Laryngeal response to nasal ventilation in nonsedated newborn lambs

François Moreau-Bussière,* Nathalie Samson,* Marie St-Hilaire, Philippe Reix, Joëlle Rouillard Lafond, Élise Nsegbe, and Jean-Paul Praud

Neonatal Respiratory Research Unit, Departments of Pediatrics and Physiology, Université de Sherbrooke, Quebec, Canada

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First published March 1, 2007; doi:10.1152/japplphysiol.00891.2006.—Although endoscopic studies in adult humans have suggested that laryngeal closure can limit alveolar ventilation during nasal intermittent positive pressure ventilation (nIPPV), there are no available data regarding glottal muscle activity during nIPPV. In addition, laryngeal behavior during nIPPV has not been investigated in neonates. The aim of the present study was to assess laryngeal muscle response to nIPPV in nonsedated newborn lambs. Nine newborn lambs were instrumented for recording states of alertness, electrical activity [electromyograph (EMG)] of glottal constrictor (thyroarytenoid, TA) and dilator (cricothyroid, CT) muscles, EMG of the diaphragm (Dia), and mask and tracheal pressures. nIPPV in pressure support (PS) and volume control (VC) modes was delivered to the lambs via a nasal mask. Results show that increasing nIPPV during wakefulness and quiet sleep led to a progressive disappearance of Dia and CT EMG and to the appearance and subsequent increase in TA EMG during inspiration, together with an increase in trans-upper airway pressure (TUPA). On rare occasions, transmission of nIPPV through the glottis was prevented by complete, active glottal closure, a phenomenon more frequent during active sleep epochs, when irregular bursts of TA EMG were observed. In conclusion, results of the present study suggest that active glottal closure develops with nIPPV in nonsedated lambs, especially in the VC mode. Our observations further suggest that such closure can limit lung ventilation when raising nIPPV in neonates.

MATERIALS AND METHODS

Experiments were conducted in nine mixed-breed term lambs aged from 3 to 5 days and weighing 4.2 kg (SD 1.2; range 3.1–7) on the day of the experiment. All lambs were born at term by spontaneous vaginal delivery and housed with their mother in our animal quarters. The study protocol was approved by the ethics committee of the Université de Sherbrooke for animal care and experimentation.

Surgical Preparation

Surgery was performed 1–3 days after birth under general anesthesia (1–2% isoflurane + 30–50% N2O + 48–68% O2). Intramuscular atropine sulfate (0.1 mg/kg) and ketamine (10 mg/kg) were injected before endotracheal intubation. Vital sign monitoring included electrocardiogram, rectal temperature, pulse oximetry, end-tidal CO2 (PETCO2), venous pH, and glycemia. A mixture of 5% dextrose, 3 meq·kg⁻¹·day⁻¹ NaCl, 1.5 meq·kg⁻¹·day⁻¹ KCl, and 2 meq·kg⁻¹·day⁻¹ Ca²⁺ was systematically infused perioperatively. Bipolar enameled chrome wire electrodes were inserted into the TA, CT, and diaphragm (Dia) muscles for recording EMG activity (20). Custom-designed electrodes for EEG, electrooculogram (EOG), and ECG recordings were also implanted as previously described (39). A custom catheter was inserted between the third and fourth tracheal rings to record subglottal pressure (26). Leads from each electrode were subcutaneously tunneled to exit on the back of the lambs. Finally, an arterial catheter (Insyte, 18GA, Infusion Therapy Systems, Sandy, UT) was inserted into a radial artery for blood sampling and gas analysis.

Postoperative care included daily intramuscular injection of penicillin G (Dulpocillin, 0.05 ml·kg⁻¹·day⁻¹) and gentamicin (5 ml·kg⁻¹·day⁻¹) until the end of the experimentation. The arterial catheter was flushed daily with heparin solution. Lambs were euthanized at the end of experiments by pentobarbital overdose. Correct electrode positioning was systematically verified at autopsy.

Experimental Equipment

Ventilatory equipment. Nasal ventilation was performed with a Siemens Servo 300 ventilator and Servo Screen (Siemens, New York) with heated (33°C) and humidified air. A custom-made nasal mask was built from a plaster shell filled with dental paste to fit the muzzle of each lamb as previously described (42). Briefly, the mask included...
a double nasal cannula, a nasogastric tube, and a plastic catheter for \( P_{\text{ET}}\text{CO}_2 \) sampling.

