Measurement of Lung Microvascular Pressure in the Intact Anesthetized Rabbit by the Micropuncture Technique

Sunita Bhattacharya, Matthew R. Glucksberg, and Jahar Bhattacharya

We have developed a micropuncture technique for the measurement of microvascular pressure in the intact lung of rabbit. We anesthetized 16 rabbits (halothane, 0.8%) and mechanically ventilated them through a tracheotomy. Then, we exposed the right lung by rib resection over the right anterior chest. We measured pulmonary artery, left atrial, and systemic pressures by direct catheterization and cardiac output by the thermodilution technique. For micropuncture, we stabilized the lung on a shelf and stopped ventilation for 3–4 minutes at an airway pressure of 7 cm H2O. We injected pancuronium intravenously to paralyze the diaphragm and intercostal muscles. Of the total pulmonary vascular pressure drop, 52% occurred in the microvascular segment between arterioles and venules of 20 µm diameter, 28% occurred in the arterial segment, and 20% occurred in the venous segment. We conclude that in the intact lung of rabbit, the major pressure drop occurs in the microvascular segment. (Circulation Research 1989;64:167–172)

Recently, we and others have used the micropuncture technique for measurements of lung microvascular pressures.1–4 These pressures crucially affect lung liquid filtration; hence, their determination is essential. We believe that among the methods available, only the micropuncture technique affords direct microvascular pressure measurement at determined anatomical sites. Consequently, the profile of pressures can be traced across the microvascular bed from arterioles to venules. However, for successful micropuncture, the lung must be free of motion. This condition is best met in the isolated perfused lung, which has therefore been the preparation of choice for lung micropuncture measurement.1–4

Despite its suitability for micropuncture, the isolated perfused lung differs from the intact lung in several important respects. In the isolated lung, vascular resistance is usually greater,1,4 vascular reactivity to vasoconstrictors is altered,2,5 nerve and lymphatic supplies are lacking and microvascular permeability is increased.5 To avoid these limitations of the isolated lung preparation, we believe it is important to develop the lung micropuncture technique in intact animals. Thereby, direct micropuncture measurements of microvascular pressures will be possible in intact lungs of living animals. Moreover, we expected that preparation of the intact lung would be much less traumatic than that of the isolated lung, since blood loss and lung excision can be avoided. We report an intact lung preparation developed in the anesthetized rabbit for microvascular pressure determination by the micropuncture technique.

Materials and Methods

Surgery

We induced anesthesia in 16 New Zealand White rabbits (weight, 3–4 g) initially with 5% halothane by face mask. Then, through a tracheotomy, we mechanically ventilated each animal with a mixture of 0.8% halothane in 100% O2. To record systemic arterial blood pressure and to withdraw samples for arterial blood gas analysis (Corning Blood Gas Analyzer Model No. 178, Corning, New York), we placed a catheter in the carotid artery. We set the ventilator (Harvard Instruments, South Natick, Massachusetts) to establish arterial PCO2 of 20–25 mm Hg and pH of 7.4–7.5. Usually this required tidal volume of 30 ml at respiratory frequency of 21/min. Arterial PO2 always exceeded 400 mm Hg. Body
temperature was maintained at 39–40°C by means of a heating blanket (Harvard Instruments), and the right jugular vein was cannulated to deliver intravenous infusions. Plasma total protein concentration and blood hematocrit were determined at the beginning and end of the experiment.

To expose the right lung, we removed ribs three to eight over the right chest. Right thoracotomy revealed the entire right ventricle and its outflow tract as well as the right margin of the left atrium, which lay immediately dorsal to the sternum. To record pressures, catheters were inserted into the pulmonary artery and the left atrium. While pressure was continuously monitored, a catheter was directed into the pulmonary artery through a right ventricular incision. Catheter placement was confirmed by appearance of the pulmonary artery waveform in the recorded tracing. The pulmonary artery catheter possessed a sleeve 2 cm from the tip to prevent it from slipping out of the ventricle. We secured the catheter by means of a purse-string suture on the ventricle. Next, we pulled up the left atrium and inserted a catheter into the left atrial cavity through a left atrial incision.

