Hypoxemia and low Crs in vagally denervated lambs result from reduced lung volume and not pulmonary edema

SALIM LALANI, JOHN E. REMMERS, YOLANDA MACKINNON, GORDON T. FORD, AND SHABIH U. HASAN
Department of Pediatrics, Respiratory Research Group, Faculty of Medicine, The University of Calgary, Calgary, Alberta, Canada T2N 4N1

Received 14 September 2001; accepted in final form 16 April 2002

Lalani, Salim, John E. Remmers, Yolanda MacKin- non, Gordon T. Ford, and Shabih U. Hasan. Hypoxemia and low Crs in vagally denervated lambs result from reduced lung volume and not pulmonary edema. J Appl Physiol 93: 601–610, 2002. First published April 19, 2002; 10.1152/japplphysiol.00949.2001.—Vagal denervation performed in the intrathoracic region in newborn lambs leads to hypoxemia and decreased respiratory system compliance (Crs), which could result from atelectasis and/or pulmonary edema. The objective of the present study was to quantify the relative roles of alveolar derecruitment and pulmonary edema as underlying cause(s) of respiratory failure. Vagal denervation was performed in the intrathoracic region and below the recurrent laryngeal nerves in six newborn lambs within 24 h of birth, whereas six were sham operated. Pre- and postinfation Crs was measured to investigate the presence of alveolar derecruitment. Pulmonary edema was assessed with lung wet-dry-to-wet and lung tissue wet-to-dry ratios, total protein, and FITC-BSA recovery in lung tissue and bronchoalveolar lavage. Compared with that in the sham-operated animals, Crs was significantly lower in vagally denervated animals. However, postinflation, pulmonary system compliance obtained by quasi-static lung inflation and deflation to 30 cmH2O showed no significant difference between the sham-operated and denervated lambs. The lung wet-dry-to-wet and lung tissue wet-to-dry ratios, total protein, and FITC-BSA recovery in lung tissue and bronchoalveolar lavage were similar in denervated and sham-operated groups. We provide evidence that reduced lung volume and not pulmonary edema is associated with intrathoracic vagal denervation and is the likely underlying mechanism for hypoxemia and low Crs.

TO ESTABLISH AN INDEPENDENT life at birth, the newborn must initiate and maintain continuous breathing and pulmonary gas exchange. Previous studies have shown that vagal innervation of the lung plays a key role in this vital process, and postnatal vagal denervation produces deleterious effects on ventilation as well as breathing patterns during the newborn period (10, 16, 47). However, the validity of these studies was compromised by anesthesia, tracheostomy, or possible secondary laryngeal obstruction.

Our laboratory recently developed an unanesthetized lamb model in which vagal denervation is performed prenatally and below the recurrent laryngeal nerves, thereby avoiding anesthesia, tracheostomy, and laryngeal paralysis (54). The denervated animals developed life-threatening respiratory failure shortly after birth, implying that vagal innervation of the lungs plays an essential role in establishing adequate gas exchange in the first hours after birth. However, because vagal denervation was performed prenatally, these studies do not distinguish whether the deleterious effects were derived from a pulmonary developmental deficiency or from the lack of vagal afferent feedback controlling ventilation and/or lung volume at birth (54).

Our laboratory subsequently performed vagal denervation during the early postnatal period to quantify the relative roles of prenatal vs. postnatal denervation on breathing patterns and gas exchange (32). Our laboratory’s recent observations suggested that vagally denervated animals developed hypoxemia in the immediate postoperative period followed by respiratory acidosis and apneas 16–20 h postoperatively (32). Compared with the sham-operated group, the denervated animals showed increased expiratory times and significantly lower minute ventilation, respiratory rate, and dynamic functional residual capacity (FRC). Furthermore, denervated animals showed decreased pulmonary compliance without any changes in the airway resistance (32).

The decrease in respiratory system compliance (Crs) and lung compliance (Cl) may be due to aberrations in the surfactant system, connective tissue abnormalities, alveolar derecruitment (atelectasis), and/or pulmonary edema (9, 23, 26). The biochemical, microscopic, and surface activity studies do not suggest abnormalities of the surfactant system or connective tissues to be the underlying cause(s) of decreased compliance in the postnatally denervated animals (32). Thus the decreased Cl in the denervated group might have resulted from alveolar derecruitment because of a reduction in end-expiratory lung volume toward passive compliance.
FRC, as shown in our laboratory’s previous study (32), and/or pulmonary edema (3, 22, 31, 45).

