Laryngeal and abdominal muscle electrical activity during periodic breathing in nonsedated lambs

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Kianicka, Irenej, Véronique Diaz, Sylvain Renolleau, Emmanuel Canet, and Jean-Paul Praud. Laryngeal and abdominal muscle electrical activity during periodic breathing in nonsedated lambs. J. Appl. Physiol. 84(2): 669–675, 1998.—We recently reported that glottic closure was present throughout central apneas in awake lambs. The present study tested whether glottic closure was also observed during periodic breathing (PB). We attempted to induce PB in 21 nonsedated lambs on return from hypoxic hypoxia to room air. Airflow and thyroarytenoid (n = 16), cricothyroid (n = 9), and abdominal (n = 9) muscle electrical activity (EMG) were monitored continuously. PB was observed in 16 lambs, with apneic phases in 8 lambs. Thyroarytenoid muscle EMG was observed at the nadir of PB, either throughout apnea or with prolonged expiration during the lowest respiratory efforts. Phasic inspiratory cricothyroid muscle EMG and phasic expiratory abdominal EMG disappeared at the nadir of PB. Active glottic closure at the nadir of PB, without abdominal muscle contraction, could be a beneficial mechanism, preserving alveolar gas stores for continuing gas exchange during the apneic/hypopneic phase of PB. However, consequences of active glottic closure on ventilatory instability, either enhancing or reducing, are unknown.

Materials and Methods

Animals

Twenty-one mixed-breed lambs, aged 9–25 days [mean 15.5 ± 4.3 (SD)] and mean weight 6.4 ± 1.4 kg, were involved in the study. All were born from natural full-term delivery and were housed with their mothers in our animal quarters until the experimental day. Procedures were approved by the Ethics Committee for Animal Experimentation of our institution.

Surgical Instrumentation

The lambs underwent aseptic surgery 2–4 days before study under general halothane (Fluothane)-N₂O anesthesia. Atropine sulfate (0.2 mg/kg sc) was given every 30 min during surgery. Bipolar enameled chrome wire EMG electrodes (0.1 mm diameter; Chromel, GTSM, Castelnaudary, France) were implanted into intrinsic laryngeal and abdominal muscles using established techniques, as previously reported (22). Briefly, an EMG electrode was sewn under direct vision into each thyroarytenoid muscle (TA; a glottic constrictor) via a small window on each side of the thyroid cartilage (16 lambs). Another electrode was inserted in the lateral portion of cricothyroid muscle (CT; inspiratory glottic dilator; 10 lambs) and in a right abdominal muscle (Abd; external obliquus; 9 lambs). The leads were subcutaneously tunneled to exit on the back of the animals. Finally, a catheter was implanted into the right axillary artery for blood gas sampling (10 lambs).

Measurement Apparatus

A face mask, specifically molded for each lamb, was attached to a size-0 pneumotachograph (model 21070B, Hewlett-Packard, Palo Alto, CA) to record airflow (V). Tidal volume (VT) was calculated by electronic integration (model 8815A respiratory integrator, Hewlett-Packard). Raw EMG signals were amplified and 30- to 1,000-Hz band-pass filtered (model P511 AC preamplifier and model 7 DADC drive amplifier, Grass, Quincy, MA) before undergoing 100-ms moving time averaging (Dept. of Electronics, Faculty of Medicine, Université de Sherbrooke).

VT, V, and raw and integrated EMG signals were recorded on a 10-channel polygraph (model 7D, Grass). In addition, V and integrated EMG signals were fed in parallel into an IBM-compatible microcomputer (Televideo-Telecat-286, Sunnyvale, CA), where they were digitized (sampling rate 40 Hz) and analyzed. The collected data were stored on disk for further analysis. Arterial blood gases were determined in a pH blood-gas analyzer (model 1306, Instrumentation Labora-
Hypoxic runs were adjusted empirically to obtain blood-gas values for 3 min. Each lamb was switched to 0.08 FIO2 for 10 min. At the end of the hypoxic run the lamb was abruptly switched back to room air for 5 min. Blood-gas samples were taken during baseline recordings and at the end of hypoxia. In the event that PB did not appear on return to room air, duration ran from 1 min to 10 min and FIO2 was increased from 0.08 to 0.09. Inhaled gas could be switched from room air [0.21 fraction of inspired O₂ (FIO₂)] to a hypoxic mixture (0.06–0.08 FIO₂) in <1 s by using two valves (model P314, Collins, Braintree, MA).