The catheters were connected to pressure transducers (Statham P23ID, Gould, Cleveland, Ohio), for which zero references were taken at the level of micropuncture. All pressure tracings were displayed on a multi-channel strip-chart recorder (Gould 2800S). We determined cardiac output by the thermodilution technique (Cardiac Output Computer Com-1, American Edwards, Irvine, California): saline was injected into the left atrium and temperature transients were recorded by means of a thermistor (94-030-2.5F, American Edwards) introduced into the descending aorta through a femoral artery.

**Lung Micropuncture**

Motion at the lung surface, attributable to cardiac and ventilatory movements, presented a major impediment to micropuncture of the intact lung. To reduce interference from cardiac movements, we placed the inferior margin of the right middle lobe on a shelf (Figure 1). Then, we raised the shelf 4 cm above heart level. Thus, about half of the costal surface of the right middle lobe was accessible to micropuncture. In every experiment, we confirmed that shelf placement had no effect on pulmonary

![Image of open-thorax preparation for lung micropuncture](http://circres.ahajournals.org/)

**FIGURE 1.** Open-thorax preparation for lung micropuncture. A metal shelf covered with moistened gauze lies between the heart and the right middle lobe. Zero reference of the servo-null pressure measurement system is obtained by immersing the micropuncture pipette in a pool of saline as shown. About 2 cm working distance is available between microscope objective and lung. Illumination is by means of a fiberoptic light tube. Note that about half the costal surface of the right middle lobe is accessible for micropuncture.
artery or left atrial pressures. To abolish interference from lung movements, we stopped the ventilator for up to 4 minutes at the time of micropuncture. Between successive periods of stopped ventilation, we allowed 30–40 minutes for recovery. During stopped ventilation, we infused 100% O₂ to maintain constant inflation at airway pressure of 7 cm H₂O. We induced complete neuromuscular blockade (pancuronium, 0.2 mg/kg i.v. hourly) to abolish interference from movements of the thoracic wall and the diaphragm. Despite all these procedures, however, some residual surface motion remained.

We measured microvascular pressures in subpleural microvessels of the right middle lobe by means of our lung micropuncture technique as previously described. However, differing from our previous procedures, we did not apply a suction ring on the lung surface (Figure 1). We covered the lung surface with oil at 37°C to prevent drying. We viewed the lung through a stereo microscope (X150, Olympus Model SZH) to identify 20–25 μm diameter arterioles and venules, which we micropunctured by means of glass micropipettes (tip diameter, 3 μm) connected to a servo-null pressure measurement system (IPM, Model 5A). Microvessel diameter was determined by means of a calibrated reticle in the microscope eyepiece. Zero for the micropressure system was obtained by immersing the pipette tip in a pool of saline contained in the peripheral gutter of a metal ring that was positioned at the level of micropuncture (Figure 1).

All micropuncture readings were obtained in the 3–4-minute periods of stopped ventilation and within 5 hours of surgical lung exposure. During stopped ventilation we measured cardiac output, arterial pH, Pco₂, and Po₂ before and after each micropuncture measurement. To prevent large increases of arterial Pco₂, the rabbit was hyperventilated between periods of stopped ventilation. Figure 2 shows examples of microvascular pressure tracings. We accepted micropuncture measurements that satisfied the following criteria: 1) a steady reading for at least 1 minute, 2) simultaneous transients in the pulmonary artery and microvascular pressure tracings after intravenous injection of a 2 ml saline bolus (Figure 2), 3) similar timing of pulsations in the microvessel and the pulmonary artery, and 4) matching of zero baselines before and after each reading. In a few instances, successful micropuncture was verified by means of dye injections through the micropipette. On the basis of these criteria, we had a micropuncture success rate of ~20%.

