Sites of Pulmonary Vasomotor Reactivity in the Dog during Alveolar Hypoxia and Serotonin and Histamine Infusion

JON B. GLAZIER and JOHN F. MURRAY

From the Department of Medicine and the Cardiovascular Research Institute, University of California, San Francisco General Hospital, San Francisco, California 94110

ABSTRACT In order to evaluate separately changes in vascular tone occurring in arteries and veins, we measured pulmonary capillary red blood cell (RBC) concentration under zone II (waterfall) conditions in isolated dog lungs rapidly frozen with Freon 12. The lungs were frozen while being perfused from artery to vein and from vein to artery breathing normal and hypoxic gas mixtures and during infusions of serotonin and histamine. Changes in capillary RBC concentration which occurred during the experimental conditions indicated an alteration in vascular resistance upstream from the capillaries. Alveolar hypoxia caused a significant decrease in capillary RBC concentration during forward perfusion, but no change from the control values during reverse perfusion. Serotonin infusion caused a decrease in RBC concentration during forward perfusion comparable with that of hypoxia and a small but significant decrease during reverse perfusion. Histamine infusion caused no change in RBC concentration from control values during forward perfusion, but a large decrease during reverse perfusion. We conclude that vasoconstriction occurs (*a*) exclusively in arteries during alveolar hypoxia, (*b*) predominantly in arteries but to a lesser extent in veins during serotonin infusion, and (*c*) exclusively in veins during histamine infusion.

INTRODUCTION

We have attempted to localize the site of the pulmonary vasoconstriction which occurs in response to alveolar hypoxia and to infusions of histamine and serotonin into the pulmonary circulation. The vasoconstriction that occurs with alveolar hypoxia is of particular clinical interest, and although many attempts have been made in the past to define the site of the change in vascular resistance, the results have been contradictory. The location of the response has been variously ascribed to occur in arteries (1, 2), capillaries (3), and veins (4). The reason for these discrepancies lies in the difficulty of making direct measurements of changes in vascular dimensions in different parts of the pulmonary circulation.

We have devised a new method in order to evaluate separately changes occurring in arteries and veins. We made histological measurements of the number of red blood cells in alveolar capillaries in dog lungs rapidly frozen while being perfused from artery to vein and from vein to artery. We have used the changes in the number of capillary red blood cells, which occurred during the experimental conditions, as an indicator of changes in vascular resistance occurring upstream from the capillaries.

METHODS

Preparation

Large mongrel dogs (mean 27 kg) were anesthetized with Nembutal (Abbott Laboratories, North Chicago, Ill.). A glass cannula was tied into the trachea, and a large bore polyethylene cannula was inserted into the femoral vein. The left chest was opened through the fourth interspace, and the animal then received intravenous heparin.

An initial 400 ml of blood was drained off through the femoral vein catheter (30–40 sec). The heart was then stopped by applying a current directly to the left ventricle, and the remainder of the blood was allowed to drain off. The dog was killed in this manner in order: (*a*) to collect as much blood as possible for subsequently perfusing the lungs but to avoid having the dog liberate vasoactive substances into the blood while dying slowly during exsanguination, and (*b*) to maintain a low mean circulatory pressure immediately after the heart was stopped (5).

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The entire chest wall down to the diaphragm was then removed by cutting the ribs 2 cm from the spine. Cannulas were tied into the right ventricular outflow tract and left atrium, and perfusion of the lungs was begun. From the time the heart was stopped to the time blood flow was restarted averaged 18 min (13–33 min).

