Pulmonary vagal innervation is required to establish adequate alveolar ventilation in the newborn lamb

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Wong, Kevin A., Ather Bano, Anita Rigaux, Bing Wang, Baikhunth Bharadwaj, Samuel Schurch, Francis Green, John E. Remmers, and Shabih U. Hasan. Pulmonary vagal innervation is required to establish adequate alveolar ventilation in the newborn lamb. J. Appl. Physiol. 85(3): 849–859, 1998.—To investigate the effects of bilateral intrathoracic vagotomy on the establishment of continuous breathing and effective gas exchange at birth, we studied 8 chronically instrumented, unanesthetized, sham-operated and 14 vagotomized newborn lambs after a spontaneous, unassisted vaginal delivery. Fetal lambs were instrumented in utero to record sleep states, diaphragmatic electromyogram, blood pressure, arterial pH, and blood-gas tensions. Six of eight sham-operated lambs established effective gas exchange within 10 min of birth, whereas 12 of 14 vagotomized animals developed respiratory acidosis and hypoxemia (P = 0.008). Breathing frequency in vagotomized newborns was significantly lower during the entire postnatal period compared with sham-operated newborns. Vagotomized subjects also remained hypothermic during the entire postnatal period (P < 0.05). Bronchoalveolar lavage indicated an increased minimum surface tension, whereas lung histology showed perivascular edema and partial atelectasis in the vagotomized group. We conclude that stimulation of breathing and effective gas exchange are critically dependent on intact vagal nerves during the transition from fetal to neonatal life.

blood-gas tensions; fetal sheep; intrathoracic vagotomy; respiratory failure; surfactant

BEFORE BIRTH, fetal homeostasis, including gas exchange, is supported by the maternal-placental unit. However, to establish an independent life, the newborn must adapt to the extraterine environment. One of the most vital and unique adaptations that occurs shortly after birth is the establishment of continuous breathing and adequate pulmonary gas exchange. Although the intense somatosensory stimuli (7, 18, 35) may play a role in the initiation of breathing at birth, the precise mechanisms for establishing and maintaining continuous breathing remain unknown. Evidence suggests that pulmonary vagal innervation contributes more significantly to the ventilatory control in newborns than in older animals (8, 12, 14, 44, 47). However, these studies used cervical vagotomy, an intervention that paralyzes the laryngeal muscles and may cause upper airway obstruction. In addition, these studies were not done in the immediate newborn period, and the animals were either tracheotomized or studied under general anesthesia. To circumvent the limitations of previous experiments, we investigated the role of intrathoracic vagotomy in the establishment of breathing in the unanesthetized newborn lamb, immediately after birth. This study was designed to test the hypothesis that a bilateral intrathoracic vagotomy in fetal sheep will result in respiratory failure at birth.

METHODS

Surgical preparation. Twenty-four time-dated pregnant ewes of mixed breed were operated on between 130 and 133 days of gestation (term = 147 ± 2 days), under general anesthesia, by using ketamine hydrochloride (8–9 mg/kg; Rogarsetic, Rogar/STB, London, Ontario) and 4% halothane in O2 for induction and 1.5–2.5% halothane for maintenance. With the observation of sterile techniques, the fetal head and neck were exteriorized through a midline uterine incision. An incision was made in the anterior dorsal part of the fetal neck to tunnel four pairs of electrode wires (AS 633; Cooner, Chatsworth, CA) to record 1) electrocorticogram (ECoG), 2) electrooculogram (EOG), 3) nuchal electromyogram (EMGnk), and 4) diaphragmatic electromyogram. Surgical techniques for the implantation of the electrodes have been given in detail previously (19). Briefly, to record an ECoG, a pair of electrodes was implanted bilaterally over the parietal area through two holes in the fetal skull ~2.5 cm apart, posterior to the horn buds. To record EOG, a silver-coated, spherical-shaped electrode was embedded around the lateral rectus muscle of one eye through a 5-mm incision made along the lateral bony orbital ridge. The two EMGnk electrodes were sutured in the lateral neck muscle ~5 cm apart at the level of the initial incision. A ventral incision, lateral to the trachea and just below the thyroid cartilage, was made to place polyvinyl catheters in the fetal carotid artery and jugular vein (1 mm ID, 2 mm OD; Portex, Hythe, Kent, UK). One polyvinyl amniotic catheter (3 mm ID, 5 mm OD; R3603, Tygon, Akron, OH) was sutured to the dorsal part of the fetal neck and was used to monitor the onset and progression of labor. The fetus was further exteriorized, and a thoracotomy was performed through the right fourth intercostal space. In 16 animals, the vagus and phrenic nerves were identified along the right main bronchus, and a 5-mm section of the vagus nerve was excised below the recurrent laryngeal nerve. The incision was sewn in layers, and the procedure was repeated on the opposite side. Sham-operated animals (n = 8) underwent an identical procedure but without sectioning of the vagi. After vagotomy, the fetal trunk was further exteriorized, and a 2-cm incision was made parallel to the ribs at the level of the tenth intercostal space to implant the diaphragmatic electrodes (19). All four electrode wires were soldered to a connector (Lemo), which was wrapped in silicone-filled latex to form a waterproof seal. The fetal vascular catheters were exteriorized through the left maternal flank and stored in a cloth pouch sewn adjacent to the incision site. The arterial catheter was used to draw blood samples twice daily to analyze arterial pH (pHx) and blood-gas tensions, which served as indicators of fetal well-being. After birth, this catheter was used to record blood pressure, heart rate, pHx, and blood-gas tensions, whereas the venous catheter was used to administer antibiotics and fluids postoperatively.

