Active upper airway closure during induced central apneas in lambs is complete at the laryngeal level only

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Active upper airway closure during induced central apneas in lambs is complete at the laryngeal level only. J Appl Physiol 95: 97-103, 2003. First published March 7, 2003; 10.1152/japplphysiol.00773.2002.—We tested the hypotheses that active upper airway closure during induced central apneas in nonsedated lambs 1) is complete and occurs at the laryngeal level and 2) is not due to stimulation of the superior laryngeal nerve (SLN). Five newborn lambs were surgically instrumented to record thyroarytenoid (TA) muscle (glottal constrictor) electromyographic (EMG) activity with supraglottal and subglottal pressures. Hypocapnic and nonhypocapnic central apneas were induced before and after SLN sectioning in the five lambs. A total of 174 apneas were induced, 116 before and 58 after sectioning of the internal branch of the SLN (iSLN). Continuous TA EMG activity was observed in 88% of apneas before iSLN section and in 87% of apneas after iSLN section. A transglottal pressure different from zero was observed in all apneas with TA EMG activity, with a mean subglottal pressure of 4.3 ± 0.8 cmH2O before and 4.7 ± 0.7 cmH2O after iSLN section. Supraglottal pressure was consistently atmospheric. Sectioning of both iSLNs had no effects on the results. We conclude that upper airway closure during induced central apneas in lambs is active, complete, and occurs at the glottal level only. Consequently, a positive subglottal pressure is maintained throughout the apnea. Finally, this complete active glottal closure is independent from laryngeal afferent innervation.

newborn; thyroarytenoid muscle; transglottal pressure; transpharyngeal pressure; superior laryngeal nerve

THE IMMATURE RESPIRATORY CONTROL system is responsible for frequent spontaneous apneas in newborn mammals. Recent studies suggest that central apneas, either isolated or during epochs of periodic breathing, are the most frequent types of apneas in preterm human newborns (28) and preterm lambs (27). Studies conducted in our laboratory over the past 10 years have shown that continuous thyroarytenoid (TA) muscle (a glottal constrictor muscle) electromyographic (EMG) activity is present in the vast majority (90%) of central apneas in newborn lambs, including spontaneous apneas (13, 23, 25, 27). Although endoscopic observations have shown that TA EMG activity is associated with glottal closure during induced central apneas in lambs (16), it remains unknown whether the glottis is completely closed and if so, how often. Moreover, although our laboratory has observed inferior pharyngeal constrictor EMG activity throughout spontaneous central apneas in preterm lambs (27), it is also not known whether the pharynx is open or closed during central apneas. Hence, our first objective was to test the hypothesis that transglottal pressure is different from zero (i.e., complete glottal closure) during central apneas induced in nonsedated lambs. Furthermore, it is well known that stimulation of the superior laryngeal nerve can trigger reflex central apneas with active glottal closure. Because this laryngeal chemoreflex is especially evident in the neonatal period due to immaturity of the nervous system (34, 35), involvement of this reflex in active glottal closure observed during central apneas in lambs cannot be dismissed. Therefore, our second hypothesis was that the sectioning of the internal sensory branch of both superior laryngeal nerves (iSLNs) does not prevent complete glottal closure during induced central apneas in nonsedated lambs.

MATERIALS AND METHODS

Animals

Experiments were performed on five mixed-bred lambs aged 8.4 ± 2.1 (SD) days (range 7–12 days), with a mean weight of 4.5 ± 0.6 kg (range 3.8–5.4 kg). Four of the five lambs were born at full term (147 days). One lamb (3.8 kg) was delivered 15 days prematurely by cesarean section under epidural anesthesia, after prenatal intramuscular injection of betamethasone. This lamb received standard care for preterm lambs at birth, including intratracheal surfactant (27). Lambs were housed in our animal quarters with their mothers up until the experiments. The protocol of this study was approved by our institutional Ethics Committee for Animal Research.