The perfusion circuit was primed with normal saline, and then the blood from the animal was added. As much saline as possible was displaced, and the hematocrit of the perfusion circuit averaged 39% (30–46%). Blood was pumped by a nonpulsatile roller pump through a heat exchanger which kept the blood temperature at 37°C, bubble trap, and depulsator into the pulmonary artery; blood left the left atrium and flowed into a venous reservoir and thence back to the pump. The inflow tubing just proximal to the pulmonary arterial cannula was connected to the outflow tubing just distal to the left atrial cannula by two separate pieces of tubing. These connections were left clamped during perfusion from artery to vein (forward perfusion), but the clamps could be rearranged so that blood entered the left atrium, exited from the pulmonary artery (reverse perfusion), and flowed from there into the venous reservoir. Arterial and venous pressures were measured by saline manometers connected to the circuit within 3 cm of the arterial and venous cannulas. Inflow pressure could be varied by adjusting the output of the roller pump, and outflow pressure by raising or lowering the venous reservoir. Blood flow was measured by collecting timed samples in a graduated cylinder.

The surgery was done with the dog lying supine in a stand which supported its back and held the legs directly out from the body. Once perfusion was started the stand was placed in an upright position. The lungs, which had been allowed to deflate once the dog was exsanguinated, were then slowly inflated by a positive pressure to 25 cm H_2O and were supported at the apexes by spring clips to prevent them from falling over in expiration. The lungs were ventilated with a Harvard respirator at a tidal volume sufficient to raise transpulmonary pressure from 5 cm H_2O (end expiration) to 10 cm H_2O. Every 5 min the lungs were inflated to a pressure of 20 cm H_2O to prevent atelectasis.

Ventilation was begun and continued for 5 min with a mixture of 93.5% O_2, 6.5% CO_2, balance N_2. The lungs were then ventilated with 14.5% O_2, 6.5% CO_2, and balance N_2 (control mixture) which gave alveolar gas tensions of approximately 100 mm Hg for O_2 and 40 mm Hg for CO_2. This gas mixture was used for the control measurements and during the infusion of histamine and serotonin.

For the measurements made during alveolar hypoxia the lungs breathed a mixture of 3% O_2, 6.5% CO_2, balance N_2 (alveolar gas tensions: P_{O_2} 30 mm Hg, P_{CO_2} 40 mm Hg). Using the technique described above for killing the dog and perfusing the lungs, we were able to obtain an increase in inflow pressure (range 3–17 cm H_2O) in every lung which breathed this low O_2 gas mixture.

Freezing technique. The lung was frozen with Freon 12 cooled to −150°C with liquid N_2. A brass tube 1 cm in diameter and pierced by small holes at 1 cm intervals was positioned beside the left lung so that the upper hole was oppositie the apex and the bottom hole was opposite the base. The tube was connected by plastic tubing to a funnel clamped about 1 ft above the lung. The liquid Freon was poured into the funnel and sprayed out of the holes in the brass tube rapidly freezing a strip of lung about 25 cm in length. The Freon spray onto the lung lasted about 30 sec. The lung was then rapidly cut free (about 15 sec) and placed in a box of liquid N_2. Just before the freeze the lung was marked with a soft felt pen at 5-cm intervals from apex to base so that even if the lung buckled while being cut down the original heights of the tissue relative to the vascular pressures were obvious.

Preparation of tissue. Using a band saw, blocks of tissue of approximately 1 cc were cut from the strip of pleural surface which had been frozen with Freon at 5-cm intervals down the lung from apex to base. The lung was out of liquid N_2 for only a brief period and never allowed to thaw. The tissue blocks were freeze-dried for 18 hr (Thermovac FD-1; Thermovac Industries Corp., Copiague, N. Y.) and then double embedded in a 2% celloidin solution and paraffin wax. Sections were cut to 2 μ using a rotary microtome and stained with hematoxalin and eosin.

Histological measurements. In the 2 μ section at each level sampled, the number of red blood cells was counted in each of 30 alveolar septa using a light microscope with a magnification of 400. The length of each septum was measured with an eyepiece graticule, and the number of red blood cells was expressed as red blood cells per 10 μ septum. No red cells were counted within 10 μ of the junction of two or more septa, and the length of each septum had to be at least 20 μ. All of the septa counted lay within 0.5–3.0 mm of the pleural surface which was visible in each section, i.e., we did not measure the number of red blood cells in the capillaries immediately adjacent to the pleural surface. Counting was begun at one end of the section (about 1 cm long), and each septum that met the criteria was counted as the slide was moved and the septum came into the field of view. Since alveolar size is constant in this lung preparation (6), this measurement will not be influenced by some septa having a greater density of capillaries per unit length (i.e. if some alveoli were smaller) than others.

