Differences in the time course of proximal and distal airway response to inhaled histamine studied by synchrotron radiation CT

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Bayat, Sam, Liisa Porra, Heikki Suhonen, Christian Nemoz, Pekka Suortti, and Anssi R. A. Sovijärvi. Differences in the time course of proximal and distal airway response to inhaled histamine studied by synchrotron radiation CT. J Appl Physiol 100: 1964–1973, 2006. First published February 9, 2006; doi:10.1152/japplphysiol.00594.2005.—We studied the kinetics of proximal and distal bronchial response to histamine aerosol in healthy anesthetized and mechanically ventilated rabbits up to 60 min after histamine administration using a novel xenon-enhanced synchrotron radiation computed tomography imaging technique. Individual proximal airway constriction was assessed by measuring the luminal cross-sectional area. Distal airway obstruction was estimated by measuring the ventilated alveolar area after inhaled xenon administration. Respiratory system conductance was assessed continuously. Proximal airway cross-sectional area decreased by 57% of the baseline value by 20 min and recovered gradually but incompletely within 60 min. The ventilated alveolar area decreased immediately after histamine inhalation by 55% of baseline value and recovered rapidly thereafter. The results indicate that the airway reaction to inhaled histamine and the subsequent recovery are significantly slower in proximal than in distal bronchi in healthy rabbit. The findings suggest that physiological reaction mechanisms to inhaled histamine in the airway walls of large and small bronchi are not similar.

although Airway Hyperreactivity is the hallmark of bronchial asthma, little is known about the differences in the regional kinetics of airway narrowing in the tracheobronchial tree during an asthma attack. Whether airway size plays a role in determining the local differences in reactivity and kinetics of airway response to constricting agents remains a matter of debate. Several studies in explanted lung preparations in rat (45) and human (46) and in isolated airway smooth muscle or isolated bronchus (13, 17, 18) have shown larger airway response, and faster kinetics (13, 45) in smaller distal airways. Over the past decade, high-resolution computed tomography (CT) imaging has been used in the assessment of airway response in intact lung in animal models of bronchoprovocation as well as in humans. High-resolution CT offers the possibility to study the site and heterogeneity of bronchoconstriction (5). Using high-resolution CT, Amirav et al. (1) found a trend toward a stronger response to methacholine in small airways compared with large airways in pig, although the differences did not reach statistical significance. Brown et al. (7) observed a large variability in individual airway response to methacholine in anesthetized dog, but no significant correlation between the degree of constriction and airway size. We are not aware of any specific in vivo studies of the role of airway size in the kinetics of airway response to provocation in intact lung, using an imaging technique.

We recently introduced a novel CT imaging method that uses synchrotron radiation to quantitatively image inhaled stable xenon gas within the airways with a high spatial resolution (3). In a previous study using this technique (28), we found that inhaled histamine induces large patchy defects in xenon wash-in in the peripheral lung air spaces with rapid spontaneous reversal. We hypothesized that the in vivo reactivity and kinetics of airway response to histamine inhalation could be different in distal vs. proximal airways. The goal of the present study was to measure the reactivity and kinetics of airway response to histamine inhalation in intact anesthetized rabbit lung by using synchrotron radiation CT. We directly imaged airway narrowing in proximal airways. Distal airway obstruction was indirectly quantified by measuring changes in the regional ventilated lung area on the basis of the densities of stable xenon gas used as an inhaled indicator.

METHODS

Animal Preparation

Animal care and procedures of the experiment were in accordance with the Guidelines for the Care and Use of Animals provided by the American Physiological Society and approved by the local institutional authorities. The experiments were performed on six male New Zealand rabbits (average weight: 2.45 ± 0.10 kg, Elevage Scientifique des Dombes, Chartillon sur Chalaronne, France). A catheter (22-gauge) was inserted in the marginal ear vein (Cathlon IV, Ethicon, Rome, Italy), after local anesthesia using 5% topical lidocaine (Emla, Astra-Zeneca, Rueil, France). Anesthesia was induced by intravenous injection of thiopental sodium (25 mg/kg iv, Nesdonal, Rhone-Poulenc-Rohrer, Paris, France). The animal was tracheostomized, and an endotracheal tube (no. 3, Portex, Berck sur Mer, France) was inserted and secured with a gas-tight seal. A catheter (22-gauge) was inserted into the left carotid artery for blood pressure monitoring and arterial blood sampling for blood gas measurements (Radiometer, ABL77, Copenhagen, Denmark). The lower extremities of the animal were wrapped with a bandage, and the animal was immobilized in a homemade cylindrical polyvinyl chloride holder in a vertical position. The chest wall and diaphragmatic motion were free of constraint, because the rabbit was maintained in vertical position by a plastic attachment below the lower maxillary bone, which supported the weight of the animal. There was very little stress on the forelimbs, which were taped to keep them out of the image field. The chest and...
The contrast agent is determined directly on any given point of a lung CT image, with energies (E) above and below the K-edge of xenon (EK). Absolute quantity of tomography (CT) images are acquired by using 2 X-ray beams at 2 different energies (1.0 mg/h, Norcuron, Organon, Puteaux, France). Paralysis was induced by intravenous pancuronium bromide maintained with 0.4 – 0.6% inhaled isoflurane (Forene, Abbott, Paris, France). Anesthesia was maintained in the cylindrical holder by use of foam. Anesthesia was maintained with 0.4 – 0.6% inhaled isoflurane (Forene, Abbott, Paris, France). Endotracheal pressure (P<sub>r</sub>) was monitored continuously. All monitored signals were amplified, digitized at 400 Hz (PowerLab, ADInstruments, Oxfordshire, UK), and recorded on a computer.