**Recording equipment.** Polysomnographic recordings were obtained by using our custom-designed radiotelemetry system with channels for EEG, EOG, ECG, and 4 EMGs, as previously described (28, 29). The raw EMG signals were sampled at 500 Hz, rectified, and moving-time averaged on 100 ms. Mask pressure (a measure of the level of ventilatory support) and subglottal pressure (a measure of the ventilatory support reaching the lower airways) were recorded using two calibrated pressure transducers (MP 45-30-871, Validyne, Northridge, CA). Thoracic and abdominal volume variations were qualitatively assessed with their sum using respiratory inductance plethysmography (Respitrace, NIMS, Miami Beach, FL). Arterial hemoglobin O2 saturation (\( \text{SpO}_2 \)) was monitored with a probe attached at the base of the tail (38). \( P_{\text{ET}}\text{CO}_2 \) was continuously recorded using a CO2 analyzer (Capnomac II, Datex-Ohmeda Canada, Mississauga, ON, Canada), with a 50 ml/min flow sampling rate. Arterial blood gases and pH were also measured (IL 1306; Instrumentation Laboratory, Lexington, MA) and corrected for rectal temperature of the lamb. All signals were recorded on a Power Macintosh 7300 using the Acknowledge 3.2 acquisition software (Biopac Systems, Santa Barbara, CA).

**Design of Study.**

The study was performed without sedation and at least 48 h after surgery. The lambs were comfortably positioned in a sling with loose restraints. The study was designed to allow for simultaneous recording of EEG, EOG, ECG, and EMG activity, variations of subglottal and mask pressure, respiratory movements, \( P_{\text{ET}}\text{CO}_2 \), and \( \text{SpO}_2 \) while using different levels of ventilation in the three different states of alertness. Arterial blood gases (arterial \( \text{PO}_2 \), \( \text{PCO}_2 \), and pH) were measured at each level of ventilation.

Following a first recording with the nasal mask only [no continuous positive airway pressure (CPAP), i.e., no connection to the ventilator], ventilatory support was initiated via the nasal mask at CPAP of 4 cmH2O (CPAP 4). Two ventilatory modes, i.e., PS and VC, were used in all lambs in a random order. In the PS mode (PS 10/4, PS 15/4, and PS 20/4, respectively), three different levels of positive inspiratory pressure were studied, namely, 10, 15, and 20 cmH2O, while maintaining positive end-expiratory pressure (PEEP) at 4 cmH2O, as used in a previous study in adult humans (34, 36). The trigger was adjusted in flow mode at the lowest (easiest) stable setting. In the VC mode, respiratory rate (RR) and tidal volume (VT) were initially set at the same level as when the lamb was spontaneously breathing with CPAP of 4 cmH2O (VC baseline). Minute ventilation was then successively increased three times (VC-1, VC-2, and VC-3). Following preliminary tests, VC-1 was associated with an increase in RR to 40 or 50 breaths/min [mean 42 breaths/min (SD 4.2; range 40–50)] to avoid both auto-PEEP and rebreathing. VC-2 and VC-3 corresponded to an increase in VR with 15- or 20-ml increments (depending on the lamb’s weight) to a maximum of 23 ml/kg (SD 3.2; range 18–25). PEEP was maintained at 4 cmH2O throughout the VC-mode experiments. Since stable ventilation has been shown to be difficult to obtain in the pressure control mode in a previous study (36) and during our preliminary tests in lambs, this mode was not tested in the present study. Every effort was made to obtain recordings in wakefulness (W), quiet sleep (QS), and active sleep (AS) at each level of ventilation. At any given time during experiments, ventilation was stopped if the following criteria were met: 1) lamb discomfort or agitation; 2) obvious abdominal distension or presence of liquid reflux via the nasogastric tube; 3) subglottal pressure over 30 cmH2O; 4) presence of auto-PEEP; 5) inability to obtain the three states of alertness after 1 h of continuous recording.