Details of the histological techniques, the validity of the measurements (there is no reason to suspect that measurements made on alveoli close to the pleural surface are not representative of those elsewhere in the lungs), and photographs of the tissue sections exposed to different vascular pressures have been published previously (6).

Procedure. Most of the lungs (34) were frozen while being perfused under vascular waterfall conditions (zone II, analagous to flow through a Starling resistor model), that is, inflow pressure exceeded alveolar pressure, but alveolar pressure was greater than outflow pressure. Under these conditions the collapsible vessels exposed to alveolar pressure (capillaries or very small arterioles or venules depending on the direction of flow) develop a constriction in their downstream end (7, 8); this we refer to as the collapse point. Driving pressure is thus inflow-alveolar pressure rather than the conventional inflow-outflow pressure. Therefore, changes in caliber in the vessels downstream from the collapse point will not affect inflow pressure or capillary red blood cell volume so long as downstream pressure remains less than alveolar pressure (9). In order to assess changes in resistance (caliber) in the vessels upstream from the collapse point, we compared the number of capillary red blood cells for any given inflow pressure during control conditions with the number of red blood cells at the same inflow pressure under experimental conditions. If hypoxia, for example, causes vasoconstriction in the vessels upstream from the capillaries, there will be a greater pressure drop from the inflow point to the capillaries than under control conditions, i.e., less of the inflow pressure will be transmitted to the capillaries (Fig. 1). Therefore, there will be a decrease in the number of capillary red blood cells compared with that

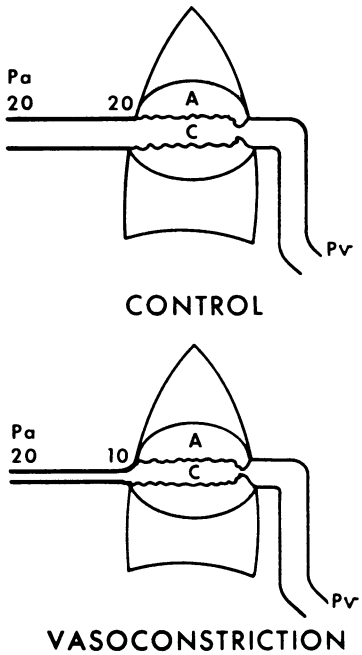

FIGURE 1 Lung perfused under vascular waterfall conditions: small vessels (C) exposed to alveolar pressure develop a constriction in their downstream end. Under control conditions, inflow pressure of 20 cm H_2O is shown as pressure at origin of capillaries. With vasoconstriction in vessels upstream from capillaries, less of the inflow pressure is transmitted to the capillaries, and consequently there is a decrease in the number of capillary red blood cells.

same inflow pressure under control conditions since the number of capillary red cells depends on capillary pressure (at similar transpulmonary pressures) (6). If hypoxia does not cause vasoconstriction, the number of capillary red blood cells will be the same for any inflow pressure as in the control measurements. We were thus able to look separately at the vessels on the arterial side of the capillaries during perfusion in the normal manner from artery to vein and at vessels on the venous side of the capillaries by reversing the direction of blood flow so that the lungs were perfused from vein to artery.

In order to determine if a vasomotor response occurred in the pulmonary capillaries during alveolar hypoxia, the number of capillary red blood cells was measured in four lungs frozen with arterial pressure equal to venous pressure 5 cm above the top of the lung (zone III, arterial and venous pressures both exceed alveolar pressure) and with blood flow stopped. Two lungs were frozen under control conditions and two during alveolar hypoxia. With these hemodynamic conditions capillary pressure is known at any level down the upright lung (it must lie between arterial and venous pressures which are virtually the same).