Pulmonary edema formation in vagally denervated animals has been reported by a number of investigators (3, 22, 31). Goldenberg et al. (22) showed that cervical vagal denervation in adult rats resulted in pulmonary vascular congestion, focal edema, atelectasis, and hemorrhage. Similarly, Kunc et al. (31) provided evidence that, by 24 h postcervical vagal denervation, adult rats had interstitial pulmonary edema and an increased mortality compared with the sham-operated group. Berry et al. (3) showed that, in adult rabbits, cervical vagal denervation resulted in decreased pulmonary compliance and arterial Po2 (PaO2).

Histological examination revealed the presence of alveolar edema, hyaline membranes, and focal hemorrhages (3). Because vagal denervation in these studies was performed in the cervical area, which would involve sectioning of the recurrent laryngeal nerves, pulmonary edema formation could result from aberrantly high negative intrathoracic hydrostatic pressures secondary to upper airway obstruction. The increased negative intrathoracic hydrostatic pressures would, in turn, augment perivascular hydrostatic pressure and fluid filtration, leading to an increased extravascular lung water (19, 33). Furthermore, a large central nervous system sympathetic discharge, secondary to hypoxemia, could lead to peripheral vasoconstriction, and a transient imbalance in right and left ventricular cardiac output would shift blood to the pulmonary circulation, thus overloading the pulmonary vascular system, causing capillary stress failure and disruption of the alveolar-capillary barrier. In addition, hypoxia-mediated myocardial depression would further diminish left ventricular cardiac output and concomitant decrease in aortic flow, and enhanced pulmonary flow would lead to a rapid increase in extravascular lung water. Thus pulmonary edema formation in vagally denervated animals may involve both hydrostatic and neurogenic mechanisms (3, 14, 19, 22, 31, 33, 36, 38, 45).

The present study was designed to quantify the relative roles of alveolar derecruitment (atelectasis) and pulmonary edema in the pathogenesis of hypoxemia, decreased Crs, and respiratory failure in the vagally denervated animals during the early newborn period. We hypothesize that intrathoracic vagal denervation in the newborn lamb is associated with alveolar derecruitment rather than pulmonary edema.

METHODS

Surgical procedures. All surgical and experimental methods and procedures conformed to the Canadian Council on Animal Care guidelines and were approved by the Faculty of Medicine Animal Care Committee. Surgery was performed on 12 newborn lambs within 24 h of birth under general anesthesia with the use of 5% halothane in 100% O2 for induction and 1.5–2% for the maintenance. Six of the twelve animals underwent intrathoracic vagal denervation below the origin of recurrent laryngeal nerve, whereas the remaining six were sham operated.

Each animal was instrumented to implant vascular catheters and diaphragmatic electrodes. The details of the surgical procedures have been given previously (35, 32). Briefly, observing sterile conditions, we implanted polyvinyl catheters (1 mm ID, 2 mm OD) in the jugular vein and the carotid artery to administer fluids and antibiotics and to draw blood samples for arterial pH (pHa) and blood-gas tensions, respectively. Thereafter, the lamb was placed on its left side, and the right vagus and phrenic nerves were exposed through a 2- to 3-cm incision made at the level of the fourth intercostal space, and a 2-cm section of the vagus nerve was removed below the origin of the recurrent laryngeal nerve. The procedure was repeated on the left side. Chest drains (8 Fr, Sherwood Medical, St. Louis, MO) were placed under water seal at −12 cmH2O (Deknatel, Fall River, MA) to minimize the occurrence of pneumothorax, as described in detail previously (32). The sham-operated animals underwent identical surgical procedures, including bilateral thoracotomy, placement of chest drains, and identification but not sectioning of the vagus nerves. Finally, three bipolar electrodes were implanted into the right costal diaphragm through a 2-cm incision made in the 10th intercostal space to record the diaphragmatic electromyogram (EMGdi). All incisions were sewn in layers, and the electrode wires and vascular catheters were tunneled subcutaneously and stored in a pouch, sutured to the dorsal neck region. Intraoperatively, heating pad temperature and ventilatory settings were adjusted to keep the rectal temperature (39°C), pHa (7.35–7.45), and blood-gas tensions [arterial Pco2 (PaCO2) = 35–45 Torr, Pao2 = 90–110 Torr] within the normal range.

Because, in our laboratory’s previous study (32), we had investigated, in detail, a number of cardiorespiratory and behavioral responses, including the breathing patterns, sleep states, cardiovascular variables, and surfactant biochemical and biophysical activities, the animals in this study were not instrumented to measure these variables. However, postoperatively, the animals were observed for attaining an awake state, defined behaviorally as eyes open (50) and purposeful movement of limbs, including an effort to stand up.