**K-Edge Subtraction Imaging**

The K-edge subtraction (KES) method was originally developed for human coronary angiography with iodine contrast and later extended to lung imaging with stable xenon gas as contrast agent (19, 34, 40). A detailed description of the method has been published previously (3, 32). Visualization of the inhaled xenon is based on the fact that the attenuation coefficient of xenon increases by a factor of 5.4 when the energy of the incident X-ray beam crosses the energy threshold of 34.56 keV, which is called the xenon K-edge. The change in attenuation coefficients of cortical bone and lung tissue is negligible. Imaging is performed simultaneously with two X-ray beams at two slightly different energies, above and below the xenon K-edge. Subtraction of these two images on a logarithmic scale allows the observation of small anatomic structures carrying the contrast agent, while removing practically all features due to other structures (Fig. 1).

![Image](https://via.placeholder.com/150)

**Synchrotron Instrumentation**

All experiments were performed at the Medical Beamline of the European Synchrotron Radiation Facility (ESRF, Grenoble, France). A detailed description of the synchrotron instrumentation has been published previously (15). Electron energy in the storage ring of the ESRF is 6 GeV, and the maximal electron beam current is 200 mA. Synchrotron radiation is produced in a 21-pole wiggler (high magnetic field insertion device) with a maximal magnetic field of 1.4 T. In the present experiments a 60 mm magnet gap was used, and the wiggler field was 0.616 T, which corresponds to characteristic photon energy of 14.8 keV. Two exiting nearly monochromatic beams were produced from the continuous spectrum by a bent silicon crystal monochromator, and the energy difference between the beams was 250 eV. The beams focused and crossed at the animal position, beyond which they diverged and were recorded by a liquid nitrogen-cooled high-purity germanium dual-line detector (Eurisys Measure, Lingolsheim, France). The horizontal pixel size of the detector was 0.35 mm. The angle between the beams was 1 mrad, and the beam vertical width was 0.7 mm. No phase contrast effects are observed at this spatial resolution and for the sample/detector distance, which is almost 6 m. Because the X-ray beam is stationary, the animal was moved through the beams. For projection imaging the animal was moved vertically through the beams, and for CT imaging the animal was rotated through the beam around an axis perpendicular to the fan beams (6.56° off-vertical). The detector was read out synchronously with the animal motion to provide 0.35-mm vertical pixel size in the projection images and 0.5° angular resolution in the CT images. Rotation speed in CT imaging was 180°/s, and images were recorded from 720 angular projections per full 360° rotation. The centrifugal pressure due to animal rotation was 0.2 cmH<sub>2</sub>O at most, so that its effects on the regional distribution of xenon were negligible. CT reconstruction was performed using the filtered backprojection algorithm, using the Interactive Data Language (IDL, RSI, Boulougne-Bilancourt, France). The ring artifacts in images are due to defective pixels in the X-ray detector. They represent a very small fraction of the image field, and their effects on the calculated ventilated lung could be ignored.

**Mechanical Ventilation**

Details of the ventilation system were published earlier (32). The setup consisted of a custom-made apparatus using two electromagnetic valves and a T-tube, to be able to synchronize image acquisition and mechanical ventilation control precisely. The input gas flow was maintained constant at baseline, and the inspiration and expiration times were the set parameters, the tidal volume (VT) being determined by the time that the inspiration valve was open. The VT was ~7 ml/kg, and the respiratory rate was adjusted to set the arterial PCO<sub>2</sub> close to 40 Torr at baseline, then maintained throughout the study. Ventilation and the inhaled gas mixture were remotely controlled with electromagnetic valves. Gas flows were continuously measured and recorded by using mass flowmeters (Aalborg, Orangeburg, NY) and were adjusted before data acquisition. During the resting period the animal breathed air, and during imaging a mixture of xenon (Xe 70%) and oxygen (O<sub>2</sub> 30%). Ventilation was paused in inspiration or in expiration for image acquisition.