If hypoxia causes any decrease in capillary diameter (volume), the number of capillary red blood cells should be less for any capillary pressure than under control conditions. Any vasomotor changes that might occur upstream or downstream from the capillaries will not affect the measurements since flow was stopped at the time the lungs were frozen. That is, even if vasoconstriction should occur in the arteries or veins, this would not change arterial or venous pressures from the control under conditions of no flow.

Conditions during freezing

Control: forward and reverse perfusion, zone II. Eight lungs were frozen under control conditions (breathing the control mixture) while being perfused from artery to vein. These lungs and all of the others were frozen at a transpulmonary pressure of 5 cm H_2O. Pulmonary artery pressure was set to equal alveolar pressure about 5 cm above the apex of the lung. The venous reservoir was set below the base of the lung, but venous pressure was always equal to the alveolar pressure about 5 cm below the entrance of the pulmonary vein at the hilum because of the Starling resistor effect of the large veins exposed to atmospheric pressure (9), so that in any one experiment a maximum of 20–25 cm of lung tissue below the apex was available in zone II. Flow averaged 1.3 liters/min. (range 1.0–1.8 liters/min.). The lungs were then given the hypoxic gas mixture to breathe for 5 min. Within 2–3 min pulmonary artery pressure began to rise reaching its maximum after 3–5 min. The average pressure rise was 8 cm H_2O. Having shown that the hypoxic response was present in these control lungs, ventilation with the control gas mixture was then reinstituted. After another 5 min arterial pressure had returned to within 1 cm H_2O of its previous value, and the lungs were then frozen.

Four lungs were frozen while being perfused from pulmonary vein to artery (reverse perfusion). Perfusion was begun from artery to vein, and after approximately 5 min the pump was stopped for 10 sec while the clamps on the lines connecting the arterial inflow and venous outflow were repositioned. The pump was then restarted at the same speed. After several minutes of flow, pulmonary venous pressure was within 1 cm H_2O of what pulmonary artery pressure had been before the direction of flow was reversed. The lung was then given the 3% oxygen mixture to breathe for 5 min in order to demonstrate that the hypoxic response could be elicited in these control lungs during reverse perfusion. The average pressure rise was 8 cm H_2O. Ventilation with the control mixture was then restarted, and pulmonary venous pressure returned to within 1 cm H_2O of its previous reading. The lungs were then frozen.

Alveolar hypoxia, forward and reverse perfusion, zone II. Eight lungs were frozen while breathing the hypoxic gas mixture during forward perfusion and five during reverse perfusion. In each case, ventilation was begun with the 93.5% O_2 mixture for 5 min and then the 14.5% O_2 mixture for 5 min as in the control lungs. The hypoxic gas mixture was then given to the lungs, and when the inflow pressure had risen (average 8 cm H_2O during forward perfusion, 8 cm H_2O during reverse perfusion), the lungs were frozen.

Serotonin infusion, forward and reverse perfusion, zone II. The initial ventilation procedure and the setting of the pulmonary vascular pressures were the same as for the control lungs. Two lungs were frozen during forward perfusion. After 5 min of a stable pulmonary artery pressure, serotonin in a concentration of 100 μg/ml was infused continuously (Harvard infusion pump) into the circuit 1 cm proximal to the glass cannula tied into the pulmonary artery. The infusion rate was adjusted so as to produce a pressure rise similar to that obtained breathing the hypoxic gas mixture, and the lungs were then frozen. The dose of serotonin was approximately 8 μg/min per kg.

Two lungs were frozen during reverse perfusion. Perfusion was begun from artery to vein as in the control lungs

before reversing the direction of blood flow. Serotonin in the same concentration was infused into the tubing 1 cm proximal to the cannula tied into the left atrium. The lungs were then frozen during the height of the pressure rise. Almost twice the amount of serotonin (15 μg/min per kg) had to be infused into the pulmonary veins to achieve a pressure rise comparable with the pulmonary artery pressure rise during forward perfusion.

