

Hemodynamic pulmonary edema. The third left intercostal space was entered, and the left lung was ventrally retracted for exposure of the left atrium. A cuffed 10-F Foley catheter was introduced into the left atrium through a small incision in the atrial appendage and held in place with a purse-string suture. In the same manner, a 14-gauge Teflon catheter was introduced into the pulmonary artery through a small incision and held in place with a purse-string suture. The chest was then closed in layers, and the residual pneumothorax was exsufflated.

High-permeability pulmonary edema in bivagotomized lambs. A bilateral vagotomy was performed in two steps as
Vagal afferents and pulmonary edema

This two-step bivagotomy leaves the right recurrent nerve intact and allows the study of the right TA EMG.

Sustained decrease in lung volume. A catheter (PORT-A-CATH II, SIMS Delectec, St. Paul, MN) was introduced in the pleural cavity through a 1-cm incision in the fourth right intercostal space. The catheter was connected to a chamber subcutaneously sutured on the rib cage.

An intramuscular injection of buprenorphine (0.005 mg/kg) was systematically given on completion of the surgery. Furthermore, an intramuscular injection of penicillin and gentamicin was given daily until the experimental day.

Measurement Apparatus

The measurement apparatus used in the present experiments has been described previously (21). Briefly, a face mask, specifically designed for each lamb, was attached to a size-0 pneumotachograph (model 210708 plus model 8815A respiratory integrator, Hewlett-Packard, Waltham, MA). The TA and IPC EMG signals were amplified and band-pass filtered at 30–1,000 Hz (model P511, alternating-current preamplifier and 7DA direct-current driver amplifier, Grass, Quincy, MA) before undergoing rectification and 100-ms moving time averaging (Dept. of Electronics, Faculty of Medicine, Université de Sherbrooke, Sherbrooke, PQ). The integrator used had parallel outputs to a chart recorder and a computer. A 10-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 analyzed the airflow and integrated EMG signals at a 40-Hz sampling rate. Collected data were stored on disk for further analysis. Pulmonary arterial pressure was measured from the pulmonary artery catheter connected to a pressure transducer (Trantec model 60-900 physiological pressure transducer, American Edwards Laboratories, Santa Ana, CA) and displayed on the screen of a pressure monitor.

Study Design

All experiments were conducted in nonsedated conscious lambs at least 2 days after surgery. Each lamb was comfortably positioned in a sling with a face mask carefully applied on its snout and connected to a pneumotachograph (24). Each experimental procedure began with a 5-min baseline recording. Ambient temperature was kept between 20 and 22°C and humidity between 50 and 70% throughout the experiment. Lambs were systematically killed on completion of the experiments by intravenous injection of 50 mg/kg pentobarbital sodium.

Hemodynamic pulmonary edema. To verify that the expiratory upper airway closure was not specifically linked to high-permeability pulmonary edema, we recorded respiratory parameters with TA and IPC EMG during high-pressure pulmonary edema induced by inflating the cuff of the Foley catheter in the left atrium. The cuff was inflated until pulmonary arterial pressure increased over 30 mmHg. This pressure level was maintained thereafter for the remainder of the experiment.

High-permeability pulmonary edema in bivagotomized lambs. To study the role of vagal afferents from the lungs in upper airway response to lung edema, we recorded respiratory parameters with TA and IPC EMG during high-permeability pulmonary edema in five bivagotomized lambs. A venous catheter was inserted into the jugular vein. The effects of at least two intravenous injections of 0.05 ml/kg halothane were studied. Each halothane injection was followed by a recording period of not less than 30 min. An additional injection was given if the previously described expiratory upper airway closure did not appear.

Sustained decrease in lung volume. Finally, we examined whether a sustained decrease in lung volume induced a sustained expiratory upper airway closure in five lambs. After baseline recording, 50 ml of saline prewarmed to 39.5°C were infused in the right pleural cavity. Additional pleural infusions were performed if sustained expiratory upper airway closure did not appear. Each pleural infusion was followed by a recording period of not less than 15 min. Five pleural infusions, i.e., a total of 250 ml, were performed in all lambs. To ensure that pleural infusions did not elicit pain, 7 mg/kg lidocaine were added in the first infusion for the first three lambs studied; saline only was infused in the remaining two lambs.