Surgical Preparation

Initial surgery was performed 2–4 days before the experiment under general anesthesia (2% isoflurane-28% N2O-70% O2). Atropine sulfate (0.05 mg/kg), midazolam hydro-

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chloride (0.1 mg/kg), and ketamine (10 mg/kg) were injected intramuscularly before anesthesia. Antibiotics (50 mg/kg ampicillin and 2.5 mg/kg gentamicin) were administered intramuscularly, 30 min before incision and each day until the experiment. Single doses of buprenorphine (1 μg/kg subcutaneously) and dexamethasone (1 mg/kg intramuscularly) were systematically given immediately after surgery for analgesia and to limit postsurgical laryngeal inflammation, respectively.

Chronic instrumentation included placement of an electrode in both TA muscles for EMG recording (13), a tracheal, subglottal catheter (diameter 1 mm) (16) and a supraglottal catheter for monitoring transglottal pressure, and a metal wire surrounding both iSLNs for ulterior sectioning. The supraglottal catheter was adapted from an infusion catheter (Insyte, 16 gauge, Becton Dickinson Infusion Therapy Systems, Sandy, UT) and placed by using a modified Seldinger technique, as follows. First, a metal guide wire (Multilumen central venous catheterization set, Arrow, Erding, Germany) was inserted transcutaneously, 1 mm above the anterosuperior aspect of the thyroid cartilage, through the base of the epiglottis and pushed in until it exited at the mouth. Two rings were designed to hold the catheter in place, from which the syringe connector had been cut off. The first ring was made of thick, rapid-drying adhesive glue (order no. 214-00-2005, Hottinger Baldwin Messtechnik, Darmstadt, Germany) and positioned 2–3 mm from the tip of the catheter. The catheter was then slid with the help of the guide wire from the mouth, through the epiglottis and skin, until the first ring was secured on the laryngeal side of the epiglottis. The tip of the catheter was now positioned 5–7.5 mm above the glottis, while the external part of the catheter protruded at the anterosuperior aspect of the thyroid cartilage. The guide wire was then removed, and a second ring, made from the rubber end of a 3-ml syringe (Terumo Medical, Elkton, MD), was glued to the external part of the catheter. Finally, the skin was closed over this second ring, leaving 15–20 mm of protruding catheter. Loose stitching allowed for adequate epiglottal movement. Adequacy of catheter positioning was monitored throughout and at the end of the procedure by direct laryngoscopy.

Standard microsurgical instrumentation and magnifying glasses (Magni-focuser, Edroy Products, Nyack, NY) were used for placement of the metal wire around both iSLNs. Dissection was carried out lateral to the thyrohyoid muscle at the superior aspect of the thyroid cartilage. The iSLN and nearby superior laryngeal artery were identified. The superior laryngeal artery was dissected, ligated (Vicryl 5.0), and sectioned. A loose loop was made around the iSLN with a 0.4-mm-diameter, bared metal wire (no. 3165557, Medwire, Sigmund Cohn, Mount Vernon, NY) and adjusted to avoid nerve contact and compression. The two free ends of the wire were slid into a 0.1-cm-ID Silastic catheter (Microbore tubing S-54-HL, Tygon, Akron, OH). The proximal end of the silastic catheter was sutured (silk 3.0) to the thyrohyoid muscle, 0.75 cm anteroinferiorly to the iSLN. The wire was prevented from moving inside the silastic catheter by application of 1 ml of fast-setting glue (LePage Super Glue, ON, Canada) into the distal end of the catheter. The catheter was finally tunneled subcutaneously to exit on the lamb’s back.