Histamine infusion, forward and reverse perfusion, zone II. Histamine in a concentration of 155 μg/ml was infused into three lungs during forward perfusion and two lungs during reverse perfusion. A dose was selected to raise the inflow pressure approximately 8 cm H_2O, and this averaged 10 μg/min per kg. There was no difference in the amount of histamine needed during forward and reverse perfusions. During the infusion of histamine, there was no change in airway pressure with the constant tidal volume.

Alveolar hypoxia and control, zone III, no flow. Two lungs were frozen under control conditions and two during alveolar hypoxia. All of the lungs were first tested for an hypoxic response under vascular waterfall (zone II) conditions in the same way as described above. The two lungs frozen under control conditions produced pulmonary artery pressure rises of 8.5 and 12 cm H_2O, respectively with alveolar hypoxia, while the two lungs frozen during alveolar hypoxia produced pressure rises of 5 and 13 cm H_2O, respectively.

For the measurements made under control conditions venous pressure was set 5 cm above the top of the lung, the perfusion pump was turned off, and within 30 sec arterial pressure had settled to within 1 cm H_2O of venous pressure. The lungs were then frozen.

For the measurements made during alveolar hypoxia, perfusion was begun under vascular waterfall (zone II) conditions. The lungs then breathed the hypoxic gas mixture for 5 min at which time pulmonary artery pressure had risen and stabilized. Venous pressure was then raised to 5 cm above the top of the lungs, arterial flow was stopped 15 sec later, and within another 30 sec pulmonary artery pressure had settled to within 1 cm H_2O of venous pressure, and the lungs were frozen.

RESULTS

Control. Fig. 2 shows the number of capillary red blood cells for any inflow pressure during forward and reverse perfusion. The number of red blood cells per 10 μ septum was significantly greater ($P < 0.05$) during reverse perfusion than during forward perfusion only when the inflow pressure reached 20 cm H_2O. This suggests that the resistance in the vessels on either side of the capillaries is quite similar, but may be somewhat lower in the veins.

Alveolar hypoxia. Fig. 3 shows the number of red blood cells during forward perfusion for any pulmonary artery (inflow) pressure when the lungs were breathing either the control or hypoxic gas mixture. The number of red blood cells was significantly decreased during alveolar hypoxia compared with the control measurements at all inflow pressures: 2.5 cm H_2O, $P < 0.01$; 10, 15, 20, and 25 cm H_2O, $P < 0.001$.

This smaller number of red blood cells in the capillaries must indicate an increase in vascular resistance (vasoconstriction) in the vessels upstream from the capillaries or in the capillaries themselves; that is, there is a greater pressure drop from the inflow point to the capil-

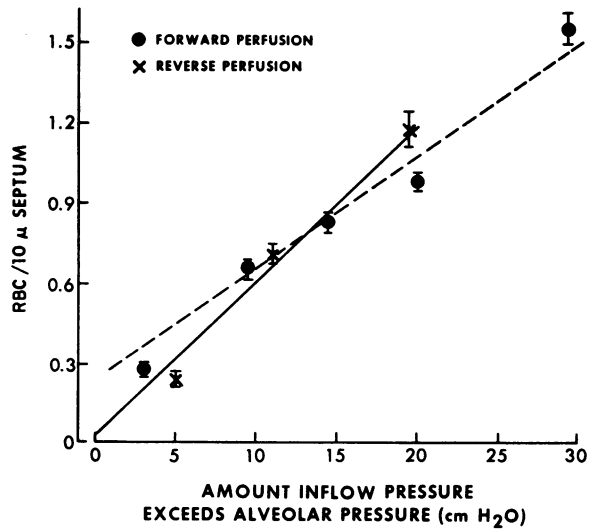

FIGURE 2 Number of red blood cells per 10 μ of alveolar septum plotted against amount inflow pressure exceeds alveolar pressure during forward and reverse perfusion. Increase in the number of capillary red blood cells with increasing inflow pressure is presumably due to increase in capillary pressure. Each point is the mean and standard error for 90–180 measurements (30 measurements in each dog), except for 20 cm reverse perfusion (60 measurements) and 30 cm forward perfusion (30 measurements).