<table>
<thead>
<tr>
<th>Condition</th>
<th>pH</th>
<th>Pco₂ (mm Hg)</th>
<th>Po₂ (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>7.4±0.1*</td>
<td>27±6</td>
<td>&gt;400†</td>
</tr>
<tr>
<td>Stopped ventilation</td>
<td>7.2±0.1$</td>
<td>62±16$</td>
<td>&gt;400†</td>
</tr>
</tbody>
</table>

*Mean±SD for 16 experiments.†Inflation with 100% O₂.‡Duration, 4 minutes.§p<0.05 compared with top row.
TABLE 2. Effect of Arrested Ventilation on Hemodynamic Variables in Anesthetized Rabbit

<table>
<thead>
<tr>
<th>Condition</th>
<th>Heart rate (beats/min)</th>
<th>Cardiac output (l/min)</th>
<th>Pulmonary artery</th>
<th>Left atrial</th>
<th>Aortic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>270±41*</td>
<td>0.4±0.1</td>
<td>18±4.3</td>
<td>0±2.3</td>
<td>93±20</td>
</tr>
<tr>
<td>Stopped ventilation†</td>
<td>250±43‡</td>
<td>0.3±0.2‡</td>
<td>15±4.2‡</td>
<td>0±3.2</td>
<td>113±18‡</td>
</tr>
</tbody>
</table>

*Mean±SD for 16 experiments.  
†Duration, 4 minutes (inflation with 100% O2).  
‡p<0.05 compared with top row.

In one group of 11 rabbits, either only arterioles or only venules were micropunctured in each experiment. In another group of 5 rabbits, data were obtained from an arteriole and a venule in every experiment. Since the measurements were similar in both groups, all data were analyzed together. At the end of the experiment, the lungs were removed for determination of extravascular lung water content by techniques that are standard in our laboratory.1

Data Analysis

The pressure data was analyzed as described previously.7 The pressure gradients from pulmonary artery to arteriole and venule to left atrium were averaged and the gradient means were added to the mean pulmonary artery and the mean left atrial pressures, respectively, to compute mean arteriolar and venular pressures. Data are expressed as mean±SD. We used the t test for all paired comparisons and accepted a significance level of p<0.05.

Results

Throughout the experimental period, systemic blood pressure remained steady. Hematocrit fell from 38±3% to 31±3%, and plasma protein concentration from 4.8±0.5 to 3.2±0.5 g/dl. Since there was practically no blood loss, we attribute these changes to hemodilution caused by the saline injections required for vascular transients and cardiac output measurements. Mean extravascular lung waters were similar for the right (3.7±0.6 g/g dry wt) and the left (3.8±0.6 g/g dry wt) lungs. Therefore, our procedures did not make the lungs edematous.

Tables 1 and 2 show the effects of 4 minutes of stopped ventilation on blood gases and general circulatory variables. The fall in arterial pH and the rise in arterial Pco2 were both significant. However, pulmonary artery pressure fell by only 16%. In addition, heart rate and cardiac output fell and systemic arterial pressure rose slightly.

Table 3 shows the distribution of mean vascular pressures from 33 microvessels from 16 experiments. Of the total pressure drop of 13.8 cm H2O, 28% occurred in the arterial segment, 52% occurred in the microvascular segment between arterioles and venules of 20–25 μm diameter, and 20% occurred in the venous segment.

Discussion

This is the first report of micropuncture data from the lung of anesthetized rabbit. We chose the rabbit because the species is extensively used for studies of lung liquid balance. Moreover, we wished to develop a small animal model for intact lung micropuncture. Our experiments demonstrate the feasibility of lung micropuncture in the living rabbit for the measurement of microvascular pressure. The preparation remained stable for at least 5 hours after thoracotomy. The surgical procedures caused practically no blood loss, and they did not adversely affect lung fluid balance because extravascular lung water was normal in both lungs.

The main difficulty was that ventilation had to be stopped to reduce lung motion; hence, only 3–4 minutes were available for micropuncture. The absence of lung motion is a critical requirement for successful lung micropuncture. In anesthetized dog, the left and right lungs can be ventilated separately.7 Thus, one lung can be held at constant inflation for micropuncture while the other is continuously ventilated to maintain the preparation. This strategy proved unworkable in rabbit because the narrow airway did not accommodate separate ventilation lines to each lung.