**Measurement Apparatus**

Leads from the EMG electrodes and a custom-made nasal thermocouple (27) were connected to a transmitter attached to the lamb’s back just before the experiment. Raw EMG signals and nasal flow were transmitted by radiotelemetry to a receiver (17). Thoracic and abdominal volume variations were qualitatively assessed by using respiratory inductance plethysmography (Respitrace, NIMS, Miami Beach, FL). No attempts were made to quantify tidal volume. Sub- and supraglottal pressures were recorded by using the two air-filled catheters attached to two calibrated pressure transducers (MP 45-30-871, Validyne, Northridge, CA). Raw and moving-time-averaged (100 ms) EMG signals, thoracic and abdominal volume variations and their sum, sub-, and supraglottal pressures and their sum and transglottal pressures, as well as nasal flow were continuously recorded on an Apple Macintosh microcomputer, using the Acknowledge 3.2 software (Biopac System, Santa Barbara, CA). The collected data were stored on compact disk for further analysis.

**Design of the Study**

The study was designed to allow for simultaneous recording of TA EMG activity; variations of sub-, supra-, and transglottal pressures; nasal flow; and thoracic and abdominal movements during central apneas induced in nonsedated lambs. Ambient temperature was maintained between 20 and 22°C throughout the experiment. A catheter was introduced into the external saphenous vein of a posterior limb before each recording. The nonsedated lamb was then placed in a sling with loose restraints and studied in prone position, during quiet wakefulness (Fig. 1). Careful adjustment of the lamb’s head in a comfortable position on the adjustable head holder prevented any pressure on the laryngeal region and twisting of the pressure catheters. A sealed face-mask (anesthesia mask no. 8292, CDMV, Sainte-Hyacinthe, PQ, Canada) was applied over the lamb’s muzzle for hypoxic breathing. The lamb was allowed an adjustment period of ~10 min preceding baseline recording. Hypocapnic central apneas were then repeatedly induced by transient inhalation of pure O2 for five breaths (Dejours’ test) during a 15-min, hypocapnic, steady-state hypoxic run (inspired O2 fraction = 0.08) (23). Subsequently, central apneas were induced to confirm that the observations made after Dejours’ test during hypocapnic hypoxia were reproducible. Nonhypocapnic apneas were induced by using intravenous injection of dopamine (10 μg/kg) (5, 18). In addition, because previous observations revealed that central apneas frequently developed in lambs after the tachypneic response to intravenous injection of capsaicin (10 μg/kg) (unpublished observations), these apneas were also studied as well.

The above experiment was repeated 24 h later, 1 h after iSLN sectioning, performed as follows. The distal end of the silastic catheter, which contained the glue, was cut, allowing...
for free movement of the metal wire into the catheter. The free ends of the metal wire were attached to an electrocauter (model 770, set at cutting power 3/11, Electrosectia, Britcher, CA). Traction was then applied to the wire during electrocautery, thus sectioning the iSLN. The procedure was completed in <5 s and resulted in minimal discomfort for the lamb. Complete, bilateral iSLN section was always verified at autopsy.

**Data Analysis**

Central apneas were defined by the absence of nasal flow with no thoracic or abdominal movements for at least 3 s. Periodic breathing was defined as a series of more than three contiguous breaths alternating with apneas or hypopneas (<50% preceding breaths) during more than 2 s. Apnea duration; presence of continuous TA EMG activity; and mean sub-, supra-, and transglottal pressures were recorded for each apnea. Because the objective of the study was only to test the hypothesis that transglottal pressure was different from zero, whatever the method used to induce apnea, and independently of afferent iSLN activity, only descriptive statistics were used. Values obtained for each type of apnea were first averaged in each lamb, then for all lambs as a whole, both before and after iSLN sectioning. Mean values are reported as means ± 95% confidence interval.