Experimental Design

The study was designed to monitor glottic and abdominal muscle EMG during induced PB immediately after abrupt return from hypoxic to room-air breathing, as described previously (5). Each awake, nonsedated lamb was studied in the prone position while supported in a sling. After application of the face mask, the head of the lamb was carefully secured in a naturally adopted position on an adjustable head holder, with care taken to avoid compression of the hypopharyngeal region. After baseline room-air breathing was recorded for 3 min, each lamb was switched to 0.08 FIO₂ for 10 min. At the end of the hypoxic run the lamb was abruptly switched back to room air for 5 min. Blood-gas samples were taken during baseline recordings and at the end of hypoxia. In the event that PB did not appear on return to room air, duration ran from 1 min to 10 min and FIO₂ was increased from 0.08 to 0.09. Inhaled gas could be switched from room air [0.21 fraction of inspired O₂ (FIO₂)] to a hypoxic mixture (0.06–0.08 FIO₂) in <1 s by using two valves (model P314, Collins, Braintree, MA).

Analysis

For the purpose of our study, the following definitions were used. An episode of PB was defined as a series of more than three contiguous breaths alternating with apneas (>2 s) or hypopneas (VT <50% baseline VT for ≥3 respiratory cycles) (9, 28). Regarding glottic closure, our study design did not enable us to ascertain whether the glottis was completely or only partially closed when TA EMG was present and V̇ was nil. Consequently, the term “glottic closure” was used in its broader sense, without the inference that it was complete or partial. In addition, the presence of glottic closure did not exclude the possibility of simultaneous (active or passive) pharyngeal obstruction. Expiratory airflow braking was defined as a brisk decrease in expiratory airflow (often to zero or near-zero values) before the transition from expiration to inspiration. Finally, tonic EMG activity referred to a permanent EMG activity bearing no relation to the different phases of the respiratory cycle and over which a phasic (synchronous with inspiration or expiration) EMG discharge could be superimposed.

All recorded signals were analyzed during the 5-min post-hypoxic period. The number of cycles and total duration of each PB episode were calculated. Laryngeal and Abd EMGs were carefully observed during baseline room-air breathing, at the end of hypoxia, and during the 5-min posthypoxic period. VT, V̇, breathing frequency (averaged in 15-s epochs), Pao₂, and Paco₂ were measured during baseline room-air breathing and just before return from hypoxic to room-air breathing in each lamb; average values were then calculated for all lambs as a whole. Values are means ± SD.

Finally, we studied whether CT inspiratory and expiratory Abd EMG amplitude changed in parallel with VT during PB episodes. We performed a regression analysis between integrated CT EMG (expressed as percentage of maximal CT EMG observed in the recording) and VT (expressed as percentage of the maximal VT observed) throughout PB episodes in each animal. A similar regression analysis was performed for Abd EMG and VT throughout PB episodes in each animal. P < 0.05 was considered significant.

RESULTS

Although 21 lambs were initially involved in the study, data from 5 lambs could not be analyzed because of agitation and/or failure to induce PB despite several attempts. We were able to analyze TA EMG in all 16 remaining lambs, CT EMG in 10 lambs, and Abd EMG in 9 lambs.

Baseline Room-Air Breathing

Quiet ventilation was interrupted only by brief swallowing or body movements. Average values for respiratory parameters are reported in Table 1. No TA expiratory EMG was recorded in any of the 16 lambs (Fig. 1A). Phasic inspiratory and tonic expiratory (9 of 10 lambs) and only tonic (1 of 10 lambs) CT EMG were recorded. Consistent phasic expiratory Abd EMG was observed in all nine lambs studied (Fig. 1A).