**Data Analysis.**

**States of alertness.** Standard electrophysiological and behavioral criteria were used to define W, QS, and AS from EEG, EOG, and continuous observation (39). Arousal from QS was characterized by sudden disappearance of high-amplitude, low-frequency waves on the EEG trace, together with sudden appearance of any EMG activity and increase in heart rate, whereas arousal from AS was recognized by direct observation of the lamb and disappearance of intense EOG activity.

**Respiratory parameters.** For each state of alertness and every ventilatory level, an observer blinded to the goal and hypothesis of the study selected 20 consecutive breaths, which had to be preceded and followed by 20 s of stable respiratory pattern. Thereafter, respiratory parameters (inspiratory moving time average amplitude of CT, TA, and Dia EMG, RR, mask and subglottal pressures, and \( P_{\text{ET}}\text{CO}_2 \)) were quantified, analyzed, and averaged on the 20 selected breaths, using the Acknowledge (version 3.7.0, Biopac Systems) and Microsoft Excel software. In the present study, the qualifier “inspiratory” was used for Dia, CT, and TA muscle EMG activities during nIPPV, when they occurred simultaneously with lung inflations, even when there was no evidence of central inspiratory drive, i.e., no visible Dia EMG activity. For both Dia and CT muscles, the inspiratory EMG maximal amplitude measured during W with no CPAP was averaged and used as reference value (100%) for subsequent calculations in the different ventilatory modes and states of alertness in each lamb. Since no TA EMG was recorded during inspiration in spontaneous, baseline breathing, the averaged EMG maximal amplitude recorded during five swallows was chosen as the reference value (100%). In addition, the

**Table 1. Respiratory parameters during no CPAP, CPAP 4 cmH2O, and nasal intermittent positive pressure ventilation in wakefulness**

<table>
<thead>
<tr>
<th>CT Inspi EMG</th>
<th>TA Inspi EMG</th>
<th>Dia Inspi EMG</th>
<th>Inspi TUAP, cmH2O</th>
<th>RR, breaths/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>No CPAP</td>
<td>1</td>
<td>0</td>
<td>−0.2 (0.5; −1.1 to +0.6) &amp; d,e</td>
<td>40 (11; 24–56) &amp; d,e</td>
</tr>
<tr>
<td>CPAP 4</td>
<td>0.58 (0.44; 0.18–1.54) &amp; d,e</td>
<td>0</td>
<td>0.9 (0.2; 0.7–1.3) &amp; d,e</td>
<td>1.1 (0.4; 0.5–1.7) &amp; d,e</td>
</tr>
<tr>
<td>PS 10/4</td>
<td>0.34 (0.23; 0.11–0.78) &amp; d,e</td>
<td>0.07 (0.05; 0.03–0.20) &amp; d,e</td>
<td>0.7 (0.3; 0.3–1.1) &amp; d,e</td>
<td>1.1 (1.0; 1.0–3.1) &amp; d,e</td>
</tr>
<tr>
<td>PS 15/4</td>
<td>0.27 (0.18; 0.12–0.68) a</td>
<td>0.14 (0.09; 0.06–0.34) b,e</td>
<td>0.4 (0.2; 0.2–0.7) b</td>
<td>1.5 (2.5; 0.0–8.1) b</td>
</tr>
<tr>
<td>PS 20/4</td>
<td>0.25 (0.09; 0.17–0.46) a</td>
<td>0.29 (0.17; 0.06–0.53) a</td>
<td>0.5 (0.2; 0.2–0.7) a</td>
<td>2.3 (1.6; 0.2–5.4) a</td>
</tr>
<tr>
<td>VC base</td>
<td>0.59 (0.45; 0.10–1.40) &amp; b,h,i</td>
<td>0.10 (0.08; 0.03–0.24) a</td>
<td>0.8 (0.3; 0.4–1.3) b,h,i</td>
<td>3.9 (2.4; 1.4–9.7) b,h,i</td>
</tr>
<tr>
<td>VC-1</td>
<td>0.38 (0.41; 0.11–1.22) b,h,i</td>
<td>0.17 (0.16; 0.04–0.45) a</td>
<td>0.4 (0.2; 0.2–0.7) a</td>
<td>6.7 (4.8; 2.6–18.1) a</td>
</tr>
<tr>
<td>VC-2</td>
<td>0.18 (0.10; 0.08–0.37) a,b</td>
<td>0.20 (0.14; 0.04–0.44) a</td>
<td>0.4 (0.1; 0.1–0.6) a</td>
<td>10.4 (2.6; 6.7–14.3) a</td>
</tr>
<tr>
<td>VC-3</td>
<td>0.21 (0.08; 0.03–0.45) a,b</td>
<td>0.26 (0.17; 0.05–0.53) a</td>
<td>0.5 (0.3; 0.1–1.2) a</td>
<td>17.5 (5.5; 10.6–25.8) a</td>
</tr>
</tbody>
</table>

