Coordination between glottic adductor muscle and diaphragm EMG activity in fetal lambs in utero

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Kianicka, Irenej, Véronique Diaz, Dominique Dorion, and Jean-Paul Praud. Coordination between glottic adductor muscle and diaphragm EMG activity in fetal lambs in utero. J. Appl. Physiol. 84(5): 1560–1565, 1998.—It has previously been reported that active glottic adduction is present during prolonged apneas but absent during periods of breathing movements in fetal lambs in utero. The present study was aimed at examining the precise coordination between fetal breathing movements [diaphragm electromyographic (EMG) activity (Di EMG)] and glottic adduction [thyroarytenoid muscle EMG activity (TA EMG)].

Electrodes for electroencephalogram, eye movements, TA EMG, and Di EMG and an arterial catheter were surgically implanted in fetal lambs 123–142 days postconception. Polygraphic recordings were performed without sedation while the ewe breathed room air (n = 11) or various gas mixtures (hypoxia, n = 5; hyperoxia, n = 4; hypercapnia, n = 5; hypercapnia + hypoxia, n = 5). Tonic TA EMG was observed throughout >90% of apneas (>6 s) in both non-rapid-eye-movement and rapid-eye-movement sleep, and when Di EMG frequency decreased in rapid-eye-movement sleep. In all but two fetuses, TA EMG was immediately inhibited when Di EMG appeared. Altering blood gases did not modify these results. In conclusion, Di EMG and TA EMG are well coordinated in late gestation.

For several years now, we have been studying laryngeal control in relation to neonatal apneas by using a newborn lamb model. Recent results in nonsedated lambs have shown that tonic glottic closure was frequent throughout artificially induced central apneas, either posthyperventilation (11, 15) or after an injection of pentobarbital sodium (17). Relevance of these results to spontaneous apneas is suggested by preliminary identical observations of tonic active glottic closure with spontaneous central apneas in newborn lambs (Ref. 16 and unpublished observations). Of particular interest is the observation that a precise coordination between glottic muscle control and inspiratory efforts invariably allowed the resumption of breathing without glottic obstruction in term lambs. It is not clear, however, whether neonatal conditions such as prematurity could be responsible for a lack of coordination between glottic muscles and inspiratory efforts leading from central to mixed apneas, as previously proposed in preterm infants by Ruggins (18). Such a mechanism could explain tonic glottic closure observed endoscopically during severe central and mixed apneas in preterm infants (19).

In utero, the fetal glottis is closed during prolonged apneas in non-rapid-eye-movement (NREM) sleep and reopens during periods of fetal breathing movements (FBM) (7). The purpose of the present study was to test the hypothesis that a precise coordination between a glottic adductor muscle (the thyroarytenoid muscle (TA)) and the diaphragm was sometimes missing in the intact, nonsedated fetus in utero during late gestation. If verified, this observation would suggest that mixed/obstructive apneas of prematurity may, at times, be due to a lack of coordination between glottic constrictor muscles and the diaphragm.

MATERIALS AND METHODS

Animals

Eleven mixed-breed pregnant sheep were involved in the study 123 to 142 days after mating (term is 145 ± 5 days). The procedures used were approved by the Ethics Committee for Animal Experimentation of our institution.

Surgical Instrumentation

After 48 h of fasting, the ewe underwent strict aseptic surgery under general isoflurane-N₂O anesthesia. Premedication consisted of ketamine (10 mg/kg) and acepromazin (0.1 mg/kg im) and atropine sulfate (0.05 mg/kg sc). After midline incision of the maternal abdominal wall, the fetus was approached by hysterotomy of the pregnant horn in a region free from cotyledons and major blood vessels. Loss of amniotic liquid was prevented throughout fetal surgery by clamping together the uterine wall, amniotic membranes, and fetal skin by using Babcock forceps. Two bipolar enameled chrome wire electromyographic (EMG) electrodes (0.1 mm diameter, Chromel GTSM, Castelnaudary, France) were first inserted through a small window on either side of the thyroid cartilage, as described previously (11). Monitoring Ag-AgCl electrodes (Neotrode 1731–003, Conmed, Utica, NY) were placed biparietally (one on each side) under the scalp.
to record an electroencephalogram (EEG), with a reference electrode positioned in the frontal region. A monopolar chrome wire electrode with a 1-cm uninsulated tip was sewn into the superior and inferior eyelids for recording eye movements (electrooculogram; EOG). All the electrodes were secured in place by one drop of surgical glue (Vetbond Tissue Adhesive no. 1469, 3M Animal Care Products, St. Paul, MN). Leads were then tunneled to exit on the back of the fetus. A small polyethylene catheter (19 gauge) was inserted into the carotid or femoral artery for blood-gas measurement.