**RESULTS**

**Before iSLN Sectioning**

A total of 116 central apneas, with a mean duration of 7.3 ± 1.05 s, were induced in the five lambs. Although expiratory TA EMG activity was frequently observed during baseline room air breathing in quiet wakefulness, it was consistently absent in hypoxia. Continuous TA EMG activity was observed in 88% of the apneas where the TA electrodes were functional. Whereas supraglottal pressure was consistently atmospheric throughout all apneas, subglottal pressure was maintained above atmospheric pressure (mean = 4.3 ± 0.8 cmH2O) in 95% of all apneas. Moreover, the transglottal pressure was different from zero throughout all apneas where TA EMG activity was observed, suggesting that active glottal closure was complete for the duration of the apneas. Subglottal pressure was virtually atmospheric (−0.1 ± 0.1 cmH2O) in apneas without TA EMG activity (n = 12).

**Hypoxic runs.** Seventy-seven central apneas (mean duration = 7.8 ± 1.4 s) were induced by the Dejours’ test during eight hypoxic runs in the five lambs (Fig. 2). The TA electrodes were not functional during 9 of 77 apneas. Moreover, TA EMG activity was absent in 5 of the remaining 68 apneas. Mean subglottal pressure was measured at 4.9 ± 0.9 cmH2O with TA EMG activity and −0.3 ± 0.1 cmH2O without TA EMG activity. Periodic breathing episodes occurred on return to air breathing after three of the eight hypoxic runs in three lambs (Fig. 3). A total of 11 apneas (mean = 4.5 ± 0.7 s) occurred during the episodes of periodic breathing. TA EMG activity was present throughout all apneas, with a mean subglottal pressure of 2.6 ± 0.8 cmH2O.

Apneas after tachypnea induced by intravenous capsaicin. Intravenous injection of capsaicin was responsible for the full classic pulmonary chemoreflex, including an initial apnea followed by rapid shallow breathing, in 13 of 17 tests in the 5 lambs. TA EMG activity was present throughout 6 of 13 initial apneas (mean duration = 8.8 ± 3.6 s). Mean subglottal pressure was 6.8 ± 2.6 cmH2O when continuous TA EMG activity was present, and it was 0 cmH2O when TA EMG activity was absent. A tachypneic period, with at least a threefold increase in respiratory frequency, followed all 17 capsaicin injections in the 5 lambs. Moreover, a secondary, presumably hypocapnic, apnea (mean duration = 7.5 ± 1.8 s) was observed 141 ± 59 s after 8 capsaicin injections in 3 lambs, just before resumption of baseline breathing. TA EMG activity was present in all secondary apneas, with a mean sub-glottal pressure of 6.4 ± 0.7 cmH2O (Fig. 4).

**Intravenous dopamine injections.** While intravenous dopamine consistently induced ventilatory depression in all five lambs, only seven central apneas (mean duration 6 ± 1.2 s) were observed in two lambs, for a total of 13 injections. Continuous TA EMG activity was present throughout all apneas, and the average subglottal pressure was measured at 1 ± 0.3 cmH2O (Fig. 5).

**After iSLN Sectioning**

A total of 58 central apneas were induced in the 5 lambs after bilateral iSLN section, including 37 apneas after the Dejours’ test performed during a hypoxic run, 1 apnea during periodic breathing, 8 apneas immediately after intravenous capsaicin injection, 4 apneas occurring 102 s (range 50–270 s) after intravenous injection of capsaicin, and 8 apneas induced by intravenous injection of dopamine. The proportion of apneas with functional TA electrodes and presence of contin-

