Micropuncture Measurement of Lung Microvascular Pressure Profile during Hypoxia in Cats

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SUMMARY. To determine the lung microvascular pressure profile during hypoxia, we micropunctured the subpleural microcirculation of isolated perfused cat lungs. Our procedures involved exsanguinating a cat, then cannulating its pulmonary artery, left atrium, and trachea. Using the cat's own blood, we perfused the lungs at pulmonary artery and left atrial pressures of 18 and 9 cm water, respectively, to obtain lung blood flow of 81 ± 29 ml/(kg body weight x min), which we held constant throughout the experiment. We stabilized the lung surface with a vacuum ring and micropunctured 30- to 50-μm arterioles and venules to measure microvascular pressure by the servo-null method. During micropuncture, we held the lungs at constant inflation using airway pressure of 8 cm water. We varied the oxygen concentration of the inflation gas from 30% during baseline to 2% during hypoxia. We studied groups with high (>7.5) or normal pH. During normoxia, 27, 44, and 29% of the pressure drop occurred in the arterial, capillary and venous segments, respectively. During hypoxia, the increase in pulmonary vascular resistance, which was marked in both groups, was significantly greater in the normal pH group. All segmental pressure drops increased significantly during hypoxia. However, the predominant increase occurred in the arteries where segmental pressure drop increased by 148% and 210%, respectively, in the high and normal pH groups. We conclude that the major site of hypoxic vasoconstriction is in the pulmonary arteries. (Circ Res 54: 90-95, 1984)

THE subject of hypoxic pulmonary vasoconstriction (its existence, site, and mechanism) has evoked continual controversy, since its modern description nearly 40 years ago (Von Euler and Liljestrand, 1946). This paper deals only with the site of the hypoxia vasoconstriction.

Although evidence in favor of small muscular pulmonary artery constriction predominates (Bland et al., 1976; Dawson et al., 1978, 1979; Fishman, 1976; Kato and Staub, 1966; Sackner et al., 1966), there is some support for pulmonary venous constriction. Bressack and Bland (1980) suggested that venous constriction may occur in the hypoxic newborn lamb because lung liquid filtration is increased, contrary to what occurs in the adult sheep.

The hypothesis that an extravascular mechanism exists has achieved some favor. Kapanci and associates (1974, 1975) proposed that contractile interstitial cells in the alveolar wall are responsive to low Po2. They believed the cells contracted, causing folding of the alveolar walls and an increase in capillary resistance. Mazzzone (1980) obtained ultrastructural evidence to support that view. Mitznner and Sylvester (1981) studied pressure-flow relationships and liquid filtration rates in the isolated, perfused pig lung and proposed a Starling resistor model in which hypoxic vasoconstriction is interpreted in terms of a change in critical closing pressures. The Starling resistor appears to be located at the alveolar level.

Hakim et al. (1983) have interpreted their arterial and venous occlusion experiments in terms of a mechanism that does not involve vascular smooth muscle contraction, but, rather, contractile elements within the alveolar wall, which caused alveolar wall folding and increased capillary resistance.

We have used direct lung micropuncture in the perfused cat lung to determine the longitudinal distribution of vascular pressure during normoxia and hypoxia. Acute alveolar hypoxia caused mainly arterial vasoconstriction, but there was a small component of venous constriction.

Methods

We used 30 cats of either sex (body weight 3.4 ± 1.4 kg) anesthetized with pentobarbital sodium (50 mg/kg, ip). We performed a tracheostomy and ventilated each animal, cannulated both carotid arteries, injected heparin intravenously (1000 U/kg), and exsanguinated each animal through one carotid artery, while we simultaneously infused 250 ml of 5% albumin and lactated Ringer's solution through the other artery. This technique allowed us to collect approximately 350 ml of blood (hematocrit 11%, total protein 5 g/dl) to use in our perfusion apparatus.

Following exsanguination, we made a median sternotomy and cannulated the main pulmonary artery via the right ventricle and the left atrial appendage with 6 mm o.d. plastic tubing. To avoid introducing air into the blood vessels during cannulation, we occluded the vessel distal
to the cannulation site with a bulldog clamp. Using 5% albumin in saline, we briefly washed out the pulmonary circuit via the left atrium (retrograde) to remove any pulmonary arterial emboli. We then started lung perfusion in the normal direction. The total time from opening the rib cage to the onset of perfusion was less than 15 minutes. The lungs were not removed from the cat.