Postoperatively, to help maintain normal body temperature (39.0°C), the animals were transported from the operating room to the laboratory with a neonatal transport incubator (Air Shields) and a heat lamp during observations in the laboratory. The establishment of spontaneous breathing was defined as a respiratory rate of at least 15 breaths/min. Supplemental oxygen and/or manual positive pressure ventilation were administered if the animals were unable to establish effective pulmonary gas exchange and spontaneous breathing. Effective pulmonary gas exchange was defined as a pHa > 7.30, Pao2 < 50 Torr, and Pao2 > 50 Torr.

To prevent hypoglycemia and dehydration, 10% dextrose in water and 0.45% NaCl were continuously infused intravenously at 90–120 ml·kg−1·day−1. Blood glucose and electrolytes were monitored at 4-h intervals. Antibiotic prophylaxis consisted of two intravenous doses of cefazolin sodium (25 mg/kg) and gentamicin sulfate (2.5 mg/kg), administered at 8-h intervals.

Experimental design. Postoperatively, arterial blood samples were drawn every 60 min or more frequently if clinically indicated for measurement of pHa, and blood-gas tensions were corrected for rectal temperature (IL 1312). The rectal temperature was monitored with a Physi-temp temperature monitor (Physi-temp Instruments, Clifton, NJ). Arterial blood samples were not obtained while animals were receiving supplemental oxygen. EMGdi, esophageal pressure, and rectal temperature were recorded once per hour for a period of 5 min. The EMGdi signal was amplified and filtered appropri-
ately with a frequency range of 50 Hz–1 kHz. Esophageal pressure was obtained by placing an 8-Fr polyvinyl tube in the midesophagus and was recorded with a pressure transducer (Statham P23 ID; Gould). all bioelectric signals were displayed on an eight-channel chart recorder (Gould Brush 2800S), digitized, and stored on a videocassette with an eight-channel Neurorecorder (DR-886; Neurodata, New York, NY).

Breathing patterns were recorded 1 h before surgery and 1 h (recovery), 6 h, and 18 h postoperatively with a well-sealed face mask connected to a Fleisch pneumotachograph (size 00), a Hans-Rudolph flow occluder (Hans Rudolph, Kansas City, MO), and Validyne pressure transducers (DP45-32-A-3-5-S-4-D and DP45-14-A-3-5-S-4-D, Validyne Engineering, Northridge, CA). The postoperative recovery period was defined as the time immediately after surgery when the animal was breathing spontaneously with a respiratory rate >15 breaths/min with or without requirement for supplemental oxygen. The recorded respiratory variables were stored on a personal computer (Dell 233 MHz) and later analyzed with the Anadat, Labdat, and Auto programs (version 5.2, RHT-Info Dat, Montreal, Quebec).

Either 20 h postoperatively or earlier if the lambs were experiencing respiratory distress (as indicated by a pH < 7.1, Paco2 > 50 Torr, and PaO2 < 50 Torr), arterial blood samples were drawn to analyze complete blood count and blood wet-to-dry ratio, which were used in the determination of lung wet-to-dry ratios.

To determine capillary-alveolar leak, 100 mg/kg body wt FITC-labeled albumin (FITC-BSA) diluted in 10 ml normal saline were infused intravenously over 15 min. One hour post-FITC-BSA administration, 15 ml of blood were withdrawn, and plasma was separated by centrifuging the blood sample for 10 min at 140 g. Plasma was stored at −70°C until fluorescence analysis was performed, as described below.

Once the blood samples were collected, the lambs were endotracheally intubated with a 5-Fr polyvinyl endotracheal tube under chloral hydrate sedation (25 mg/kg, orally). The endotracheal tube was connected to a Fleisch pneumotachograph (size 00), a Hans-Rudolph flow occlusion valve (Hans Rudolph), and Validyne pressure transducers (DP45-32-A-3-5-S-4-D and DP45-14-A-3-5-S-4-D, Validyne Engineering) to obtain preindflation Crs. Crs measurements were based on the principles described by Lesouef et al. (34) and have been given in detail previously (32). Briefly, the airway of the animal was occluded with the occlusion valve at end inspiration for 300 ms, followed by exhalation against ambient air through the flowmeter. The Crs was calculated by using the pressure signal obtained during occlusion and the flow signal during the exhalation periods. The data were recorded for 4 s at a sampling rate of 250 samples/s. The flow signal was integrated into volume, and a flow-volume curve was constructed. The slope of the descent of the curve was established by using regression analysis (75% of the slope and regression coefficient >0.99) at the appropriate part of the curve. Compliance was calculated as volume divided by plateau pressure at end occlusion. The data were analyzed by using the Anadat, Labdat (version 5.2, RHT-Info Dat), and Auto programs (courtesy of Dr. A. Charles Bryan and Helena Frndova, The Hospital for Sick Children, Toronto, Ontario).