After the fetal head was positioned back into the uterus, 500 mg of ampicillin were instilled into the amniotic cavity and the uterus was closed. The leads and catheter were tunneled subcutaneously to exit in the left flank of the ewe and were protected by a pouch sutured to the flank. The abdominal wall was surgically closed.

After surgery, the ewe was maintained in an individual, specially designed cage, with free access to food and water. Antibiotics [ampicillin (1 g) and gentamicin (5 mg/kg im)] were given daily until the experimental day. The fetal catheter was continuously infused with heparinized saline (25 IU/ml).

Measurement Apparatus

Raw EMG signals were amplified and 30- to 1,000-Hz band-pass filtered (Grass P511 alternating current (AC) preamplifier and polygraph direct current (DC) driver amplifier, Quincy, MA) before undergoing 100-ms moving time averaging (Dept. of Electronics, Faculty of Medicine, Université de Sherbrooke). EEG and EOG were both amplified and filtered by using a Grass AC/DC EEG wide-band amplifier (0.1–30 Hz) and low-level DC preamplifier, respectively. Raw and integrated EMG signals, EEG, and EOG were recorded on a 10-channel Grass 7D polygraph. Arterial blood gases and pH were determined in a pH blood-gas analyzer (model 1306, Instrument Laboratory, Lexington, MA) and corrected for life temperature of the ewe (Mon-A-Therm 6500, St. Louis, MO) (1).

Experimental Design

The study was designed so that we could monitor glottic constrictor muscle (TA) and diaphragm EMG activity (Di EMG and TA EMG, respectively) during naturally occurring behavioral states in the fetus. Each nonsedated fetal lamb was studied in utero 2–7 days after surgery. The ewe was allowed to adopt a natural position in the cage, with free access to food and water all times. Ambient temperature was maintained at 21°C.

Before each experiment, a fetal arterial blood sample was drawn for determination of blood gases and pH. TA EMG and Di EMG were continuously recorded together with EEG and EOG during at least two periods of each fetal behavioral state (REM sleep-like and NREM sleep-like; see below). Duration of total recording time was between 1.5 and 9 h. In addition, the effects of varying blood-gas conditions on TA EMG activity during periods of apneas and periods of FBM were also studied when possible, i.e., after 120 min of good-quality recordings, including at least two periods each of NREM and REM sleep. Fetal hypoxia, hypercapnia, hyperoxia, or hypercapnia+hyperoxia conditions were induced by varying the O2 (FIO2) and/or CO2 (FICO2) inspiratory fraction (8–10% FIO2, 5–7% FICO2, 100% FIO2, or 95% FIO2+5% FICO2, respectively) to the ewe. Changes in fetal parameters were considered after a 10-min equilibration period of altered inspiratory gas fractions.

Analysis of Results

Behavioral states. Two behavioral states were recognized: a NREM sleep-like state characterized by high amplitude, low-frequency EEG, and absence of rapid eye movements, and a REM sleep-like state characterized by low-amplitude, high-frequency EEG and presence of rapid eye movements. Total recording times and percentages of each behavioral state were calculated for each recording and averaged for the 11 lambs as a whole. In the absence of nuchal EMG recording, a few episodes of wakefulness were probably included in REM sleep-like episodes; hence their proportion may be slightly overestimated (10). Portions of recordings with artifacts related to ewe feeding and/or movements were not analyzed.

Respiratory muscles. A period of FBM was defined by the presence of at least three bursts of phasic Di EMG within 9 s. Apnea was recognized when no phasic Di EMG was observed for at least 6 s (20). Total time with FBM and total time in apnea were calculated in each lamb as a percentage of each behavioral state time and then were averaged for the 11 lambs as a whole. Presence of Di EMG and TA EMG was carefully noted throughout each FBM and apnea period in both REM and NREM sleep states, with special attention to any occurrence of phasic Di EMG (“inspiratory” effort) when TA EMG was present. Amplitude of tonic TA EMG during apneas was expressed as a percentage of the maximal TA EMG amplitude observed during each recording (apart from TA EMG activity during swallowing, which was usually out of range). In agreement with previous reports in fetal (5) and newborn (6, 12) sheep, swallowing activity was identified when a characteristic burst(s) of brief, high-amplitude TA EMG was observed. Recordings performed while O2/CO2 conditions were changed were analyzed separately.