The animal was placed on the micropuncture table and perfused at constant flow according to the circuit shown in Figure 1. Briefly, blood returned by gravity to the venous reservoir, was propelled via a roller pump (model 3500, Sarns) through a heat exchanger (Miniprime, 57 ml, Travenol Labs), a microfilter (Ulripore, Pall Products), and, finally, through a bubble trap back to the pulmonary artery. We monitored blood temperature (37°C ± 1°C) using a thermometer in the pulmonary artery line near the lung and blood flow using an ultrasonic flow meter (Parks Electronics). To measure vascular pressures, we placed catheters near the tips of the pulmonary artery and left atrial cannulas and connected them to strain gauges.

An additional feature of this circuit is the presence of a Starling resistor between the arterial line and the venous reservoir. The resistor was surrounded by a pressure of 60 cm H2O. The resistor allowed us to stop blood flow into the lung for our zero flow test during micropuncture without stopping flow in the external circuit. Under normal operating flow conditions, there was essentially no flow through the Starling resistor.

The lungs were ventilated with appropriate gas mixtures between micropuncture attempts. During micropuncture, the lungs were held inflated at a constant airway pressure of 8 cm H2O. Blood was sampled hourly through T-pieces into the lung for our zero flow test during micropuncture.

Lung Micropuncture

To measure lung microvascular pressures, we micropunctured the lung as we have previously described (Bhattacharya and Staub, 1980; Bhattacharya et al., 1982). Briefly, we used beveled micropipettes with 2-μm tip diameter, filled with 2 M NaCl colored with green dye. The micropipette was attached to a holder in a micromanipulator (Leitz). Pressures were measured by the servonull technique (model 3, Inst. Physiol. Med.).

The key to successful lung micropuncture was stabilization of the lung surface (usually, the right middle or either lower lobe) with a vacuum ring. We then viewed the lung surface through a stereomicroscope (120X, Leitz). We filled the vacuum ring with saline and micropunctured subpleural arterioles and venules (30-50 μm in diameter) under direct vision. These vessels were chosen because they are relatively easy to find and to puncture. Micropuncture measurements at these sites permitted us to clearly distinguish segmental pressure drops among arterial (pulmonary artery to arteriole), alveolar wall (arteriole to venule), and venous (venule to pulmonary vein) segments.

Since flow is constant and subpleural microvessels appear to represent lung microvessels in general (Overholser et al., 1982; Linehan et al., 1982), the segmental pressure drops are equivalent to segmental resistances.

We leveled all strain gauges to the micropipette tip and further ascertained the validity of each micropuncture pressure measurement, using standard criteria (Bhattacharya et al., 1982) and a new zero flow test.

An example of the zero flow test is shown in Figure 2. We clamped both arterial and venous lines, which caused rapid equilibration of pressure throughout the system. At this point, the intravascular pressure at three points, namely, pulmonary artery, pulmonary vein, and micropunctured vessel, should be equal. By this test, we can guarantee the validity of each microvascular pressure reading. The Starling resistor bypass made this test simple to do. If the micropressure deviated from arterial and venous pressures by more than 1 cm H2O, that datum was rejected. For smaller deviations, the micropressure was corrected by the offset value. Corrections were necessary in about 30% of the measurements. The average correction was <0.5 cm H2O. Occluded arterial and venous pressures never differed from each other by more than 0.5 cm H2O.
Experimental Protocol

We ventilated the lung at 20/5 cm H_2O (inspiratory/expiratory pressure) with 30% O_2, 6% CO_2, and 64% N_2 (normoxic gas) for several minutes, then switched to constant airway pressure equal to 8 cm H_2O. We adjusted flow until pulmonary arterial pressure was approximately 18 cm H_2O. The venous line was adjusted to an outflow pressure of 9 cm H_2O. Once established, flow was maintained constant throughout the experiment. We then micropunctured the lung and measured pressure in arterioles and venules, attempting to obtain at least two arterioles and one venule in each lung. The duration of normoxic micropuncture averaged about 1.5 hours.

For hypoxia, we ventilated the lung at the same airway pressures with the gas mixture containing 2% O_2, 6% CO_2, and 92% N_2 (hypoxic gas). We required that, at constant flow, the driving pressure (pulmonary vascular resistance) should increase at least 50% and achieve a sustained plateau. We tried to puncture the same or adjacent arterioles and venules during the hypoxic period, as we had in normoxia. This portion of the experiment required 1.5-2 hours. On the average, blood flow was 81 ± 29 ml/kg body weight per min (249 ± 66 ml/min total).