**Histamine Challenge**

Histamine aerosol was administered by use of an ultrasonic nebulizer (SAM LS2000, Villeneuve sur Lot, France). The mass median aerodynamic diameter (MMAD) of the aerosol particles was 3.5 μm with a geometrical standard deviation of 2.0, as determined by laser optical diffraction using the Malvern SprayTec, according to the manufacturer. A 125 mg/ml histamine solution (Sigma, St. Quentin Fallavier, France) in normal saline was administered continuously for 4 min. The delivered histamine dose was 875 μg·min<sup>-1</sup>·l<sup>-1</sup> inspired air.

**Data-Acquisition Protocol**

Each rabbit served as its own control, as baseline images were acquired before inhalation of histamine. Animal position was checked from an anteroposterior thoracic reference image. On the basis of the reference image, three different cross-section levels were selected at the fourth (upper), sixth (middle), and eighth (lower) thoracic vertebral levels for tomographic image sequences. All cross-section levels were caudal to the carina because the size of major airways was larger caudal to the carina, making caliber analysis based on the images more accurate. Cardiac gating was not used, because it has been
verified that cardiac motion causes only minor artifacts on the sequential CT images (32). For the best matching of the anatomical structures, the baseline images were acquired at two extra levels on each side of the nominal upper, middle, and lower cross-section levels.

The imaging sequence described in Fig. 2 was repeated every 3 min at the upper, middle, and lower lung cross-section levels for the first 30 min after histamine inhalation, then every 10 min up to 60 min. Airways whose lumens could be directly measured from the CT images are referred to as “proximal.” In the beginning of the imaging sequence, the inhaled gas was switched from air to the Xe-O$_2$ gas mixture. After a preset number of four ventilatory cycles used to prime the ventilation circuit, a deep inspiration was administered at a deeper inspiration (DI) of 1.3 s. A CT image was acquired during a breath hold of 4.0 s, indicated by shading. After the first image, ventilation was resumed, and after 6 ventilatory cycles, imaging was performed at end expiration during a breath hold of 4.0 s. At the end of the imaging sequence, the inhaled gas was switched back to air. The rapid oscillations in Ptr are artifacts due to rotation of the animal. After histamine, Ptr increases and Vt decreases.

3-Dimensional Reconstruction of the Bronchial Tree

To obtain a visual assessment of the ventilated bronchial tree, three-dimensional (3D) images were reconstructed from 80 successive KES-CT images in one rabbit before and 1 h after histamine inhalation. The CT images were acquired on inspiration, when the bronchi were filled with the Xe-O$_2$ gas mixture, by a modified imaging sequence. This imaging sequence consisted of one CT image at inspiration, and between the CT acquisitions xenon was flushed out from the lungs. The image acquisition started at the upper extremity, and the vertical step between images was equal to the beam height, i.e., 0.7 mm. The KES-CT images were segmented based on the xenon density within the airways, and the surface rendering of the 3D image was performed by use of specific software developed with the MatLab programming package (MathWorks, Natick, MA).

Image Analysis

The luminal surface area of the two largest bronchi in each lung slice was calculated by fitting ellipses to contours of the xenon distribution in the bronchi. A total of 36 airways (6 per animal) were analyzed. The two larger airways on each image were chosen because the measurement of airway luminal area and caliber could be most accurately performed in airways larger than 2 mm in diameter, as explained in the following. Initial segmentation was done manually. The centroid of the segmented region was used as an initial estimate for the ellipse center. The distances from the center were calculated for each pixel at the edge of the segmented region, and the farthest point was used for approximating the orientation and length of the major axis, whereas the nearest point was used to estimate the length of the minor axis. The center point location, axis lengths, and orientation of the ellipse were refined by a minimization procedure using the Nelder-Mead simplex method (30). In most cases the axes of the ellipse were almost equal, indicating that the bronchus was nearly perpendicular to the image plane. The minor axis is the radius of a circular lumen of bronchus, and this radius was used to calculate the measurement of airway luminal area and caliber could be most accurately performed in airways larger than 2 mm in diameter, as explained in the following. Initial segmentation was done manually. The centroid of the segmented region was used as an initial estimate for the ellipse center. The distances from the center were calculated for each pixel at the edge of the segmented region, and the farthest point was used for approximating the orientation and length of the major axis, whereas the nearest point was used to estimate the length of the minor axis. The center point location, axis lengths, and orientation of the ellipse were refined by a minimization procedure using the Nelder-Mead simplex method (30). In most cases the axes of the ellipse were almost equal, indicating that the bronchus was nearly perpendicular to the image plane. The minor axis is the radius of a circular lumen of bronchus, and this radius was used to calculate the luminal area given in Table 1 and in Figs. 5 and 6.