After acquiring the pulmonary mechanics, intravenous pancuronium bromide (0.15 mg/kg) was administered to construct quasi-static pressure-volume (P-V) curves. The endotracheal tube was connected to a pressure transducer (Statham, P-23), and the lungs were inflated with room air in increments with a 60-ml syringe to a maximum pressure of 30 cmH2O, representing total lung capacity. Pressures were recorded with a Gould 2800 polygraph chart recorder. At each successive inflation increment, a pressure value was determined after 6 s to account for "creep," and a P-V curve was constructed. Once lungs were inflated to 30 cmH2O, a deflation curve was constructed by deflating the lungs in decrements to 0 cmH2O. To prevent air trapping and alveolar collapse, deflations <0 cmH2O were not performed. A total of three P-V curves were made for each animal.

The animals were killed with Euthanyl (pentobarbital sodium, 240 mg/ml), and the sectioning of both vagi and integrity of the phrenic nerves were confirmed. The right main bronchus was tied, and ~1-cm3 sections from the right upper, middle, and lower lobes were removed, weighed, and placed in preweighed aluminum dishes for determination of lung wet-to-dry weight ratios. The wet-to-dry ratio was corrected for lung blood by using the methods described by Bland et al. (5).

Bronchoalveolar lavage (BAL), with the use of 100 ml/kg body wt of normal saline, was performed in four aliquots on the left lung by using the gravity method at 20 cmH2O and was aspirated with a 60-ml syringe (92). The lavage gate was centrifuged for 8 min at 150 g, and the supernatant was used in the quantification of alveolar edema. Subsequently, the left lung was sectioned into small pieces, frozen in liquid nitrogen, and stored at −70°C until analysis of lung tissue FITC-BSA content could be performed.

To determine the FITC-BSA content in plasma, lung tissue, and bronchoalveolar lavage, samples were thawed at 4°C, the lungs were homogenized in twice the volume of double-distilled water, and the total homogenate volume was measured. The homogenate was centrifuged at 2,000 rpm for 10 min (53) to separate particulate matter, and aliquots of the supernatant were used to measure FITC-BSA and total protein content. Hemoglobin content of the supernatant from the lung homogenate was measured, and the intravascular blood content of the homogenate was estimated from the hemoglobin content of the final blood sample (5). Lung tissue fluorescence was corrected for the contribution from intravascular blood (27).

Determination of FITC-BSA in the homogenized lung, BAL, and plasma samples was performed with a Perkin-Elmer luminescence spectrometer (model LS5B). FITC-BSA standards of different concentrations (0.005–1 μM) were prepared to 2.0 ml in 0.01 M Tris buffer, pH 8.0, and a standard curve was constructed by plotting fluorescence (arbitrary units) against the volume of standard stock solution present in the 2-ml sample. The BAL, homogenate, and plasma samples were diluted to give readings within the range of the standard curve (Fig. 1A). A linear relationship was observed with sample fluorescence and the volume of sample added. The slopes of the standard curve and of the sample curves were used to determine the content of FITC-BSA in the plasma, BAL, and lung homogenate (Fig. 1, B–D). Thus the ratio of the slope of the sample curve (S) to the standard curve (S0) is equal to the ratio of the FITC-albumin concentration in the undiluted sample (C) to that in the 1 μM FITC-BSA albumin stock solution (C0) = (C = S/S0 × 1 μM). To obtain the percent recovery, concentration of FITC-BSA in the undiluted sample was multiplied by the volume of the BAL obtained or by the total volume of the diluted homogenate to determine the total FITC-albumin recovered from the animal. Percent recovery was then calculated as follows: percent recovery = FITC albumin in sample (mg)/total FITC albumin given (mg) × 100. Total plasma proteins were determined with Lowry’s method (37).

Statistical analysis. The effects of intrathoracic vagal denervation and sham surgery on breathing patterns and pul-

J Appl Physiol • VOL 93 • AUGUST 2002 • www.jap.org
monary mechanics were analyzed with ANOVA. If a significant difference was observed, Tukey’s test was performed to determine whether the differences were across time but within a given group. Differences in quasi-static P-V curves, albumin levels, and wet-to-dry ratios were analyzed with Student’s t-test. All values are given as means ± SD, and statistical significance was considered as P < 0.05.

RESULTS

All sham-operated animals were able to establish spontaneous breathing by 9 ± 7 (mean ± SD) and 10 min (median) postoperatively and did not require manual positive pressure ventilation. In contrast, five of the six denervated animals required manual positive pressure ventilation and took a significantly longer time to establish spontaneous breathing (73 ± 51 and 90 min; mean ± SD and median, respectively) postoperatively (Table 1). During the postoperative recovery period, the animals exhibited brief, intense gross motor activity of all four limbs, but complete behavioral arousal was not observed. The intermittent gross motor activity was followed by total cessation of respiratory effort. Additionally, vagally denervated animals could not maintain their body temperatures and had to be warmed with a heat lamp, whereas sham-operated animals were able to maintain normal body temperatures over the study course. Toward the end of the study, denervated animals exhibited prolonged apneic periods, gasping, and respiratory failure, whereas sham-operated animals were able to maintain normal breathing patterns.

