Strain-induced growth of the immature lung

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Zhang, Shaoping, Vicki Garbutt, and John T. McBride, Strain-induced growth of the immature lung. J. Appl. Physiol. 81(4): 1471–1476, 1996.—To investigate the relationship between strain and postnatal lung growth, two groups of weanling ferrets were tracheotomized: the study group was exposed for 2 wk to a continuous positive airway pressure (CPAP) of 6 cmH2O and the other group was exposed to atmospheric pressure (control). Total lung capacity after 2 wk was 40% higher in the CPAP-exposed animals than in the control animals (n = 19 for the control group and 18 for the study group; P < 0.01). CPAP exposure was also associated with increases in lung weight and total lung protein and DNA contents. Lung recoil, measured in a subgroup of animals, was characterized by air-filled and saline-filled static expiratory pressure-volume curves. Neither in the air-filled lungs nor in the saline-filled lungs was there a significant difference between CPAP-exposed and control animals in lung recoil at equal fractions of total lung capacity. These data indicate that mechanical strain was associated with an acceleration of lung growth in immature ferrets. The preservation of volume-corrected lung recoil and the expected contribution of surface forces and tissue forces to lung recoil in CPAP-exposed animals suggest that this response did not involve simple lung distension but included a remodeling of the lung parenchyma.

mechanical stress; pulmonary mechanics; continuous positive airway pressure; ferret; alveolar development

There is abundant evidence that mechanical factors influence lung growth. Diaphragmatic hernia, a decreased volume of amniotic fluid, and failure of fetal breathing are all associated with deficient prenatal lung growth and differentiation (pulmonary hypoplasia), presumably because of underdistension of the lung (21). In vitro studies have demonstrated that the proliferation and metabolic activity of fetal lung cells are stimulated by strain (13). The hypothesis that mechanical factors are also important in postnatal lung growth is supported by many clinical observations, including the fact that the lungs grow to fit the distorted chest in patients with scoliosis (7). A role for distension in postnatal lung growth is supported by the well-documented role of mechanical factors in the growth of other tissues, notably the myocardium (17) and bone (20). Mechanical strain has long been considered a primary stimulus for the growth of the remaining lung after unilateral pneumonectomy (6, 18), a widely used model of postnatal lung growth (5).

Despite the preponderance of evidence that strain influences postnatal lung growth, few studies have directly tested this hypothesis. In 1972, Buhain et al. (3) reported an increase in total lung capacity (TLC) in tracheotomized adult dogs that breathed for 2 wk through valves that obstructed expiration and raised functional residual capacity (FRC). However, because that study was designed to investigate the effect of chronic distension on the elastic properties of the lung, the extent to which the increase in lung volume represented tissue growth was not measured.

To investigate the influence of mechanical strain on postnatal lung growth, we developed a method of delivering continuous positive airway pressure (CPAP) to unanesthetized animals for prolonged periods. CPAP has been used clinically to raise lung volume and improve gas exchange in critically ill patients for over 25 years. Although the short-term effects of CPAP on cardiovascular and respiratory functions have been widely studied, we were unable to identify previous studies on the effect of the long-term administration of CPAP on lung growth. We have adapted a tethering system so that tracheotomized ferrets can be exposed to CPAP for prolonged periods without restricting their activity. With this system, we have been able to document that exposure of immature animals to a modest level of CPAP for 2 wk is associated with a burst of lung growth that includes increases in lung volume, mass, and cellularity.

METHODS

Animals

The study protocol was approved by the University of Rochester Committee on Animal Resources. Fifty young male ferrets (6–8 wk old) were purchased from Marshall Farms (North Rose, NY). Mean body weight on arrival was 350 ± 9 (SE) g (body weight at maturity ~1,500 g). Two animals were used to study the acute effects of CPAP on ventilation. The other animals were assigned to a CPAP or control group on the basis of body weight and studied for periods of 4 wk in groups of four or eight. During the first week, the animals were acclimatized to the environment and accustomed to the jackets used in the tether system. In the second week, the ferrets were tracheotomized, and humidified air was delivered to the tracheostomy at ambient pressure (see Administration of CPAP). From the beginning of the third week to the end of the fourth week, the animals breathed either against a CPAP of 6 cmH2O above atmospheric pressure or against atmospheric pressure. After a tracheostomy was performed, the animals were given daily intramuscular injections of amoxicillin (11 mg/kg). The animals were killed at the end of the fourth week.