Fig. 2. Hypocapnic central apnea induced by 5 inhalations of pure O2 (Dejours’ test) during a hypoxic run (inspired O2 fraction = 0.08). TA, raw thyroarytenoid muscle electromyographic activity; JTA, moving-time-averaged (time constant: 100 ms) TA; Supra, supraglottal pressure (cmH2O); Sub, subglottal pressure (cmH2O); Flow, nasal flow, inspiration upward; Sum, variations in tidal and apneic lung volume, assessed from respiratory inductance plethysmography.
uous TA EMG activity was identical before and after iSLN section (88 and 87% of apneas, respectively). Supraglottal pressure was always atmospheric. Conversely, subglottal pressure for all apneas as a whole was maintained above atmospheric pressure (4.7 ± 0.7 cmH$_2$O) during apneas with TA EMG activity. More specifically, mean subglottal pressure with TA EMG activity was 4.6 ± 0.6 cmH$_2$O during apneas (n = 32) induced by Dejours' test during hypoxia and 4.5 cmH$_2$O for the apnea observed during periodic breathing. The mean subglottal pressure during apneas with TA EMG activity immediately after intravenous capsaicin injections was 6.8 ± 2.6 cmH$_2$O (n = 3), whereas during the apneas after the tachypneic response induced by capsaicin injection it was 7.9 ± 0.4 cmH$_2$O (n = 4). Lastly, the mean subglottal pressure with TA EMG activity during the apnea induced by dopamine injections was 5.2 cmH$_2$O. In contrast, the mean subglottal pressure observed during apneas without TA EMG activity was 0.2 ± 0.2 cmH$_2$O (n = 6). The transglottal pressure gradient was different from zero in all apneas where TA EMG activity was observed.

**DISCUSSION**

The present study clearly demonstrates the completeness of active glottal closure during the vast majority of central apneas induced in awake, nonsedated lambs, regardless of the method used to induce apnea. The completeness of this closure is evidenced by a transglottal pressure gradient different from zero.
throughout virtually all apneas with TA EMG activity. This complete active glottal closure does not originate from iSLN stimulation. Moreover, the presence of atmospheric supraglottal pressure (= zero transpharyngeal pressure) from the outset of all these apneas excludes simultaneous pharyngeal closure. Results of the present study bring new light to the neural control of the upper airways during neonatal central apneas.

**Transglottal Pressure Measurements**

Transglottal pressure measurements have been previously performed by using various different techniques in several anaesthetized or decerebrate animal models, such as cats (2, 33), rats (9, 21), dogs (1), and piglets (6). Much of these models involved functional isolation of the larynx during spontaneous breathing of the animal through a tracheostomy tube, with the aim of assessing laryngeal resistance under various respiratory conditions. Other than measuring laryngeal resistance during vocalization, measurement of transglottal pressure has rarely been assessed in nonsedated humans (4, 26). In preterm infants, differences between esophageal and pharyngeal pressure measurements using transnasal catheters have been used to assess the site of upper airway closure during apnea and respiration (19). However, the inability to control the position of the catheter tip relative to the larynx when a nasopharyngeal catheter is used can lead to interpretative errors. Finally, although transupper airway pressure has been measured in lambs to assess upper airway closure, this did not allow to discriminate between pharyngeal and/or laryngeal closure (9). To our knowledge, the present study is the first attempt to measure transglottal pressure directly in awake, non-sedated newborn animals to assess laryngeal closure. Our unique technique for supraglottal catheter placement is simple, well tolerated, and allows for chronic measurements of both transglottal and transpharyngeal pressures in the same animal under various conditions and without sedation. Reliability of the technique is shown by the ability to measure transglottal pressure in 77% of all apneas.

**Complete Glottal Closure During Central Apneas in Lambs**

Presence of glottal closure during apneas of prematurity was first suggested by Milner in 1980 (20). Previous results from our laboratory have shown that continuous TA EMG activity was present throughout induced central apneas in nonsedated lambs (13, 23), and endoscopic observations have suggested completeness of glottal closure during such apneas in lambs (16). Results of the present study are definitive proof that active glottal closure is complete during induced central apneas in lambs when TA EMG activity is observed, i.e., in 88% of apneas. Interestingly, complete glottal closure was observed, regardless of the method used to induce apnea. Central apneas after Dejours’ test applied during hypopancnic hypoxia are mainly secondary to loss of chemical drive from both peripheral and central chemoreceptors. Dopamine-induced, nonhypopancnic central apneas have been shown to be secondary to peripheral D2-receptor mechanisms, through inhibition of carotid sinus nerve activity and other ill-defined mechanisms (stimulation of area postrema?) (22). We presume that central apneas observed after the tachypneic response to capsaicin injection are primarily secondary to hypopacnia. However, the mechanisms involved are probably much more complex, e.g., potentially involving the ventilatory inhibitory effect of capsaicin acting on vanilloid receptors located in the nucleus tractus solitarius (7). Differences in the transglottal pressure observed from one type of apnea to another are not readily explained because our study was not designed to address this specific query. Given that our laboratory has previously reported that abdominal muscle activity is absent during central apneas induced in lambs (12, 24), the most likely explanation is that higher subglottal pressures were due to onset of apnea at a higher lung volume (greater lung recoil pressure).

