Respiratory mechanics and lung development in the rat from early age to adulthood


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IMMATURE SUBJECTS ARE KNOWN to be more susceptible to developing airways hyperresponsiveness than are adults (17, 24, 25, 40, 44, 45, 47). Although the mechanisms are still unclear, some studies have identified age-related differences in sensitivity to certain agonists, the relative amounts of airway smooth muscle, and differences in the site of action of certain drugs (10, 44, 49). However, the airway hyperresponsiveness of immaturity has also been related to anatomic and mechanical factors, such as the smaller lumen size of peripheral airways and the relatively greater airway and chest wall compliances (24). In particular, recent studies in rabbits challenged with methacholine have shown that the airways of younger animals close at higher transpulmonary pressures than those of older animals and that airway closure is prevented during tidal ventilation (45, 47). This implicates reduced mechanical interdependence between airways and parenchyma in young animals as being a causative factor (48).

It is thus clear that, to understand why young lungs have a propensity to be hyperresponsive, we must have an improved understanding of how the link between respiratory function and lung structure changes with development. As airway-parenchymal interdependence almost certainly plays a role in this link, it is particularly important to evaluate the influence of lung volume (Vl) on respiratory mechanics at different stages of lung growth. The purpose of the present study was, therefore, to establish how the mechanical properties of the respiratory system depend on the level of lung inflation during development from the early postnatal period to adulthood and how lung structure changes within this period. In particular, we addressed the hypothesis that airway-parenchymal interdependence increases during maturation. We undertook this study in the rat because of the similarities between major aspects of rat lung development and those of other mammals (1, 6, 7, 21, 32), their ease of reproduction and rapid growth, and the extensive information available on lung structural and cellular events occurring during the early postnatal period in this species (6, 7, 19, 34).

MATERIALS AND METHODS

Animal preparation. We studied seven groups of Sprague-Dawley rats between 10 days and 3 mo of age (Table 1). No distinction regarding the sex of the animals was made before 25 days of age, because it has been shown that there are no differences in lung development between sexes during the early postnatal period (7). However, the older groups of animals were composed of male rats. Adult rats (group VII) were sedated with xylazine (14 mg/kg ip) and anesthetized with a single bolus of pentobarbital sodium (45 mg/kg ip). To avoid an overdose in the younger groups of rats, we gave an initial dose of pentobarbital sodium of 25 mg/kg to groups I and II, 30 mg/kg to groups III and IV, and 40 mg/kg to groups V and VI, followed by smaller doses of the drug, administered every 10 min, until the animals were deeply anesthetized. The total dosage of pentobarbital sodium never exceeded 45
mg/kg. The animals were tracheostomized, and the trachea was cannulated with a snug-fitting metal needle. The cannula was then connected to a computer-controlled small-animal ventilator (SAV) developed for the assessment of respiratory mechanics in small animals (37). The rats were mechanically ventilated in a quasi-sinusoidal fashion with a respiratory frequency of 170 breaths/min in groups I–IV, 120 breaths/min in groups V and VI, and 90 breaths/min in group VII. The rats were then paralyzed with succinylcholine chloride (groups I–III: 1 mg ip; group IV: 1.5 mg ip; group V: 2 mg ip; group VI: 3 mg ip; group VII: 8 mg ip).

**Experimental protocol.** Measurements of respiratory system mechanics were made using a forced-oscillation technique against five different levels of PEEP (0–7 hPa) applied in random order. The procedure for each PEEP level took 2–3 min and began with the establishment of regular mechanical ventilation against the PEEP. Volume history was then standardized by inflating the lung to total lung capacity three times, achieved by closing the expiratory line of the ventilator to a water trap. Breathing frequencies were set to 170 breaths/min in groups I–IV, 120 breaths/min in groups V and VI, and 90 breaths/min in group VII. The rats were then paralyzed with succinylcholine chloride (groups I–III: 1 mg ip; group IV: 1.5 mg ip; group V: 2 mg ip; group VI: 3 mg ip; group VII: 8 mg ip).

The central 8 s of R (~t) and E (~t) (corresponding to the original Pao-Vl pair) were calculated from each Pao-Vl pair by fitting the model

\[
\text{Pao}(t) = R(t)V(t) + E(t)V(t) + K
\]

by recursive multiple linear regression using an exponential memory time constant (22) equal to the oscillation period in each case. V is flow obtained by numerical differentiation of Vl, t is time, and K is a baseline pressure. Because recursive linear regression begins with initial parameter estimates (assumed here to be zero) and updates them at each data sampling instant, this yielded R and E as functions of time t.