Respiratory rate was similar in both groups preoperatively. However, during the postoperative period, the respiratory rate was significantly lower in vagally denervated animals compared with the sham-operated group. The respiratory rates during the recovery period and 6 and 20 h postoperatively were 69 ± 13 vs. 30 ± 10, 65 ± 10 vs. 35 ± 10, and 57 ± 9 vs. 31 ± 9 breaths/min in sham-operated vs. denervated animals, respectively (means ± SD; P < 0.05). The baseline inspiratory and expiratory times and expiratory time-to-total time ratio were similar in both sham-operated and denervated animals. The postoperative inspiratory

Table 1. Postoperative Course

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Denervated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset of Spontaneous Breathing, min</td>
<td>9.2 (6.5)</td>
<td>73.3 (51)</td>
</tr>
<tr>
<td>n</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>Time of Extubation, min</td>
<td>18.3 (11.7)</td>
<td>111.2 (83.1)</td>
</tr>
<tr>
<td>Awake and Alert, min</td>
<td>45 (24.3)</td>
<td>174.5 (125.4)</td>
</tr>
<tr>
<td>n</td>
<td>6 (6)</td>
<td>6 (6)</td>
</tr>
</tbody>
</table>

n = 6 sham and 6 denervated animals.
and expiratory times and the ratio of expiratory time to total time remained unchanged compared with the baseline values in the sham-operated group. However, in the denervated group, the inspiratory and expiratory times increased two- to threefold compared with the baseline as well as the values observed in the sham-operated group. The ratios of expiratory time to total time were also significantly higher in the denervated group (0.7 ± 0.06, 0.68 ± 0.11, and 0.64 ± 0.04) compared with the sham-operated animals (0.56 ± 0.04, 0.58 ± 0.02, and 0.57 ± 0.03) during recovery and at the 6- and 20-h postoperative periods, respectively (Fig. 2; \( P < 0.05 \)).

In the denervated group, \( \text{PaO}_2 \) remained persistently low throughout the entire duration of study compared with that in the sham-operated animals (43.3 ± 4.8 vs. 82.6 ± 13.9 Torr, respectively; means ± SD, \( P < 0.01 \)). Similarly, compared with the sham-operated group, \( \text{pH} \) was lower in the denervated animals (7.34 ± 0.03 vs. 7.28 ± 0.1), and \( \text{PaCO}_2 \) was higher (39 ± 4.1 vs. 49 ± 17 Torr, respectively; \( P < 0.05 \)).

Representative pre- and postlung inflation and slope (coefficient of the first order of regression) of the P-V curves in sham-operated and vagally denervated animals are shown in Figs. 3 and 4, respectively. The preinflation P-V curve and its slope were similar to the postinflation values in the sham-operated animals (Fig. 3), whereas, compared with the preinflation values, the postinflation slope was markedly higher in the denervated animal (Fig. 4). The mean preinflation \( \text{Crs} \) was significantly lower in the denervated group compared with the sham-operated values (\( P < 0.05 \); Fig. 5A). However, postinflation, \( \text{Crs} \) per kilogram body weight showed no significant difference between the sham and denervated lambs (Fig. 5B).

No significant differences were observed in lung wet-to-dry ratio between the two groups (0.82 ± 0.01 vs. 0.84 ± 0.03, sham vs. denervated; \( P = 0.2 \)). Similarly, the lung wet-to-dry ratio, after correction for pulmonary blood volume, showed no significant difference between sham-operated and vagally denervated animals (\( P = 0.2 \); Table 2).

The percentage of FITC-BSA recovery and total plasma protein levels per kilogram body weight in the lung tissue showed no significant differences between the denervated and sham-operated animals, which suggested the absence of interstitial edema (Table 2). Similarly, FITC-BSA amounts recovered from the BAL, representing alveolar edema, were similar between the vagally denervated and sham-operated animals. Total protein levels in the BAL and lung homogenate of sham and denervated lambs were also similar (Table 2). FITC-BSA percent recovery from BAL was 1.4 ± 0.8 vs. 1.0 ± 0.5% in sham vs. denervated animals, whereas lung homogenate FITC-BSA percent recovery was 5.6 ± 4 vs. 3.5 ± 3% in sham vs. denervated lambs. These data indicate that neither alveolar nor lung tissue edema levels increased as a result of vagal denervation (Table 2).