The method for estimating the airway luminal area was validated by using a Plexiglas phantom. The phantom consisted of six tubes with various diameters (2.0, 4.0, 6.0, 8.0, 10.0, and 12.0 mm). The tubes were filled with the same Xe-O$_2$ gas mixture that was used in the animal experiments. The luminal areas were calculated from the CT images by using the method described above. The values were compared with the true cross sections. Differences were less than 5.0%.

The kinetics of distal airway response to histamine was studied indirectly by imaging the area of xenon-filled air spaces in the lung

<table>
<thead>
<tr>
<th>Level</th>
<th>Area, mm$^2$</th>
<th>Diameter, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper</td>
<td>$13.1 \pm 2.7$</td>
<td>$4.1 \pm 0.4$</td>
</tr>
<tr>
<td>Middle</td>
<td>$9.3 \pm 2.6$</td>
<td>$3.4 \pm 0.4$</td>
</tr>
<tr>
<td>Lower</td>
<td>$7.6 \pm 1.5$</td>
<td>$3.1 \pm 0.3$</td>
</tr>
</tbody>
</table>

Values are means ± SD from all animals ($N = 6, 2$ airways per animal per cross-section level).
fields in each KES-CT image (named “ventilated alveolar area”) using specifically developed software using the MatLab programming package (MathWorks). Before analysis, the proximal airways were eliminated from the original image by masking. The distribution of xenon density in baseline KES-CT images was unimodal. Ventilated alveolar area was calculated by thresholding based on the xenon density. The upper and lower threshold density limits were defined as the mode ± 2 SD based on the xenon distribution in the baseline image. The ventilated alveolar area was calculated as the total area of pixels comprised within the threshold limits. Within-subject heterogeneity of ventilation was calculated from the coefficient of variation (CV) of xenon density in the lungs. Results from different animals were compared with the baseline values.

Lung Mechanics Parameters

The overall respiratory system resistance was calculated using a multiple linear regression method using the following mathematical model:

\[ \text{Ptr} = P_0 + EV + RF \]  

(1)

where \( \text{Ptr} \) (cmH\(_2\)O) is the tracheal pressure, \( P_0 \) (cmH\(_2\)O) is the dynamic positive end-expiratory pressure, \( E \) (cmH\(_2\)O/ml) is respiratory system elastance, \( V \) (ml) is the lung volume, \( R \) (cmH\(_2\)O/s·ml\(^{-1}\)) is respiratory system resistance, and \( F \) (ml/s) is ventilation flow. \( P_0 \), \( E \), and \( R \) are estimated for each respiratory cycle by multiple linear regression based on selected parts of the flow signal (14). The respiratory system conductance \( (G) \) is defined as \( 1/R \). Results are expressed as relative conductance \( (G/G_0) \).

Statistical Analysis

Data are expressed as the mean values ± SD. The significance of the results between the groups was tested with Student’s \( t \)-test (paired two samples for mean), and correlation between parameters was estimated by Pearson’s correlation coefficient. The kinetics of proximal airway luminal area at the lower cross-section level was tested vs. ventilated alveolar area after histamine inhalation by repeated-measures ANOVA. A \( P < 0.05 \) was considered significant.

RESULTS

Our results show that using the KES-CT technique, both the magnitude and kinetics of the alterations in regional lung ventilation induced by histamine bronchoprovocation can be quantified. For an overall view of the effects of histamine provocation, a 3D image of the bronchial tree was obtained based on serial CT images of the lung, performed before and after histamine inhalation. Such image reconstructions allow a global assessment of the effectively ventilated proximal airways. An example of a 3D reconstruction of the bronchial tree in one rabbit is shown in Fig. 3.

Luminal Areas of Individual Proximal Airways

At each cross-section level, the two largest airways on opposite lung fields were chosen from all imaging cross-section levels for a detailed analysis. In the baseline images, the lumens of these airways ranged from 5.9 to 18.4 mm\(^2\), corresponding to diameters of 2.7–4.8 mm. These airway dimensions correspond to approximately the sixth and the second airway generations (33). The airway areas were largest at the upper cross-section level and smallest at the lower cross-section level, as successively further generations of the bronchial tree were imaged. Baseline airway diameters and luminal areas are summarized in Table 1.

Kinetics of Airway Narrowing in Proximal and Distal Airways

Kinetics of histamine-induced bronchoconstriction was studied up to 60 min after histamine inhalation. An example of the effect of histamine on proximal airway diameters and on ventilated alveolar area measured based on regional xenon density in the CT images in one animal are shown in Fig. 4. Luminal areas of individual proximal airways reached a minimum on the average 22 min after histamine inhalation (Fig. 5, Table 2). Airways in the upper slice showed a significantly less constriction than airways in the middle and lower slices, and in one-half of the animals no constriction was observed at this cross-section level. There was no significant difference in the constriction of middle and lower lung levels. The difference in time to maximal constriction was not significant between any of the levels.

