Impact of elevated pulmonary blood flow and capillary pressure on lung responsiveness

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There is ample literature evidence that changes in pulmonary hemodynamics markedly affect the mechanical properties of the lung (1, 3, 7, 9, 11, 12, 19, 20, 25, 26). Elevation of the pulmonary vascular pressure (11, 20) and/or blood flow (Qp) (7, 14) is followed by compromised lung function, due to the increased capillary filling, leading to a decrease in resting lung volume (7) and/or stiffening of the alveolar wall (20). However, the results of these previous studies did not permit consistent conclusions as to whether the pulmonary vascular pressure or Qp exerts the predominant effect on the lung mechanics. Our laboratory recently reported experimental evidence that, after acute changes in the pulmonary hemodynamics, primarily the filling pressure in the pulmonary capillaries is responsible for the lung mechanical changes, with the altered Qp playing no part in this respect (20).

Besides the basal lung mechanical changes, a few previous clinical studies and case reports have suggested the presence of bronchial hyperreactivity (BHR) subsequent to alterations in the pulmonary hemodynamics (1, 16, 17, 22–24). However, all of these previous observations were based on lung function measurements in children with congenital heart diseases, where the complex nature of the heart diseases precluded a systematic evaluation of the particular roles of altered Qp and/or pulmonary pressures on the enhanced lung reactivity to constrictor stimuli. In the present study, therefore, we set out to explore the separate roles of altered pulmonary filling pressures and Qp in changing the lung responsiveness in an experimental setting that allows independent manipulations of each hemodynamic parameter before the performance of the broncho-provocation tests. Identification of the pulmonary hemodynamic parameter primarily responsible for BHR is of major importance, concerning the prevention of lung function exacerbation in patients with abnormal pulmonary hemodynamics.

Methods

Animal Preparations

Forty-seven adult male Sprague-Dawley rats (weighing 356–454 g) were anesthetized with isoflurane (3–4% induction dose). The rats were tracheotomized (14-gauge polyethylene cannula; Braun, Melsungen, Germany) and mechanically ventilated (7 ml/kg, 70–80 breaths/min) with a constant volume-cycled rodent ventilator (model 683, Harvard Apparatus, South Natick, MA), while a positive end-expiratory pressure of 2.5 cmH2O was applied. Maintenance of anesthesia was ensured with 1.4% isoflurane (one minimum alveolar concentration for rats) during the surgical preparation and interrupted when the heart-lung blocks were excised to avoid the potential bronchodilatation effect of the volatile agent during the study period. The femoral vessels were prepared surgically and then cannulated for blood sampling and continuous arterial blood pressure monitoring. Blood gases from arterial blood samples were analyzed regularly (UltimaTM, Datex/Instrumentarium, Helsinki, Finland). Airway pressure (Paw) was monitored continuously (Validyne DP 45, Northridge, CA). The rats were fully anticoagulated (heparin, 1.5 IU/g iv), and 20 ml blood were then gently withdrawn via the arterial cannula, with the collected blood continuously replaced by intravenous infusion of colloid solution (6% hydroxyethyl-starch). This resulted in the collection of 20-ml diluted blood with a hematocrit of ~25%, which served as priming volume for the isolated perfusion circuit. The experimental protocol was approved by the Animal Ethics Care Committee of the Canton of Geneva, Switzerland.

Preparation of Isolated Lungs

The preparation of the animals and the harvesting and perfusion of the isolated rat lungs were carried out similarly, as detailed previously.
(20). A median sternotomy was performed, and the main pulmonary artery was cannulated via the right ventricular outflow tract (14-gauge; Braun, Melsungen, Germany). Another catheter was placed in the left ventricle through the left ventriculotomy, in which a Combiﬁx adapter (Braun) was tightly ﬁxed and connected to medical-grade silicone tubing. A third catheter was placed directly in the left atrium [polyethylene tubing, inner diameter (ID) 0.88 mm, Portex, Hythe, UK]. The lungs and the heart were excised and extracted in a single block, dissected free of adjacent tissue, and weighed. The net lung weight was obtained by subtracting the weight of the instrumental components (cannula for the vessels and the trachea) from the total weight of the preparation.