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After instrumentation was completed, the fetus was returned to the uterine cavity, and all incisions were sewn in layers. A polyvinyl catheter was placed in the maternal jugular vein for infusion of antibiotics and fluids (3 mm ID, 5 mm OD; Tygon). The ewes were housed in large (1.8 x 2.4 m, custom-made) individual cages with free access to food and water. Daily care included administration of 125 mg (for fetuses) and 375 mg (for ewes) of cefazolin sodium in saline (Ancef, SmithKline Beecham Pharma, Oakville, Ontario), 20 mg (for fetuses) and 80 mg (for ewes) of gentamicin sulfate (Garamycin Injectable, Schering Canada, Pointe-Claire, Quebec) twice daily for 5 days, and heparinized flush for patency of the vascular catheters. In addition, 20 mg of gentamicin were instilled in the amniotic cavity.

Experimental design. The ewes were observed continuously by one of the investigators for signs of the onset of labor, including general discomfort, frequent changes in posture, passage of mucous plug(s), and the “bloody show.” Once these were detected, the amniotic line was then intermittently connected to a pressure transducer to monitor the frequency and amplitude of uterine contractions as a measure of the progress of labor. When delivery appeared imminent, the stopcocks and needles attached to the vascular catheters were removed to facilitate an unobstructed delivery. The catheters were knotted and reconnected to stopcocks after birth. All lambs were born through spontaneous, unassisted vaginal delivery without pharmacological interventions to either augment or postpone the onset or progression of labor.

After birth, the fetal electrode wires and arterial vascular catheter were immediately connected to the recording apparatus (Neurolog System, Medical Systems, Greenvale, NY). The ECoG, EOG, EMGk, and diaphragmatic electromyogram signals were amplified and filtered appropriately with the ECoG, EOG, EMGk, and diaphragmatic electromyogram (Neurolog System, Medical Systems, Greenvale, NY). The right main bronchus was then intubated, and a bronchoalveolar lavage (BAL) was performed by using 30 ml of HEPES solution. The lavaged material from each lung was centrifuged at 500 g for 5 min to bring down a pellet of cells and debris. The supernatant was then placed in the bubble chamber of a captive-bubble surfactometer (45, 46).

In addition, a fluid-filled esophageal catheter, connected to a pressure transducer, was inserted to measure intrapleural pressure. In accordance with the animal care guidelines, the animals were killed once they reached the established end-point of pHa ≤ 7.0. An autopsy was performed on all animals to verify the integrity of the phrenic nerves and complete sectioning of the vagi. The right main bronchus was then intubated, and a bronchoalveolar lavage (BAL) was performed by using 30 ml of HEPES solution. The lavaged material from each lung was centrifuged at 500 g for 5 min to bring down a pellet of cells and debris. The supernatant was then placed in the bubble chamber of a captive-bubble surfactometer (45, 46).

The bubble surface tension and area were determined from the height and diameter of the recorded bubble images displayed on the video screen and by using the approximations described previously (45). Approximately 1.5 ml of the surfactant suspension were placed in the bubble chamber, and the temperature was raised to 37°C within 5 min by a warm airflow. To assess the surface activity of the surfactant suspension, the film formation by adsorption, minimum surface tension on film compression, and area compression required to reach minimum surface tension for a maximum of 70% change in the bubble volume were determined as follows.