RESULTS

Baseline Conditions

A total of 16 recordings in baseline conditions was performed in 11 fetuses over several days. Three animals underwent two or three recordings. After rejecting recording periods contaminated with ewe body movements, agitation, and feeding, we finally analyzed 92 periods of REM sleep and 90 periods of NREM sleep. Total recording duration in baseline conditions was 39.8 h for the 11 fetuses (Table 1).

FBM were present during 39.2% of total recording time for the 11 lambs as a whole (range for each lamb, 28.2–49.5 %).

Table 1. Effect of gestational age on REM sleep duration and percentage of REM sleep time with fetal breathing movements

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<thead>
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<th>Parameter</th>
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<th>≤130 (n = 6)</th>
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<td>Tr, min</td>
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<td>REM, %Tr</td>
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<td>FBM, %REM</td>
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Values are means ± SD with range in parentheses. n, No. of recordings; REM, rapid eye movement; Tr, total time of recording analyzed; REM (%Tr), REM sleep duration, expressed as %Tr; FBM (%REM), fetal breathing movements expressed as %REM sleep.
13.4–52.4%). Nearly all FBM periods (99.4%) occurred during REM sleep periods.

NREM sleep periods. Time spent in NREM sleep represented 50.3% of total recording time. Mean duration of NREM periods was 14.4 ± 4.6 min. Time spent with FBM represented only 0.6 ± 0.1% of NREM sleep time, with a mean FBM period duration of 7.6 ± 9.7 s. A total of 102 apneic periods of 13.1 ± 10.7-min duration (range 3.4–85.5 min) were analyzed in NREM sleep.

DI EMG AND TA EMG. Di EMG was absent during NREM sleep, apart from rare and short FBM periods observed in five fetuses. Mean frequency of phasic Di EMG during these FBM periods was 26.9 ± 16.6 episodes/min. Moreover, large and prolonged nonrespiratory Di EMG bursts (3–6 s), accompanied by high-amplitude phasic TA EMG bursts, occurred in all observed animals with a mean frequency of 0.6 ± 0.3 discharges/min (Fig. 1).

Continuous TA EMG was present throughout 97 of 102 apneas (95.1%), with a mean amplitude of 10.1 ± 4.1% of maximal activity. Continuous TA EMG remained unaltered throughout the apneic period in the majority of cases, being eventually interrupted at the moment of Di EMG discharge. In 26% of apneic periods (11 recordings), however, amplitude of tonic TA EMG tended to diminish before the end of the apneic period; this occurred in fetuses with a gestational age ranging from 124 to 140 days and did not appear to bear any relationship to fetal maturation in the studied age range.

Three recordings in two fetuses (124–125 and 131 days of gestation, respectively) showed a few episodes of phasic Di EMG with concurrent continuous TA EMG (Fig. 2A).

REM sleep periods. Time spent in REM sleep represented 49.7% of total recording time, with a mean duration period of 13.4 ± 3 (SD) min. Time spent with FBM represented 75.8 ± 15.4% of REM sleep time (Table 1), and the mean FBM duration period was 9.2 ± 2.1 min. We analyzed 1,168 apneic events (13.7 events/period of REM sleep) longer than 6 s (mean 14.5 ± 12.8 s, range 6–143 s) in REM sleep.

DI EMG AND TA EMG. Phasic Di EMG discharges had a mean frequency of 46.1 ± 26.4 discharges/min (range 6–106 discharges/min) in REM sleep. First Di EMG discharges were usually of low amplitude and low frequency and were interrupted by apneas. Both amplitude and frequency then progressively increased to reach a maximum and thereafter diminished progressively at the end of REM sleep periods. In most cases, REM sleep periods ended by a large burst of simultaneous Di EMG and TA EMG (up to 10-s duration).

