Active glottal closure during central apneas limits oxygen desaturation in premature lambs

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The immature respiratory control system is responsible for frequent spontaneous apneas in newborn mammals. Repetitive oxygen desaturation and bradycardias secondary to apneas and periodic breathing remain a highly significant problem in neonatal care (19). This is especially true in premature newborns, with 75% of those born at 27 wk presenting apneas of prematurity (7). Interestingly, and for yet-unknown reasons, the magnitude of oxygen desaturation after neonatal apneas or periodic breathing is highly variable (22). In previous experiments conducted in lambs (including preterm lambs), our laboratory found that 90% of central apneas, either isolated or within periodic breathing, are characterized by active glottal closure and maintenance of high-lung volume (inspiratory breath holding) (8, 9, 15, 18). This led us to hypothesize that maintenance of a high lung volume would increase alveolar oxygen stores during apneas and limit postapneic oxygen desaturation.

METHODS

The experiments were conducted in four preterm lambs with a postconceptional age of 132 days (normal gestation 147 days) and a mean birth weight of 3.1 ± 0.2 kg (range 2.9–3.3 kg). The protocol of the study was approved by the University of Sherbrooke’s Ethics Committee. Preterm lamb model. Three lambs were delivered vaginally after prenatal lung maturation, as previously described (18). One lamb was delivered by cesarean section under epidural anesthesia with 5 ml of 2% lidocaine. Exogenous surfactant (10 ml of BLES, London, ON) was given to the lamb by transcutaneous, intratracheal injection immediately after birth and repeated 24 h later. Standard care for the first postnatal hours systematically included nasal continuous positive airway pressure for 2 h (Bourns-BP200, Life System, Riverside, CA) and supplemental oxygen to maintain transcutaneous oxygen saturation >95%, the use of an incubator to maintain rectal temperature >38.5°C, and dextrose intravenous supplementation to maintain glycemia >2.3 mmol/l. Continuous nasogastric feeding with natural ewe’s milk was started after 3–4 h of life and replaced by discontinuous gastric feeding after 1–2 days. The nasogastric tube was systematically removed for polysomnographic recordings.

Surgical preparation. Surgery was performed 2–3 days after birth under general anesthesia (1–2% isoflurane + 30% N2O + 68% O2). Atropine sulfate (150 μg/kg subcutaneously) was given preoperatively with 5 mg/kg ketamine and 100

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μg/kg midazolam intramuscularly. Bipolar enameled chrome wire electrodes were inserted into the thyroarytenoid (TA) and diaphragm muscles for recording electromyographic (EMG) activity (8), together with custom-made electrodes for EEG, electrooculogram, and ECG (18). Leads from each electrode were subcutaneously tunneled to exit on the back of the lambs. Furthermore, a tracheostomy with no intratracheal tube was performed between the fifth and sixth tracheal rings (9). Postoperative care included intramuscular butpronorphine (50 μg/kg, one dose at the end of surgery) and intramuscular injection of 50 mg/kg ampicillin and 2.5 mg/kg gentamicin daily thereafter. Lambs were euthanized at the end of the experiments by an overdose of pentobarbital, and correct electrode positioning was verified at autopsy.

**Recording equipment.** Prolonged recordings were obtained by using our custom-designed radiotelemetry system comprising eight differential channels for nasal flow, ECG, electrooculogram, EEG, and four EMG recordings (11). The raw EMG signals were rectified, integrated, and moving time averaged (100 ms). Nasal airflow was recorded by using a thermocouple. Furthermore, we used our newly developed pulsed radiotelemetry system with a pressure transducer (MP-45-30-871; Validyne, Northridge, CA). All signals were recorded on a Power Macintosh 7300 with the use of the Acknowledge 3.2 acquisition software (Biopac Systems, Santa Barbara, CA). Validity of the data was assessed with their sum by using respiratory inductive plethysmography (Respitrace, NIMS, Miami Beach, FL). In two lambs, subglottal pressure was also monitored. Subglottal pressure was measured by using a pressure catheter connected laterally to the tracheostomy. It was subsequently connected to a pressure transducer (MP-45-30-871; Validyne, Northridge, CA). All signals were recorded on a Power Macintosh 7300 with the use of the Acknowledge 3.2 acquisition software (Biopac Systems, Santa Barbara, CA).