A bubble, ~5 mm in diameter, was introduced into the chamber by drawing air through the bottom inlet into the chamber. Adsorption at 37°C was started immediately by pulsating the bubble at 10 cycles/min for 3 min with only 30% changes in the bubble volume. After the adsorption period, a surface tension between 24 and 40 mN/m was obtained at maximum bubble volume for the samples from sham-operated or vagotomized animals. The adsorption period was followed immediately by 20–30 dynamic cycles at 20 cycles/min. Bubble area at a surface tension of 25 mN/m, minimum surface tension after film compression, and the area compressions required to compress the bubble from 25 mN/m to minimum surface tension were calculated in percentage of the bubble area at the surface tension of 25 mN/m. The minimum surface tension and the area compressions as described above were determined for cycles 5, 10, and 20 of the consecutive dynamic

<table>
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<tr>
<th>Table 1. Effect of CPAP and 50–100% O2 on pHa and blood-gas tensions in vagotomized newborn lambs</th>
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<tr>
<td>pHa</td>
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<tr>
<td>PCO2, Torr</td>
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<td>Pao2, Torr</td>
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<td>Pco2, Torr</td>
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Values are means ± SE. Continuous positive airway pressure (CPAP) at 4–6 cmH2O with 50–100% O2, pHa, Pco2, and Pao2; arterial pH, Pco2, and Pao2, respectively. *P < 0.05.
pulsations. The calculated relative error ranged from \( \sim 10\% \) for surface tensions \(< 5.0 \text{ mN/m} \) to \( \sim 6\% \) for surface tensions \( > 30 \text{ mN/m} \). The uncertainty in the area was 5–10\%. Each minimum surface tension and percent area compression value represents the average of independent measurements. Each measurement is the average obtained from two bubbles formed in the same sample fluid. A series of 20–30 pulsations was performed on one bubble. This bubble was then squeezed out of the bubble chamber, and a second bubble was formed and subjected to 20–30 pulsations. A rationale and discussion of the surface tension and area calculations are given elsewhere (46).

Subsequently, the left lung was cannulated and inflated with Zamboni's fixative at 25 cmH2O pressure. After fixation, samples of tissue were taken for light, transmission, and scanning electron microscopy. For light microscopy, tissues were processed through paraffin, and standard 5-µm sections were stained with hematoxylin and eosin. For transmission electron microscopy, the lung tissue was cut into small pieces and fixed in 2.5% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.4) for 2 h at 4°C. After being washed three times with the same buffer, the specimens were postfixed in 1% osmium tetroxide buffered with cacodylate for 2 h at 4°C, dehydrated in a graded series of acetone, and embedded in Epon 812, an epoxy resin (Canemco, Quebec, Canada). Sections were cut \( \sim 1 \mu m \) thick, stained with toluidine blue, and examined by light microscopy to select appropriate areas. Ultrathin sections were cut with a diamond knife, stained with uranyl acetate and lead citrate, and examined with an Hitachi H-7000 electron microscope. For scanning electron microscopy, specimens were postfixed with 1% osmium tetroxide in 0.05 M cacodylate buffer (pH 7.4) for 1 h at 4°C. They were dehydrated through a graded alcohol series and dried by the critical-point drying method with liquid CO2. Dried specimens were coated with gold palladium in an ion coater. The metal-coated specimens were observed with an Hitachi S-450 electron microscope. The investigators performing the surface activity and histological studies were blinded to the samples from the experimental groups.

Lung wet-to-dry-weight ratios were determined by using the methods described by Alcorn et al. (2). Briefly, three pieces of lung tissue from identical lobes were tied and dissected from both sham-operated and vagotomized animal, weighed, and dried in preweighed aluminum containers for 3 days at 120°C.

Statistical analysis. The effects of intrathoracic vagotomy and sham surgery on pulmonary gas exchange, breathing patterns, temperature, heart rate, and blood pressure and the surface activity of the BAL were analyzed by using repeated measures of the ANOVA across time. For the purpose of analyses, various variables were divided into one prebirth, if applicable, and four postnatal time bins (0–10, 10–30, 30–60, and 60+ min after birth). The 60+ min time was \(< 70\)-min (63–69) postnatal age. The time bins were similar for both sham-operated and vagotomized animals. If a significant effect of time was observed, Tukey's test was done to determine where the differences were across time but within a given group. Gestational ages and birth weights were compared by the nonpaired \( t \)-tests. Fisher's exact test was used to compare the development of respiratory failure and survival rates between the two groups. All values are given as means \( \pm \text{SE} \). The study protocol was approved by the Animal Care Committee of the University of Calgary.

RESULTS

Neonatal outcome. The gestational ages of the sham-operated (142 ± 1 days) and vagotomized ani-

mals (140 ± 2 days) were similar (\( P = 0.18 \)). Complete section of the vagal nerves and integrity of the phrenic nerves were confirmed at autopsy in 14 of 16 vagotomized animals, whereas vagotomy was incomplete (unilateral) in two animals. The two unilaterally vagotomized animals were excluded from the statistical analyses. All eight sham-operated animals had bilaterally intact vagal and phrenic nerves. Twelve of fourteen vagotomized animals developed respiratory failure, whereas two of eight sham-operated animals were unable to establish effective gas exchange (\( P = 0.008 \)). Of the 12 vagotomized subjects that died, three did so within 5 min of birth. Both partially vagotomized animals established effective pulmonary gas exchange.