Physiological relevance of complete glottal closure observed during induced central apneas herein is suggested by the following observations. First, TA EMG activity was present throughout 88% of spontaneous central apneas in nonsedated preterm lambs (27). Second, glottal closure was observed endoscopically during spontaneous central apneas in preterm infants and during an episode of periodic breathing during quiet sleep in an infant (29, 30). Moreover, we have observed maintenance of high lung volume during central apneas in a few preterm human infants (ongoing study). Thus, although there may be interspecies differences with respect to the proportion of central apneas involved, some central apneas in human infants are also characterized by complete glottal closure and maintenance of high lung volume. The importance of active glottal closure during early expiration is well established in the newborn to delay lung deflation and preserve an elevated end expiratory lung volume, which in turn is beneficial for gas exchange (8). Our results suggest that central apneas in the newborn are frequently characterized by the presence of a continuous positive pressure in the subglottal airways, which should be beneficial for continuation of gas exchange during apneas.

Recent reviews on apneas of prematurity tend to suggest that pharyngeal closure is present during central apneas in preterm infants (28). Such belief relies on previous measurements of pharyngeal pressure by using nasopharyngeal catheters (19) or on the absence of cardiac artifacts on airflow recording (10). However, our direct measurements of transglottal pressure show for the first time that upper airways are in fact not closed at the pharyngeal level in induced central apneas. Although the present results may seem contradictory with our laboratory’s previous observations of continuous inferior pharyngeal constrictor EMG activity during spontaneous central apneas in preterm lambs (27), it has been suggested that contraction of the inferior pharyngeal constrictor contributes to ac-
active glottal closure (14) The intriguing absence of (passive) pharyngeal closure during induced central apneas in lambs warrants further confirmation, however, in spontaneous central apneas.

Glottal Closure, iSLN, and Neonatal Central Apneas

Mechanical or chemical stimulation of laryngeal receptors or direct iSLN stimulation can induce a reflex central apnea with active glottal closure (3, 11, 15). Inhibition of the brainstem “respiratory centers” during this laryngeal chemoreflex is especially powerful in the immature newborn (11, 15, 32). It is currently widely acknowledged that this reflex is frequently responsible for apparently life-threatening events in infants and may be involved in some cases of sudden infant death syndrome (35). Our present findings show that deprivation of laryngeal afferent innervation by sectioning the iSLN does not prevent the complete, active glottal closure observed during induced central apneas in lambs. This, along with other data, which do not suggest a role for vagal and chemical afferent messages (23), support the hypothesis that active glottal closure during central apneas in the neonatal period can also occur independently of peripheral afferent messages. This is reminiscent of the active glottal closure present when respiratory movements are absent in the fetus (13) and of the basic respiratory pattern in vertebrates, alternating respiratory movements and prolonged inspiratory breath holding with the glottis closed (31).

In conclusion, results of the present study in lambs constitute further demonstration of the importance of the larynx for neonatal respiration and challenge previous notions on pharyngeal behavior during central apneas. Although it must be remembered that interspecies differences can exist, our findings do suggest a novel understanding of the neural control of the upper airways during neonatal apneas.

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