**Design of the study.** Each lamb was studied without sedation, at least 48 h after surgery. The telemetry transmitters were connected to electrode leads and to the oximeter probe and attached to the lamb’s back before each recording session. Lambs were studied in an incubator for 4–8 h daily. Central apneas with closed glottis (and closed tracheostomy) and maintenance of a high-apneic lung volume were compared with apneas with opened tracheostomy (surrogate for open glottis) and low lung volume. Recording sessions were divided into periods of 30–60 min in duration, alternating periods with tracheostomy continuously closed with a cap (Cl-Trach) and periods with tracheostomy opened (Op-Trach) during apneas. For the later periods, while the tracheostomy was kept tightly closed with a finger during regular breathing, it was quickly opened at the beginning of apneas and resealed as soon as breathing resumed (see Fig. 1).

**Data analysis.** Standard electrophysiological and behavioral criteria were used to define wakefulness (W), quiet sleep (QS), and active sleep (AS)(18). Central apneas were defined by the absence of airflow for at least 3 s, with no respiratory efforts and no diaphragmatic EMG. Periodic breathing was defined as alternating series of contiguous breaths and apneas (≥3 s) or hypopneas. Central apneas with motion arti-

**RESULTS**

Total duration of recordings in the four lambs was 83 h, with a mean total recording time of 8.3 ± 5.0 h in W, 7.0 ± 4.3 h in QS, and 4.4 ± 2.0 h in AS. The apnea index was 11.6 ± 9.6 h⁻¹ during W, 12.4 ± 10.8 h⁻¹ during QS, and 9.7 ± 7.1 h⁻¹ during AS. A total of 2,163 apneas were recorded, including 2,105 central apneas and 58 obstructive and mixed apneas. From 1,623 central apneas, which were further analyzed, TA EMG was continuous throughout 90% of apneas (including 84% in AS) present, but discontinuous in 7% of apneas, and absent in 3% of apneas. A total of 1,452 central apneas with continuous TA EMG were analyzed, including 333 apneas during W (156 isolated, 177 during periodic breathing), 1,010 apneas during QS (248 isolated, 762 during periodic breathing), and 109 isolated apneas during AS. Regardless of the state of alertness, respiratory breath holding was considered present when the sum signal of the respiratory inductive plethysmograph during a central apnea was maintained above the preceding end-expiratory lung volume and the subglottal pressure was maintained above atmospheric pressure (n = 2) (Fig. 1). The consequences of apnea on SpO₂ were assessed as follows: the highest pre-SpO₂ and the lowest postapneic SpO₂ (post-SpO₂) values were measured for each apnea. The slope of oxygen desaturation (∆SpO₂/∆t; where t is time; in %/s) was calculated as follows, (pre-SpO₂ - post-SpO₂)/time between pre-SpO₂ and post-SpO₂, for each duration group, state of alertness, and tracheostomy status. Results are reported as means ± SD. Comparisons were performed by using Student t-test for unpaired comparisons and two-factor ANOVA (SuperANOVA, Abacus Concepts, Berkeley, CA). A P value < 0.05 was considered statistically significant, and the Bonferroni correction was used when applicable (21).