Pulmonary gas exchange. There were no statistically significant differences in the prebirth \( \text{pH}_a \), \( \text{PaCO}_2 \), or \( \text{PaO}_2 \) between the sham-operated and the vagotomized fetuses (Fig. 1). \( \text{pH}_a \) decreased in both sham-operated...
and vagotomized animals within 10 min of birth compared with the prebirth values. However, pH values returned to the normal range (7.32 ± 0.03) in the sham-operated group by 30 min of age, whereas there was a further decrease in the vagotomized group (P < 0.05). Furthermore, this decrease in pH was also significant compared with the sham-operated animals (P < 0.05).

\( P_{aO_2} \) in the vagotomized animals increased significantly from the mean prebirth value of 47 ± 1 to 72 ± 6 Torr within 10 min after birth (P < 0.05) and remained elevated (71 ± 10 Torr) for the duration of the study. However, in sham-operated animals, no significant change in \( P_{aCO_2} \) was observed during the postnatal period. Furthermore, postnatal \( P_{aCO_2} \) values in the vagotomized animals were significantly higher than in the sham-operated group (P < 0.05) for the duration of the experiment.

\( P_{ao} \) did not change significantly from 19 ± 2 Torr prebirth to 17 ± 3, 21 ± 3, 24 ± 6, and 27 ± 6 Torr at 10, 30, 60, and 60+ min of age, respectively, in vagotomized animals. In contrast, \( P_{ao} \) in the sham-operated animals rose sharply from 24 ± 2 Torr prebirth to 47 ± 4, 49 ± 3, 56 ± 3, and 52 ± 2 Torr at 10, 30, 60, and 60+ min of age, respectively. The postnatal increase in \( P_{aao} \) values for the sham-operated animals was significantly higher than that observed in the vagotomized animals for the entire duration of the experiment.

Both bicarbonate (\( HCO_3^- \)) and base deficit showed significant decreases in the vagotomized group by 10 min of age. By 30–60 min of age, these changes were also significantly different than those observed in the sham-operated animals (Fig. 2).

Breathing patterns. Breathing frequency gradually decreased from 82 ± 6 breaths/min at 10 min of age to 63 ± 7 breaths/min by 1 h of age (P < 0.05) in the sham-operated animals while remaining unchanged from 32 ± 8 to 29 ± 6 breaths/min, respectively, in the vagotomized group. The frequency of breathing was significantly higher in the sham-operated group compared with the vagotomized animals during the entire postnatal period (P < 0.05; Fig. 3). The number of augmented breaths per hour was significantly higher in the sham-operated animals compared with the vagotomized animals (13 ± 1 vs. 0.8 ± 0.1 breaths/h, respectively; P < 0.05).

Body temperature. The rectal temperature decreased in vagotomized animals shortly after 10 min of age compared with the at-birth values, whereas no significant differences were observed within the sham-operated group. Despite attempts at rewarming the animals, rectal temperature gradually decreased to 34.2 ± 1.3°C in the vagotomized animals by 1 h of age (P < 0.05), whereas the sham-operated animals were able to maintain their temperature within normal range (39.2 ± 0.2°C; Fig. 4). The decrease in rectal temperature in vagotomized fetuses was also significant compared with the sham-operated group (P < 0.05).

BAL fluid surface activity. The minimum surface tensions and the percent area compressions required to reach the minimum surface tension of 25 mN/m, the equilibrium surface tension of normal pulmonary surfactant, are given in Fig. 5. The surface tension of the BAL in sham-operated animals was significantly lower (2.17 ± 0.93 mN/m) compared with the vagotomized group (18.7 ± 4.2 mN/m; P < 0.05) at five pulsations. The surface tension remained significantly higher in vagotomized animals at 10 and 20 pulsations.

Lung wet-to-dry-weight ratios and histology. The lung wet-to-dry-weight ratios in sham-operated and vagotomized animals were 9.71 ± 0.18 and 9.86 ± 0.14, respectively (P = 0.52). Gross examination of the lungs...
from vagotomized animals showed large areas of segmental and lobar atelectasis, whereas the lungs from the sham-operated group appeared homogeneously aerated.