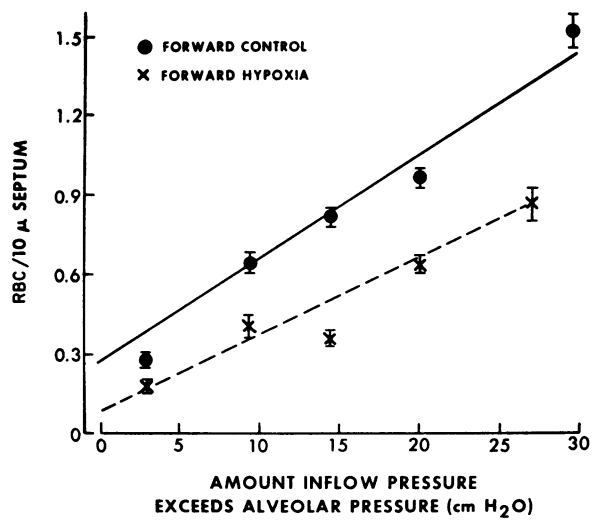

FIGURE 3 Number of red blood cells per 10 μ septum plotted against the amount inflow pressure exceeds alveolar pressure. Measurements made during forward perfusion with the lung breathing control and hypoxic gas mixtures. Each noncontrol point in this and subsequent graphs represents the mean and standard error of 60–180 measurements.

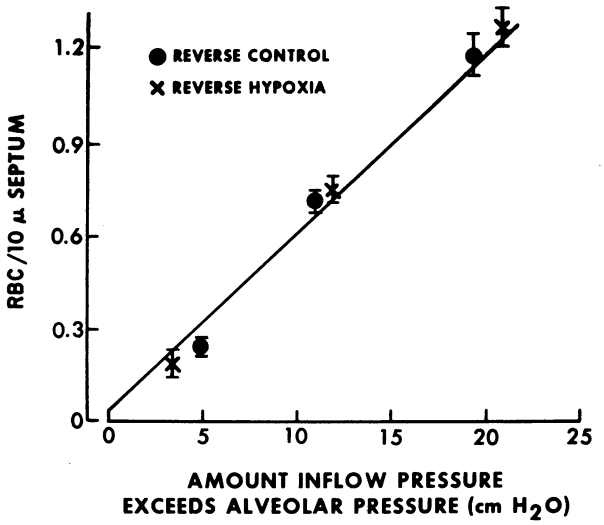

FIGURE 4 Number of capillary red blood cells during reverse perfusion with the lung breathing control and hypoxic gas mixtures. Note that during alveolar hypoxia there was no significant change in the number of red blood cells from control values.

lary, and thus there is a corresponding decrease in the number of capillary red blood cells compared with the same inflow pressure under control conditions. Note that the lines of best fit for the two gas mixtures are not parallel. This divergence in the slopes can be explained by the increase in blood flow as inflow pressure is increased: that is, for the same degree of vasoconstriction there will be a proportionally greater pressure drop from the inflow point to the capillary as flow increases.

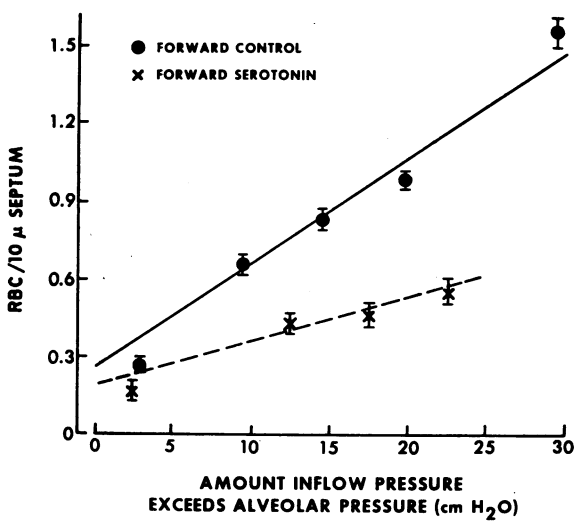

FIGURE 5 Number of capillary red blood cells under control conditions and during serotonin infusion into pulmonary artery during forward perfusion.