Tracheostomy and Tethering

After the animals were anesthetized with ketamine and xylazine (25 and 2.5 mg/kg im, respectively), the trachea was exposed through a midline incision midway between the cricoid cartilage and the jugular notch, and a window was made by excision of the ventral one-third of two cartilage rings. A modified size 0 Shiley pediatric tracheostomy cannula (5 mm OD, 3.4 mm ID) was positioned in the trachea, and the incision was closed around the cannula with sutures and wound clips. The cannula was capped, and the animals
were allowed to breathe through a 3-mm-ID plastic T tube incorporated in the adapter of the tracheostomy tube through which a bias flow of humidified warmed air was maintained. The animals were dressed in jackets that fit snugly around the forelegs and the shoulders. The jacket held the cannula and T tube in position and anchored one end of a 12 × 0.25 in. hollow plastic tether that connected to a swivel in the middle of the cage roof. The tubing and swivel provided tethering while allowing free movement of the animal within the cage and the delivery of humidified air with or without CPAP. After the tracheostomy was performed, airway patency was maintained by instilling 1 ml of sterile saline and suctioning accumulated secretions from the cannula every 6–10 h.

Administration of CPAP

Airflow and airway pressure were provided by an adjustable-flow air pump and humidifier that delivered heated humidified air to the tethering system, a water seal with which pressure in the inspiratory tubing was adjusted, and a resistance in the expiratory limb of the breathing circuit that could be adjusted to allow adequate airflow to prevent rebreathing at the desired pressure. The pressure was set by adjusting the water seal while measuring end-expiratory pressure at the tracheal cannula. In the animals exposed to CPAP, the tracheostomy tube was permanently capped except during suctioning. In the control animals, the expiratory resistance was omitted, and a perforated tracheostomy tube cap was used to ensure both adequate humidification and maintenance of ambient pressure at end expiration.

In Vivo Measurement of Lung Volumes

Lung volumes and body weights were measured at the initiation of CPAP and 1 and 2 wk thereafter. Lung volumes were measured with an integrated-flow plethysmograph with a flat-frequency response to 20 Hz. Plethysmographic pressure and airway pressure were measured by two MP-45 pressure transducers (range ±2 and ±50 cmH₂O, respectively; Validyne, Northridge, CA). An FV-156 analog integrator (Validyne) was used for the integration of flow to obtain a volume change in the plethysmograph. The animals were anesthetized with ketamine and xylazine (25 and 2.5 mg/kg IM, respectively); the tracheal cannula was replaced by a 3.5-mm (OD) cuffed endotracheal tube, and the animal was placed in the plethysmograph. To measure FRC by the application of Boyle’s law, the animals were briefly hyperventilated to apnea, the endotracheal tube was occluded at FRC, and changes in pressure and volume with subsequent respiratory efforts were displayed on an oscilloscope. Vital capacity (VC) was measured by inflation of the lungs to 25 cmH₂O and deflation to −25 cmH₂O. Expiratory reserve volume was measured by deflation of the lungs from FRC to residual volume (RV; −25 cmH₂O). Measurements were repeated three times and averaged (coefficient of variation < 2%). After the testing, the modified tracheostomy tube was reinserted, and the animal was reconnected to the tethering system after it recovered from the anesthesia.

Excised-Lung Measurements

At the end of the second week of CPAP, the animals were given a lethal intraperitoneal dose of pentobarbital sodium (100 mg/kg) and ventilated. The thorax was opened through a midsternotomy incision, and a tie was placed around the pulmonary artery and aorta. The tie was abruptly secured, and the animal was exsanguinated by withdrawal of blood from both ventricles.

Air-filled pressure-volume (P-V) curve. With the chest open, the lungs were inflated with air to 25 cmH₂O above atmospheric pressure for 1 min, then slowly deflated in 15 equal steps of volume to atmospheric pressure at a rate of 0.15 ml/s by a syringe pump (Harvard, Millis, MA). Static pressure at the airway opening was measured between the intervals of lung deflation by an MP-45 Validyne transducer (+50 cmH₂O) and digitized by a DT 2826 analog-to-digital converter (Data Translation, Marlboro, MA). The volume withdrawn was corrected for gas compression. RV of the lungs at zero transpulmonary pressure was measured by subtraction of the lung weight from the submerged lung weight.