By light microscopy, sections of lungs from sham-operated animals showed no histological abnormalities apart from mild patchy atelectasis of the subpleural and paraseptal zones. Sections from the vagotomized lambs were similar to the sham-treated animals in most respects. However, the degree of peripheral atelectasis was greater in these animals, and there was evidence of mild perivascular edema, not present in sham-operated animals (Fig. 6). By transmission electron microscopy, sections of alveolar wall showed similar numbers of alveolar type II cells and apical mature lamellar bodies in both vagotomized and sham-operated animals (Fig. 7).

Cardiovascular responses. Heart rate and blood pressure for the two groups are shown in Tables 2 and 3, respectively. Although there was a tendency toward higher heart rates in the sham-operated group after birth, the differences were not significant. However, there was a significant increase in heart rate in both groups 10 min after birth. No significant differences were observed in the mean blood pressures in either group.

Neonatal behavior and sleep states. The ECoG activity remained in an indeterminate state, and no coincidence of ECoG, EOG, and EMGnk was observed to define a given sleep state (19) in vagotomized animals. Vagotomized subjects were unable to stand unsupported and displayed periods of weak, uncoordinated neck flexions. The neck flexions represented muscle weakness rather than seizure or spasmodic activity. In contrast, the sham-operated subjects were able to stand, feed, and walk within 20 min of birth.

DISCUSSION

We provide evidence that pulmonary branches of the vagal nerves are critical for the establishment of continuous breathing and effective pulmonary gas exchange at birth. Bilateral, intrathoracically vagotomized subjects developed impaired gas exchange as evidenced by respiratory acidemia and hypoxemia. Our data also show that vagotomized animals had higher surface tension and greater perivascular edema and peripheral atelectasis compared with the sham-operated group. The vagotomized subjects were unable to maintain normal body temperature despite attempts to rewarm. With regard to physical behavior, vagotomized subjects showed little activity aside from periodic neck flexion and could not stand unsupported, whereas sham-operated animals could do so within 20 min of birth.

$\text{pH}_a$ decreased in both sham-operated and vagotomized animals within 10 min of birth; however, it returned to normal values by 1 h of age in the sham-operated subjects. $\text{Pa}_\text{O}_2$ rapidly increased and plateaued by 1 h of age in sham-operated animals. These changes are qualitatively similar to those reported previously in both human infants and newborn piglets and lambs (6, 37, 42). However, we observed neither an increase in base deficit just before parturition nor a marked increase in $\text{Pa}_\text{CO}_2$ after birth, as reported previously (6, 37). Furthermore, the decrease in $\text{pH}_a$ was quantitatively more marked in the human infants and newborn lambs, compared with those observed in our present study, and may reflect the fetal compromise or higher baseline levels just before birth (37). The decrease in $\text{pH}_a$ after birth, without a significant increase in $\text{Pa}_\text{CO}_2$ in our studies, is likely due to metabolic acidosis, because an increase in blood lactic acid concent-
tration during the immediate newborn period has been reported by Randall (42).

We have previously shown that fetoplacental gas exchange, acid-base balance, incidence of fetal breathing movements, and sleep states are not affected by vagotomy during fetal life (19). The effects of postnatal vagotomy on neonatal breathing have previously been studied by several other investigators (8, 12, 14, 44, 47). Coombs and Pike (8) found that, in anesthetized newborn to 10-day-old kittens, bilateral cervical vagotomy led to a slowing of respiration, followed by death within a few hours, with some subjects displaying gasping and dyspnea before death. Furthermore, vagotomy appeared to affect breathing in the kitten more than in the adult cat, which could survive indefinitely after vagotomy. Schwieler (47) also observed more profound deleterious effects on the ventilation, breathing patterns, and acid-base status in anesthetized kittens and neonatal rabbits compared with adult animals after cervical vagotomy. Duron and Marlot (12) found that, in the anesthetized newborn kitten, bilateral cervical vagotomy resulted in a decrease in respiratory frequency and an increase in both inspiratory and expiratory times, followed by apnea and eventual respiratory failure. Furthermore, they found the effects of vagotomy on breathing to be progressively less pronounced in the course of the postnatal period. Their data support the hypothesis that vagal afferents play a
vital role in the control of neonatal breathing and that this influence decreases with age. However, these studies, often performed with the animal under anesthesia, involved cervical vagotomy, which leads to denervation of the laryngeal musculature and possible upper airway obstruction. Furthermore, the studies were either acute experiments in tracheotomized animals, involving sectioning of the sinus nerves, or not done in the immediate newborn period (8, 12, 14, 44, 47).