Values are means (SD; range). RR, respiratory rate; EMG, electromyogram; Dia, CT, and TA Inspi EMG, diaphragm, cricothyroid, and thyroarytenoid phasic inspiratory electrical activity, respectively; TUAP, trans-upper airway pressure; CPAP, continuous positive airway pressure; PS, pressure support; VC, volume control ventilation. For further description of PS modes (10/4, 15/4, 20/4) and VC modes (baseline, VC-1, VC-2, VC-3), see Design of Study. For both Dia and CT muscles, the inspiratory EMG maximal amplitude measured with no CPAP was averaged and used as reference value of 1 for subsequent calculations of values in the different ventilatory modes. For TA muscle, the averaged EMG maximal amplitude recorded during 5 swallows was chosen as the reference value of 1. All superscript letters are \( P < 0.05; \) vs. no CPAP; & vs. CPAP 4 cmH2O (CPAP 4); & vs. PS 10/4; & vs. PS 15/4; & vs. PS 20/4; & vs. VC-1; & vs. VC-2; & vs. VC-3.
pressure difference between mask and subglottal pressures, i.e., the TUAP, was calculated and analyzed on the same 20 breaths during baseline breathing. Analysis of the relationship between TUAP and TA EMG was conducted in each lamb as follows. Both TA EMG and TUAP were measured at two discrete time points during each lung inflation in the VC mode, at the highest level of ventilation (VC-3), when TA EMG was present. As airflow, by definition, is constant in the VC mode, any increase in TUAP indicated an increase in trans-upper airway resistance. Finally, one additional lamb was further instrumented with a chronic catheter positioned just above the glottis to directly measure transglottal pressure (TGP) (10). The latter parameter, together with measurement of airflow (Hewlett-Packard 21070–60040 pneumotachograph interposed between the ventilator and nasal mask) enabled us to study the relationship between transglottal resistance (TGR = TGP/airflow) and TA EMG in VC-3.

Statistical analysis. Statistical analyses were performed using the SAS software version 9.1 (SAS Institute, Cary, IL). Results were first averaged in each lamb, then in all lambs as a whole, and expressed as means (SD). Normality was first tested using the Shapiro-Wilks test. Blood gases, which assumed a normal distribution, were analyzed using ANOVA with repeated measures. All the other analyses (CT, TA, and Dia EMG, TUAP, and RR) were performed using the Poisson regression model with repeated measures (GENMOD procedure).

Power analysis was performed for each variable using the Nquery 4.0 software. Unless specified, all nonstatistically significant results given in this report have been tested beforehand for at least 80% power. Finally, regression analysis (REG and MIXED procedures) were also performed for testing the relationship between TUAP and TA EMG in the VC-3 mode. All results with P value <0.05 were considered as statistically significant.

RESULTS

Of the nine lambs that underwent surgery, CT and TA muscles could be analyzed in eight lambs only, due to displacement of the electrodes observed at autopsy in one lamb. Total duration of polysomnographic recordings analyzed was 2,151 min, with a mean recording time of 239 min (SD 60; range 149–369). Mean duration of states of alertness in each lamb was 187 min (SD 63; range 103–330) in W, 47 min (SD 20; range 18–74) in QS, and 4 min (SD 5; range 0–13) in AS.

Baseline Breathing With No CPAP in Wakefulness

Regular phasic inspiratory Dia and CT EMG were consistently observed in all lambs during baseline recording with no
CPAP, i.e., with the nasal mask in place but without the ventilator. In addition, phasic CT EMG was observed during the second part of expiration (E2) in four of eight lambs, while consistently absent in the first part of expiration (E1 or postinspiratory period). No tonic CT EMG was present during baseline breathing with no CPAP. While phasic expiratory TA EMG was observed in E1 in four of the eight lambs studied, TA EMG was consistently absent during both inspiration and E2 in all lambs. Values for the various respiratory parameters measured during baseline breathing and during different ventilatory support modes are given in Table 1.

