Maturational changes in responses of tissue and airway resistance to histamine

ISMAIL A. DRESHAJ, MUSA A. HAXHIU, CHARLES F. POTTER, FATON H. AGANI, AND RICHARD J. MARTIN
Departments of Pediatrics and Medicine, School of Medicine, Case Western Reserve University, Cleveland, Ohio 44106

Dreshaj, Ismail A., Musa A. Haxhiu, Charles F. Potter, Faton H. Agani, and Richard J. Martin. Maturational changes in responses of tissue and airway resistance to histamine. J. Appl. Physiol. 81(4): 1785–1791, 1996.—We determined how postnatal maturation affects the relative contributions of airways and lung parenchyma to pulmonary resistance (R L) and whether there are developmental differences in their respective responses to constrictive agents. We studied open-chest ventilated anesthetized piglets of three ages: 2–4 days, 2–3 wk, and 10 wk. R L was partitioned into tissue (Rti) and airway (Raw) resistance by means of alveolar capsules under baseline conditions and after intravenous histamine. Postnatal maturation was associated with a progressive decline in Rti, Rti, and Raw and with an increase in the contribution of Rti to R L from 38 ± 8% at 2–4 days to 72 ± 2% at both 2–3 and 10 wk. Histamine caused Rti to increase at all ages. When partitioned into Rti and Raw, the percent increase in Rti significantly exceeded that of Raw at both 2–4 days and 2–3 wk. In contrast, the percent increase in Raw significantly exceeded that of Rti at 10 wk. Administration of atropine before histamine in piglets aged 10 wk reduced the response of Rti and Raw to histamine. Histamine-induced responses of R L were blocked by prior H1-receptor blockade with pyrilamine (2 mg/kg). These results indicate that 1) the contribution of Rti and Raw to R L changes during maturation and that 2) contractile responses to exogenous histamine are manifest predominantly in most distal airways and lung parenchyma during early postnatal life; with advancing maturation there is greater contribution of airways to the increase in R L induced by histamine.

l lung mechanics; alveolar capsules; tissue visance; development; cholinergic transmission; piglet

IT HAS BECOME WIDELY RECOGNIZED that both lung parenchymal structures and airways contribute to lung resistance (R L). Alveolar capsules applied to the lung surface have been utilized to measure the tissue component of lung resistance (Rti) in a variety of open-chest animals at various ages (6, 11, 12, 14, 16, 19, 23). These studies have revealed that Rti comprises at least 50% of R L in most species, depending on baseline experimental conditions. Earlier studies performed nearly 30 years ago estimated that average Rti in spontaneously breathing newborn infants represents a higher proportion of R L than is the case in normal adults (17). However, there has been no systematic comparison of the quantitative contribution of Rti to R L at different postnatal ages in any one species by employing currently available techniques.

The effect of postnatal maturation on the relative contributions of airway resistance (Raw) and Rti to the increase in R L induced by neurochemical stimuli has also not been studied. Histamine is an inflammatory mediator that has been widely implicated in enhancing airway contractile responses under various pathophysiological conditions (2). It is thought to induce bronchoconstriction primarily by a direct effect on H1 receptors on airway smooth muscle, although histamine may also enhance cholinergically mediated contractile responses via reflex or other mechanisms (2, 21). Administration of inhaled histamine to mature open-chest dogs caused Rti, Rti, and Raw to all significantly increase (12). In contrast, Sly and Lanteri (23) observed that in 8- to 10-wk-old puppies, inhaled histamine caused an increase in R L that was almost entirely caused by an increase in Rti, with a negligible response of Raw. In addition, it was found that histamine given intravenously caused a greater degree of airway smooth muscle contraction in adult than in immature guinea pig airways (1). In this study we, therefore, sought to systematically characterize the effect of postnatal maturation on the airway and tissue responses induced by exogenous histamine administration in developing piglets. We hypothesized that there would be a differential distribution of contractile responses at various ages with a dominant effect of exogenous histamine on distal lung regions during early postnatal life.

METHODS

Experimental preparation. Experiments were performed on a total of 28 piglets and young pigs of either sex at three ages (2–4 days, n = 6; 2–3 wk, n = 11; and 10 wk, n = 11). The piglets were initially sedated with intramuscular ketamine hydrochloride (14 mg/kg) and xylazine (2.8 mg/kg) and were anesthetized with intravenous α-chloralose (24 mg/kg) and urethan (120 mg/kg). A femoral artery was cannulated for measurement of blood pressure and for blood-gas sampling, and an external jugular vein was cannulated for administration of further anesthesia, histamine, and fluids.