**Apneas during QS.** Overall, 659 Cl-Trach apneas were compared with 351 Op-Trach apneas in QS (see examples in Fig. 1). Apnea duration and pre-SpO₂ were not significantly different between Cl-Trach and Op-Trach apneas (7.6 ± 2.3 vs. 7.4 ± 1.8 s; P = 0.12, and 93.8 ± 3.6 vs. 93.7 ± 2.7%; P = 0.53).
Similarly, the time lapse between pre-SpO₂ value and apnea onset was not significantly different between Cl-Trach (6.3 ± 1.6 s) and Op-Trach apneas (6.2 ± 1.6 s; \( P = 0.7 \)). However, time lapse between apnea termination and post-SpO₂ value was shorter for Op-Trach (3.2 ± 0.8 s) than for Cl-Trach apneas (4.3 ± 1.4 s; \( P = 0.0009 \)). Overall, \( \Delta \text{SpO}_2/\Delta t \) was significantly lower after Cl-Trach than after Op-Trach apneas (2.0 ± 0.7 vs. 2.4 ± 0.7%/s; \( P = 0.0001 \)). This was true for both isolated apneas and those during periodic breathing (\( P = 0.0001 \)). Moreover, a significantly lower \( \Delta \text{SpO}_2/\Delta t \) after Cl-Trach was found for 3- to 6-s apneas (\( P = 0.0001 \)) and 6- to 9-s apneas (\( P = 0.0001 \)). Whereas a similar trend was observed for 9- to 12-s apneas (\( P = 0.022 \)) and >12-s apneas (\( P = 0.28 \)), differences did not reach statistical significance (\( P < 0.0125 \) expected with Bonferroni correction) (Fig. 2A).
Apneas during AS. During AS, 109 apneas were analyzed, including 79 Cl-Trach and 30 Op-Trach apneas. Overall, δSpO₂/Δt and apnea duration were not different after Cl-Trach apneas and Op-Trach apneas (1.7 ± 0.7 vs. 1.7 ± 0.9%/s; \( P = 0.88 \) and 5.4 ± 2.8 vs. 5.1 ± 1.9 s; \( P = 0.7 \)). The time lapse from pre-SpO₂ value to apnea onset was not statistically different between Cl-Trach (5.9 ± 2.3 s) and Op-Trach (5.2 ± 1.4 s; \( P = 0.5 \)). However, time lapse between apnea termination and post-SpO₂ value was again shorter for Op-Trach apnea (3.2 ± 0.4 s) than for Cl-Trach (5.6 ± 2.7 s; \( P = 0.0094 \)). Unfortunately, the low number of apneas in each duration category precluded further statistical analysis.

Apneas during W. Overall, 242 Cl-Trach apneas and 91 Op-Trach apneas were compared during W. Duration of Cl-Trach apneas (7.6 ± 3.1 s) was significantly shorter than duration of Op-Trach apneas (8.5 ± 3.6 s; \( P = 0.03 \)). The time lapse between pre-SpO₂ and apnea onset was 6.7 ± 1 s for Op-Trach and 7.2 ± 1.7 s for Cl-Trach (\( P = 0.25 \)). Time lapse between apnea termination and post-SpO₂ value was again shorter for Op-Trach (3.2 ± 0.6 s) than for Cl-Trach (4.5 ± 1.9 s; \( P = 0.0004 \)). Whereas pre-SpO₂ was not significantly different for Cl-Trach and Op-Trach apneas (\( P = 0.28 \)), δSpO₂/Δt was significantly lower after Cl-Trach apneas than after Op-Trach apneas (1.9 ± 0.6 vs. 2.5 ± 0.9%/s; \( P = 0.0001 \)). This was true for both isolated apneas (\( P = 0.0001 \)) and apneas during periodic breathing (\( P = 0.0001 \)). Moreover, a significantly lower δSpO₂/Δt after Cl-Trach was found in the 6- to 9-s apneas (\( P = 0.0001 \)) and 9- to 12-s apneas (\( P = 0.002 \)). For both the 3- to 6-s apnea and >12-s apnea groups, δSpO₂/Δt was not found to be statistically different (\( P = 0.55 \) and 0.54, respectively) (Fig. 2B).