The maximal magnitude of constriction in proximal airways relative to the luminal area at baseline and the time required for maximal airway narrowing are summarized in Table 2. There was a significant correlation \((R = 0.54, P < 0.001)\) between the baseline airway luminal area and the airway area at maximal constriction (Fig. 6).

Behavior of distal airways was studied indirectly by imaging the ventilated alveolar area. Figure 7 shows the kinetics of ventilated alveolar area and the relative respiratory system conductance \( G/G_0 \) after histamine inhalation. This figure also includes two data points taken from a separate study with four rabbits under the same experimental conditions, except that no deep inspiration was included in the sequence. Unlike the reduction in proximal airway size, the drop in ventilated alveolar area was immediately maximal, with the first data points acquired 6 min after the start of the histamine inhalation. Because no significant difference was found between the three studied lung cross-section levels, the ventilated alveolar area data were pooled and presented as a single curve. Ventilated alveolar area and lung mechanics parameters, \( \text{Ptr} \), \( \text{Vt} \), and \( R \), are summarized in Table 3. The maximal relative change in ventilated alveolar area averaged over the three imaged cross-section levels was 55% after histamine inhalation, which was
similar in magnitude to that in proximal airways of 57% (Tables 2 and 3).

The increase of the respiratory system resistance was accompanied by a marked increase of the heterogeneity of regional xenon density, as shown in Fig. 8. Like ventilated alveolar area, the heterogeneity of xenon density was immediately maximal and showed fast recovery.

The recovery rate in the ventilated alveolar area was much faster than that observed in proximal airways, with values close to the baseline as soon as 25 min after histamine inhalation. Examination of the kinetics of the overall respiratory system resistance showed that it was also maximal immediately after the histamine inhalation. Unlike the ventilated alveolar area, the respiratory system resistance was still significantly elevated at 20 min after histamine inhalation and showed a slower decay with values still significantly above baseline at 60 min. Proximal and distal airways had a combined impact on the respiratory system conductance (G/G0); however, with significantly different kinetics (P < 0.0001 by repeated-measures ANOVA); initially dominated by distal airway obstruction followed by proximal airway narrowing, as presented in Fig. 9.

DISCUSSION

In this study we have assessed the role of airway size in the kinetics and magnitude of airway response to histamine inhalation in healthy rabbits. This is the first study in which bronchoconstriction after histamine inhalation has been quantitatively imaged at the level of individual bronchi in vivo in a small-animal model.

Technical Considerations

We used a novel method, KES CT imaging, to assess the changes in bronchial luminal areas after bronchoconstriction induced by inhaled histamine. A detailed discussion of the advantages and limitations of the method has been presented recently (32). Previously, videomicrometry in lung slices maintained in culture media has been used to study reactivity in both proximal and distal airways, including in small-animal models such as rat (11, 25). A major advantage of these techniques is that airways of all generations can be studied with little limitation in image resolution. On the other hand, central nervous system and circulatory influences are absent, and the load on airway smooth muscle is theoretically smaller in...
isolated than in intact lung (46). A significant advantage of the KES-CT technique is that it allows for the noninvasive study of several generations of conducting airways in the rabbit model in vivo. This is due to a spatial resolution that is sufficient for the study of bronchial reactivity in individual airways down to \( \sim 2.0 \) mm in diameter. Unlike videomicrometry, KES-CT does not allow direct measurement of small distal airway reactivity. However, by using this technique, regional lung xenon density can be directly quantified in airways and alveoli. As we showed previously, regional kinetics of xenon density vs. time during Xe-O2 wash-in allow measurements of regional-specific lung ventilation using dynamic KES-CT imaging (32). In the present study, dynamic imaging was not performed because this would have increased data-acquisition time, reducing the number of data points after histamine administration. However, the regional xenon density at the FRC image acquired after 11 respiratory cycles with Xe-O2 is directly determined by the local lung ventilation. Because distal airway obstruction induces significant alterations in the distribution of alveolar ventilation (20), we assumed that the measurement of disturbances in regional lung ventilation can indirectly quantify distal airway obstruction. Previous studies by us (27, 28), using 2D and 3D KES-CT in rabbit lung after histamine inhalation, and by others (35, 42, 43) have shown that bronchoconstriction produces large ventilation defects. If proximal conducting airway obstruction were the only cause of such regional defects in lung xenon filling, the abnormalities would theoretically appear anatomically systematic (21) and the drop in xenon density due to the decreased local ventilation would be uniform throughout the defects. Venegas et al. (42), using positron emission tomography in human and sheep, observed large ventilation defects with small-scale heterogeneity and bimodal distribution of ventilation within the defects. Similar results were also found in our study (Fig. 4). This suggests that the defects in regional lung ventilation causing the changes in the ventilated alveolar area resulted mainly from clusters of constricted distal airways (42). Figure 4 illustrates this observation in one animal. Six minutes after histamine inhalation, although narrowing in proximal airways was moderate, marked patchy defects in regional xenon density were observed. However, in later images, e.g., at 24 min after histamine when a substantial recovery in regional alveolar ventilation was seen in this animal, proximal airways were most constricted.