The cause of respiratory failure in our vagotomized animals is unclear, but three major possibilities exist: 1) elimination of vagal feedback of lung volume and respiratory pattern, leading to expiratory braking; 2) a

Table 2. Heart rate in sham-operated and vagotomized newborn lambs

<table>
<thead>
<tr>
<th>Group</th>
<th>Prebirth</th>
<th>0–10 min</th>
<th>10–30 min</th>
<th>30–60 min</th>
<th>60+ min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated</td>
<td>128±15</td>
<td>167±21</td>
<td>193±11*</td>
<td>201±10*</td>
<td>186±17*</td>
</tr>
<tr>
<td>Vagotomized</td>
<td>132±21</td>
<td>160±28</td>
<td>182±12*</td>
<td>187±14*</td>
<td>180±19*</td>
</tr>
</tbody>
</table>

Values are means ± SE in beats/min. *P = 0.05, compared with prebirth values within the group.

Fig. 7. Electron micrographs of type II pneumocytes from sham-operated (A) and vagotomized (B) newborn lambs. Both cells show mature lamellar bodies of approximately equal no. and size. Original magnifications, ×25,000.
Table 3. Blood pressure in sham-operated and vagotomized newborn lambs

<table>
<thead>
<tr>
<th>Group</th>
<th>Prebirth</th>
<th>0–10 min</th>
<th>10–30 min</th>
<th>30–60 min</th>
<th>60+ min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated</td>
<td>70 ± 12</td>
<td>75 ± 9</td>
<td>74 ± 7</td>
<td>70 ± 6</td>
<td>71 ± 7</td>
</tr>
<tr>
<td>Vagotomized</td>
<td>59 ± 11</td>
<td>81 ± 10</td>
<td>76 ± 12</td>
<td>66 ± 13</td>
<td>65 ± 16</td>
</tr>
</tbody>
</table>

Values are means ± SE in mmHg.

decrease in the synthesis, secretion, and/or dysfunction of the surfactant system; and 3) a lack of an increase in pulmonary blood flow normally observed shortly after birth. In addition, ventilatory-metabolic interaction was likely affected by persistent hypoxia, hypocarbia, and hypothermia.

Fedorko and colleagues (14) investigated the effects of bilateral cervical vagotomy on respiration in anesthetized and unanesthetized newborn and adult rats, under normal and hypoxic conditions. After cervical vagotomy in unanesthetized newborns, there was a significant decrease in minute ventilation during air breathing, a threefold increase in tidal volume, significant increases in both inspiratory and expiratory times, and a concomitant decrease in breathing frequency. Under hypoxic conditions, unanesthetized newborns also showed a decrease in minute ventilation after vagotomy. However, this decrease was not as great as that seen in the air-breathing group, suggesting a vagally mediated chemoreceptor feedback. In anesthetized newborn and adult rats, vagotomy led to similar proportionate changes in breathing variables, with increases in tidal volumes and inspiratory times. However, expiratory times increased far more in the newborns, and only the newborns showed a fall in minute ventilation. The data support the hypothesis that vagal afferent input from pulmonary mechanoreceptors provides the positive feedback in newborns necessary for the maintenance of adequate respiratory center drive, affecting not only the breathing pattern but also ventilation. Cross et al. (10) have also shown vagi to modulate the phrenic nerve output and the volume-related feedback to be excitatory to the inspiratory activity.

Rapid respiratory rate (60–90 breaths/min), as observed in our sham-operated animals, and reduction of expiratory flow (expiratory braking) are characteristic features of neonatal breathing in an effort to maintain the lung volume above the functional residual capacity (32). During the first few hours after birth, the lung volume is maintained by the inspiratory muscle activity during expiration combined with large reductions in expiratory flow. In the first 10 min of life, the interruption of expiratory flow occurs as frequently as 50% of the time and may be reduced to near zero values. The number of augmented breaths was significantly less than that observed in the sham-operated group and could have contributed to pulmonary atelectasis. It is likely that the vagotomized animals in our present study were unable to maintain or establish an effective lung volume because the neural pathway to control the lung volume appeared to be vagally mediated (32).

Evidence suggests that the change in breathing pattern and, in particular, the changes in tidal volume and inspiratory time after vagotomy in neonates may be attributed to the loss of afferent information generated by the pulmonary stretch receptors and relayed by the vagus (Hering-Breuer reflex). Olinsky et al. (36) showed that occluding the airway at end expiration and comparing the duration of inspiratory effort during occlusion to the duration of the preceding unoccluded inspiration caused significant increases in the duration of inspiration in both full-term and preterm infants, although quantitative differences existed, confirming the previous studies conducted in neonatal animals.