The piglets were placed on a heating pad to maintain body temperature between 37.5 and 38.5°C. After a high cervical tracheostomy, the piglets were artificially ventilated with 100% inspired O2 through a tightly fitting tracheal cannula made of stiff plastic, with a side tap connected to a volume ventilator (model 55-0798, Harvard Apparatus) that delivered a cycled volume of 10–14 ml/kg. The ventilator rate was set at ~25 cycles/min (range 21–30 cycles/min) to maintain an arterial PCO2 of 30–35 Torr and an arterial PO2 of >300 Torr during baseline conditions. Blood-gas tensions and pH were determined with an automated blood-gas analyzer (Radiometer, Copenhagen, Denmark).

A midline sternotomy was performed, and the chest was widely retracted. Lightweight round-base capsules (10-mm diameter) were applied to the pleural diaphragmatic surface of two different lobes by means of cyanocrylate glue, as previously described (6, 7, 12, 14). The pleura under each capsule was retracted to leave a 1- to 2-cm space that was filled with saline. This was done to ensure that the capsule was not compressed by the underlying lung parenchyma.

The effect of histamine on Rti and Raw was measured after administration of histamine intravenously (0.025 mg/kg). Histamine was administered in 25- and 50-µg increments. Histamine was administered only once during each experiment. The contractile responses of both Rti and Raw to histamine were measured 5 min after the intravenous administration of histamine. Histamine administration was repeated in each piglet at each age (2–3 wk and 10 wk) to confirm the results obtained.

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Postnatal maturation from 2–4 days to 2–3 wk and 10 wk was associated with a progressive decline in baseline \( R_L \), \( Rti \), and Raw as shown in Table 1. The increase in RL between piglets of 2–4 days and 2–3 wk was mainly due to the decline in Raw (Table 1) because Rti did not fall until 10 wk. Under these baseline conditions before histamine administration, the proportion of RL that comprised Rti and Raw differed between ages (Fig. 1). At 2–4 days of age, Rti comprised 38.2 ± 6.6% of RL. This was significantly lower than at 2–3 wk and 10 wk when Rti comprised 72.0 ± 1.8% and 71.9 ± 8.5% of RL. This was significantly lower than at 2–3 wk and 10 wk when Rti comprised 72.0 ± 1.8% and 71.9 ± 8.5% of RL.

Table 1. Effect of maturation on baseline measurements of lung mechanics measured in open-chest piglets

<table>
<thead>
<tr>
<th></th>
<th>2–4 days</th>
<th>2–3 wk</th>
<th>10 wk</th>
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<tbody>
<tr>
<td>( R_L ), cmH( _2 )O·l(^{-1} )·s(^{-1} )</td>
<td>14.0 ± 2.9</td>
<td>7.3 ± 0.4</td>
<td>2.3 ± 0.5</td>
</tr>
<tr>
<td>( Rti ), cmH( _2 )O·l(^{-1} )·s(^{-1} )</td>
<td>5.6 ± 1.7</td>
<td>5.3 ± 0.4</td>
<td>1.7 ± 0.4</td>
</tr>
<tr>
<td>( Raw ), cmH( _2 )O·l(^{-1} )·s(^{-1} )</td>
<td>8.5 ± 2.1</td>
<td>2.0 ± 0.2</td>
<td>0.6 ± 0.1</td>
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</tbody>
</table>

Lung mechanics values are means ± SE. \( R_L \), lung resistance; \( Rti \), tissue resistance; \( Raw \), airway resistance. Kruskal-Wallis nonparametric test with adjustments made for multiple comparisons revealed a significant effect of age on \( R_L \) (\( P < 0.001 \)), \( Rti \) (\( P < 0.005 \)), and \( Raw \) (\( P < 0.001 \)).

RESULTS

Experimental protocol. We performed noncumulative concentration-response curves of RL, Raw, Rti, and dynamic compliance during intravenous administration of histamine. Histamine hydrochloride in amounts of 0.2, 0.6, 2, 6, and 20 µg/kg body wt was given as a bolus in 1 ml of saline. After administration of the first concentration, the next higher concentration was administered when resistance had returned to within 10% of its baseline value and at the same time period after lung inflation. Measurements of resistance and \( Edyn \) were averaged over three to five ventilatory cycles before histamine administration and at peak response to each histamine dose, defined by maximum change in RL. To examine whether readministration of the same concentrations of histamine caused tachyphylaxis, we compared the concentration-response curves of two consecutive trials in four studied piglets. In 11 piglets (6 at 2–3 wk and 5 at 10 wk), concentration response-curves were also performed before and after intravenous administration of pyrilamine (2 mg/kg), an \( H_1 \)-receptor blocker.