Xenon is a denser gas than air, which may have an effect on respiratory system mechanics (2). Data from our laboratory in identical baseline experimental conditions show that, after 12 tidal cycles of ventilation with xenon, the respiratory system resistance increases by 15%. Whether the increase in the inhaled gas density may cause any further alterations in gas exchange in bronchoconstricted lung is unlikely because of the short duration of the imaging sequence. Anesthesia with 70% xenon causes no gas-exchange abnormalities in human patients, despite slight deterioration in lung mechanics, making it a safe anesthetic, including in patients with chronic lung disease (23).

### Effect of Airway Size on the Magnitude and Kinetics of Airway Response to Inhaled Histamine

In this study, we found a significant correlation between baseline airway caliper and reactivity to histamine provocation in proximal airways down to \( \sim 2.7 \) mm in diameter, corresponding approximately to the sixth generation in rabbit (33). The measurement of the drop in ventilated alveolar area was used to obtain an estimate of distal airway response, which is a mean value for all distal airways leading to alveoli within a CT image. Although it is difficult to directly compare luminal narrowing in proximal airways and the drop in ventilated

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**Figure 4**: Proximal airway luminal area at maximal constriction expressed as percent baseline area plotted against baseline airway luminal area. Each point represents a single airway; results are from all 6 rabbits.

**Figure 6**: Proximal airway luminal area at maximal constriction expressed as percent baseline area plotted against baseline airway luminal area. Each point represents a single airway; results are from all 6 rabbits.
alveolar area on an absolute scale, the maximal relative change in both parameters was similar in magnitude on the average over the three imaged cross-section levels. Also, the regional distribution of xenon gas within the lung fields became very inhomogeneous after histamine aerosol challenge (Fig. 4), suggesting significant alveolar ventilation heterogeneity (Fig. 8).

**Topographical differences.** Topographical differences in bronchial response to histamine have previously been reported by others. Fujiwara et al. (18) found that, in isolated smooth muscle strips prepared from rabbit trachea and bronchi, in vitro response to histamine gradually increased down to the fifth generation. Also, Fleisch and Calkins (17) reported a much greater lung capacity. Whether the deeper inspiration before the initial image in the sequence has induced bronchodilation, because the amplitude of volume oscillations can suppress airway response to a bronchoconstricting agent.

In the present study, there were significant differences in the kinetics of airway response to histamine among proximal airways down to 2.7 mm in baseline diameter, with smaller airways responding faster than larger more proximal airways (Tables 2 and 3; Fig. 9). Moreover, response in proximal airways was considerably delayed compared with that of distal airways as estimated from the ventilated alveolar area measurements, with a time lag of ~20 min to reach maximal response in proximal airways. Unlike proximal airways, in distal airways response to histamine appeared maximal in the first data points acquired 6 min after provocation, with a much more rapid recovery (Fig. 9).

**Effect of inspired gas volume.** In the image-acquisition protocol shown in Fig. 2, the initial image was acquired after a single deep inspiration maneuver. A potential bias is that a deep inspiration may have opened constricted peripheral airways, thereby enhancing the arrival of xenon in constricted lung regions. The impact of this factor is limited, however; first, because the single deep inspiration was only at 57 and 69% total lung capacity, at 6 and 22 min, respectively, after histamine and, second, the ventilated alveolar area was not measured on the basis of the inspiration image, but from the second image in the sequence obtained at FRC, six respiratory cycles after the deep inspiration. The total contribution of the deep inspiration to lung ventilation with Xe-O2 during the image sequence up to the FRC image was ~16%. The second issue is whether the deeper inspiration before the initial image in the sequence has induced bronchodilation, because the amplitude of volume oscillations can suppress airway response to a bronchoconstrictor agent.

Figure 8. Heterogeneity of regional ventilation presented as the coefficient of variation (CV) of xenon density within masked CT images. Data points are mean ± SD values from 6 rabbits (N = 18 images). Dashed line represents the baseline value. CV remained significantly higher than the baseline value 60 min after histamine inhalation; P < 0.05.

Figure 9. Relative changes in G/G0, luminal area of proximal airways at the lower cross-section level where the largest response to histamine was observed (% of baseline), and ventilated alveolar area after inhalation of histamine aerosol (% of baseline). The same data points and their error bars are presented in Figs. 5 and 7. Data are means from all 6 rabbits.