The central 8 s of R(t) and E(t) (corresponding to the original 8-s data records before edge extension) were isolated. The R and E values that we report in this study are the averages of these isolated segments between 4 and 5 s.

Lung tissue (Rti) decreases hyperbolically with frequency, approaching zero at frequencies above ~4 Hz (13, 15, 39, 46). R at 4.8 Hz is a good approximation to airway R in rats (15) because the Newtonian component of chest wall R is essentially negligible. We presume that this is true at 4.8 Hz (although it is possible that the chest wall does make a contribution to R at this frequency). We, therefore, estimated Rti at 0.9 Hz by subtracting R obtained at 4.8 Hz from R at 0.9 Hz. This assumes that regional ventilation heterogeneity is not important in normal mice and that the low-frequency dependence of R is due to tissue rheology. We also calculated hysteresivity (η) (12) as

\[
\eta = \frac{\text{Rti}}{E}
\]

where ω is the angular frequency.

**Morphometric analysis.** At the end of the experiment, the rats were killed with an overdose of pentobarbital sodium.
and exsanguinated by transection of the abdominal aorta and inferior vena cava. The lungs were removed en bloc from the thorax, the heart, thymus, and other pulmonary structures were dissected away. The lungs were weighed (Table 1). The left lung was then isolated and fixed in 10% formalin solution at a distending pressure of 20 hPa for at least 24 h. Left Vl (Vlₜ) was then measured by water displacement (36) (Table 1), and three blocks were obtained from the cranial, middle, and caudal regions of the left lung perpendicular to the main lung axis. The blocks were dehydrated, embedded in paraffin, cut into four 5-µm-thick histological sections, and stained with hematoxylin-eosin for light microscopy (model Dialux 20, Leitz microscope).

In each section, we studied microscopic fields that contained no major airways or vessels. Colored images of these fields, measuring 624 × 832 µm, were captured by a Panasonic videocamera (model DIGITAL 5100) connected to a personal computer employing the National Institutes of Health Image software interface. Measurements of tissue density (TD), mean linear intercept (Lₘ), mean air space chord (MAC) length, and mean air space wall thickness (AWT) were obtained using software that we developed using MATLAB (The Mathworks, Natick, MA). This software converts a digitized colored image into an intensity image with 256 levels of gray. By choosing a threshold value of gray with the aid of an image histogram, we transformed the intensity images into black and white images. These black and white images were filtered by removing white patches having areas smaller than a specified threshold value and similarly for black patches. This eliminated small speckles that we considered to be due to noise. TD was then calculated as the percentage of black pixels in the filtered image. MAC was determined as the mean distance across a white patch (corresponding to an air space) in the image. This was achieved by scanning every alternate line of pixels, both horizontally and vertically, excluding patches at the edge of the image. Similarly, AWT was determined as the mean distance across a black patch (corresponding to an air space wall). Lₘ was obtained as the ratio of the total length of the scan lines to the number of times that they intersected a white-black-white transition in the image. An estimate of the total air space surface area (Sₐ) in the left lung was obtained from an estimate of the volume occupied by the air spaces (Va) and MAC. Thus (21)

\[ Sₐ = 4 \frac{Vₐ}{MAC} \]  

(3)

where

\[ Va = (1 - TD)VLₜ \]  

(4)

Sₐ was normalized to the two-thirds power of both Vlₜ and Va to determine whether lung growth in rats between 10 and 90 days of age is isotropic or not. If normalized Sₐ does not change with age, then this suggests that growth occurs mainly by simple distension of the air spaces. Increasing normalized Sₐ with age indicates instead a multiplication of the number of structures and/or a change in air space shape.

Statistical analysis. ANOVA was performed to compare values of R and E among different age groups and also to determine the significance of differences due to the effects of PEEP, the interaction between PEEP and age, and of a particular animal in one group. As the interactions between age group and PEEP turned out to be statistically significant, we used one-way ANOVA to investigate the effects of PEEP in each age group. We also studied the differences in the parameter values among age groups for each PEEP. One-way ANOVA was also used to compare the lung structure parameters among the various age groups. When appropriate, Tukey's honestly significant difference or Dunn's test for multiple pairwise comparisons was performed. Statistical significance was considered when \( P < 0.05 \).