It should be noted that at the level of micropuncture, zone 2 conditions (alveolar>left atrial pressures) prevailed. Since the micropuncture level

TABLE 3. Distribution of Vascular Pressures in Lung of Anesthetized Rabbit

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pulmonary artery</th>
<th>Arteriole (20–25 μm)*</th>
<th>Venule (20–25 μm)*</th>
<th>Left atrium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure† (cm H2O)</td>
<td>13.9±5.5‡</td>
<td>10±3.3</td>
<td>2.9±2.3</td>
<td>0±2.7</td>
</tr>
<tr>
<td>No. of measurements</td>
<td>14</td>
<td>16</td>
<td>17</td>
<td>16</td>
</tr>
</tbody>
</table>

* Diameter.  
† Data from 16 rabbits.  
‡ Mean±SD.
was 4 cm above the left atrium, we expect that the entire right middle and upper lobes and a major part of the right lower lobe were also in zone 2. However, at the most dependent parts of the lung, where zone 3 conditions (alveolar < left atrial pressures) may have existed, the lung vascular pressure profile may have differed from our measurements.

Microvascular Pressure Profile

Our results indicate that of the total pressure drop in the intact rabbit lung, 52% occurred between arterioles and venules of 20 µm diameter. Therefore, the major vascular pressure drop occurred in the microvascular segment. Previous micropuncture data from isolated lung indicate that of the total pressure drop, the microvascular pressure drop is 60% in rabbit, 78% in dog, and 44% in cat. Thus, according to the micropuncture data from several species, the largest pressure drop in the pulmonary circulation occurs in the microvascular segment. However, based on indirect measurements, others disagree. We cannot be sure that the micropuncture pressure measurements represent vascular pressures in the lung interior. However, recent estimates of the lung microvascular pressure drop obtained by indirect methods agree with findings from micropuncture.

The possibility should be considered that during the 3–4 minutes of stopped ventilation, a redistribution of lung microvascular pressures occurred secondary to the changes in PCO2 and pH of arterial blood, but cardiovascular changes were minor. Although arterial PCO2 increased on average to 62 mm Hg during stopped ventilation, heart rate and cardiac output only fell slightly and systemic blood pressure remained stable. Recordings of pressure transients indicate that after a step increase of PCO2 to above 70 mm Hg, pulmonary vasoconstriction does not occur for 5 minutes. Therefore, it is unlikely that in our experiments, significant changes in pulmonary vascular tone occurred in the 3–4 minutes of stopped ventilation. In fact, during stopped ventilation, pulmonary vascular resistance (calculated as the difference between pulmonary artery and left atrial pressures divided by cardiac output) increased by only 11%. We confirm a fact that has been long known, that present levels of arterial PCO2 and pH have minor effects on lung hemodynamics. We point out that micropuncture readings were obtained toward the beginning of the period of stopped ventilation, whereas blood gas samples were obtained 1–2 minutes after micropuncture. Therefore, the PCO2 and acidosis we recorded for stopped ventilation probably overestimate values at the time of micropuncture. Moreover, our pressure measurements agree with similar data obtained at normal values of arterial PCO2 and pH. Therefore, in the micropuncture period, we do not believe that a significant redistribution of lung vascular pressures occurred.

Our present determinations of the arterial and microvascular pressure drops in the intact rabbit lung are similar to those reported by Raj et al for isolated rabbit lung. Raj et al used papaverine to fully vasodilate the isolated rabbit lung. The similarity of our respective data might suggest that the arterial and microvascular segments of the intact lung are practically devoid of vascular tone. In the venous segment, however, we recorded a pressure drop which was almost three times that reported by Raj et al. Our data were obtained in zone 2, whereas Raj et al obtained theirs in zone 3 conditions. We have reported elsewhere that the pulmonary venous pressure drop is higher in zone 2 than in zone 3. Hence, the difference in the venous segmental pressure drops may be attributable to the differences in zonal conditions.

In anesthetized dog, we have reported a lung vascular pressure profile for zone 2 conditions that is remarkably similar to the present data. In rabbit and dog, respectively, the pressure drops are 3.9 and 3.2 cm H2O from pulmonary artery to arterioles, 7.1 and 5.7 cm H2O from arterioles to venules, and 2.8 and 6.2 cm H2O from venules to left atrium. The main difference, which we cannot explain, seems to be that the postvenular pressure drop in dog is approximately double that of rabbit. However, the similarities in the arterial and the microvascular segmental pressure drops suggest that despite disparities in lung size, vascular network geometries in the two species are alike.

Acknowledgment

We thank Terecita Cruz for technical assistance.

References


**KEY WORDS** • intact lung • lung microvascular pressure • lung micropuncture • pulmonary circulation
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