We will report two series of experiments. The initial group, although suitably hypoxic, were alkalemic (pH > 7.50). This was in part because of CO_2 diffusion through the lung surface and because the venous reservoir was open to air. In the second series, we wrapped the lung in gas-tight plastic, covered the venous reservoir with parafilm, and flushed it with hypoxic gas mixture and used degassed saline in the vacuum ring over the micropuncture site.

Data Analysis

To compare segmental pressure drops, we used an analysis of variance, then applied the Neuman-Keul's test. To compare differences between normoxic and hypoxic groups, we used the paired t-test, and to compare the high and normal pH groups we used an unpaired t-test. We accepted P < 0.05 as indicating statistical significance.

Results

Pulmonary Microvascular Profile in the Normoxic Cat

We measured the microvascular pressure profile in 30 normoxic cats, of which 15 also had measurements made during hypoxia. The other 15 include animals with unsustainable hypoxic vasoconstriction or that had served as time controls in which microvascular pressures and flow were measured for 3-4 hours.

The data are summarized in Table 1. Forty-four percent of the total pulmonary vascular pressure drop is located between the arterioles and venules, that is, within the alveolar wall microvessels (small arterioles, capillaries, smallest venules). Under the conditions of our experiment, the pressure drops through the arterial and venous segments are similar.
TABLE 4
Blood Gases during Micropuncture Experiments in Perfused Cat Lungs (Normal pH)

<table>
<thead>
<tr>
<th>Condition</th>
<th>No.</th>
<th>$P_{O_2}$ (mm Hg)</th>
<th>$P_{CO_2}$ (mm Hg)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoxia</td>
<td>6</td>
<td>170 ± 2</td>
<td>32 ± 4</td>
<td>7.40 ± 0.07</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>6</td>
<td>37 ± 5</td>
<td>34 ± 5</td>
<td>7.39 ± 0.06</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD.

Micropuncture Records

As already mentioned, Figure 2 shows one example of a successful puncture of a venule, 50 μm in diameter. The duration of puncture was 2 minutes. Two tests of micropipette location and calibration are shown. At the first vertical dashed line, the venous reservoir was elevated slightly for 10 seconds. It is necessary that the micropipette respond promptly and return to the baseline level, when the reservoir is lowered. The second vertical dashed line shows the zero flow calibration test, as already described.

Pulmonary Vascular Pressure Profile in Hypoxia

Alkalosis

The blood gas data for the nine experiments in this group are shown in Table 2. During both normoxia and hypoxia, pH was high and $P_{CO_2}$ was low.

The pulmonary vascular pressure profiles are summarized in Table 3. The nine cats are a subgroup of the 30 cats used in the entire study. There are no significant differences between the normoxic values of this table and those of Table 1.

During hypoxia, total lung vascular resistance always increased, the average increase being 74%. Likewise, there were increases in pressure drop in all segments. The pressure difference between the pulmonary artery and the arterioles increased 148%, the pressure difference from arterioles to venules increased 26%, and the pressure difference from venules to vein (left atrium) increased 91%. Blood flow through the lungs averaged 78 ± 28 ml/kg per min.

Normal pH

The blood gas data for the six experiments in this group are shown in Table 4. pH averaged 7.4 and $P_{CO_2}$ averaged 33 mm Hg.

The pulmonary vascular pressure profiles are summarized in Table 5. The six cats are a subgroup of the 30 cats used in the entire study. The results during normoxia are not different from those shown in Tables 1 and 3. During hypoxia, the whole lung vascular resistance always increased: the average resistance increase was 110%, which is a significantly greater increase than in the alkalotic group. Likewise, the pressure drop through each segment increased. The pulmonary artery to arteriole pressure drop increased 210%, arteriole to venule by 55%, and the venule to vein (left atrium) by 67%. The absolute increases in all segmental pressure drops are greater than in the high pH (alkalosis) group (Table 3), even though the percent increase in the venous segment pressure drop is less. Blood flow through the lungs in the normal pH group averaged 80 ± 20 ml/kg per min.

Discussion

The reason we used cats in these experiments is that they gave more consistent and sustained hypoxic vasoconstriction than we could achieve in dogs. We did not use any arachidonate cascade inhibitors, as has been suggested (Newman et al., 1980).

The pressure profile in zone III normoxic cats differs somewhat from that in the dog (Bhattacharya et al., 1980). In the cat, there is a greater pressure drop (resistance) in the arterial and venous segments. We believe these are real differences between the dog and the cat, especially since the isolated cat lung showed a much better and more sustained hypoxic vasoconstriction than did the isolated dog lung.

