Maturational changes in responses of tissue and airway resistance to histamine

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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 (RL) 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. RL 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 RL 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 Raw were blocked by prior H3-receptor blockade with pyrilamine (2 mg/kg). These results indicate that 1) the contribution of Rti and Raw to RL 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 RL induced by histamine.

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 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 cyanoacrylate glue, as previously described (6, 7, 12, 14). The pleura under each...
capsule was punctured to a depth of 2–3 mm four to fivetimes
with a 20-gauge needle to bring the underlying alveoli into
communication with the capsule chamber. Pressures in the
capsules and in the tracheal cannula were measured with
miniature piezoresistive pressure transducers (model 8510
B-2, Endevco, San Juan Capistrano, CA). We considered that
capsules were not successfully installed if the magnitude of
the alveolar pressure swing did not match that of the tracheal
pressure swing during slow tidal ventilation. The animals
were all mechanically ventilated at a positive end-expiratory
pressure (PEEP) of 3 cmH2O to prevent closure of the most
distal airways as well as atelectasis. In addition, the lungs
were inflated every 10 min by occluding the expiratory line of
the ventilator for two to three consecutive volume cycles.
Each hyperinflation was performed at an identical time
before any experimental maneuver (e.g., histamine adminis-
tration) to avoid confounding effects of different volume
histories. The expiratory line was reopened when transpulmo-
nary pressure reached 25 cmH2O. Histological analysis in the
study by Dreshaj et al. (6) showed no distortion or bleeding within
parenchymal structures at the sites of capsule application.

Tracheal flow signals were obtained with a Fleisch pneumo-
tachograph and were electronically integrated to derive volume.
Pressure and flow signals were filtered electrically with
matched 15-Hz low-pass filters and were recorded on
a six-channel recorder (Gould, Cleveland, OH) along with vol-
ume and arterial blood pressure. Tracheal and alveolar
pressures were displayed against lung volume on a Tektronix
storage oscilloscope. All data were recorded on an FM mag-
cnetic tape for later playback and analysis. Dynamic matching
of the transducers was tested, and there was no phase or
amplitude distortion of signals in the range of applied pres-
sure and frequency.

A custom-made computer program was used to calculate
resistance on the basis of the method of Von Neergaard and
Wirz (25), as previously described by Dreshaj et al. (6). Total
RL and Rti were calculated from tracheal flow and from
tracheal and alveolar pressure changes, respectively. Raw
was derived as the difference between RL and Rti. Dynamic
lung elastance (Edyn) was calculated as a function of change
in airway pressure per volume. The tissue elastance values
calculated from the alveolar pressure were compared with
those calculated from airway pressure. The data obtained
from two capsules were averaged to obtain a single value of
Rti and Raw for each concentration of histamine in five
piglets (2 piglets of each younger group and 1 animal of 10
wk). The two capsules gave comparable results when concen-
trations of histamine up to 20 µg/kg were used. Therefore, in
the remaining experiments, data from only one capsule were
used for analysis.

Experimental protocol. We performed noncumulative con-
centration-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 re-
turned 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
to examine whether readministration of the same concen-
trations 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 H1-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 nonpara-
metric test with adjustments made for multiple comparisons
as well as two-way analysis of variance when appropri-
ate. Differences were considered significant at P < 0.05.

RESULTS

Postnatal maturation from 2–4 days to 2–3 wk and
10 wk was associated with a progressive decline in
baseline RL, Rti, and Raw as shown in Table 1. The
decrease 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 propor-
tion of RL that comprised Rti and Raw differed between
ages (Fig. 1). At 2–4 days of age, Rti comprised 38.2 ±
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 ±

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>
</tr>
</thead>
<tbody>
<tr>
<td>RL, cmH2O·l·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, cmH2O·l·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, cmH2O·l·s⁻¹</td>
<td>8.5 ± 2.1</td>
<td>2.0 ± 0.2</td>
<td>0.6 ± 0.1</td>
</tr>
</tbody>
</table>

Lung mechanics values are means ± SE. RL, lung resistance; Rti,
tissue resistance; Raw, airway resistance. Kuksral-Wallis nonpara-
metric test with adjustments made for multiple comparisons re-
vealed a significant effect of age on RL (P < 0.001), Rti (P < 0.005),
and Raw (P < 0.001).

Fig. 1. Contribution of tissue resistance (Rti; □) and airway resis-
tance (Raw; △) to lung resistance (RL) (expressed as percentage of RL)
in piglets of 3 different ages [2–4 days (d), 2–3 wk (w), and 10 wk].
2.1\% \text{of RL}, \text{respectively} (P < 0.001 \text{for 2–4 days vs. 2–3 wk, and } P < 0.001 \text{for 2–4 days vs. 10 wk}). The effects of intravenous administration of increasing concentrations of histamine on RL, Rti, and Raw were assessed in each individual group. Increasing doses of histamine caused RL 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 RL, when expressed as percent change from baseline RL, appeared to increase with advancing age. When this response of RL was partitioned into Rti 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 Rti 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 Rti 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 Rti 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 Rti at 10 wk (P < 0.05; all 2-way analyses of variance).

At a higher concentration of histamine (20 µg/mg), the contribution of Rti to RL tended to increase in piglets of 2–4 days and 2–3 wk of age. In the control period before histamine administration, Rti contributed 38.2 ± 8.5 and 72.0 ± 1.8\% \text{, respectively. During the peak response to 20 µg/kg histamine, Rti accounted for 56.6 ± 16.6 and 83.0 ± 2.1\% \text{of RL at 2–4 days and 2–3 wk, respectively. In contrast, in piglets of 10 wk of age, the contribution of Rti to RL decreased after histamine administration; after histamine administration, Rti accounted for 60.0 ± 2.8\% \text{of RL compared with 72.0 ± 2.0\% in the control period. In five piglets of 10 wk of age, atropine methylnitrate (1 mg/kg) was administered before histamine. Prior administration of atropine decreased the responses of RL, Rti, 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

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig2.png}
\caption{Example of effects of intravenous histamine (20 µg/kg; at arrow) on tracheal (Ptr) and alveolar (Palv) pressures measured simultaneously from right and left lungs.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig3.png}
\caption{Effect of increasing concentrations of histamine on RL (○), Rti (□), and Raw (△) in piglets of 3 ages [2–4 days (A), 2–3 wk (B), and 10 wk (C)].}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig4.png}
\caption{Effect of intravenous administration of histamine on RL, Rti, and Raw in piglets of 3 ages [2–4 days (A), 2–3 wk (B), and 10 wk (C)].}
\end{figure}
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 cmH₂O·l⁻¹·s with the first administration and by 8.5 ± 3.3 cmH₂O·l⁻¹·s after the second administration. The same amount of histamine increased Raw by 2.0 ± 1.1 cmH₂O·l⁻¹·s with the first trial and by 1.7 ± 0.7 cmH₂O·l⁻¹·s after the second administration performed 30 min later.

Prior administration of the H₁-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 H₁ 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 maturation.
In this study we have demonstrated that postnatal 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 (pyrilamine; 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).

Clerici 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 heterogenous 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 effector 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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