Lung Perfusion

The setup used for the lung perfusion has been detailed previously (20). The heart-lung block was placed in a humidified box (590 ml) and ventilated in the same manner as in the chest, except that 5% CO2 (38 mmHg) was added to the inspired air to avoid hypocapnea. A series of hyperinﬂations (peak pressure 25–30 cmH2O) was applied to standardize the lung history by eliminating the atelectatic areas. Lung perfusion was then started by driving the blood in the cannula of the pulmonary artery by a roller pump (Ismatec Pump, Glattburg, Zurich, Switzerland). The initial perfusion parameters were set to achieve a Q˙p of 5 ml/min, a pulmonary arterial pressure (Ppa) of 17.5 mmHg, and a left atrial pressure (Pla) of 5 mmHg. The latter was attained by placing the distal extremity of the left ventricular outﬂow cannula at an appropriate height. The zero pressure reference in the pulmonary vasculature was located at the level of the left atrium. Both Pla and Ppa were greater than the mean Paw, so the lungs were maintained under West’s zone 3 conditions [Ppa > Pla > mean (Paw)]. The blood dripping from the left ventricular outﬂow cannula was collected in a cylinder and aspirated from this reservoir with polyethylene tubing passing through another roller pump. The Ppa and Pla values were recorded continuously (Honeywell, model 156-PC 06-GW2), and a transit-time ﬂowmeter (T-201 CDS, Transonic Systems, Ithaca, NY) situated between the perfusion reservoir and the catheter cannulating the main pulmonary artery measured Q˙p continuously. The pulmonary vascular resistance (Rv) was calculated by dividing the pressure difference between Ppa and Pla by Q˙p. Pulmonary capillary pressure (Pc) was estimated by applying the Gaar equation [Pc = Ppa − 0.44 × (Ppa − Pla)] (10) and was used to assess the capillary ﬁlling pressure before the maneuvers. Paw, Ppa, Pla, Pc, Q˙p, and the lung weight were recorded and stored on a microcomputer at a sampling rate of 50 Hz via an analog-to-digital interface converter (Biopac, Santa Barbara, CA).

Lung Weight Changes

The heart-lung block was suspended from an isometric force displacement transducer (Grass FT03, Quincy, MA) that allowed continuous monitoring of the lung weight gain (WG) during the experiments. The inferior lobe of each lung was weighed (wet weight) and then dried in an oven (Memmert, Schwabach, Germany) at 60°C for 2 days, after which it was weighed again to determine the wet-to-dry lung weight ratio. These measurements allowed the exclusion of the influence of edema on the findings.

Lung Mechanical Measurements

The mechanical parameters were estimated from the input impedance (ZL) measured by forced oscillation with the wave tube technique, as previously described (21). Briefly, at end-expiration, the mechanical ventilation was paused. During these apneic periods, a loudspeaker and the tracheal cannula were connected through a polyethylene wave tube (length = 102 cm, ID = 0.2 cm), and a small-amplitude (1 cmH2O peak to peak) forcing signal was delivered into the trachea. The loudspeaker was driven by a computer-generated pseudorandom signal containing 23 components ranging from 0.5 to 21 Hz, which is a suitable frequency range via which to obtain both tissue and airway parameters. Lateral pressures were measured at both ends of the wave tube with miniature side-arm transducers (ICS 33N000D). These pressure signals were low-pass filtered (<25 Hz) and digitized at a sampling frequency of 128 Hz. The pressure transfer function was created by fast Fourier transformation from the 8-s recording. The ZL was computed from the pressure transfer function, as the load impedance of the wave tube, by using the transmission line theory (8).