Saline-filled P-V curve. The excised lungs were submerged in saline in a vacuum chamber, and the pressure was reduced to the vapor pressure of water for 30 s. The degassing cycle was repeated three times. The lungs were then immersed in saline, inflated with saline to the previously measured air-filled TLC, and slowly deflated in 15 equal steps of volume to atmospheric pressure. The static recoil pressure was measured as described in Air-filled pressure-volume (P-V) curve. Using a fluid-filled pressure transducer (164 PC01d37, Microswitch, Freeport, IL). The residual fluid volume was calculated by subtracting the initial lung weight from the weight after deflation was completed.

Lung protein and DNA contents. The lungs were separated; the hilar structures of the four lobes of the right lung were trimmed to the pleura; and the lobes were blotted dry, weighed, and homogenized. Colorimetric/fluorometric techniques were employed to measure total lung DNA (11) and protein (modified from Lowry et al. [14]).

Data Analysis

TLC and RV were calculated from measured FRC, VC, and expiratory reserve volume values. Student’s t-tests of paired comparisons (before and after CPAP) were employed to compare control and CPAP animals. Lung volume for the P-V curves were normalized to 100% full inflation volume. Recoil pressure at equal fractions of TLC of air- or saline-filled curves were analyzed as dependent variables by analysis of variance for repeated measures. The statistical model included the variation among ferrets, the effect of lung volume, and the effect of CPAP and the possible interactions among the above factors.

RESULTS

Acute Effects of CPAP on Ventilation

In two anesthetized animals, initiation of CPAP resulted in an immediate increase in end-expiratory volume as expected (Fig. 1). In addition, however, tidal volume increased and breathing frequency decreased. Arterial blood gases measured in unanesthetized animals after 30 min of control or CPAP breathing demonstrated no change in alveolar ventilation in association with CPAP administration; pH = 7.4 ± 0.02 (SE), arterial Pco₂ = 30.3 ± 1.81 Torr, and arterial Po₂ = 97.5 ± 3.11 Torr for the control group; pH = 7.38 ± 0.17, arterial Pco₂ = 32.5 ± 1.48 Torr, and arterial Po₂ = 101.5 ± 3.4 Torr for the CPAP group (P > 0.3). None of the CPAP animals developed clinical evidence of respiratory distress.

Chronic Effects of CPAP

Of the 48 animals used to study the effect of prolonged CPAP exposure, 37 survived without problems...
until the end of study (19 ferrets in the control group and 18 in the CPAP group). One animal died during surgery, six of plugging of the tracheostomy tube, and four of gastric distension. Measurements of body weights, lung volumes, and protein and DNA contents of excised lungs were available for all 37 animals. Air- and saline-filled P-V curves were measured in 18 animals (9 control and 9 CPAP exposed). The increase in body weight over the 3 wk after the tracheostomy was somewhat less than expected in both groups of animals compared with unoperated and untethered animals of the same age but was not different between the two study groups (Fig. 2).

In vivo lung volumes. TLC measured in the two groups at baseline and for 2 wk thereafter is shown in Fig. 3. After 2 wk, mean TLC of the CPAP group was ~40% larger than that of the control group (P < 0.01). VC, FRC, and RV changed approximately in proportion to TLC; when these volumes were normalized to TLC, there were no significant differences between the groups.

Lung weight and total lung protein and DNA contents. Lung weight was significantly higher for the CPAP group than for the control group; total protein and DNA contents of the lungs showed similar changes (for all comparisons, P < 0.01; Fig. 4).

Lung recoil (Fig. 5). Neither in the air-filled lungs nor in the saline-filled lungs was there a significant effect of CPAP on lung recoil (P > 0.05 by analysis of variance). At no lung volume below 95% of TLC did mean lung recoils differ between the two groups by >0.5 cmH₂O.