Finally, 104 Cl-Trach isolated apneas with continuous TA EMG (closed glottis) were compared with 86 Cl-Trach isolated apneas with noncontinuous TA EMG (open glottis) during W. Although pre-SpO₂ was significantly lower in the apneas with continuous TA EMG than in the apneas with noncontinuous TA EMG (95.6 ± 2.6 vs. 97.4 ± 2.9%; \( P = 0.0001 \)), δSpO₂/Δt was significantly lower after apneas with continuous TA EMG (1.7 ± 0.6% vs. 2.1 ± 0.9%/s; \( P = 0.0026 \)). Moreover, apnea duration was longer when TA EMG was noncontinuous (7.9 ± 2.3 s) than when TA EMG was continuous (6.4 ± 2.9 s; \( P = 0.0001 \)).

DISCUSSION

The present study presents convincing evidence that active glottal closure during central apnea limits postapneic oxygen desaturation in nonsedated preterm lambs. Although several studies have brought evidence that active expiratory glottal closure protects against oxygen desaturation during breathing, to our knowledge such protective effects on postapneic oxygenation have not been reported previously.

In 1980, Milner et al. (12) were the first to speculate that the glottis was closed during some spontaneous apneas in preterm newborns. Recurrent glottal closure was subsequently observed by using endoscopy during central apneas within a prolonged episode of periodic breathing in one human infant (20). During the past few years, our group has demonstrated that TA EMG was present throughout the vast majority of induced (8, 9, 15) and spontaneous central apneas (18) in full-term and preterm lambs, irrespective of the state of alertness. Continuous TA EMG was shown to be associated with complete glottal closure and maintenance of lung volume in an inspiratory position throughout central apneas (9,
18). Results from the present study confirm our previous findings and demonstrate that inspiratory breath holding due to active glottal closure limits postapneic desaturation in W and QS, especially for apneas with a duration between 6 and 12 s. The inability to reach identical conclusions for apneas in AS may be related to the few Op-Trach apneas available for comparison in AS.

Recent studies suggest that central apneas, either isolated or within epochs of periodic breathing, are the most frequent types of apneas in preterm human newborns (10) and preterm lambs (18). A few previous observations have suggested the importance of lung volume for determining desaturation after central apnea. Severe desaturation after apneas of prematurity was previously shown to be linked to low end-expiratory lung volume (1, 14). Conversely, using a mathematical model of neonatal respiration, it was suggested that the presence of a high lung volume provided a buffer for gas exchange during the short central apneas often observed in infants (23). Also, lung volume was shown to be the most important determinant of $\text{SpO}_2$ after voluntary central apnea in awake human adults (4). The use of active glottal closure to maintain high lung volume is especially relevant in neonatal respiration, because of low pulmonary compliance and high chest wall compliance, both of which tend to decrease lung volume (13). This was first demonstrated by the dramatic decrease in arterial oxygenation secondary to bypassing the glottis by tracheal intubation in grunting premature newborns (6). Later on, it was shown that by bypassing the glottis by opening a tracheostomy led to reflex activation of glottal adductor muscles in adult cats (17), lambs (5), and puppies (3), presumably to prevent a decrease in lung volume and the consequent oxygen desaturation. Our findings extend those previous observations to spontaneous central apneas in preterm lambs.

Finally, the importance of the Hering-Breuer reflex in neonates might have suggested that active inspiratory breath holding leads to prolongation of apneas (13), which would be deleterious for gas exchange. Interestingly, that apneas with high lung volume (Cl-Trach apneas) were of shorter (W) or equal (QS) duration than apneas with low lung volume (Op-Trach apneas), such deleterious effects were not observed in the present study. Personal results on the Hering-Breuer reflex in preterm lambs, showing that occlusion at end inspiration inhibits inspiration for <2 s (2), suggest that this would not be appreciable during apneas >3 s (as in the present study).

In conclusion, the present study demonstrates that the larynx fulfills an important role for preserving neonatal oxygenation during spontaneous central apneas in preterm lambs. Further studies are ongoing to test the hypothesis that this mechanism is highly prevalent in human newborns and limits oxygen desaturation after isolated apneas and during periodic breathing.

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