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**Table 3. Ventilated alveolar area, coefficient of variation, airway pressure, tidal volume, and respiratory system resistance at baseline, 6 min, 22 min, and 60 min after histamine inhalation**

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Ventilated Alveolar Area, % baseline</th>
<th>CV, SD/mean</th>
<th>Ptr, cmH2O</th>
<th>Vt, ml/kg</th>
<th>R, cmH2O·s·ml⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Upper</td>
<td>Middle</td>
<td>Lower</td>
<td>Overall</td>
<td>Upper</td>
</tr>
<tr>
<td>Baseline</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>6 min</td>
<td>49.8 ± 22.7*</td>
<td>52.8 ± 22.8*</td>
<td>64.7 ± 19.8*</td>
<td>0.34 ± 0.05*</td>
<td>0.37 ± 0.06*</td>
</tr>
<tr>
<td>22 min</td>
<td>89.1 ± 15.1</td>
<td>91.3 ± 9.5</td>
<td>92.9 ± 6.5</td>
<td>0.22 ± 0.06</td>
<td>0.21 ± 0.04*</td>
</tr>
<tr>
<td>60 min</td>
<td>90.2 ± 17.7</td>
<td>92.1 ± 9.6</td>
<td>93.4 ± 8.8</td>
<td>0.21 ± 0.05</td>
<td>0.21 ± 0.04*</td>
</tr>
</tbody>
</table>

Values are means ± SD from all animals (N = 6). CV, coefficient of variation; Ptr, airway pressure; Vt, tidal volume; R, respiratory system resistance. *Significant difference compared with baseline (P < 0.05).
response to methacholine in rabbit, supposedly by the direct effect of stretch on force generation by the airway smooth muscle. The results of a separate study in identical experimental conditions in four rabbits, in which no deep inspiration was administered and all the images were obtained at FRC and in which the drop in respiratory system conductance in response to inhaled histamine was similar to this study, show that the reversal of peripheral lung fill-in defects after histamine administration is equally fast in the absence of any deep inspiration during the imaging sequence (Fig. 7). In other words, the deeper inspirations did not seem to be the cause of the rapid recovery of xenon filling in the peripheral lung after histamine administration.

Changes in the inspired gas volume just before the inspiratory image could induce differences in proximal airway cross-sectional area measurements, which were based on the inspiratory image, i.e., in the first of the two images acquired in each sequence. Our data show that there were no significant differences in the inspired gas volume before the inspiratory image, at baseline. Immediately after histamine, the inspired gas volume was reduced. It is seen in Table 3 that, at 6 min posthistamine when the first posthistamine images were acquired, the drop in the inspired volume before the image was maximal, whereas the decrease in proximal airway caliber was small as shown in an example in Fig. 4 and in Fig. 5. On the contrary, 22 min after histamine (average time to maximum for all airways), when the inspired volume was not significantly different than baseline, the narrowing in proximal airways was maximal. These observations suggest that the impact of the initial decrease in inspired gas volume after histamine on proximal airway cross-sectional area measurements was minimal.

**Mechanisms of Differences in the Kinetics of Bronchoconstriction**

The degree of airway narrowing during bronchoconstriction is a balance between the force generated by the airway smooth muscle and the elastic forces that limit airway smooth muscle shortening (24). The size-dependence of the response to histamine in proximal airways and the differences in the kinetics of bronchoconstriction after histamine administration may be due to several mechanisms.

**Airway wall structure.** Structural differences, namely the presence and the relative amount of cartilage as well as airway smooth muscle, can determine the mechanical properties of the airway wall. The elasticity of airway cartilage tends to limit airway smooth muscle shortening. Moreno et al. found that softening of the airway cartilage by intravenous treatment with papain enhances the maximal response to intravenous acetylcholine in rabbit (29). Distal airway wall contains less cartilage and is presumably more pliable (18). Airway smooth muscle density is significantly higher in terminal bronchioles in rabbit compared with monkey (39), which may explain at least in part the observed differences between distal and proximal airway reactivities in the two species. Morphometric analysis of the airway wall structure has shown that the percentage of airway wall cartilage rapidly decreases from ~60% to zero at the eighth generation in mature rabbit, whereas the percent smooth muscle content increases from 10 to 30% (33). Using computational modeling, Ramchandani et al. (33) predicted that both the increased proportion of airway smooth muscle and the increased airway wall compliance due to less cartilage results in increases in lung resistance during bronchoconstriction. On this basis, it can be hypothesized that airway narrowing would be faster in distal airways. The greater amount of smooth muscle may alone be responsible for the difference in the response to histamine in distal airways, because it has been demonstrated that cartilage does not prevent airway narrowing to the point of complete collapse, even in very large airways given a large enough stimulus (8). The observation that response and recovery are faster in small distal airways is in line with previous findings by Wohlsen et al. (45) in sensitized rat lung explants provoked with ovalbumin. They observed not only that smaller airways contracted more strongly and quickly, but that they also relaxed faster, suggesting that smaller airways are more reactive. Duguet et al. (13) found that in rabbit lung explants, in airways of 3.48 to 0.12 mm² cross-sectional area, both the magnitude and velocity of airway narrowing increased as airways became smaller. Therefore, structural differences could potentially explain both the greater magnitude and higher velocity of airway constriction in smaller airways.