DISCUSSION

Exposure of immature ferrets to a CPAP of 6 cmH₂O for 2 wk was associated with increases in lung volume, lung weight, and total lung protein and DNA contents.
might decrease. A consistent relationship between lung CPAP-associated increase in lung size involved alveolar volume decreases. It might be postulated that if the each other, whereas with aging, the ratio of mass to mass and volume increases more nearly in proportion to volume with growth (1, 19, 22). Later in childhood, increases at a slower rate than the increase in lung growth. In early postnatal life, lung tissue mass in-. 

...about these aspects of the pattern of strain-related changes before maturity, we cannot address whether strain altered the lung permanently or simply accelerated growth that would otherwise have occurred over a longer time period. Although the possibility that strain is associated with important alterations in postnatal lung growth is not surprising, this hypothesis has not previously been studied so directly. The physiological importance of the relationship between the size of the lungs and that of the chest wall and the consistency of pleural pressures beyond early infancy suggest that the size of the chest wall must influence the size of the lungs. There are other clinical observations that support this hypothesis. In addition to the effects on lung shape and size in individuals with abnormal chest walls, it has been suggested that short- and long-term alterations in lung volume in individuals with asthma or other diseases associated with chronic airway obstruction might reflect strain-induced alterations in lung structure.

Our data are relevant not only because they document that strain influences lung growth but also because they provide insight into the aspects of postnatal lung development influenced by strain. Immature ferrets breathing with CPAP for 2 wk had increases in lung volume, lung weight, and total lung protein and DNA contents. The fractional changes in these various parameters were similar, suggesting cellular hyperplasia rather than hypertrophy and the maintenance of a constant ratio of lung mass to lung volume. However, the variability of the measurements and the small number of animals studied preclude firm conclusions about these aspects of the pattern of strain-related growth. In early postnatal life, lung tissue mass increases at a slower rate than the increase in lung volume with growth (1, 19, 22). Later in childhood, mass and volume increase more nearly in proportion to each other, whereas with aging, the ratio of mass to volume decreases. It might be postulated that if the CPAP-associated increase in lung size involved alveolar distension alone, the ratio of lung mass to lung volume might decrease. A consistent relationship between lung tissue mass and lung volume has been documented during the lung growth that follows unilateral pneumonectomy (18), a phenomenon in which strain is likely to be a major stimulus.

Our measurements of air-filled and saline-filled lung recoil in association with CPAP exposure in these ferrets differ from the findings of Buhain et al. (3), who made similar measurements in the study of tracheotomized dogs breathing through expiratory retard valves mentioned in the introduction. In that study, the difference between air-filled and fluid-filled lung recoils was decreased in animals with large lungs due to prolonged expiratory obstruction. Their finding of a smaller contribution of surface forces to lung recoil after strain-related remodeling would be consistent with a degree of alveolar distension or enlargement (9). In our study, air-filled and fluid-filled lung recoils, and the difference between these values, remained unchanged after CPAP exposure. The most likely explanation for the difference between these two observations is that we studied immature animals and they studied mature animals. Studies of postpneumonectomy lung growth have concluded that this phenomenon is less complete or extensive in adult animals as opposed to immature animals (4). However, Hsia et al. (10) have documented morphometrically that postpneumonectomy growth includes parenchymal remodeling and alveolar "multiplication" even in mature dogs after a right pneumonectomy. The preservation of a normal relationship between the contribution of surface forces and tissue forces to lung recoil in CPAP-exposed animals suggests that strain-induced lung growth did not occur by simple distension in these immature animals. Improved definition of the structural consequences of strain-related growth and of the influence of age awaits further study.

The application of CPAP represents a tonic strain reflected by an increase in end-expiratory lung volume, and it seems likely that the influence of CPAP on lung growth was related to this tonic strain. It is possible, however, that some other consequence of CPAP was involved. We documented that CPAP exposure was not associated with changes in blood gases and somatic growth in weanling ferrets, and it is unlikely that this level of distending pressure had important cardiovascular effects. CPAP did alter respiratory pattern. Although this was not evident to casual observation, anesthetized animals exposed to CPAP acutely breathed more slowly and erratically and with greater tidal volumes. There is a possibility that this increased phasic strain contributed to CPAP-induced lung growth rather than or in addition to the tonic increase in end-expiratory lung volume. Increased phasic strain without changes in tonic strain is observed whenever minute ventilation changes without a change in mean lung volume. The influence of minute ventilation on lung growth is controversial (8, 12). In studies on the lung growth that follows unilateral pneumonectomy, McBride (15) and others (2) have found that blocking the increase in end-expiratory lung volume by replacing the resected lung with a space-occupying substance blunts the postpneumonectomy increase in lung volume but does not eliminate concomitant cellular prolif-