RESULTS

Both R and E decreased considerably with age, as would be expected with an increase in lung size. In 10-day-old rats, E and R at 0.9 Hz were up to 27- and 17-fold greater, respectively, than in adult animals, whereas, at 4.8 Hz, R was an order of magnitude greater in the youngest rats. Therefore, to compare respiratory parameters among different age groups, we normalized by the amount of tissue in the lungs by multiplying R, Rti, and E by lung weight, yielding NRₐLW, NRtiLW, and NEₐLW, respectively. These normalized parameters also decreased progressively and markedly with age (Fig. 1), suggesting that the intrinsic mechanical properties of the lungs were changing with age.

Figure 1 also shows the PEEP dependence of both NEₐLW and NRtiLW at 0.9 Hz and NRₐLW at 4.8 Hz in the seven groups of rats studied, as well as the contribution of Rti to total respiratory R at 0.9 Hz. In groups I–IV, NEₐLW had a minimum at 2–3 hPa, followed by a dramatic increase with increasing PEEP in the two youngest groups and a much smaller increase in the 18- and 21-day-old groups. However, this positive dependence of NEₐLW on PEEP was virtually absent in animals >21 days of age. NRtiLW followed a similar pattern, although the two youngest groups of animals had no significant change from 0- to 2-hPa PEEP. In contrast, NRₐLW at 4.8 Hz decreased progressively with PEEP in all age groups. The contribution of Rti to R at 0.9 Hz was slightly lower in adult rats than in younger animals and increased very slightly with PEEP in all groups.

Figure 2 shows \( \eta \) as a function of PEEP in all groups studied. In animals under 14 days of age, \( \eta \) was lowest at a PEEP of 7 hPa. In all other groups, \( \eta \) either remained constant or increased slightly with PEEP.

Figure 3 shows the percent change in R at 4.8 Hz as a function of PEEP for all seven groups of animals. The curves in Fig. 3 appear to lie in two groups, with animals >25 days of age having a greater decrease in percent change in R at 4.8 Hz than the younger animals. The percent change in E at 0.9 Hz (\( \Delta E_{0.9\ Hz} \)) with PEEP also shows a marked dependence on age (Fig. 4). In animals <18 days of age, \( \Delta E_{0.9\ Hz} \) is negative until a PEEP of ~4 hPa, above which \( \Delta E_{0.9\ Hz} \) is markedly positive. In contrast, in animals 18 days of age and older, \( \Delta E_{0.9\ Hz} \) is always negative and increases in absolute value with age.

Figure 5 shows TD, Lₘ, AWT, and MAC, obtained from three different regions of the left lung, as a function of age. TD and AWT decreased markedly with age until 21 days, after which changes were minor. In contrast, Lₘ and MAC increased with age until day 30. Although there were no differences in Lₘ between 10
and 14 days of age, MAC increased significantly between these two ages.

$S_a$ of the left lung increased progressively and substantially with age (Fig. 6). However, when $S_a$ was normalized to either $V_{l,2/3}$ or $V_{a,2/3}$, it did not vary between 10 and 21 days of age but did increase modestly between days 21 and 25 of postnatal age, remained constant until day 30, and then increased substantially in adult animals.

**DISCUSSION**

Our study was motivated by the fact that children have a greater propensity for airways hyperresponsiveness than do adults and that mechanical differences in the mature vs. immature lung have been proposed to

Fig. 1. Normalized respiratory system elastance ($E$) at 4.8 Hz to lung weight ($NE_{lw,0.9}$, Hz, $A$), normalized tissue resistance ($R_{ti}$) at 0.9 Hz to lung weight ($NR_{ti,lw,0.9}$, Hz, $B$), normalized resistance ($R$) at 0.9 Hz to lung weight ($NR_{lw,0.9}$, Hz, $C$), and contribution of $R_{ti}$ to total respiratory $R$ at 0.9 Hz ($D$) as a function of positive end-expiratory pressure (PEEP). Values given are means ± SE calculated for PEEP levels ranging between 0 and 7 hPa in each age group. d, Days of age.

Fig. 2. Hysteresivity ($\eta$) in rats between 10 and 90 days of age. Values given are means ± SE calculated for PEEP's ranging between 0 and 7 hPa in each age group.

Fig. 3. Percent decrease in $R$ at 4.8 Hz ($\Delta R_{4.8Hz}$) as a function of PEEP (relative to $R$ at 0 PEEP) in the 7 groups of rats. Significant difference ($P < 0.05$) between age groups marked with the same symbol (*, ++); age groups within the same bracket are significantly different from any other age group in the other bracket. Values are means ± SE.
play a role in this phenomenon. The results of our study show that the rat respiratory mechanics undergo substantial maturational changes, which may be important in explaining the hyperresponsiveness of immaturity.