Hypoxic Runs

Inhalation of the hypoxic mixture (0.06–0.08 FIO₂) elicited augmented respiratory efforts in all the lambs, including the typical diphasic response when hypoxic runs lasted for >3 min. Average values for respiratory parameters at the moment of return from hypoxia to room-air breathing are given in Table 1.

No TA EMG was present during hypoxia (apart from infrequent swallowing movements and agitation) in 11 of 16 lambs (Fig. 1B). Consistent increased phasic inspiratory TA EMG was observed with hypoxia in the remaining five lambs; in one of these lambs, phasic expiratory TA EMG was also present. Hypoxia elicited an increase in phasic inspiratory CT EMG in all 10 lambs, paralleled by increased expiratory activity in 8 of 10 lambs. Augmented phasic expiratory Abd EMG was observed during hypoxia in all nine lambs, although this was not always sustained throughout the hypoxic runs (Fig. 1B).

Posthypoxia-Induced PB

Fifty-nine episodes of PB were observed on abrupt return to room air in 16 lambs. In eight lambs (16 episodes of PB), apneas (2–10 s) were present at the nadir of PB. In the remaining episodes the nadir of PB corresponded to hyponeas with (35 episodes in 11 lambs).

Table 1. Respiratory parameters during baseline room-air breathing and at onset of periodic breathing (end of hypoxia)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>End of Hypoxia</th>
</tr>
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<tbody>
<tr>
<td>f, breaths/min</td>
<td>44.3 ± 11.2</td>
<td>66.6 ± 21.4</td>
</tr>
<tr>
<td>VT, ml/kg</td>
<td>11.8 ± 2.1</td>
<td>14.3 ± 3.1</td>
</tr>
<tr>
<td>V̇E, ml·min⁻¹·kg⁻¹</td>
<td>502 ± 7</td>
<td>918 ± 246</td>
</tr>
<tr>
<td>Pao₂, Torr</td>
<td>93.7 ± 5</td>
<td>26.7 ± 3.5</td>
</tr>
<tr>
<td>Paco₂, Torr</td>
<td>39 ± 2.8</td>
<td>29.4 ± 2.2</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 16 lambs. f: Breathing frequency; VT: tidal volume; V̇E: minute ventilation; Pao₂: arterial PO₂; Paco₂: arterial PCO₂.
lambs) or without (8 episodes in 4 lambs) expiratory braking. Total duration of PB episodes varied from 25 to 235 s \[65 \pm 25\ (SD)\ s\], with 2–13 cycles per episode \[6 \pm 6.5 (SD)\].

In the eight lambs with apneas during PB, continuous TA EMG was consistently present throughout apneas and absent during respiratory efforts (Figs. 1C and 2). During hypopneas at the nadir of PB, expiratory TA EMG was also observed with expiratory braking (Fig. 3); during respiratory efforts, this expiratory TA EMG abruptly disappeared or progressively decreased when ventilation increased. In two cases, ventilatory efforts were accompanied by phasic inspiratory TA EMG, which disappeared on resumption of regular breathing.

CT EMG (Fig. 3) was absent during apneas and hypopneas, with expiratory braking in all lambs but two where low-amplitude, tonic EMG was present. Phasic inspiratory CT EMG consistently reappeared and increased with respiratory efforts (Fig. 3). Regression analysis showed a highly significant relationship between inspiratory CT integrated EMG amplitude and VT throughout PB episodes in each animal (\(P < 0.001, n = 32\) of 35 PB episodes; \(P < 0.05, n = 3\) of 35 PB episodes), attesting to the parallel changes in inspiratory CT EMG and inspiratory efforts. Phasic inspiratory and tonic expiratory CT EMGs consistently returned on resumption of regular breathing.

Abd EMG disappeared completely after return to room air in six of nine lambs (Fig. 2). In the remaining three lambs, low-amplitude, expiratory Abd EMG cycled with respiratory efforts, Abd EMG being absent in two lambs (Fig. 3) or tonically active in the other lamb (Fig. 1C) during apneas. Regression analysis showed a significant relationship (\(P < 0.05\)) between Abd expiratory EMG and VT changes throughout six of nine PB episodes.