**DISCUSSION**

The objective of our present study was to investigate the relative roles of pulmonary atelectasis and edema formation in the pathogenesis of reduced \( \text{Crs} \) in vagally denervated animals. Using quasi-static inflation-deflation curves, we have shown that reduced \( \text{Crs} \) in the intrathoracically denervated newborn animals can be reversed by lung inflations, thus providing evidence that lack of vagal afferent feedback leads to reduced lung volume or alveolar derecruitment. A similar recovery of fluorescein-labeled albumin in bronchoalveolar lavage and lung tissue, lung wet-to-dry ratios, and total protein concentrations in sham and vagally denervated animals do not suggest the presence of significant pulmonary edema in the denervated lambs.

Several investigators have studied the association and mechanisms of pulmonary edema formation in vagally denervated animals of various species (3, 6, 14, 15, 22, 31, 36, 45). Studies performed by Farber (14, 15) in adult rabbits and guinea pigs showed severe interstitial and alveolar edema formation and high mortal-
ity within 24 h postdenervation. However, vagal denervation was performed in the cervical area, which could lead to vocal cord paralysis and upper airway obstruction, leading to pulmonary edema formation (19, 33). To investigate whether upper airway obstruction was the underlying mechanism of pulmonary edema, Lorber (36) and Reichsman (45) performed right-sided intrathoracic and left cervical vagal denervation in adult rats, guinea pigs, and rabbits, thereby sparing the right recurrent laryngeal nerves. In the study by Lorber (36), no pulmonary edema was observed in the guinea pigs; however, all denervated rats and two of three rabbits developed pulmonary edema or marked pulmonary congestion, respectively. Similarly, Reichsman (45) showed that three of six rats developed moderate or marked pulmonary edema. It, therefore, appears that the incidence of pulmonary edema formation decreased but was not prevented by right-sided intrathoracic and left cervical vagal denervation compared with the bilateral cervical denervation. In contrast to a number of studies (3, 14, 22, 31, 36, 45), no pulmonary edema was observed in our animals. The likely reasons for the discrepancy in pulmonary edema formation between our present study and previous studies include the site of vagal denervation, species, and the postnatal age of the animals. We performed bilateral vagal denervation in the intrathoracic region and below the origin of the recurrent laryngeal nerves, whereas, in the above-mentioned studies, vagal denervation did not completely (unilateral) avoid the vocal cord paralysis, and pulmonary edema was observed in some but not in all of the animals (36, 45). Furthermore, in the previous studies, neither the lung wet-to-dry ratios nor alveolar-capillary leak studies were consistently performed, to either confirm or refute the presence of pulmonary edema in intrathoracically denervated animals. Finally, studies by Goldenberg et al. (22), Kunc et al. (31), and Berry et al. (3) focused on the effects of vagal denervation on the mechanism of surfactant dysfunction and not the pathogenesis of pulmonary edema formation. We, therefore, provide new and unequivocal evidence that bilateral intrathoracic vagal denervation performed in the newborn lambs does not lead to pulmonary edema formation.

To investigate the possible presence of pulmonary edema in vagally denervated animals, we utilized three techniques: lung wet-to-dry ratios, lung wet-dry-to-wet ratios, and quantification of fluorescein-labeled albumin in the BAL and lung tissue. The lung tissue wet-dry-to-wet ratio provides the fraction of wet weight, whereas normalization to the dry lung weight of the lung weight is expressed as wet-to-dry ratio (46). Because the extravascular lung water may be overestimated by 20–30% because of the presence of residual blood (49), the wet-to-dry ratios were corrected for the lung blood, as described by Bland et al. (5). In view of the potentially toxic effects of radioactive compounds (125I or 131I), as used in previous studies (27, 29), we

![Fig. 3. Representative preinflation and postinflation pressure-volume curves (A) and slope of the curves (B) in a sham-operated animal. No significant change was observed in the postinflation curve compared with the preinflation values.](image-url)
opted to utilize FITC-BSA to quantify protein leak from the vasculature into the pulmonary interstitium and alveoli of newborn lambs to assess the presence and extent of edema in vagally denervated and sham-operated animals. Similar agents have previously been used to investigate the progression of pulmonary edema and alveolar flooding in the rat and transport from the alveolar space to the vascular space in isolated perfused hamster lungs (17, 52). An appropriate dose of FITC-BSA and frequency range of fluorescence to investigate the alveolar-capillary leak were validated in our laboratory, as discussed in METHODS.