A model containing an airway and a constant-phase tissue compartment was ﬁtted to the averaged ZL data in each condition by minimizing the squared sum of weighted differences between the measured and the modeled ZL data. The airways were characterized by a frequency-independent airway resistance (Raw) and inertance, while the tissue compartment was described by the parenchymal damping (G) and elastance (H) (13). The airway parameters were corrected for the resistance and inertance of the endotracheal tube.

Methacholine Challenges

Constrictor responses of the lungs were provoked by infusing methacholine (MCh) into the pulmonary artery by a constant-ﬂow infusion pump (ID2S, model ID 2 ET, Asnieres, France) at doses of 2, 6, and 18 μg · kg−1 · min−1 (body weight). The infusion rate was set at from 0.5 to 4.5 ml/h (ranging from 0.08 to 1.5% of the total Q˙p) from a MCh solution containing 1 mg/ml MCh. Each infusion level lasted for ~20–25 min, which resulted in the administration of 2–2.5 ml MCh solution in total.

Study Protocol

A 15-min period of lung perfusion was necessary to establish steady-state conditions before the start of the protocol on the isolated lungs. After the steady-state conditions had been reached, the lungs were randomly assigned to one or another of the following seven protocol groups (Fig. 1).

Group 1 (n = 10). Pc was lowered to 5 mmHg and maintained at this level throughout the whole protocol, whereas Q˙p was altered from 5 to 10 ml/min and then returned to 5 ml/min.

Group 2 (n = 7). Pc was maintained at 10 mmHg, whereas Q˙p was altered from 5 to 10 ml/min and then returned to 5 ml/min.

Group 3 (n = 6). Pc was increased to 15 mmHg and maintained at this level throughout the whole protocol, whereas Q˙p was altered from 5 to 10 ml/min and then returned to 5 ml/min.

Group 4 (n = 6). Q˙p was decreased from 10 to 5 mmHg and then returned to 10 mmHg.

Group 5 (n = 5). Q˙p was decreased from 10 to 5 mmHg and then returned to 10 mmHg.

Group 6 (n = 6). Q˙p was increased to 15 mmHg and maintained at this level throughout the whole protocol, whereas Pc was decreased from 10 to 5 mmHg and then returned to 10 mmHg.

Group 7 (n = 7). Q˙p was decreased from 10 to 5 mmHg and then returned to 10 mmHg.

The protocol groups were designed to allow the assessment of altered Q˙p at constant low ([group 1], normal ([group 2], or high Pc levels ([group 3]). The last four groups were designed to study the effects of increased ([groups 5 and 7]) or decreased Pc ([groups 4 and 6]), while a constant normal ([groups 4 and 5]) or high Q˙p ([groups 6 and 7]) was persistently maintained. Three to four ZL recordings were collected at each Q˙p ([groups 1–3]) and Pc ([groups 4–7]) level under the baseline conditions and during each infusion of MCh and ensemble averaged. To exclude a possible time effect, the inﬂuence of alterations in the hemodynamics was estimated by comparing the mechanical and pulmonary vascular parameters after the changes in Q˙p (in [groups 1–3]) or Pc (in [groups 4–7]) with the averages of the values obtained before and after the changes.
Statistical Evaluation

Since the procedural data proved to be normally distributed, as analyzed by the Shapiro-Wilk test, data are reported as means ± SE. Two-way repeated-measures ANOVA, using a general linear model, was used to test the significance with fixed factors: the hemodynamic condition and the MCh infusion level. For pairwise comparisons, 95% confidence intervals for the differences were computed by taking into account the significant interactions between the factors. The Student-Newman-Keuls test was used for post hoc comparisons. The statistical tests were performed by using the SigmaStat statistical program package (Systat Software). $P < 0.05$ was considered significant.

RESULTS

No differences in body weight ($P = 0.30$) or the wet-to-dry ratio ($P = 0.78$) of the isolated lungs were observed between the protocol groups (Table 1). Moreover, edema formation was not observed during the perfusions, as WG was not statistically significant from zero in any protocol group (ranging from $-1.0 \pm 1.4$ to $0.8 \pm 2.0$ mg/s, $P = 0.23$) during the protocol.