**Breathing With CPAP 4 During Wakefulness**

A small but statistically significant decrease in RR was observed with CPAP 4 compared with no CPAP ($P < 0.0001$). No changes in inspiratory Dia EMG were observed from breathing with no CPAP to CPAP 4 ($P = 0.32$). In contrast, a significant decrease in inspiratory CT EMG was observed from no CPAP to CPAP 4 ($P = 0.03$). Inspiratory CT EMG even disappeared in two of the eight lambs when breathing with CPAP 4. Small-amplitude, phasic inspiratory TA EMG was observed in one of the eight lambs with CPAP 4. A significant decrease in expiratory TA EMG was observed with CPAP 4 compared with no CPAP ($P = 0.03$). Finally, a small but significant increase in inspiratory TUAP was observed from CPAP 0 to CPAP 4 ($P < 0.0001$) (Table 1).

**PS Mode in Wakefulness**

A progressive decrease in RR was observed with each step increase in ventilatory support. Overall, a 58% decrease in RR was observed with PS 20/4 compared with no CPAP ($P < 0.0001$). A progressive decrease in Dia EMG was observed from no CPAP and from VC baseline to VC-3. Also, inspiratory TA EMG appeared in seven of eight lambs and increased progressively from VC baseline to VC-3 ($P = 0.006$). Expiratory (E1) TA EMG was still present in four lambs in VC-3 (3 of which already had E1 TA EMG activity with no CPAP). A major increase in TUAP was progressively observed with increasing VC, culminating at 17.5 cmH2O on average in VC-3 ($P < 0.0001$) (see Table 1). Interestingly, the pattern of inspiratory TA EMG was different in VC compared with PS. Indeed, the slope of the increase in TA at the onset of inspiration was less abrupt in VC than in PS. Also, during PS mode, following the early peak of activity at onset of lung inflation, a decrescendo in inspiratory TA EMG was observed, as opposed to a more sudden decrease in VC mode (Fig. 3).

Further analysis showed that the increase in TUAP was significantly correlated with TA EMG in VC-3 in each lamb,
indicating that trans-upper airway resistance increased simultaneously with TA EMG (*P* < 0.001) in the VC mode in each lamb (Fig. 4).

**End-Tidal CO₂ and Arterial Blood Gases**

A slight but statistically significant decrease in PCO₂ and PETCO₂ was observed when increasing nasal ventilation in both PS and VC (Table 2). While averaged values remained within normal physiological ranges, PCO₂ was outside the normal range in some lambs. One hypercapnic lamb during no CPAP (PCO₂ = 50 Torr) decreased its PCO₂ to 45 Torr in VC-3. Two other lambs went from normocapnia to PCO₂ = 32 Torr. A fourth lamb remained hypercapnic throughout the entire experiments (maximal PCO₂ = 49 Torr). However, neither TA nor CT EMG evolved differently in lambs with PCO₂ values out of the normal range.

**Apneas**

Twenty-nine central apneas were recorded during the experiments (0.8 apnea/h), with a mean duration of 8.7 s (SD 2.9; range 3–14.9). Most apneas occurred in W (25/29) in the PS mode (15/29) and were preceded by a sigh (24/29). Seven apneas occurred during no CPAP, five during CPAP 4, and finally two in VC mode. No episodes of periodic breathing were observed in any of the lambs or ventilatory modes.

**Influence of the States of Alertness**

Overall, the majority of results obtained in PS and VC modes in QS were identical to those obtained in W, including a significant decrease in inspiratory Dia and CT EMG and a significant increase in both inspiratory TA EMG and TUAP (see Table 3). However, low statistical power precluded any possible comparison of glottal muscle EMG response between QS and W for the same level of nIPPV in a given ventilatory mode.