Data Analysis

Minute ventilation (Ve), VT, respiratory frequency (f), and duty cycle (Ti/Tt) were computed for each breath in all lambs. Averages were calculated over 15 s during baseline recordings and at preset intervals during the experimental procedure, i.e., at 15-, 30-, 60-, and 90-min marks during the two first experiments and 15 min after each pleural infusion during the third experiment. We looked for expiratory airflow braking on instantaneous airflow traces during the above-defined 15-s epochs in each lamb. TA and IPC EMG were carefully observed throughout the experimental procedure. The percentage of breaths with expiratory airflow braking and the percentage of breaths with expiratory TA EMG was calculated during the above-defined 15-s epochs in each lamb. The expiratory IPC EMG 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 average value during baseline room air breathing (taken as 100%).

At the end of the experiment, the lambs were killed and pulmonary edema was evaluated by macroscopic observation and by measurement of lung-to-body weight and wet-to-dry lung weight ratios (29).

Statistical analysis. Above-defined 15-s averaged values were calculated for all lambs as a whole (22) and compared by analysis of variance for repeated measures, completed by contrast comparison when appropriate (SuperANOVA 1989, Abacus Concepts, Berkeley, CA). Lung-to-body weight and wet-to-dry lung weight ratios were compared with a control group [n = 10, mean age 19 ± 11 (SD) days, weight 7.4 ± 2.5 kg] by a Mann-Whitney U-test.

RESULTS

Hemodynamic Pulmonary Edema

Five lambs aged 10–18 days and weighing 5.7–7.3 kg were studied.

Baseline room air breathing. Values for ventilation and its component variables are illustrated in Fig. 1A. Neither expiratory TA EMG nor airflow braking was observed (n = 5; Figs. 1B and 2A). Non-respiratory-related bursts of TA EMG were consistently recorded,
however, with swallows in each lamb. Expiratory phasic IPC EMG (n = 4) was consistently observed during baseline room-air breathing (Fig. 2A). Pulmonary arterial pressure varied from 14 to 20 mmHg.

Hemodynamic pulmonary edema. Cuff inflation of the Foley catheter produced a steady increase in pulmonary arterial pressure above 30 mmHg (range 30–48 mmHg), which was responsible for a pulmonary edema, as evidenced by characteristic macroscopic findings (focal hemorrhages and congestion, luminal foam, and bloody pleural liquid) and a significant increase in lung-to-body weight ratio (P < 0.05; n = 3; Fig. 1C). A significant increase in f (F = 11.69, P < 0.002) was observed from 15 min after cuff inflation, but VE, VT, and Ti/Tt did not change significantly (Fig. 1A). An early expiratory airflow braking and TA EMG appeared 15–90 min after cuff inflation. Simultaneously, phasic expiratory IPC EMG increased (Fig. 1, B and C). Once present, this active expiratory upper airway closure steadily persisted throughout the recording period (up to 180 min in 2 lambs).

Conclusion. Pulmonary edema can be triggered by impeding pulmonary venous return in nonsedated lambs. This hemodynamic pulmonary edema invariably induces a sustained, active expiratory upper airway closure.

High-Permeability Pulmonary Edema in Bivagotomized Lambs

Five lambs aged 9–28 days and weighing 5.1–13.5 kg were studied. Two doses of halothane (0.05 ml/kg) were
injected in all five lambs. A third injection was given in three lambs, and a fourth injection was given to one lamb to ensure that the lack of expiratory upper airway closure was not due to an insufficient pulmonary edema.

Baseline room-air breathing. Respiratory parameter values are illustrated in Fig. 3A. Low-amplitude expiratory TA EMG was consistently observed in three of five lambs; this was accompanied by expiratory airflow braking in only one lamb (Fig. 2B). Non-respiratory-related bursts of TA EMG were consistently observed with swallows in the five lambs, attesting to the integrity of the right recurrent nerve. Expiratory IPC EMG (n = 4) was consistently observed in baseline conditions.

High-permeability pulmonary edema. Halothane injections induced a high-permeability pulmonary edema, as evidenced by characteristic macroscopic findings observed at systematic postmortem examination. Increased lung water content was confirmed by a significant increase in both lung wet-to-dry weight and lung-to-body weight ratios (n = 5; P < 0.05; Fig. 3C). A period of hypopnea without apnea (individual Ve < 50% of the mean Ve recorded in the minute preceding the injection) with expiratory airflow braking and increased expiratory TA and IPC EMG was inconsistently observed immediately after the injection, lasting <30 s. Apart from the first minute, no significant change in VT, V̇e, or Ti/TT was observed after halothane injection, whereas f progressively increased, reaching statistical significance at 30 min (F = 6.49, P < 0.003; Fig. 3A).