The effects of maturity on lung mechanics have been investigated quite extensively by previous workers. For example, Nardell and Brody (32) investigated the relationship between lung mechanics and the deposition of extracellular connective tissue during rat lung development. Others have addressed age-related changes in the viscoelastic properties of the rat respiratory system from birth to puberty (42, 43) and in lung static elastic properties from puberty to older age (35). However, these previous experiments were performed either in vitro, in excised water-filled lungs, or in situ after the animals were killed. Ours is the first study in living animals that investigates the effects of maturity on the frequency and PEEP dependencies of respiratory mechanics. The mechanical impedance of the respiratory system in humans and other species, including rats, is highly dependent on the frequency of ventilation and on Vl (3, 13–15, 18, 27, 28, 30, 31, 33, 38). In particular, R decreases markedly with frequency such that, by 4.8 Hz, it reflects something close to a pure airway R (15), whereas at 0.9 Hz it consists of a combination of airway R and Rti. Thus by measuring impedance simultaneously at these two frequencies, we were able to partition total R at 0.9 Hz into its airway component (R at 4.8 Hz) and its tissue component (the remainder).

Our results showed that both R and E at all frequencies decrease with age as a natural consequence of animal growth and increase in lung size. However, when these measurements are normalized to lung weight (Fig. 1), they also show that the intrinsic properties of the respiratory tissues change with maturation, as has been shown previously (32, 42, 43).
assumes, of course, that mechanical heterogeneity is not important in determining the low-frequency dependence of $R$ in normal mice.) Light and electron microscopic studies in the rat (2, 5–7, 34) have documented the extensive remodeling of the lung parenchyma that takes place after birth. The enlargement of air spaces, formation of alveoli, thinning of the interstitium, and changes in the capillary network occur in three phases. From days 1 to 4, the lung grows primarily by distension of the existing air spaces with little increase in tissue volume. Between days 4 and 13, there is intense cell production and structural reorganization, leading to a large increase in the surface area for gas exchange. Between days 13 and 21, there is a long period of equilibrated growth in which both $V_L$ and $S_a$ increase at a slower rate, cell proliferation decreases, and the alveolar walls become thinner. Although alveolarization in rats is thought to occur mainly during the phase of tissue proliferation, it has been shown that $S_a$ increases as a result of both formation of new alveoli and expansion of the preexistent air spaces between 14 and 60 days after birth and perhaps longer (4, 16). A fourth phase in rat lung development between 12 and 20 days of age in which elastin accumulates has also been described (32). Indeed, elastin accumulation parallels the phase of rapid alveolar multiplication through septation of primary saccules; thus it is believed that elastin may play an important role in alveolar formation (5, 6, 8).

The nature of the PEEP dependence of respiratory mechanics in our rats changed substantially as the animals aged, most particularly in the tissues (Fig. 1). Whereas older animals showed a slight decrease in NRtLW and NEtLW with PEEP <2 hPa, in the youngest animals NRtLW exhibited no significant variation from 0 to 2 hPa, and NEtLW exhibited minima at 2–3 hPa beyond which both NRtLW and NEtLW increased substantially. In contrast, the older animals showed almost no changes in these parameters >3-hPa PEEP. Although the age differences in the PEEP dependencies of NRtLW and NEtLW could have been contributed to by the chest wall (15), the contributions were likely minor because the chest wall in very young animals, with the exception of piglets and foals, is highly compliant (11, 20, 39, 41). Also, chest wall tissue $R$ in rats has been shown not to vary with lung inflation at relatively low $V_L$ values (15). Our laboratory has previously observed a negative PEEP dependence of NRtLW and NEtLW below ~2 hPa in other rodents (13, 15), presumably due to closure of air spaces resulting from decreased parenchymal interdependence forces that would otherwise tend to maintain patency. This negative dependency in the present study was more pronounced in older animals (Figs. 1 and 4), suggesting that the structural changes taking place with maturation endow the lungs with a greater propensity for closure at low PEEPs. Although we have not ascribed much mechanical importance to the chest wall so far, if there is any, it is likely to increase with age as the chest wall becomes stiffer. A stiffer chest wall would presumably tend to protect the older lungs from collapse; thus our conclusion that the older lungs have an increased tendency for closure further supports the notion that intrinsic changes in the maturing lung tissue must have taken place.