No statistical analysis could be performed in AS due to a lack of sufficient data, including four of nine lambs, who did not sleep in AS. Semiquantitative observations suggested that Dia EMG was increased in CPAP 4, PS, and especially in the VC mode. In addition, while CT EMG was clearly increased in both inspiration and expiration in all five lambs in AS, TA EMG did not appear to change, aside from bursts of TA EMG resembling bursts of nonnutritive swallows. Interestingly, the latter occasionally occurred simultaneously with ventilator insufflations, leading to a total glottal blockage of ventilation, with a marked increase in trans-glottal pressure and an absence of inspiratory volume variation on the respiratory plethysmography in VC mode (see Fig. 5). Complete glottal closure in such cases induced a marked elevation in mask pressure in the VC mode, from 15 to 20 cmH₂O in normal breaths to as high as 55 cmH₂O. The VC mode was also associated with asynchronism between respiration and the ventilator in five of eight AS episodes in the VC mode, due to irregular respiratory pattern of the lambs in AS.

**DISCUSSION**

The present results in lambs indicate that raising nIPPV progressively inhibits phasic laryngeal dilator EMG and trig-
The onset of phasic laryngeal constrictor EMG during lung inflations in wakefulness and quiet sleep. In addition, our observations further suggest that modifications in laryngeal muscle EMG are responsible for active glottal narrowing, which limits lung inflation during nIPPV. The observation that nIPPV is accompanied by modifications of laryngeal muscle activity is a unique finding and furthers previous endoscopic reports of glottal narrowing in adult humans. We believe that such findings are of high physiological interest and may bear significant consequences for neonatal respiratory care.

**Inspiratory Glottal Muscle Electrical Activity During nIPPV**

TA muscle inspiratory activity. When present, phasic respiratory contraction of the TA muscle normally occurs in early expiration (13, 23, 31, 32, 41, 47). This has been shown to be...
Table 2. Arterial blood gases (pH, $P_{CO_2}$, $P_{O_2}$, and $HCO_3^-$) and $PET_{CO_2}$, relative to each ventilatory mode

<table>
<thead>
<tr>
<th>pH</th>
<th>$P_{CO_2}$, Torr</th>
<th>$P_{O_2}$, Torr</th>
<th>$HCO_3^-$, mmol/l</th>
<th>$PET_{CO_2}$, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>No CPAP</td>
<td>7.37 (0.04)$^{a,b}$</td>
<td>42 (5)$^{b,c,d,e}$</td>
<td>90 (19)</td>
<td>24 (4)</td>
</tr>
<tr>
<td>CPAP 4</td>
<td>7.37 (0.04)</td>
<td>42 (5)</td>
<td>95 (17)</td>
<td>24 (4)</td>
</tr>
<tr>
<td>PS 10/4</td>
<td>7.37 (0.05)$^d$</td>
<td>41 (5)</td>
<td>94 (17)</td>
<td>24 (5)</td>
</tr>
<tr>
<td>PS 15/4</td>
<td>7.38 (0.05)</td>
<td>41 (5)</td>
<td>90 (13)</td>
<td>24 (5)</td>
</tr>
<tr>
<td>PS 20/4</td>
<td>7.37 (0.06)</td>
<td>40 (6)</td>
<td>94 (13)</td>
<td>23 (6)</td>
</tr>
<tr>
<td>VC base</td>
<td>7.38 (0.06)</td>
<td>40 (6)</td>
<td>93 (13)</td>
<td>24 (5)</td>
</tr>
<tr>
<td>VC-1</td>
<td>7.38 (0.06)</td>
<td>41 (5)</td>
<td>95 (15)$^b$</td>
<td>24 (4)</td>
</tr>
<tr>
<td>VC-2</td>
<td>7.39 (0.05)</td>
<td>40 (5)</td>
<td>94 (15)</td>
<td>24 (5)</td>
</tr>
<tr>
<td>VC-3</td>
<td>7.37 (0.05)</td>
<td>39 (5)</td>
<td>100 (15)</td>
<td>22 (5)</td>
</tr>
</tbody>
</table>

Values are expressed as means (SD). $P_{CO_2}$ and $P_{O_2}$ are arterial $P_{CO_2}$ and $P_{O_2}$, respectively; $PET_{CO_2}$, end-tidal $P_{CO_2}$; pH, arterial pH. All superscript letters are $P < 0.05$; $a$ no CPAP vs. VC-1; $b$ no CPAP vs. VC-3; $c$ PS 10/4 vs. VC-2; $d$ no CPAP vs. VC base; $e$ no CPAP vs. PS 20/4; $f$ VC-1 vs. VC-2; $g$ CPAP 4 vs. PS 20/4; $h$ CPAP 4 vs. VC-1; $i$ CPAP 4 vs. VC-2; $j$ PS 10/4 vs. VC-1; $k$ PS 15/4 vs. VC-1; $l$ PS 15/4 vs. VC-2; $m$ VC base vs. VC-2.