To determine whether some of the airway response to histamine was mediated through cholinergic pathways in piglets of 10 wk of age, atropine methyl nitrate (1 mg/kg) was administered before histamine in five animals.

Statistics. All averaged data are presented as means ± SE. Statistical comparisons employed Kruskal-Wallis nonparametric test with adjustments made for multiple comparisons (22) as well as two-way analysis of variance when appropriate. Differences were considered significant at \( P < 0.05 \).
2.1% of R₇, respectively (P < 0.001 for 2–4 days vs. 2–3 wk, and P < 0.001 for 2–4 days vs. 10 wk).

The effects of intravenous administration of increasing concentrations of histamine on R₇, R₈, and Raw were assessed in each individual group. Increasing doses of histamine caused R₇ to increase in all three age groups in a concentration-dependent fashion. Examples of the effects of 20 µg/kg histamine on tracheal and alveolar pressures are shown in Fig. 2. Changes in measured pressures from different capsules in the same animal were identical.

The relative magnitude of the response of R₇, when expressed as percent change from baseline R₇, appeared to increase with advancing age. When this response of R₇ was partitioned into R₈ and Raw, the increase in Raw was minimal at 2–4 days and 2–3 wk but became prominent by 10 wk. In contrast, a clear increase in R₈ was apparent at each age. The average results for each group studied are shown in Fig. 3. To systematically compare the relative responses of Raw and R₈ to histamine infusion at different ages, we expressed the increase in these parameters as a percentage of control values. As seen in Fig. 4, the percent increase in R₈ significantly exceeded the increase in Raw at 2–4 days (P < 0.05) and at 2–3 wk (P < 0.001). In contrast, the percent increase in Raw exceeded the increase in R₈ at 10 wk (P < 0.05; all 2-way analyses of variance).

At a higher concentration of histamine (20 µg/mg), the contribution of R₈ to R₇ tended to increase in piglets of 2–4 days and 2–3 wk of age. In the control period before histamine administration, R₈ contributed 38.2 ± 8.5 and 72.0 ± 1.8%, respectively. During the peak response to 20 µg/kg histamine, R₈ accounted for 56.6 ± 16.6 and 83.0 ± 2.1% of R₇ at 2–4 days and 2–3 wk, respectively. In contrast, in piglets of 10 wk of age, the contribution of R₈ to R₇ decreased after histamine administration; after histamine administration, R₈ accounted for 60.0 ± 2.8% of R₇ compared with 72.0 ± 2.0% in the control period.

In five piglets of 10 wk of age, atropine methyl nitrate (1 mg/kg) was administered before histamine. Prior administration of atropine decreased the responses of R₇, R₈, and Raw when compared with the responses of the non-atropine-treated piglets of 10 wk (Fig. 5). Differences between dose-response curves were statistically significant (P < 0.01; Kruskal-Wallis test).

Intravenous administration of histamine caused a concentration-dependent increase in Edyn in all three studied groups. The tissue elastance values derived from the tracheal pressures were largely comparable to the results calculated from alveolar capsules. In only
two animals, after administration of 20 µg/kg of histamine, did Edyn differ by >10% between calculated values (15.7% in 1 piglet and 11.9% in another). The relationship between Rti and Edyn was significant at all ages studied (Fig. 6).

In four piglets aged 2–3 wk, repeat administration of the same concentrations of histamine resulted in a slightly lower increase in Rti and Raw. For example, histamine given at a concentration of 20 µg/kg caused Rti to increase by 11.1 ± 3.9 cmH\textsubscript{2}O·l\textsuperscript{-1}·s with the first administration and by 8.5 ± 3.3 cmH\textsubscript{2}O·l\textsuperscript{-1}·s after the second administration. The same amount of histamine increased Raw by 2.0 ± 1.1 cmH\textsubscript{2}O·l\textsuperscript{-1}·s with the first trial and by 1.7 ± 0.7 cmH\textsubscript{2}O·l\textsuperscript{-1}·s after the second administration performed 30 min later.

Prior administration of the H\textsubscript{1}-receptor blocker pyrilamine (2 mg/kg) in five piglets studied at 10 wk of age almost completely abolished the histamine-induced changes in Rl, Rti, and Raw, respectively (Fig. 7). Similarly, pyrilamine blocked the response to histamine in six piglets of 2–3 wk of age (data not shown). There were no differences in the effectiveness of the H\textsubscript{1} blocker in the two age groups.