Neither expiratory airflow braking nor increase in TA or IPC EMG was recorded up to 30 min after the second halothane injection in four lambs (Figs. 3B and 4). The remaining lamb, which already exhibited mild expiratory airflow braking with TA and IPC EMG in baseline conditions, maintained the same breathing pattern, without any enhancement of TA and IPC EMG throughout the experiment (Fig. 3B). No expiratory airflow braking was observed initially in the three lambs that were given three or four halothane injections. However, the last (3rd or 4th) injection induced a breathing pattern characterized by irregular breathing, prolonged Te, and marked expiratory upper airway closure, leading to prolonged apneas with TA and increased IPC EMG and death 25–30 min after the last injection. This sequence of events occurred without any agitation in any of the three lambs.

Conclusion. Sustained active upper airway expiratory closure is not observed in bivagotomized, nonse-
dated lambs during halothane-induced, high-permeability pulmonary edema. The late upper airway closure during central apneas immediately preceding death is not related to vagal afferent activity from bronchopulmonary receptors.

Sustained Decrease in Lung Volume

Five lambs aged 8–16 days and weighing 4.4–5.4 kg were studied.

Baseline room air breathing. Respiratory parameters are illustrated in Fig. 5A. None of the five lambs exhibited expiratory airflow braking or TA EMG activity and of amplitude of IPC EMG expressed as percentage of baseline value. Each symbol represents a different animal.

Pleural infusions. After the five pleural infusions, none of the lambs exhibited any signs of discomfort. Although lidocaine (7 mg/kg) was added to the first saline pleural infusion in the first three lambs studied, no difference in their TA and IPC response was noted compared with the two remaining lambs that were given no local anesthesia. A significant decrease in VT value was observed 15 min after the fifth pleural infusion (Fig. 5A). Neither expiratory airflow braking nor increased expiratory TA or IPC EMG was observed after saline infusion (Fig. 6B), aside from a few cycles in the first minutes after infusion in three lambs. The volume of saline recovered from the right pleural space just after completion of the study was between 200 and 250 ml in all five lambs.

Conclusion. Decreasing lung volume with a 250-ml right hydrothorax did not induce any sustained active expiratory upper airway closure in nonsedated lambs.
DISCUSSION

The results of the present study in nonsedated lambs shed some light relative to the mechanisms potentially linking pulmonary edema and expiratory upper airway closure in lambs. Our unique results establish that a sustained, active expiratory upper airway closure is present not only in high-permeability pulmonary edema but also in hemodynamic pulmonary edema and is related to vagal afferents originating from bronchopulmonary receptors, and finally, our results suggest that this expiratory upper airway closure is not primarily due to the sustained decrease in lung volume accompanying pulmonary edema.

Apart from the direct noxious effect of halothane on pulmonary C-fiber endings, several reflex mechanisms may account for the enhancement of upper airway constrictor muscle EMG, which we previously observed in lambs with high-permeability pulmonary edema. Indeed, increased extravascular lung water per se is known to stimulate vagal afferent activity from all known lung receptors, including C-fiber endings, rapidly adapting receptors, and slowly adapting receptors (27). Also, reflexes from pulmonary vessel receptors (3), chest wall receptors (26), or upper airway receptors (10) could theoretically be involved. It should be pointed out that previous results did not suggest a significant role for chemoreceptor afferent activity in expiratory upper airway closure observed during pulmonary edema (5, 22).

Hemodynamic Pulmonary Edema

The observation of an active expiratory upper airway closure during hemodynamic pulmonary edema strongly suggests that it is not related to the direct effect of halothane on C-fiber endings. Such effects were previously questioned for other noxious substances producing high-permeability pulmonary edema with lung injury (27). The present results further support our hypothesis that active expiratory upper airway closure is related to the increase in extravascular lung water per se. However, chemicals such as prostacyclin released in the edematous lung (31) may still play a part through stimulation of C-fiber endings (28).

Vagotomized Animals

The observation of baseline phasic expiratory TA EMG in three of five lambs, accompanied by expiratory airflow braking in one lamb, has been previously reported (10, 21) and attributed to absent vagal afferent...
information from the pulmonary stretch receptors (10). However, the absence of enhanced TA EMG in two of five lambs (and more so the absence of glottic closure in 4 of 5 lambs) underlines that the relief of tonic TA EMG inhibition by suppressing vagal afferent information can be masked by other unrelated factors. It has been previously shown that expiratory TA EMG during baseline room air breathing is also under the influence of numerous factors unrelated to pulmonary vagal afferents, such as drowsiness and quiet sleep (3, 26a) and afferent information from upper airways (10) and chest wall (26).

Despite this baseline expiratory TA EMG, the present results show that vagotomy prevented active expiratory upper airway closure induced by pulmonary edema in intact lambs. This supports our previous hypothesis that modifications in vagal afferent activity play a major role in triggering active expiratory upper airway closure associated with pulmonary edema (5, 22).

