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

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Submitted 26 August 2002; accepted in final form 2 January 2003

Reix, Philippe, Julie Arsenault, Valérie Dôme, Pierre-Hugues Fortier, Joëlle Rouillard Lafond, François Moreau-Bussière, Dominique Dorion, and Jean-Paul Praud. Active glottal closure during central apneas limits oxygen desaturation in premature lambs. J Appl Physiol 94: 1949–1954, 2003.—Our laboratory previously reported that active glottal closure was present in 90% of spontaneous central apneas in premature lambs while maintaining a high-apneic lung volume (Renolleau S, Letourneau P, Niyonsenga T, and Praud JP. Am J Respir Crit Care Med 159: 1396–1404, 1999.) The present study aimed at testing whether this mechanism limits postapnea oxygen desaturation. Four premature lambs were instrumented for recording states of alertness, thyroarytenoid muscle and diaphragm electromyographic (EMG) activity, nasal airflow, lung volume changes, and pulse oximetry. One thousand four hundred fifty-two spontaneous central apneas (isolated or within periodic breathing) were analyzed in nonsedated lambs. Apneas, with high lung volume maintained by active glottal closure, were compared with apneas, with a tracheostomy opened at apnea onset. Oxygen desaturation slopes were lower after isolated apneas with continuous thyroarytenoid EMG during wakefulness, compared with apneas and noncontinuous thyroarytenoid EMG (≈ glottis opened shortly after apnea onset). These results highlight the importance of maintaining high-alveolar oxygen stores during central apneas by active glottal closure to limit desaturation in newborns.

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 tracheostomy 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 μg/kg fentanyl. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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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. The aim of the present study was to test the hypothesis that active inspiratory breath holding limits oxygen desaturation after spontaneous central apneas in preterm lambs. This was accomplished by comparing oxygen desaturation after apneas with closed glottis and maintenance of a high lung volume, as opposed to oxygen desaturation after apneas with opened tracheostomy (surrogate for open glottis) and a low lung volume.

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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 bufrenorphine (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 pulsatil radiotelemetry system to record arterial oxygen saturation (SpO2) continuously. This oximeter was built by using the OEM board and probe of commercially available pulse oximeters (Nonin Medical, Plymouth, MN). The only addition was the telemetry transmitter and receiver, which did not affect the basic functioning of the oximeter. The averaging time was 4 s. In our experience with lambs, the best signal was obtained by using a reflectance probe (8000R reflectance sensor, Nonin Medical) placed at the base of the tail. This conclusion was reached after extensive recording sessions for validating the device (16). Values contaminated by movement artifacts were readily recognized by simultaneous recording of the pulse wave and systematically rejected. Thoracic and abdominal volume variations were assessed with their sum by using respiratory inductance plethysmography (Respirtrace, 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 (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 artifacts or preapneic SpO2 (pre-SpO2) < 85% were not considered. The apnea index (number of central apneas per hour) was calculated for each state of alertness and averaged for the four lambs. Obstructive and mixed apneas were not analyzed further. Central apneas with continuous TA EMG from the four lambs were classified, depending on their duration, i.e., between 3 and 6, 6 and 9, 9 and 12, or >12 s, and their tracheostomy status, i.e., Op-Trach or Cl-Trach apneas, and were pooled in each state of alertness. The few apneas with absent TA EMG were included in the Op-Trach group, considering that the glottis was open during these apneas. Moreover, there was a sufficient number of isolated central apneas with noncontinuous TA EMG (i.e., present during less than one-third of the duration of the apnea) during W to allow us to compare oxygenation between these apneas (open glottis) and apneas with continuous TA (closed glottis).

Inspiratory 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 (ΔSpO2/Δt; 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).

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, inspiratory breath holding was present in apneas with continuous TA EMG. Moreover, continuous TA EMG was not affected by opening the tracheostomy (Fig. 1). Finally, post-SpO2 values obtained in Op-Trach and Cl-Trach conditions were, respectively, 83 ± 6.7 and 85 ± 7.6% (P = 0.0001).

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, ∆Spo2/∆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 ∆Spo2/∆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).

**Fig. 1.** Example of recordings obtained in a 10-day-old preterm lamb during quiet sleep. Isolated apneas with opened (A) and closed (B) tracheostomy are illustrated, as well as apneas during periodic breathing (C). Tracheostomy was opened (*) and closed (†). Dashed line, end-expiratory lung volume. The highest value of arterial oxygen saturation from pulse oximetry (Spo2) preceding oxygen desaturation and the lowest Spo2 value are indicated. TA, thyroarytenoid muscle electromyographic activity; fTA, moving-time-averaged TA; Dia, diaphragm muscle electromyographic activity; fDia, moving-time-averaged Dia; F, nasal airflow, inspiration upward; Sub-P, subglottal pressure; Sum, sum signal of respiratory volume measured by respiratory inductive plethysmography; EOG, electrooculogram.
Apneas during AS. During AS, 109 apneas were analyzed, including 79 Cl-Trach and 30 Op-Trach apneas. Overall, $\Delta \text{SpO}_2/\Delta t$ 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-Sp$_2$ 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-Sp$_2$ 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-Sp$_2$ 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-Sp$_2$ was shorter for Op-Trach (3.2 ± 0.6 s) than for Cl-Trach (4.5 ± 1.9 s; $P =$ 0.0004). Whereas pre-Sp$_2$ was not significantly different for Cl-Trach and Op-Trach apneas ($P =$ 0.28), $\Delta \text{SpO}_2/\Delta t$ 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 $\Delta \text{SpO}_2/\Delta t$ 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, $\Delta \text{SpO}_2/\Delta t$ 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-Sp$_2$ 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), $\Delta \text{SpO}_2/\Delta t$ 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 SpO2 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 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, given 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.

The authors thank Bruno Gagné, Christophe Grenier, and Christine Mayrand-Charette for technical assistance. The authors also acknowledge the generous donation of surfactant by BLES Inc., London, ON, Canada.

The research was supported by Canadian Institutes of Health Research Grant MT15558 and the Fonds de la Recherche en Santé du Québec. P. Reix is a scholar of the Société de Pneumologie de Langue Francaise. J.-P. Praud is a senior scholar of the Fonds de la Recherche en Santé du Québec.

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