DISCUSSION

Limitations of the technique used to measure Rti. There are significant limitations to the use of alveolar capsules during bronchial challenge if airway constriction is induced by drugs used in high concentrations. Nagase et al. (16) have demonstrated by physiological and morphometric analyses the presence of marked
heterogeneity in peripheral airway narrowing during induced bronchoconstriction. Hence, when airways are constricted, alveolar capsules placed on the pleural surface may not adequately reflect intrapulmonary inhomogeneity. In the studies reported here, histamine-induced inhomogeneity due to significant bronchoconstriction could have interfered with the interpretation of the results in older piglets but not in animals of 2–4 days and 2–3 wk of age. In younger piglets during the peak response to 20 µg/kg of histamine, the increase in Raw was insignificant compared with changes observed in 10-wk-old piglets. Because of these limitations in the alveolar capsule technique, in the present study we avoided amounts of histamine that may cause severe bronchoconstriction and inhomogeneity.

Contribution of Rti and Raw to RL during matura-
tion. In this study we have demonstrated that postna-
tal maturation in the piglet substantially influences the contributions of Rti and Raw to RL under baseline conditions. To our knowledge, systematic study of age-
related changes in the distribution of airway and parenchymal components of RL has not been previously performed. Our findings provide new insight into the relationship between the airways and most distal lung units and into the potential sites of action for substances that cause changes in pulmonary function in early life.

The relative contributions of Rti and Raw to RL have been quantified in many recent studies. At physiological tidal volumes and frequencies, Rti accounts for ~60–80% of RL in open-chest adult dogs, depending on the level of applied PEEP (3, 7, 12). Similar data have been observed by Sly and Lanteri (23) in 8- to 10-wk-old puppies and by Dreshaj et al. (6) and Martin et al. (14) in 2- to 3-wk-old piglets. Comparable data were observed in our present study in both 2- to 3-wk-old and 10-wk-old piglets. However, in the newborn piglets, Rti contributed significantly less to RL. This could be partly due to lower elastic recoil, which increases during the

Fig. 6. Relationship between Rti and dynamic lung elastance (Edyn) at baseline and peak responses to increasing concentrations of histamine in piglets at 3 ages [2–4 days (A), n = 36 data points; 2–3 wk (B), n = 66 data points; and 10 wk (C), n = 54 data points].

Fig. 7. Effects of prior administration of H1-receptor blocker (pyril-
amine; 2 mg/kg) on responses of Rti (A) and Raw (B) to increasing concentrations of histamine in five 10-wk-old piglets. Open symbols, control; solid symbols, pyrilamine.
1st postnatal mo and is associated with air expansion. In the 2nd and 3rd mo, septal proliferation occurs and there is an increase in arithmetic mean septal thickness, but elastic recoil does not change further (13). The considerably higher Raw in the newborn animals, than in those of 2–3 and 10 wk of age, is related to the size of central airways but not to differences in cholinergic tone (10).

It is well documented that ventilatory parameters (notably tidal volume, frequency, and level of functional residual capacity) substantially alter the ratio between Raw and Rti when alveolar capsules are employed in open-chest animals. Large volumes and slow rates will increase Rti relative to Raw (3). Hence, in the present study, frequency was comparable between ages, PEEP remained at 3 cmH₂O throughout, and tidal volume was kept at 10–14 ml/kg. Therefore, the observed maturational changes are not due to variations in tidal volume and frequency between animals.

In 1966, Polgar and String reported that lung Rti constitutes ~40% of total Rl (excluding nasal resistance) when estimated by subtraction of airway from total Rl in spontaneously breathing newborn infants (17). Despite the different measuring techniques employed, such a contribution of Rti to Rl is similar to that observed in the current study employing placement of alveolar capsules. Relative changes in Rti and Raw during development may be related to maturational changes in the depth and rate of breathing aimed to maximize gas exchange and to reduce work of breathing and energy expenditure.

Maturational changes in the site of action of histamine. The second major finding of our present study is that postnatal development is associated with maturational changes in the site of action for histamine. While the response of Rti to infused histamine was strong at all ages, the response of Raw was negligible in the two groups of young piglets. Maturation-related changes in airway responses to histamine have been studied in several animal species, and the results are at times conflicting. Our studies are in agreement with results obtained in puppies showing that airway reactivity increases as puppies approach adulthood (18). Similarly, responsiveness of the airways to aerosolized histamine increases with age in healthy lambs from 1 mo of age to adulthood (20).