Figure 2 demonstrates the Raw under the baseline conditions (solid symbols) and during infusions of the two highest doses of MCh, where Qp was altered while a constant Pc was maintained in the lungs involved in protocol groups 1–3. Alterations of Qp had no significant effects on the baseline values of the Raw ($P = 0.18$) and did not affect the basal values of G ($274 \pm 11$ and $278 \pm 16$ cmH2O/l at Qp 5 and 10 ml/min, respectively, $P = 0.11$) and H ($2,032 \pm 79$ and $1,975 \pm 99$ cmH2O/l at Qp 5 and 10 ml/min, respectively, $P = 0.12$). Doubling Qp enhanced the MCh-induced Raw responses in the lungs with normal Pc ($P = 0.004$ at 10 mmHg), and even more profoundly in the lungs with high Pc ($P < 0.001$ at 15 mmHg). This enhancement was not apparent at low Pc (5 mmHg, $P = 0.92$). The responses in G were also affected by the elevations of Qp during the highest dose of MCh ($1,098 \pm 47$ and $1,351 \pm 73$ cmH2O/l, $P < 0.001$), while the changes of Qp had no effects on the MCh-induced increases in H (data not shown).

Table 1. Body weights and wet-to-dry ratios of the lung weights determined in the protocol groups

<table>
<thead>
<tr>
<th>Protocol Group</th>
<th>Body Weight, g</th>
<th>Wet-to-Dry Ratio</th>
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<tbody>
<tr>
<td>1</td>
<td>400 ± 7</td>
<td>6.6 ± 0.4</td>
</tr>
<tr>
<td>2</td>
<td>414 ± 8</td>
<td>6.2 ± 0.4</td>
</tr>
<tr>
<td>3</td>
<td>410 ± 14</td>
<td>6.8 ± 0.2</td>
</tr>
<tr>
<td>4</td>
<td>420 ± 7</td>
<td>7.3 ± 0.5</td>
</tr>
<tr>
<td>5</td>
<td>421 ± 12</td>
<td>6.7 ± 0.5</td>
</tr>
<tr>
<td>6</td>
<td>412 ± 7</td>
<td>6.4 ± 0.6</td>
</tr>
<tr>
<td>7</td>
<td>406 ± 7</td>
<td>6.0 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE.
Figure 3 depicts the Raw under the baseline conditions and during the infusions of the two highest doses of MCh, when Pc was altered during maintenance of a constant Qp in the lungs involved in protocol groups 4–7. Raw was not affected by the changes of Pc at any stage of the experiments (P = 0.23). In groups 5 and 7, mild but statistically significant increases were observed in the baseline values of G (309 ± 17 and 390 ± 28 cmH2O/l for group 5; 350 ± 28 and 393 ± 54 cmH2O/l for group 7, P < 0.001 for both) and H (2,530 ± 122 and 2,869 ± 218 cmH2O/l for group 5; 2,440 ± 149 and 2,745 ± 262 cmH2O/l for group 7, P < 0.001 for both) with rising Pc, but these minor changes did not affect the lung responsiveness to MCh (1,315 ± 78 vs. 1,304 ± 95 cmH2O/l for G, and 4,251 ± 182 vs. 4,390 ± 258 cmH2O/l for H at Pc = 5 and 10 mmHg, respectively, during 18 µg·kg⁻¹·min⁻¹·MCh). Decreasing Pc in groups 4 and 6 had no effects on the mechanical parameters or on the lung responsiveness.

The relative changes in the Raw following the pulmonary hemodynamic changes in all of the protocol groups are demonstrated in Fig. 4 under baseline conditions and during MCh infusion. Increasing Qp in groups 1–3 augmented the MCh-induced increases in Raw, with these effects becoming more pronounced as the constant Pc level increased. In contrast, changes in Pc in groups 4–7 had no detectable effect on the lung responsiveness to MCh.