Previous studies suggested that the decreased Crs and poor gas exchange observed in vagally denervated animals might result from the presence of alveolar plasma proteins secondary to the capillary-alveolar leak causing surfactant system dysfunction (3, 31). However, in our laboratory’s recent study (32), vagal denervation during the postnatal period did not cause any adverse effects on the surfactant system, as evidenced by the absence of changes in biochemical (total surfactant pool size, absolute concentrations, and ratios of large and small surfactant aggregates) and physical properties of the BAL and presence of normal pulmonary type II epithelial cells. Nor did we observe increased plasma protein concentrations in lung tissue or BAL. The likely reason for the lack of any effects on the surfactant system in our laboratory’s study (32) is the absence of pulmonary edema, which was observed in previous studies (3, 22, 31). Notwithstanding the effects of pulmonary edema, our results on the surfactant system are in agreement with those reported by Berry et al. (3). In a previous study from our laboratory, Wong et al. (54) observed increased surface tension in vagally denervated animals; however, they performed vagal denervation 10–14 days before birth, and the animals were studied in the immediate postnatal period. During the transitional period from fetal to neonatal life, a number of cardiorespiratory adaptations occur, including lung liquid clearance, an increase in pulmonary blood flow, and several changes in surfactant secretion and metabolism (28). It is, therefore, likely that the poor surface activity might have resulted from 1) decreased lung expansion leading to lowered surfactant secretion and recycling, and 2) delayed lung liquid absorption resulting in surfactant dysfunction secondary to the lack of establishment of normal breathing patterns at birth, because the histological appearance of lamellar bodies was identical in the sham and denervated animals (54). Therefore, the studies from our laboratory provide evidence that vagal denervation has no apparent effects on the lamellar bodies or the total surfactant pool size (32, 54).

In our laboratory’s previous study (32), we did not observe histological evidence of pulmonary atelectasis, which is probably due to the pulmonary reexpansion secondary to BAL (100 ml/kg body wt) performed on both lungs to analyze large and small surfactant ag-

Fig. 4. Representative preinflation and postinflation pressure-volume curves (A) and slope of the curves (B) in a vagally denervated animal. In postinflation, the respiratory system compliance improved compared with the preinflation values.
gregates, total proteins, and surface tension properties. Also, it would have been difficult to assess pulmonary edema on lavaged lungs (32). Similarly, in our present study, we were unable to perform histological examination to delineate atelectasis, as BAL was performed on one lung, whereas the second lung was homogenized to investigate the FITC-BSA recovery in the lavageate and lung tissue.

In our study, Crs that includes both chest wall compliance and CL was measured, as it is relatively easier than measuring CL. Because the chest wall compliance is five times higher than CL in the newborn, Crs measurements reflect CL (40). However, the neonatal compliant chest wall also leads to chest wall distortion, defined as an inward movement of the rib cage during inspiration (35), resulting in an uneven intra-alveolar gas distribution. Second, although we made a very small intercostal incision to perform intrathoracic denervation, it is possible that some chest wall edema was present, which could affect the Crs measurements. However, the Crs in the vagally denervated animals was compared with that in the sham-operated group, which had also undergone bilateral thoracotomy. Furthermore, in our laboratory’s previous study (32), post-thoracotomy compliance measurements in the sham group did not show any change compared with the preoperative values, suggesting absence of significant chest wall edema.

To elucidate the presence of progressive alveolar derecruitment, we compared Crs both before and after lung inflation 20 h postoperatively in vagally denervated and sham-operated lambs. Before lung inflation, vagally denervated animals had lower Crs than the values observed in the sham-operated group. However, the P-V curves obtained via quasi-static lung inflation to 30 cmH₂O and deflation to 0 cmH₂O showed no significant difference in Crs between the two groups. The postinflation improvement in Crs suggests the presence of atelectasis and alveolar derecruitment in denervated animals. The progressive alveolar derecruitment was the likely cause of low Crs and relatively late onset of hypercapnia and respiratory acidosis. In newborn rabbits, Mortola et al. (42) observed an increase in CL that was attributed to changes in lung volume history determined by an increased tidal volume. In 1990, Pisarri (44) showed that, in spontaneously breathing adult dogs, rapidly adapting receptors provide afferent feedback inversely proportional to changes in dynamic CL, indicating a role of vagal afferents in the maintenance of CL. This observation is supported by Mills et al. (39) and Sellick and Widdicombe (48), who have shown that background discharge of rapidly adapting receptors increases when the lungs become less compliant.