DISCUSSION

The present study provides unique data on laryngeal and Abd muscle activity during posthypoxia-induced PB in lambs. We observed active glottic closure (continuous TA with silent CT EMG) and inhibition of Abd EMG recurring consistently with each apnea (or hypopnea) during posthypoxia-induced PB.

PB in Neonates

PB, which consists of alternating series of contiguous breaths and apneas (or hypopneas), has been frequently reported in human infants, especially in preterm babies (9). Occurrence of PB is dependent on age [rare during the first days of life (3)], health [decreased in infants with bronchopulmonary dysplasia or respiratory tract infection (9)], and sleep state [more frequent in active sleep in full-term infants and in quiet sleep in preterm infants (1); increased after sleep deprivation (6)]. Most studies have failed to find a prognostic value of PB for the later development of severe apnea of infancy, apparent life-threatening events, or sudden infant death syndrome (9). However, PB can lead to significant hypoxemia in preterm infants and has been reported to be accompanied by obstructive apneas (15).

Mechanisms responsible for PB in infants are mostly still a matter of debate. Although clinical evidence points to a role of hypoxia in promoting respiratory
instability in neonates (7), present knowledge of factors promoting ventilatory instability mainly stems from theoretical models (12, 14) and from experimental data in adult animals and humans (8, 16, 28). It is generally accepted that a PB (Cheyne-Stokes type of breathing) cycle is initiated when a transient change in alveolar gas tension is allowed to induce a delayed ventilatory overshoot followed by an undershoot, reflecting chemoreceptor instability. This arises when overall chemoreceptor gain is increased (e.g., during hypoxia), when the time delay between lung and chemoreceptors is increased (e.g., during congestive heart failure), or when damping of an alveolar gas tension change is decreased (e.g., decreased end-expiratory volume) (14, 25). Recent reviews on PB have clearly emphasized the extreme complexity of ventilatory stability, which involves multi-
multiple mechanisms. Accordingly, numerous factors can induce or maintain PB, among which sleep-state instability, upper airway instability, and altered postdischarge phenomenon seem especially important (8, 16, 29).

In contrast to results in human neonates, spontaneous PB has not been described in newborn mammals; however, we have been able to consistently induce PB in lambs at 10 days of age, a time when peripheral chemoreceptors are functionally mature (5). These data and observations by others that PB in human infants is rare in the first 48 h of life (3), at a time when peripheral chemoreceptors are functionally immature, support the hypothesis that mature peripheral chemoreceptors are important for PB development in neonates.

### Laryngeal Muscle EMG and PB

The present results clearly show that expiratory/continuous TA EMG was enhanced at the nadir of PB in awake, nonsedated lambs, whereas phasic inspiratory and tonic CT EMG were inhibited. These results are in agreement with our previous results in isolated, induced central apneas. Indeed, we observed continuous glottic constrictor EMG (TA) with simultaneous inhibition of phasic inspiratory and tonic glottic dilator EMG (CT and posterior cricoarytenoid) in posthyperventilation apneas (13, 18). Taken together, our observations show that induced central apneas, either isolated or in PB, are associated with active glottic closure (complete or partial) in nonsedated lambs. The relevance of these data is emphasized by preliminary identical observations from polysomnographic recordings in spontaneous central apneas in sleeping lambs (20; unpublished observations).

The mechanism(s) responsible for active glottic closure at the nadir of PB is not known, and the present data do not allow us to draw any firm conclusion. However, these and our previous results do provide some insight as to potential mechanisms involved.

### Chemical control

In the present study, initiation of PB was probably related to the presence of a critical combination of hypocapnia and hypoxia, together with an abrupt increase in FIO₂ (5). However, hypocapnia, known to enhance glottic closure (4), is not a prerequisite for glottic closure throughout central apneas (21). Abrupt relief from hypoxia, which has been suggested to enhance TA EMG by relieving the inhibitory influence of peripheral chemoreceptors on TA (18), is also not a prerequisite: indeed, continuous TA EMG is also present throughout central apneas, which develop during hyperoxia (PaO₂ > 400 Torr) (13, 21). This suggests that mechanisms other than chemical influences could be responsible for the development of active glottic closure during PB.