Vagotomized animals in our study remained hypoxic and breathed at a much lower rate than did the sham-operated animals. Many animal species, including the neonatal lamb, exhibit a characteristic biphasic ventilatory response to acute hypoxia consisting of a brief, initial increase in minute ventilation followed by a depression to near or below baseline values, in a gestation-dependent manner (11, 13, 26). The mechanisms of the hypoxic respiratory depression have been discussed by several investigators (11, 13, 33, 34, 40). Although there is no general consensus, the likely mechanisms include central inhibition, reduction in metabolic rate, and immaturity of peripheral chemoreceptors (11, 13, 33, 34, 40, 43). Such a biphasic response was reported to be maintained in vagotomized animals, even though ventilation decreased and the breathing frequency declined to apnea followed by a gasping-type breathing pattern that required supplemental O2 in 48-h-old primates, suggesting a significantly decreased flow and volume-mediated sensory feedback (26).

In another study (11), even though the biphasic response to hypoxia was observed in 10-day-old conscious lambs, vagotomy blunted the characteristic increase in ventilation to hypoxia. However, no significant changes in blood-gas tensions were reported. The most likely reasons for the conflicting data include the differences in postnatal ages, species, and experimental conditions. The animals in the study by Laframboise et al. (26) were younger and were a species less mature at birth than those studied by Delacourt (11). Similarly, studies by Bureau et al. (5) have also suggested gestational age-dependent qualitative and quantitative differences in ventilatory responses to hypoxia. Despite the methodological differences in these studies, all have consistently shown hypoxia to depress the ventilatory drive in the newborn. Therefore, persistent hypoxia may have added to the already depressed ventilation in vagotomized animals. Hyperventilation is the general response to hypercapnia and, in contrast to hypoxia, has no metabolic component (33). In our animals, paradoxically, breathing frequency was lower in vagotomized animals despite a significant increase in $P_{aco_2}$, suggesting an overriding influence of the absence of positive vagal feedback and central depressive effects.

Pulmonary surfactant (a lipoprotein composed of ~80% glycerol phospholipids, 10% cholesterol, and 10% protein) plays a critical role in the transition from the fluid-filled lungs to the aerated newborn lungs (20, 23).
Our results indicate poor surface activity on the BAL fluid obtained from the vagotomized animals compared with the sham-operated group. The poor surface activity may be due to either decreased surfactant synthesis, secretion, or dysfunction (2, 4, 17, 20, 22, 23, 25, 38).

A considerable body of inferential evidence suggests that vagotomy may compromise surfactant synthesis or release (2). We did not observe any morphological differences in the degree of maturity of the lung as determined by alveolar and airway structure and ultrastructure or density of the alveolar type II cells in the vagotomized animals. In contrast, studies by Alcorn et al. (2) have suggested ultrastructural differences in the inclusions of the fetal type II cells of the antenatally cervical-vagotomized animals that may reflect an alteration in the pulmonary surfactant production. However, the vagotomized lambs in their studies were delivered by cesarian section, were not allowed to breathe air, and were preterm, ranging from 130 to 133 days of gestation (full term ~145–150 days). Studies by Mescher et al. (31) have shown that surfactant secretion rapidly increases after 135 days of gestation and does not reach the mature newborn values until 140 days of gestation. Postnatal studies by Massaro and Massaro (30) have suggested that cholinergic stimulation may be involved in the secretion of surfactant and showed lung inflations to be potent stimuli of surfactant secretion. The cholinergic (vagal) innervation of the airways in the young has been reported by Fisher et al. (15). In a study by Lawson et al. (28), surfactant concentrations increased after 30 min of air and nitrogen breathing, whereas, in the animals with occluded trachea, little or no increase was observed. They speculated that the possible mechanisms for surfactant release might be mediated by vagal nerves. However, animals in all three groups had previously been ventilated with room air for 30 min. Oyarzun and Clements (38) have shown that increased minute ventilation augments the air space phospholipids and that such release may be mediated via cholinergic mechanisms. Studies have consistently shown the minute ventilation to be decreased in vagotomized animals. In another study, Oyarzun et al. (39) also showed that, in collapsed lungs, the lung phospholipid profile changes its composition toward the fetal pattern. A recent study by Kitterman et al. (24) has shown that physical factors influence the maturation of pulmonary surfactant. Studies by Corbet et al. (9) and Hildebran et al. (21) also support a role for lung expansion in surfactant secretion. We have no evidence of the adequacy or the extent of lung expansion in our neonatal model. It is possible that, on the basis of histological evidence, lung distension was not uniform, and the poor surface activity of the BAL was due to an attenuated secretion of surfactant.