Cleridi et al. (4) observed that newborn guinea pigs required a higher dose of infused histamine than did adult animals to induce an equivalent increase in resistance. This was associated with lack of a cholinergic component of the histamine-induced contractile response in young but not in adult animals. Such a cholinergic contribution to histamine-induced bronchoconstriction has been shown in adult dogs to comprise both central and local reflex components (21). Similarly, in the 10-wk-old piglets, atropine given before histamine administration reduced the histamine-induced increase in Rti and Raw. The contribution of histamine-induced reflex responses to the increase in Rti in younger animals was not examined. However, based on an earlier study in newborn piglets, reflex responses are weaker than in adult animals (9). Nonetheless, differences in maturation of cholinergically mediated reflex responses to histamine may contribute to, but are not the sole cause of, developmental differences in the site of responses to histamine observed in the piglets. The results of this study in 10-wk-old piglets suggest that not only the airway but also the parenchymal reflex component are under cholinergic control. This is supported by recent study showing the presence of vagal control of Rti in piglets (14). We conclude that histamine may reflexly elicit a cholinergically mediated increase in Rti, which, like the Raw of Rl, may be attenuated by prior blockade of muscarinic receptors.

Lung parenchyma contains heterogeneous contractile elements. The close correlation found in these studies between Rti and Edyn is consistent with recent findings of Fredberg and Stamenovic (7) and Hantos et al. (8). The increases in Rti and Edyn can be caused by contraction of alveolar interstitial contractile elements or periductal smooth muscle. Differences in parenchymal and airway responses during maturation could be associated with alterations in receptor distribution or affinity and partly due to age-related changes in blood flow and resultant distribution of agonist.

Murphy et al. (15) studied the topographic distribution of airway bronchoconstrictor responses to histamine infusion in 2- and 10-wk-old piglets by in vivo measurements of Rl combined with bronchograms to assess airway diameter. They found that mature animals had relatively less histamine-induced narrowing of the smallest (highest generation) airways compared with the younger piglets. Baseline values of Rl were higher than in our present study, which may be related in part to our use of open-chest animals. Differences in tidal volume and frequency may also have contributed to different values for pulmonary function.

Available in vitro data from developing pig airways suggest that tissue from 20- to 26-wk-old animals exhibits less tracheal but more bronchiolar constriction in response to histamine compared with tissue from 4-wk-old animals (24). While these data appear consistent with our own findings, we recognize that extrapolation of these in vitro data to our in vivo experiments, performed in animals of different ages, is difficult.

Histamine-induced changes in Rti and Raw were abolished by the H₁-antagonist pyrilamine, indicating that these responses are H₁-receptor mediated. Blockade of the histamine-induced changes could not be attributed to the slightly lower responses observed after the second administration of histamine. The apparent increase in the airway contractile response to histamine from birth to 10 wk of postnatal life could be due to differences in the density of H₁ receptors or to differences in H₁-receptor/effecter affinity via G-protein coupling, as Haxhiu-Poskurica et al. (10) have shown for cholinergically mediated responses during early development.

In 8- to 10-wk-old puppies, Sly and Lanteri (23) documented that aerosolized histamine also increased Rl almost exclusively by an increase in tissue viscoelastic properties. These findings in young animals of
different species indicate that they are not limited to a single species or route of histamine administration. Development of a mature response, comprising contributions from both Rti and Raw, by 10 wk in the piglet is consistent with the more rapid postnatal neural maturation of that species (5) than the dog. We speculate that the observed results can be attributed to a progressive increase in H1-receptor density from distal lung to larger airways during postnatal maturation.

Conclusions. The results of this study showed that in piglets, postnatal maturation was associated with a progressive decline in Rl, Rti, and Raw. Furthermore, the contribution of Rti and Raw to Rl changed with advancing postnatal age, such that in the newborn piglets, Rti contributed significantly less to Rl than in 2- to 3- and 10-wk-old animals. Furthermore, postnatal development was associated with maturational changes in the site of action of histamine. The response of Rti to infused histamine was significant at all ages; however, the response of Raw was negligible in the two groups of younger piglets. We speculate that the observed results may be attributed to maturational changes in the distribution or function of H1 receptors throughout the respiratory system.

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Address for reprints: M. A. Haxhiu, Dept. of Medicine, School of Medicine, Case Western Reserve Univ., 10900 Euclid Ave., Cleveland, OH 44106 (E-mail: mah10@po.cwru.edu).

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