Our pre- and postinflation quasi-static data suggest that the abnormalities in pulmonary gas exchange and compliance in the vagally denervated animals are likely due to reduced lung volume, resulting from the

Table 2. Lung tissue wet-to-dry ratios, total plasma protein, and FITC-BSA recovery in lung tissue and bronchoalveolar lavage

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sham operated</th>
<th>Vagally denervated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung wet-dry-to-wet weight ratio</td>
<td>0.82 ± 0.008</td>
<td>0.84 ± 0.037</td>
</tr>
<tr>
<td>Lung wet-to-dry weight ratio</td>
<td>5.46 ± 0.817</td>
<td>6.71 ± 1.83</td>
</tr>
<tr>
<td>Lung tissue FITC-BSA recovery, %</td>
<td>5.56 ± 3.5</td>
<td>3.45 ± 2.95</td>
</tr>
<tr>
<td>Lung tissue total plasma protein, mg/kg body wt</td>
<td>371 ± 57.8</td>
<td>363 ± 122</td>
</tr>
<tr>
<td>Bronchoalveolar lavage FITC-BSA recovery, %</td>
<td>1.40 ± 0.78</td>
<td>0.96 ± 0.47</td>
</tr>
<tr>
<td>Bronchoalveolar lavage total plasma protein, mg/kg body wt</td>
<td>45.73 ± 32.8</td>
<td>43.8 ± 23.8</td>
</tr>
</tbody>
</table>

Values are means ± SD.
absence of afferent vagal feedback, which plays a critical role in the maintenance of lung volume and breathing patterns in both term and preterm neonates (7, 8, 13, 16, 30, 32). To meet the high-metabolic demands and overcome the adverse effects of a very compliant chest wall, the neonates use various strategies to defend lung volume, including rapid breathing rates, long expiratory time constants relative to the expiratory time, high-dynamic FRC, and higher frequency of augmented breaths (11, 40). Evidence suggests that diaphragmatic postinspiratory activity, along with intercostal muscle and laryngeal adductors activation (expiratory braking) during expiration, helps maintain the end-expiratory lung volume in both neonates and adults (8, 12, 13, 18, 20, 43). Furthermore, in the neonate, expiratory braking assists in keeping the end-expiratory lung volume above the passive FRC (7).

Several studies have shown that augmented breaths mediated via vagal afferents play an important role in preventing alveolar derecruitment, and, for the reasons discussed above, the frequency of augmented breaths is higher in infants compared with their occurrence in adults (2, 51). However, in our laboratory’s previous study (32), the frequency of augmented breaths increased toward the end in the vagally denervated animals, which is in contrast to the previous observations (2, 21). The most likely reasons for the intriguing persistence of augmented breaths in our vagally denervated animals (32) include the site of vagal denervation, postnatal age, nonanesthetized state, and intact peripheral chemoreceptors and would require further studies.

One of the most consistently severe and deleterious effects of vagal denervation in previous (32) and the present study has been the profound postoperative respiratory depression. The slow postoperative recovery cannot be explained on the basis of anesthesia or surgical procedures, as both sham and denervated animals underwent a similar duration of anesthesia, intraoperative homeostasis, and instrumentation, including bilateral thoracotomy. Similar dramatic effects of vagal denervation or nerve blockade on respiratory drive have recently been reported in unanesthetized ground squirrels (Spermophilus lateralis) by Harris and Milsom (24).

Although the precise mechanisms for the severe postoperative suppression of the respiratory drive and hypothermia in vagally denervated animals remain unknown, these could reflect the hypoxia-mediated decreased metabolic rate. Because the metabolic rate in the neonates is higher than in the adults, the hypoxic hypometabolic response would be exaggerated in the neonate (41). Moreover, the lack of vagalafferent feedback, which plays a critical role in respiratory drive, would further depress the metabolic ventilatory drive (41), resulting in prolonged respiratory depression.

Recent studies have shown the presence of $\mu$-opioid receptors along plasma membranes of vagal afferent terminals and the medial nucleus tractus solitarius (NTS). The $\mu$-opioid receptors modulate both presynaptic and postsynaptic vagal input in the NTS and release of excitatory neurotransmitters from vagal afferents. Because both afferent and efferent vagal axonal transport of $\mu$-opioid receptors and accumulation of opioid receptors proximal to the vagal nerve ligature have been reported by Young et al. (55), it is possible that vagal denervation or blockade could lead to a transient accumulation of endogenously produced opioids, resulting in respiratory depression (4). Furthermore, studies have shown glutamate to be the primary neurotransmitter released by vagal afferents in the NTS; lack of glutamatergic excitatory input could have further attenuated postoperative resumption of breathing in the denervated animals (1).

In summary, our present study confirms and extends previous observations that, in vagally denervated newborn lambs, progressive alveolar derecruitment is the underlying mechanism for the decreased Crs and hypoxemia. We provide evidence that vagal denervation performed in the intrathoracic region does not lead to pulmonary edema and is not the cause of reduced Crs in newborn animals. Finally, this communication shows that FITC-labeled albumin is a valid method to assess alveolar permeability in large animals. The delayed onset of the awake and alert behavioral state and the need for extended duration of assisted ventilation and provision of supplemental oxygen after vagal denervation continue to be intriguing, and its mechanisms need to be investigated.

REFERENCES