The observation of dramatic expiratory upper airway closure with progressively developing fatal apneas triggered by repeated halothane injections in vagotomized lambs might be taken as further evidence that active upper airway closure during central apneas is explained by other mechanisms than vagal afferent activity from lung receptors. Previous studies from our laboratory in lambs during actively or passively induced prolonged (even fatal) central apneas (22, 23) had already led us to speculate that an intrinsic brain stem mechanism was likely involved in the consistent upper airway closure observed in induced central apneas in lambs. This awaits further confirmation. Alternatively, repeated intravenous injections of halothane might have several untoward consequences related to factors such as chemical makeup or osmolality. Furthermore, sympathetic lung afferents (2) might be involved in such reflex activity in vagotomized animals.

**Decreased Lung Volume**

It is currently accepted that active laryngeal expiratory airflow braking is an essential mechanism for preventing the decrease in functional residual capacity in newborn mammals. Several peculiarities of the
neonatal period, including high chest wall compliance and low lung compliance, would render such an energy-efficient mechanism especially well suited for the neonate (8, 10, 13, 19). To our knowledge, however, it has not been firmly established that a sustained decrease in lung volume, such as that encountered during pulmonary edema (20), would trigger a sustained active expiratory upper airway closure in awake, nonsedated newborn animals.

Progressive infusion of saline into the right pleural cavity allowed us to study different degrees of decrease in lung volume in each nonsedated lamb, beginning with a volume of liquid grossly reproducing the volume of pleural effusion generally observed at autopsy in lambs with pulmonary edema. Unexpectedly, we found that the sustained decrease in lung volume induced in the above-described manner did not elicit a sustained enhancement of expiratory upper airway constrictor muscle EMG in any of the awake lambs studied, even when lidocaine was absent from pleural infusion. Although these highly reproducible results argue against a crucial role for the decrease in lung volume in active expiratory upper airway closure present during pulmonary edema in awake lambs, reconciliation with previous results obtained by others in lambs (9) or dog pups (7) is not evident. Differences in the methods of inducing a decrease in lung volume (9), use of light anesthetics (1), bypass of the upper airways (7), and observations limited to a few cycles after decrease in lung volume (7) could account for these discrepancies. Alternatively, a unilateral decrease in lung volume as performed in the present study could conceivably induce contralateral compensatory mechanisms such as an increase in contralateral lung volume. This in turn could offset the enhancement of expiratory TA EMG secondary to the unilateral decrease in lung volume. Finally, the significant decrease in VT after a 250-ml pleural infusion (Fig. 5) may have been responsible for an increase in arterial PCO₂, which has been reported to decrease expiratory TA EMG (17). Hence, our present data in lambs do not rule out a significant role of expiratory laryngeal braking in preserving lung volume in neonates. However, they have the merit to underline the fact that the link between sustained decreased lung volume and upper airway dynamics in the neonatal period is perhaps not so obvious as generally stated. Accordingly, future studies will have to examine this link more thoroughly.

Finally, the bronchopulmonary receptor type responsible for active expiratory upper airway closure consistently observed in awake lambs with pulmonary edema has yet to be found. Previous data in either anesthetized (1, 14, 18, 30) or decerebrate (12) adult animals have shown that stimulation of C-fiber endings induces

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**Figure 6.** Recording in 1 intact lamb in baseline condition (A) and after a pleural infusion of 250 ml of saline without lidocaine (B). See Fig. 2 for definitions of abbreviations. Saline infusion is responsible for an increase in respiratory rate and decrease in VT. Note absence of both expiratory upper airway closure on airflow trace and significant increase in TA EMG activity during edema (compare with Fig. 1, B and C).
an increase in laryngeal resistance and/or in expiratory TA EMG. These data, together with results of the present study, lead us to speculate that stimulation of C-fiber endings known to occur during pulmonary edema (27) is primarily responsible for active expiratory upper airway closure observed in awake, non-sedated lambs. However, because some data on C-fiber ending stimulation suggest a weaker response in newborn than in adult mammals (6, 15), this hypothesis remains to be tested. Alternatively, stimulation of rapidly adapting receptors, which have previously been shown to be stimulated during pulmonary congestion (28), or sympathetic lung receptors (2), might have a role in the active expiratory upper airway closure observed during pulmonary edema.

Conclusion

The active expiratory upper airway closure consistently observed in non-sedated lambs during pulmonary edema appears to be related to vagal afferent traffic from bronchopulmonary receptors. The speculated role of C-fiber endings is addressed in our companion paper (4).

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