**Airway-parenchymal interdependence.** The forces of interdependence between the airways and the surrounding lung parenchyma have been proposed as a factor limiting airway narrowing (12). Lung volume can thereby affect airway narrowing. In the present experimental setup, the input gas flow was maintained constant at baseline, and the inspiration and expiration times were the set parameters. The gradual increase in VT after its initial drop after histamine administration could potentially attenuate the constriction of the airway lumen. On the other hand, airway pressure rose significantly after histamine administration (see Table 3), concomitant to the drop in VT, followed by a gradual decrease thereafter. This increase in airway pressure should act oppositely to the change in VT and limit airway narrowing. However, despite the nearly twofold increase in peak airway pressure during tidal breathing measured at 6 min posthistamine when the first image data were acquired (Table 3), the decrease in ventilated alveolar area due to distal airway constriction was maximal. On the basis of a computational model in mature rabbit lung (24) when transmural pressure increases from 10 to 20 cmH2O, normalized lumen area increased by less than 20% in airway generations 1 to 9, the change being largest for generation 9 and least for generation 1. In the present study, 6 min posthistamine, Pţ was on the average 14% (or 3.4 cmH2O) higher compared with 22 min posthistamine, when the constriction was maximal in proximal airways. This pressure difference is too small to have prevented narrowing of larger airways in the early phase after histamine inhalation. Therefore, the drop in VT and the concomitant elevation in Pţ most likely due to the elevation in resistance observed in the immediate phase after histamine, were the consequence rather than the cause of distal airway constriction.

**Airway vascular engorgement and permeability.** Airway vascular engorgement has been proposed as a mechanism causing airway wall thickening, encroachment of the airway wall lumen, and loss of parenchymal tethering. Blosser et al. (4) have shown that extended increased bronchial perfusion causes significant airway wall thickening, but these changes were insufficient to cause changes in large or small airway...
resistance or airway reactivity. Vascular engorgement per se therefore seems to play a negligible role in determining airway resistance (44). Slow narrowing and recovery in response to histamine in proximal airways may also be due to mucosal edema formation and removal. This hypothesis is suggested by the fact that histamine is one of the inflammatory mediators involved in asthma that can induce airway vasodilatation and mucosal thickening (6, 10) as well as increased vascular permeability (36). Also, the effect of histamine on airway vascular permeability may show regional differences. Evans et al. (16) found that histamine causes transient Evans-blue extravasation in trachea and main bronchi but not in intrapulmonary airways via H1 receptor stimulation in guinea pig. Therefore the delayed maximal response to histamine and the slow recovery observed in the main bronchi in the present study may possibly be due to submucosal edema formation followed by gradual removal. The presence of luminal edema fluid cannot be confirmed on the basis of the present data, and further studies are needed to determine the role of blood and fluid kinetics in the airways during bronchoconstriction.

Aerosol deposition. Predominant aerosol deposition in peripheral airways would deliver a larger histamine dose in these airways. The maximal magnitude of response in proximal airways, i.e., at the lower lung level, is quite significant: the luminal area dropping to 38% of the baseline value (Table 2), which is not in favor of a small histamine dose being deposited in the proximal airways. Uneven aerosol distribution has been suggested as a potential cause of the heterogeneity of regional ventilation induced by histamine. However, Brown and Mitzer (8) found that aerosol and intravenous routes of histamine administration produced equal heterogeneity in individual airway narrowing in dog, suggesting that the heterogeneity in histamine response is predominantly controlled by local mechanisms. Aerosol deposition depends on the MMAD of the aerosol particles, the smaller aerosol particles depositing more easily in the peripheral lung (38). In a study with a similar aerosol particle MMAD of 3.3 μm in tracheostomized and ventilated rabbit (31), the administered aerosol dose deposited in the trachea was more than twice that in the peripheral lung. In this study, the MMAD of the aerosol particle was 3.5 μm. Therefore, the earlier response in the peripheral airways does not appear to be due to a larger dose delivery to the peripheral vs. central airways.

In conclusion, we have used an original CT imaging method to assess the role of airway size in the kinetics and magnitude of airway response to histamine inhalation in healthy rabbits. Our results indicate significant differences in the kinetics of histamine response in proximal vs. distal airways, as well as differences in airway reactivity as a function of airway size in proximal airways: larger proximal airways reacted and recovered more slowly than smaller distal airways. The findings suggest that changes in respiratory system conductance after inhaled histamine result from combined reactions in proximal and distal airways. However, the relative contribution of these components seems very unequal in time after histamine inhalation. These findings may be important for studies of the pathophysiology and treatment of asthma.

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