Table 3. Respiratory parameters during no CPAP, CPAP 4 cmH$_2$O, and nasal intermittent positive pressure ventilation in quiet sleep

<table>
<thead>
<tr>
<th>CT Inspi EMG</th>
<th>TA Inspi EMG</th>
<th>Dia Inspi EMG</th>
<th>Inspi TUAP, cmH$_2$O</th>
<th>RR, breaths/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>No CPAP</td>
<td>0.85 (0.18)</td>
<td>0.66–1.07$^{b,c,d,e}$</td>
<td>0$^b$</td>
<td>0.7 (1.2); -2.5$^c$; +0.6$^{b,c,d,e}$</td>
</tr>
<tr>
<td>CPAP 4</td>
<td>0.41 (0.27)</td>
<td>0.16–0.92$^{e,d}$</td>
<td>0$^e$</td>
<td>0.8 (0.2); 0.6–1.2$^{c,d,e}$</td>
</tr>
<tr>
<td>PS 10/4</td>
<td>0.26 (0.25)</td>
<td>0.10–0.85$^e$</td>
<td>0.07 (0.06); 0.02–0.22$^a$</td>
<td>0.6 (0.2); 0.3–0.9$^a$</td>
</tr>
<tr>
<td>PS 15/4</td>
<td>0.22 (0.08)</td>
<td>0.11–0.36</td>
<td>0.13 (0.08); 0.05–0.29</td>
<td>0.5 (0.3); 0.1–1.1</td>
</tr>
<tr>
<td>PS 20/4</td>
<td>0.21 (0.13)</td>
<td>0.04–0.35</td>
<td>0.20 (0.19); 0.02–0.48</td>
<td>0.4 (0.3); 0.1–0.7</td>
</tr>
<tr>
<td>VC base</td>
<td>0.52 (0.50)</td>
<td>0.11–1.35$^{b,i}$</td>
<td>0.08 (0.06); 0.03–0.20$^{a,i}$</td>
<td>0.9 (0.5); 0.3–1.7$^{b,i}$</td>
</tr>
<tr>
<td>VC-1</td>
<td>0.39 (0.57); 0.09–1.67</td>
<td>0.13 (0.10); 0.04–0.31$^i$</td>
<td>0.10 (0.02); 0.09–0.13$^a$</td>
<td>0.3 (0.2); 0.1–0.5$^a$</td>
</tr>
<tr>
<td>VC-2</td>
<td>0.21 (0.08); 0.09–0.28$^{a,i}$</td>
<td>0.10 (0.02); 0.09–0.13$^a$</td>
<td>0.3 (0.2); 0.1–0.5$^a$</td>
<td>9.8 (9.19); 7.7–11.9$^{a,i}$</td>
</tr>
<tr>
<td>VC-3</td>
<td>0.18 (0.06); 0.09–0.21$^{a,i}$</td>
<td>0.29 (0.17); 0.17–0.48$^a$</td>
<td>0.3 (0.1); 0.1–0.4$^a$</td>
<td>17.6 (4.4); 13.4–23.6$^a$</td>
</tr>
</tbody>
</table>

Values are means (SD; range). For both CT and Dia muscles, the inspiratory EMG maximal amplitude measured during wakefulness with no CPAP was averaged and used as reference value of 1 for subsequent calculations of values in the different ventilatory modes and states of alertness. For TA muscle, the averaged EMG maximal amplitude recorded during 5 swallows was chosen as the reference value of 1. See Design of Study and Data Analysis for details. Note that the statistical power is <80% for Dia, CT, and TA Inspi EMG in VC base and VC-3. All superscript letters are $P < 0.05$; $a$ no CPAP; $b$ vs. CPAP 4; $c$ vs. PS 10/4; $d$ vs. PS 15/4; $e$ vs. PS 20/4; $f$ vs. VC base; $g$ vs. VC-1; $h$ vs. VC-2; $i$ vs. VC-3.