The vascular pressures and the Paw values were related in all protocol groups when the highest dose of MCh was administered (Fig. 5). At constant Pc in groups 1–3, Qp was altered by simultaneous opposite changes in Ppa and Pla. In groups 4–7, on the other hand, changes in Pc were achieved by simultaneous elevations (groups 5 and 7) or decreases (groups 4 and 6) in Ppa and Pla. The mean, peak, and end-expiratory Paw values were similar in the protocol groups.

MCh had no significant effect on the pulmonary hemodynamics; Rv was not affected significantly by the presence of MCh in the perfusion circuit. Table 2 lists Rv and its changes following the alterations in pulmonary hemodynamics in all protocol groups during the infusion of the highest dose of MCh.

**DISCUSSION**

In the present study, Qp and the filling pressures were altered independently with the aim of a systematic characterization of the impact of pulmonary hemodynamic changes on the lung function and responsiveness to constrictor stimuli. Increases in Pc at constant blood flow led to a deterioration of the parenchymal mechanics, but did not have a significant effect on the lung responsiveness. In contrast, increases in blood flow at constant pulmonary filling pressure did not affect the basal lung mechanics, but enhanced the lung reactivity to MCh, particularly when a high Pc was maintained in the pulmonary circulation.

**Methodological Considerations**

The tracheobronchial tree is supplied by two highly interconnected capillary networks: the bronchial and the pulmonary circulations. While the former is considered to supply the central and medium-sized airway, and the latter to dominate in support of the lung periphery, these two circulations anastomose at the level of the respiratory bronchioles and alveolar...
However, lung perfusion with a pulsatile flow was demonstrated to be not superior compared with a nonpulsatile perfusion (5), indicating that this phenomenon was not likely to have a major influence on our findings. Although the manipulations of the pulmonary hemodynamic parameters were performed in an identical manner to those detailed previously (2, 19, 20), an important new feature of the present experiments was the infusion of the constrictor agonist MCh into the pulmonary artery. Accordingly, primarily the lung periphery was accessed by this challenge with the possibility for a retrograde perfusion of the bronchial circulation, which explains the proportionally greater increases in the lung parenchymal parameters following MCh infusions than those observed in rats in vivo (15, 21).

Effects of Altered Qp on Lung Responsiveness

In agreement with our laboratory’s previous results, acute changes in Qp did not influence any of the lung mechanical parameters, while constant Pc was maintained under the baseline conditions (20). In contrast with this consistent finding, it was striking to observe the development of BHR with increasing Qp. The degree of BHR was related to the level of the capillary filling pressure: only mild elevations in responsiveness were observed at low Pc levels (5 mmHg), and a clear manifestation of BHR was present at physiological Pc (10 mmHg), whereas elevation of Pc above the physiological value (15 mmHg) led to the development of marked BHR. It is noteworthy that the influence of edema formation can be excluded from the development of BHR, since no WG was observed during the experiments, the wet-to-dry ratios of the lungs were normal and similar in all experimental groups, and the lung responses returned immediately to the initial values determined before the Qp elevations (Fig. 2).

The elevation of Qp may alter the lung reactivity via modification in the lung perfusion. One possible mechanism may be related to the changes in the relationships between the pulmonary vascular and the alveolar pressures. It is noteworthy that, in groups 2 and 3, where BHR was observed, the relationships of the perfusion and ventilation led to a large number of lung territories remaining in West zone 3 (Ppa > Pla > alveolar pressure). Increase of Qp in this scenario further extended the West zone 3 lung regions to the apex of the lung, leading to better overall lung perfusion, as suggested by the decreases in Rv. This extension in the lung perfusion may enhance MCh delivery all over the accessible lung territories, which may result in an additional access of the bronchoconstrictive agonist to those lung regions that were perfused poorly at low Qp levels.