In spite of the differences, the absolute pressure drop between the arterioles and venules is very similar to that in the dog. In the cat, 44% of the total pressure drop occurred between the arterioles and venules. We believe this can be explained mainly by the microvascular geometry (Overholser

TABLE 5
Pulmonary Vascular Pressures in Perfused Cat Lungs (Normal pH)*

<table>
<thead>
<tr>
<th>Condition</th>
<th>No.</th>
<th>Artery (30–50 μm)</th>
<th>Venule (30–50 μm)</th>
<th>Vein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoxia</td>
<td>6</td>
<td>18.2 ± 0.5</td>
<td>15.2 ± 0.7</td>
<td>12.4 ± 0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(18f)</td>
<td>(18f)</td>
<td>(11f)</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>6</td>
<td>28.3 ± 2.6</td>
<td>19.0 ± 3.7</td>
<td>145.5 ± 3.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(19f)</td>
<td>(19f)</td>
<td>(9f)</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± sd.

* Pressures in cm H2O relative to the level of the micropipette tip.

† Number of punctured vessels.
et al., 1982). The revised mathematical model of Linehan et al. (1982) brings venous occlusion results into reasonable agreement with our direct measurements under comparable conditions.

In Figure 3, we show the pressure profiles in normoxia and the two hypoxic conditions. For this graph, we combined all of the normoxia data. The slopes of the lines joining the segments reveal the relative changes.

Although the direct micropuncture data speaks for itself, a few points should be emphasized. Clearly, under hypoxic conditions, with or without pH adjustment, the predominant site of hypoxic vasoconstriction is within the arterial segment (pulmonary artery to 30- to 50-μm arterioles). This is consistent with the findings of Kato and Staub (1966), which showed quantitative histological evidence for constriction of small pulmonary arteries in the cat.

Venous constriction also occurred, although to a much lesser extent than in the arteries. Such effects are consistent with the data of Morgan et al. (1968) and of Bressack and Bland (1980).

The segment between the arterioles and venules also showed some increased pressure drop. Although this is consistent with the hypothesis of alveolar wall folding (Kapanci et al., 1974), there are also some muscular arteriolar and venular elements present. Until we do further experiments using capillary puncture, no decision about the mechanism of the alveolar wall resistance increase can be reached. However, even if all of the increase in pressure drop within the arteriole-venule segment is ascribed to interstitial cell contraction and alveolar wall plication, it is still slight relative to the major vasoconstriction in the arteries and the lesser vasoconstriction in the veins.

Finally, we come back to the question as to whether the micropuncture at the lung surface represents the whole lung. Although there are differences in the capillary density between the subpleural and the deep lung parenchyma (Miller, 1947; Guntheroth et al., 1982), in the zone III normoxic lung, the pressure profiles we have obtained by subpleural micropuncture agree well with those predicted for the whole lung (Overholser et al., 1982; Linehan et al., 1982). The possibility of oxygen and CO₂ diffusion through the pleura in these experiments poses a difficulty. Clearly, in the alkaletic group, the blood gases were not equal to the PO₂ and PCO₂ in the inspired gas mixture.

The problem of pleural gas diffusion and exchange within the perfusion circuit, which was not completely air-tight, is a technical one. It is impractical to isolate the lung completely in a chamber (unless we use an air-tight room) because that interferes with the micropuncture procedure. We believe that the second series of experiments in which we wrapped the lung in essentially gas-tight plastic wrap, except for the micropuncture site, and closed the venous reservoir greatly reduced the possibility of uneven alveolar PO₂ and PCO₂ distribution. We were also concerned about the PO₂ in the saline over the lung surface at the micropuncture site. Although the saline layer is more than 2 mm thick, and although the solubility of oxygen in saline is low, we tested the effect of filling the suction ring with degassed saline. The results were not different. We are convinced that the segments in which we measured microvascular pressure are reasonably representative of the whole lung. Our conclusions are similar to those obtained by whole lung methods (Dawson et al., 1979; Kato and Staub, 1966; Sackner et al., 1966), namely, that the major site of hypoxic pulmonary vasoconstriction is in the pulmonary arteries.

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**Figure 3.** Comparison of pressure profile through the cat pulmonary circulation in normoxia (O) (data for all 15 cats averaged), hypoxia at high pH (●) (nine cats), hypoxia at normal pH (●) (six cats). Vertical bars are 1 s.e. In hypoxia, with or without control of pH, the main site of the vasoconstriction is in the arteries, but there are also statistically significant increases in the pressure drops through the other segments.

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