Continuous tonic TA EMG was observed throughout 92% of apneas >6 s in REM sleep. In fact, tonic TA EMG was present as soon as the time between two Di EMG discharges was increased (Fig. 3), regardless of gestational age. Amplitude of tonic TA EMG reached an average of 14.2 ± 7.1% of maximal activity and was unchanged throughout each apnea. Precise coordination between Di EMG and TA EMG timing was most...
often present in all 11 fetuses, tonic TA EMG being interrupted when a Di EMG discharge occurred (Fig. 3). In the two lambs aged 124–125 and 131 days of gestation, phasic Di EMG discharges without interruption of continuous TA EMG (occasionally observed during NREM sleep) occurred in about one-half of FBM episodes during REM sleep (Fig. 2B). This concurrence of continuous TA EMG and phasic Di EMG was not observed on further recordings performed at 132 and 136 days of gestation in the latter fetus.

Brief, high-amplitude TA EMG characteristic of swallowing activity was observed in REM sleep, with a mean frequency of 0.1 to 15 episodes/min. In five periods of REM sleep (4 fetuses aged from 129 to 136 days of gestation), bouts of very-high-frequency (36–96 episodes/min) swallowing TA EMG were superimposed on FBM (Fig. 4).

Effect of fetal maturation. The effect of fetal maturation on sleep-state organization, FBM occurrence, and pattern of TA EMG and Di EMG activity was also studied. As shown in Table 1, no effect of maturation on sleep-state organization could be found. The small number of fetuses used probably explains why the decrease in percentage of time spent with FBM in REM sleep with gestational age did not reach significance levels (Kruskal-Wallis test). As for the pattern of TA EMG and Di EMG activity, whether in NREM or REM sleep, during FBM or apneic episodes, no effect of fetal maturation was identified, except possibly in one fetus. Indeed, as reported above, episodes of phasic Di EMG discharge without interruption of continuous TA EMG were observed at 131 days of gestation but not at 132 and 136 days in one fetus.

Influence of Various Fetal Arterial Po2 (PaO2) and PCO2 (PaCO2) Levels on TA EMG and Di EMG

TA EMG and Di EMG were analyzed during 10–55 min in six fetuses (gestational age 125–137 days) with altered blood gases by having the ewe breathe various gas mixtures.

Baseline PaO2 and PaCO2 levels were 23.4 ± 3.1 and 43.4 ± 3 Torr, respectively. We performed 7 recordings in hypoxia (5 fetuses), 11 recordings in hypercapnia (5 fetuses), 5 recordings in hyperoxia (4 fetuses), and 6 recordings in hyperoxia+hypercapnia (5 fetuses). Hypoxia (PaO2 = 14.6 ± 2.6 Torr) led to diminished or total cessation of FBM in all five fetal lambs. Mild hyperoxia (32 ± 3.2 Torr) and/or mild hypercapnia (48.8 ± 2.1 Torr) led to an increased occurrence of FBM without establishment, however, of regular continuous breathing movements.

None of the fetal blood-gas changes influenced the pattern of TA EMG observed under baseline conditions. Continuous tonic TA EMG was observed when FBM was absent, regardless of the sleep stage (except in 1 fetus with no TA EMG during apneas). Phasic Di EMG consistently abolished tonic TA EMG in all but the two fetuses in which phasic Di EMG was previously observed during continuous TA EMG in baseline conditions.

DISCUSSION

The unique results of the present study show that continuous active glottic constriction is present throughout apneas not only in NREM sleep but also in REM sleep in nonsedated fetal lambs. Furthermore, it appears that a precise coordination between glottic adductor muscle and diaphragm activity is generally established during late gestation in both sleep states.
However, one important observation is that the diaphragm can, at times, contract against an adducted glottis in REM sleep. Possible relevance of these results in relation to postnatal pulmonary ventilation will be addressed briefly.

### Active Glottic Adduction During Apneic Periods

It has been previously observed that the glottis is actively adducted during prolonged apneic periods in NREM sleep in fetal lambs in utero (3). The situation in REM sleep is more confusing, however. Indeed, the laryngeal constrictor muscles were at the same time reported to be “essentially inactive during episodes of FBM, except during swallowing and postural adjustments” and “to become tonically active during prolonged pauses between FBM (i.e., greater than expiratory time)” (3). To our knowledge, the latter has never been illustrated previously.