Table 2. Initial pulmonary vascular resistance and its changes following alterations in the pulmonary hemodynamics in the protocol groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Rv, mmHg·min⁻¹·ml⁻¹</th>
<th>Changes in Rv, %</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>2.48±0.15</td>
<td>2.0±3.6</td>
</tr>
<tr>
<td>2</td>
<td>2.11±0.19</td>
<td>1.2±7.6</td>
</tr>
<tr>
<td>3</td>
<td>1.85±0.26</td>
<td>-0.9±4.2*</td>
</tr>
<tr>
<td>4</td>
<td>1.61±0.21</td>
<td>36.1±8.4*</td>
</tr>
<tr>
<td>5</td>
<td>1.85±0.14</td>
<td>-5.4±2.2*</td>
</tr>
<tr>
<td>6</td>
<td>1.42±0.04</td>
<td>33.4±7.0*</td>
</tr>
<tr>
<td>7</td>
<td>1.49±0.08</td>
<td>-0.2±1.5*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Rv, pulmonary vascular resistance. *P < 0.05 vs. 0.
While this extended perfusion may favor capillary recruitment in the alveolar septa, explaining the enhanced responsiveness in the lung periphery (i.e., the excess increase in G at high Qp), the major effect of the elevated Qp was observed in Raw. Nevertheless, changes in Raw are determined by the altered tone of the bronchial smooth muscle, which are mainly supplied by the bronchial circulation with systemic origin. However, the possible retrograde perfusion of the bronchial arteries from the pulmonary circulation has been demonstrated in rats (28), and the possible involvement of this phenomenon can be anticipated, since the most pronounced BHR was observed in the presence of high Pc and Qp, where this retrograde perfusion is expected to appear. Indeed, we were able to support this hypothesis by performing experiments on two additional rats, where the heart-lung preparation was perfused identically as in the main study groups, except that the heart-lung was kept in the chest and the descending aorta was isolated and also cannulated to better characterize the potential blood return from the bronchial circulation. The appearance of blood from the aorta at Qp of 10 ml/min proved the presence of the retrograde perfusion through the bronchial circulation in the present experimental setting, since all other systemic vessels were ligated, and all of the blood returning to the left ventricle was collected. Since greater MCh delivery was demonstrated during increased bronchial blood flow to the larger airways (27), this elevated, retrograde bronchial perfusion may contribute to enhancement of the airway responses to MCh at high Qp.

**Pc and Lung Responsiveness**

The increases in the baseline parenchymal parameters at an elevated Pc level observed in the present study is consistent with our laboratory’s previous findings obtained under similar experimental conditions (20). We attributed these changes to the increased tension of the pulmonary microvasculature, leading to an enhanced stiffness and dissipation of the parenchyma via mechanical attachment and the possible loss of lung volume with filled pressurized capillaries. Although this mechanism is clearly operative in the present experiments, it does not affect the lung reactivity to MCh. This apparent controversy can be explained by the fact that an altered Pc affects the lung tissue mechanics by changing the mechanical properties of the alveolar walls, where the capillaries provide the major support for their architecture, whereas MCh acts primarily on fundamentally different lung compartments: the small airways and the terminal bronchioles. The different sites of action of the alterations of Pc and MCh may explain why the increase in the Pc, even in the presence of a high Qp in group 7, did not alter the lung reactivity to MCh.

**Summary and Implications**

The results of the present study demonstrate the development of BHR with acutely increasing Qp, particularly if this phenomenon is associated with high Pc. Various chronic lung diseases induce vascular pressure elevations in the pulmonary capillaries. Our data suggest that, under such conditions, increases in Qp per se may lead to the development of BHR. This phenomenon may contribute to the airflow limitation and airway susceptibility observed during exercise testing that elevates pulmonary vascular pressures and Qp simultaneously. Moreover, enhanced airway reactivity to constrictor stimuli with increased blood flow may contribute to the symptoms observed in patients with exercise-induced asthma or in children with congenital heart diseases (an atrial or ventricular septal defect with a left-right shunt), where Qp increases in the presence of an elevated Pc.

**REFERENCES**