CT muscle inspiratory activity. As recently shown in both humans and lambs, CT muscle acts as a glottal dilator in phase with the posterior cricoarytenoid muscle during inspiration (25, 32, 41). The present results showing disappearance of inspiratory CT EMG in newborn lambs in both PS and VC modes extend similar observations in adult humans during nIPPV in the PS mode (24). Disappearance of phasic inspiratory Dia and CT EMG is likely, or at least partly, related to the increase in vagal afferent activity from bronchopulmonary stretch receptors (2). Reflexes originating from the upper airways can also be at play, secondary to loss of negative inspiratory pressure. Indeed, this negative inspiratory pressure normally increases posterior cricoarytenoid muscle activity in eupneic breathing (44). Finally, while passive hypocapnia reduces inspiratory CT EMG (24), this mechanism is most likely not involved in the present study, as explained previously for TA EMG.

Active Inspiratory Glottal Narrowing During nIPPV

The present observations of both enhanced TA and decreased CT EMG during lung inflations strongly suggest that the glottis is actively narrowed in inspiration during nIPPV, as previously hypothesized from endoscopic observations during nIPPV in the VC mode in adult humans (40). The simultaneous increase in TA EMG and TUAP observed in all lambs at constant airflow in the VC mode indicates that upper airway resistance is increased with TA EMG during lung inflation. This increase in upper airway resistance could theoretically be related to active contraction of pharyngeal constrictor muscles, e.g., at the velopharyngeal level, or to passive mechanisms such as narrowing of the pliable laryngeal inlet due to the
Venturi effect. However, several evidences strongly suggest that active glottal closure is at least partly responsible for the increase in upper airway resistance. First, this study provides one example in which TGR increases with TA EMG during inflation in the VC mode (see Fig. 4B). Second, previous endoscopic observations in adult humans have shown that the glottis narrows in inspiration during nIPPV in the VC mode (17, 18). Finally, the existence of a complete stoppage in pressure transmission throughout the upper airways during the burst in TA EMG activity in AS, as shown in Fig. 5, strongly suggests an active mechanism.

Increased laryngeal resistance during lung inflation in nIPPV may consequently limit lung ventilation with increasing levels of nIPPV, especially in the VC mode. This is readily apparent in the one lamb illustrated in Fig. 4B during wakefulness, with lower tidal volume when both TA EMG and trans-glottal pressures are higher. In addition, bursts of TA EMG during AS were at times strong enough to totally prevent transmission of ventilator insufflations to the trachea (Fig. 5). Although such bursts of TA EMG have already been reported in adult humans during eupnea (23) and in newborn lambs during nIPPV (42), their relation with effective ventilation has not previously been discussed. Furthermore, such limitation of pressure transmission across the glottis may further increase the risk of gastric dilation and digestive perforations in newborns (11).

While we were not able to compare the PS and VC mode with regard to glottal resistance during lung inflations, some observations are nonetheless noteworthy. Indeed, as previously described, TA EMG in PS mode in all lambs was maximal in early inspiration and progressively decreased to zero before the end of inspiration (see Fig. 1), allowing prolonged transmission of constant ventilator pressure through the open glottis. Conversely, TA EMG in VC mode increased in parallel with ventilator pressure throughout inspiration, which suggests that glottal resistance was maximal when ventilator pressure peaked at the end of inspiration. In addition, pressure transmission across the glottis was further impeded in VC mode by a much shorter inspiratory duration, comparatively, than the PS mode (see Fig. 3). While these observations could explain the important differences in TUAP between the PS and VC modes (2.3 and 17.5 cmH2O, respectively; see Table 1), it is clear that a definitive assessment regarding the superiority of one nIPPV mode vs. the other in achieving lung ventilation in the newborn cannot conclusively be reached from the present results.

In conclusion, the present study shows that nIPPV, in either the PS or VC mode, induces both an inspiratory increase in glottal constrictor EMG and a decrease in glottal dilator EMG in lambs. Presence of this active glottal narrowing significantly limits lung ventilation, especially in the VC mode.

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Present address of P. Reix: Dept. of Pediatrics, Université de Lyon 1, Lyon, France.

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ACTIVE GLOTTAL CLOSURE DURING NASAL Ventilation

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