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**Fig. 5.** Group deflation static pressure-volume curves were measured in excised air- or saline-filled lungs of 9 control animals and 9 animals that had been on CPAP for 2 wk. Values are means ± SE. Analysis of variances shows no significant effect of CPAP on lung recoil in either air- or saline-filled lungs (P > 0.05).

Because we studied immature animals and assessed strain-related changes before maturity, we cannot address whether strain altered the lung permanently or simply accelerated growth that would otherwise have occurred over a longer time period.
eration and increases in lung mass. It is possible that increased minute ventilation of the remaining lung after pneumonectomy induces cellular proliferation and tissue growth without influencing lung volume.

These data on the influence of CPAP on lung growth have particular relevance for postpneumonectomy lung growth. The CPAP model was initially conceived as an approach to further investigate the role of strain in the response to partial lung resection. Because pneumonectomy involves dramatic changes in a variety of critical aspects of the physiological status of the remaining lung (increased FRC, increased minute ventilation, increased pulmonary blood flow, and decreased total pulmonary vascular bed), it was difficult to manipulate this model further to test definitively the reasonable hypothesis that the response to pneumonectomy is simply a response to strain. We reasoned that if strain was responsible for postpneumonectomy lung growth, the application of CPAP without resection should mimic this response. We selected a CPAP of 6 cmH₂O because this was associated with a fractional increase in FRC similar to that of the remaining left lung after right pneumonectomy in the ferret. Although we have inadequate data at this point to conclude that the structural and functional consequences of these two models of postnatal lung growth are identical, the similarity between the changes we observed and the extent and time course of postpneumonectomy lung growth add further weight to the hypothesis that postpneumonectomy growth is simply a response to strain.

The influence of this modest level of CPAP on lung growth has a number of important clinical implications. It is possible that CPAP, an intervention that is used clinically, has effects on lung structure beyond its acute effects on lung volume and cardiovascular function. Such effects might be beneficial or harmful. For instance, it is possible that CPAP might encourage lung growth and hasten the resolution of bronchopulmonary dysplasia or lung hypoplasia in infants. On the other hand, an increase in lung volume out of proportion to that of the chest wall might not be beneficial. The “growth” effects of an uneven distribution of strain, which occurs in many individuals with focal lung diseases or airway obstruction, is another interesting consideration. The growth or remodeling of the lung in response to strain, the reversibility of these changes, the influence of age on this response, the responses of healthy and diseased lung tissues, and ways to manipulate or block this response have important implications for many clinical situations, including volume reduction surgery in patients with chronic obstructive lung disease. Further studies of the mechanisms and structural consequences of strain-related lung growth will be necessary before the full clinical implications of this phenomenon are understood.

Our approach to the direct assessment of the relationship of strain-induced lung growth was based on the ability to deliver CPAP to unanesthetized animals for prolonged periods. Buhain et al. (3) used expiratory retard valves to raise the FRC in dogs. We were unsuccessful in applying this technique to ferrets because of their smaller size. The tethering system that we subsequently developed allows animals to be maintained on a breathing circuit while playing, eating, and sleeping. This approach might also be useful for other studies requiring chronic control of inhaled gas mixtures, aerosols, or airway pressures. This technique is not without problems. In this study, several animals died of tracheal plugs or acute gastric distension. Tracheal plugging may be minimized by improved tracheostomy care. The cause of gastric distension was not clear. The animals were unable to “sniff” after the tracheostomy, and swallowed air might contribute to gastric distension and to the general increase in the amount of gas in the small bowel that was occasionally observed on postmortem examination. Although somatic growth was not normal while the animals were maintained on this system, the animals continued to eat and gain weight throughout the study. Ferrets are particularly suited for this model because of their long neck and unusually large trachea; this technique would be more difficult in small mammals without these unusual characteristics.

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