### Vagal afferent control

It has been reported that expiratory TA EMG is consistently enhanced by a decrease in end-expiratory lung volume (10). In addition, it has been reported that end-expiratory lung volume could be decreased during episodes of PB (26). However, our previous results again suggest that continuous TA EMG can be observed in the absence of increased vagal afferents (18, 21), making decreased end-expiratory lung volume responsible for enhancing TA EMG during PB unlikely.

Finally, we hypothesize that cessation of central inspiratory drive in awake, nonsedated lambs removes the inhibition of continuous glottic constrictor muscle activity, primarily through intrinsic brain stem mechanisms. This could have ontogenetic and phylogenetic links, the glottis being known to be actively closed during prolonged apneic periods in fetuses and bimodal
animal species such as lungfish, respectively (20). Others have suggested that the propensity to develop active glottic closure when central inspiratory drive ceases continues throughout life in mammals (11).

**Abd EMG During PB**

The present study shows that Abd EMG was often absent after the abrupt return to room air, probably as a result of hypocapnia with rising PaO₂. Interestingly, in three lambs, phasic expiratory Abd EMG cycled with PB in a crescendo-decrescendo pattern: Abd EMG was absent or decreased during the apneic phase of PB and was maximal when respiratory efforts were at their highest. These data are in general agreement with previous observations in lambs, i.e., disappearance of Abd EMG during posthyperventilation (13) or barbiturate-induced central apneas (21). Hence, it appears that TA expiratory EMG is present when Abd expiratory EMG is absent and reciprocally.

Mechanisms responsible for inhibition of Abd EMG at the nadir of PB are unknown. Although hypocapnia, abrupt relief of hypoxia, or decreased vagal afferents could have a role in the present experiments, our previous results during hypercapnia (21) and in vagotomized lambs (19) suggest that none are mandatory. Again, it is possible that intrinsic brain stem mechanisms are brought into play, accounting for Abd inhibition when central inspiratory drive ceases.

**Potential Consequences on Ventilatory Stability and Genesis of Apneas of Prematurity**

Glottic closure throughout apneas, together with the absence of abdominal muscle contraction, would tend to prevent alveolar gas from flowing out of the lung. Consequent increase in end-expiratory lung volume, either above or below functional residual capacity, would likely be beneficial by increasing alveolar gas volume available for gas exchange during apnea (29).

Theoretically, this increase would prevent further ventilatory instability and, therefore, tend to decrease duration of the PB episode (25, 28). However, consequences of active glottic closure during PB might not be so favorable for ventilatory stability. Indeed, upper airway resistance at the nadir of PB has been shown to promote ventilatory instability (8, 28). Furthermore, continuous TA EMG throughout apnea might retard resumption of breathing by reciprocal inhibition of inspiratory muscles (8, 24), contributing to increased duration of PB episodes. The present results do not permit us to discern which scenario (increased or decreased ventilatory stability) is the most likely.

Previous studies have shown that apneas of prematurity preferentially occur at the nadir of PB (15, 27), which is therefore thought to play a critical role in the genesis of apnea. The observation of glottic closure at the nadir of PB leads us to hypothesize that it could precipitate mixed apnea if inspiratory diaphragmatic efforts resume without coordinated inhibition of glottic constrictors and inspiratory contraction of glottic dilators. This hypothesis is further supported by endoscopic observations of mixed apneas with actively closed glottis in preterm infants (23).

In conclusion, the present data obtained in nonseparated, awake lambs are the first report of laryngeal and abdominal muscle variations with PB. The observation of active glottic closure and the absence of abdominal muscle contraction at the nadir of PB could be of importance in regard to PB episode duration and genesis of apneas of prematurity. Future studies are needed to investigate the additional effect of sleep stage on these observations.

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