The present report is therefore the first study specifically designed to carefully examine the relationships between TA EMG and FBM. Our results clearly confirm that continuous TA EMG is present throughout >90% of apneas, regardless of sleep state, and as soon as the time interval between two “respiratory” Di EMG discharges increases. Moreover, the fact that artificial modifications of blood gases (hypoxia, hypercapnia with or without hyperoxia) did not alter continuous tonic glottic adductor EMG throughout apneas suggests that the latter is not controlled by chemical stimuli. As reviewed extensively by Harding and Hooper (3), active glottic adduction prevents pulmonary fluid efflux from the trachea when diaphragmatic contractions are absent. In doing so, glottic adduction participates in the regulation of lung liquid volume, which appears to be the major determinant of prenatal lung growth (7).

Persistence of tonic glottic adduction throughout apneas during REM sleep in fetal lambs is somewhat puzzling. Indeed, inhibition of postural muscle tonic EMG is a hallmark of REM sleep in mammals (14). Of note, we (unpublished observations) and others (4) have observed an identical tonic TA EMG during central apneas in newborn lambs during REM sleep.

### Inhibition of Glottic Adduction With Diaphragm Activity

Our observation that tonic glottic adductor EMG is transiently silenced each time a Di EMG burst is present, in both REM and in NREM sleep, extends previous knowledge of the precise temporal relationships between glottic muscles and diaphragm in fetal lambs. Indeed, inhibition of TA EMG observed in the present study, even with Di EMG discharges of very short duration (<1 s), highlights the very precise coordination between controllers of glottis aperture and diaphragm contractions usually present in both sleep states. These results suggest that central brainstem mechanisms are already functional in late gestation (at least as early as 123 days of gestation in sheep) in inhibiting continuous glottic constrictor motoneuron discharge when central inspiratory drive is present. Interestingly, an active glottic opening due to contraction of the posterior cricoarytenoid muscle, precisely coordinated with diaphragmatic phasic inspiratory EMG, is also known to be present in late gestation in fetal lambs (2, 4). Such a precise coordination between glottic muscles and diaphragm is probably already important prenatally for regulating lung liquid volume and, consequently, ensuring optimal lung growth (4).

Above all, presence of an exact coordination between glottic muscles and thoracic muscles from the moment of birth is of paramount importance to allow optimal lung ventilation.

### Possible Incoordination Between Glottic Adductor Muscles and the Diaphragm

Although our results show that a precise coordination between glottic constrictor muscles and the diaphragm is generally observed prenatally in the lamb, it appears that this coordination can sometimes be defective. Hence, phasic inspiratory Di EMGs were observed to occur in two lambs without inhibition of continuous TA EMG in fetal lambs during NREM and REM sleep. Concurrent TA EMG and Di EMG with arousal from REM and NREM sleep have previously been reported in fetal and postnatal lambs (8, 9). The unique results of the present study (see Fig. 2A) show that concurrent continuous TA EMG and phasic Di EMG can also be observed during periods without any hint of movement (or swallowing). This suggests that such a sequence of events (diaphragmatic inspiratory contractions against a glottis that remains closed) may also occur postnatally and be responsible for mixed/obstructive apneas in preterm infants. Endoscopic observation of mixed and obstructive apneas against a closed glottis in preterm infants (19) is in keeping with our hypothesis. Although no evidence is available at the present time, it is conceivable that conditions such as prematurity or perinatal apnea, which alter central neural mechanisms, may increase the incoordination between glottic dynamics and thoracic muscle activity.

Furthermore, in the present study, we observed bouts of brief, phasic TA EMG discharges superimposed on high-frequency phasic inspiratory Di EMG during periods of low-amplitude, high-frequency EEG (4 frequencies). Previous reports have linked identical TA EMG discharges to swallowing episodes in fetal (5) and term newborn lambs during REM sleep (6, 12). The fact that the swallowing episodes are also observed prenatally suggests that they are likely due to REM sleep-related phasic central mechanisms rather than to a laryngeal closure reflex triggered by stimulation of laryngeal mucosal receptors. Again, such a sequence of repetitive swallows (up to 96 episodes/min) could conceivably hinder postnatal lung ventilation.

### Conclusions

Precise coordination between glottic muscles and diaphragm contraction must be present from birth for optimal ventilation in mammals. Results of the present...
study show that this coordination is usually already present in fetal lambs in late gestation. However, some prenatal incoordination can be observed at times, leading us to suggest that central neural immaturity could be responsible for some mixed/obstructive apneas of prematurity due to tonic glottic obstruction.

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