Another important effect on tissue mechanics is seen at the high end of the PEEP spectrum where NRtLW and NEtLW start to increase in the younger animals. This likely reflects the nonlinear elastic properties of the lung and chest wall tissues that cause them to stiffen with increasing distension. Our failure to see an increase in NRtLW and NEtLW in the older animals almost certainly occurred because we did not proceed to sufficiently high levels of PEEP. Indeed, Hirai et al. (15) showed an increase in respiratory system $E$ with PEEP at 9 hPa. Nevertheless, our results demonstrate that immature lung tissue exhibits strain stiffening at lower distending pressures than does mature tissue.

The PEEP dependence of $\eta$ also changed substantially with maturation, increasing somewhat with PEEP in older animals but decreasing markedly in the youngest group (Fig. 2). The marked decrease in $\eta$ that
we found at high PEEP in the youngest animals indicates that the strain stiffening of the tissues discussed above is not accompanied by a commensurate increase in dissipative properties. The mechanisms for this dissociation between Rti and tissue E are not clear. Nevertheless, they constitute further evidence of fundamental mechanical differences between mature and immature respiratory tissues. Although previous studies in adult rats and other rodents have shown η to increase with PEEP due to the chest wall (13, 15), it has been shown that chest wall R is independent of mean Pao (15). Furthermore, chest wall E is presumed to account for only a very small portion of respiratory E in very young animals.

Stiffer lung tissue means greater changes in elastic recoil forces for a given change in Vt. As these recoil forces are mediated by the alveolar walls, this suggests that there should be increased interdependence forces between airways and parenchyma. Our results show greater stiffening at high PEEP in the immature lungs (Fig. 4), which implies that the airways of immature animals should be subjected to greater outward parenchymal tethering forces at high PEEP than those of mature animals. Immature airways should thus increase in caliber (and so decrease their R) more rapidly than mature lungs as PEEP increases. However, our experimental results show just the opposite; our measure of airway R (R at 4.8 Hz) decreased proportionately more with increasing PEEP in the older animals (Fig. 3). This indicates that parenchymal tethering actually has less of an effect on airway caliber in younger animals than in older ones. It is also possible that our results could be explained by differences in the mechanical properties of young and mature airways. That is, the young airways might reach their maximum diameters at lower transmural pressures than do the older ones. However, Mansell et al. (23) recently observed a maturation-dependent difference in parenchymal influences on bronchial caliber in piglets, which supports our interpretation of reduced parenchymal tethering.

There are a number of distinct mechanical mechanisms that can potentially affect the ability of airway smooth muscle to shorten, and significant among these is the tethering load provided by the alveolar walls that attaches to an airway (26). This load can be modulated to a large extent by changing Vt (3, 27–31). It has been shown, for example, in very old rats whose lungs have lost elastic recoil as a result of the aging process that airway responsiveness is less sensitive to changes in Vt than in younger adult animals (27). Presumably these considerations also have relevance for the bronchial responsiveness of immaturity and so lead to the hypothesis that we sought to test in the present study: immature animals have reduced airway-parenchymal interdependence compared with mature animals. Our results clearly support this hypothesis. Interestingly, we found the PEEP dependence of tissue recoil to be increased in very young animals (Fig. 4), which might be expected to lead to increased airway-parenchymal interdependence. However, the reduced effect of PEEP on airway R in the younger animals (Fig. 3) and the existence of hyperresponsiveness of immaturity both suggest that the airways and parenchyma are somehow mechanically uncoupled in very young animals. This would prevent the airways from being tethered open and so allow the airway smooth muscle to shorten relatively unhindered, thus leading to a more responsive lung.

This is not to suggest that we have identified the only possible mechanism accounting for the greater responsiveness of immature lungs. There may well be other important factors, such as differences in signal transduction (24, 44), the thickness of the airway wall internal to the smooth muscle layer (26, 40), and the amount of smooth muscle (44). Indeed, it is probably most reasonable to suppose that the hyperresponsiveness of prematurity could result from a combination of such factors.

In summary, in rats as young as 10 days of age, airway-parenchymal interdependence forces play a significant role in respiratory mechanics. Furthermore, the way in which these forces affect mechanics changes markedly with maturation, especially before ~18 days of age. These mechanical changes are accompanied by a decrease in mean TD and an increase in total alveolar surface area. Our results suggest that the airways and parenchymal tissues are relatively uncoupled in very young animals, which may play a role in the hyperresponsiveness of immaturity by removing a key mechanical load opposing the contraction of the airway smooth muscle.

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