The significant histological difference between the vagotomized and sham-operated animals was the presence of pulmonary edema. Specifically, there was evidence of interstitial and alveolar edema in the vagotomized animals, whereas the control, sham-treated animals showed no significant interstitial or alveolar edema. These findings are consistent with previous studies (4, 17, 25). Berry et al. (4) found decreased compliance and $P_{A_0}$, an 18-fold increase in radiolabeled albumin leak into the airways and alveoli, and a several fold increase in minimum surface tension as measured by the oscillating bubble technique in rabbits that had undergone bilateral cervical vagotomy, compared with the sham-operated animals. They concluded that the soluble proteins that leaked from the vascular space inhibited the surface tension-lowering properties of surfactant. Studies by Holm and Notter (22) also suggested inhibition of lung surfactant by pulmonary edema. Similarly, Kunc et al. (25) also observed an increased mortality, decreased frequency of breathing, and threefold increase in surface tension and interstitial pulmonary edema in vagotomized adult rats. Earlier studies of Goldenberg et al. (17) showed pulmonary vascular congestion, focal edema, atelectasis, and hemorrhage. Furthermore, histological changes consistent with altered surfactant metabolism were observed in the type II alveolar cells (17). Therefore, in our vagotomized fetuses, either the surfactant secretion was impaired or it lost the surface-active properties due to pulmonary edema and atelectasis.

Although histological examination showed pulmonary edema in the vagotomized animals, no significant differences were observed in the lung wet-to-dry-weight ratios. The wet-to-dry-weight ratio is a relatively insensitive technique for detecting mild interstitial edema (27). Pulmonary edema fluid has been shown to accumulate first in the peribronchovascular interstitium before flooding the alveoli. Within the interstitial space, fluid accumulation is seen primarily around arteries due to lower interstitial pressures in this compartment (41). Consequently, the observation of perivascular edema on histological sections in the face of normal wet-to-dry-weight ratios results from the relative sensitivities of the two techniques.

During fetal life, pulmonary blood flow comprises 10% of the combined ventricular output. However, an 8- to 10-fold decrease in pulmonary vascular resistance and a 10-fold increase in pulmonary blood flow are observed after birth and have mostly been attributed to lung expansion and oxygenation (1, 49). The effects of intrathoracic vagal denervation on pulmonary hemodynamics have not been studied in chronically instrumented, unanesthetized animals during transition from fetal to neonatal life. However, evidence suggests that acute hypoxia causes pulmonary vasconstriction during fetal and early neonatal periods (1, 48). Therefore, it is likely that hypoxia in our vagotomized animals did not permit the increase in pulmonary blood flow normally observed after birth, leading to persistent pulmonary hypertension of the newborn. Persistent pulmonary hypertension of the newborn is also known to be further exacerbated by surfactant system dysfunction and pulmonary edema, which were observed in our present study (48).

Although there were no significant differences in rectal temperature between the vagotomized and sham-operated animals within 10 min of birth, a significant
decrease was observed in vagotomized lambs thereafter. We utilized the heat lamp to rewarm the lambs. No other interventions, such as wrapping the animals in blankets or placing them in incubators, were instituted. This intervention could have affected the natural course of thermoregulation. However, no effects of rewarming on rectal temperature were observed. Given the degree of hypoxemia in vagotomized subjects, thermal instability was not unexpected, because acute hypoxic insults are well known to decrease the body temperature in several animal species (16, 34, 40). Metabolic-ventilatory interaction during hypoxia and hypercapnia has been extensively investigated (16, 33, 34, 40). Pedrazz and Mortola (40) have shown a decrease in body temperature and CO₂ production in 2- to 6-day-old kittens and puppies during 30-min episodes of hypoxia. It is also likely that hypoxia may decrease the metabolic rate by decreasing thermogenesis, although metabolic and ventilatory responses vary widely depending on the species and postnatal weight and age (16). Although hypometabolism is the specific response to hypoxia, hypercapnia in our animals may have caused vasodilatation-exacerbating hypothermia, because large animals are well known to decrease body temperature during hypercapnia. Furthermore, metabolic acidosis observed during hypoxemia may also contribute to the hypometabolic response (33).

Both heart rate and blood pressure were similar in sham-operated and vagotomized animals. Although heart rate tended to be higher especially after the first 10 min of life in sham-operated animals, due to the wide variability, the differences between the two groups were not significant. Both chemo- and baroreceptor activity has been observed in the fetus as well as during the first week of life, however, further maturation occurs with increasing gestational and postnatal age (29, 50). In summary, we provide new and unequivocal evidence that, in unanesthetized, untracheotomized, spontaneously delivered newborn lambs, vagal innervation is necessary for the establishment of continuous breathing and adequate ventilation at birth. Further studies are required to quantify the relative roles of the surfactant system (release vs. inhibition), volume feedback, and changes in pulmonary blood flow in the pathogenesis of vagally mediated respiratory failure in newborn animals.

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