During reverse perfusion the inflow pressure rise breathing the low O_2 mixture was the same as that during forward perfusion. There was, however, no significant difference in the number of red blood cells per 10 μ septum (Fig. 4) for any inflow pressure, indicating that there was no change in the caliber of the vessels (the veins) upstream from the capillaries.

Serotonin infusion. During forward perfusion (Fig. 5) there was a significant decrease in the number of capillary red blood cells for any inflow pressure during serotonin infusion (2.5 cm H_2O, $P < 0.01$; 10, 15, and 20 cm H_2O, $P < 0.001$. The number of red blood cells at an inflow pressure of 20 cm H_2O during serotonin infusion was comparable with that at an inflow pressure of 7 cm H_2O during control conditions.

During reverse perfusion and serotonin infusion (Fig. 6) there was a small but significant decrease in the number of capillary red blood cells compared with the control measurements at the inflow pressures of 11 cm H_2O ($P < 0.05$) and 19 cm H_2O ($P < 0.02$). This indicates that there was an increase in resistance in the vessels (the veins) upstream from the capillaries, but that the increase was very small compared with resistance change in the arterial vessels.

Histamine infusion. Figs. 7 and 8 show the measurements made with histamine infusion during forward and reverse perfusion. During forward perfusion the measurements during drug infusion did not differ significantly from the control, whereas during reverse perfusion there was a significant decrease in the number of capillary red blood cells at 11 and 19 cm H_2O ($P < 0.001$).

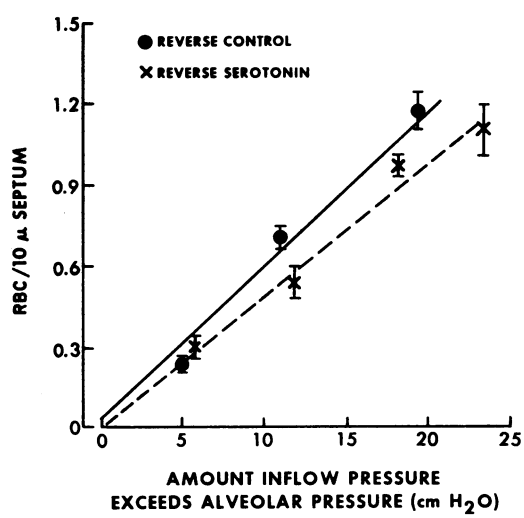

FIGURE 6 Number of capillary red blood cells during control conditions and serotonin infusion into pulmonary vein during reverse perfusion. The number of red blood cells was significantly different from control at inflow pressures of 11 cm H_2O ($P < 0.05$) and 19 cm H_2O ($P < 0.02$).

Histamine, therefore, appears to produce a vasoconstrictor response solely in the veins.

In these lungs and in the lungs in which serotonin was infused, we examined the tissue sections carefully for the appearance of an increase in width of the alveolar-capillary septum. This would have indicated pericapillary edema from changes in capillary permeability. These sections did not appear different from those in the control lungs.

Alveolar hypoxic and control, zone III, no flow. Fig. 9 shows the measurements of the number of capillary red blood cells during control and hypoxic conditions plotted against the amount arterial and venous pressures exceed alveolar pressure. The measurements made during alveolar hypoxia did not differ significantly from the control indicating that there was no decrease in the volume (vasoconstriction) of the capillaries. This interpretation assumes that the vasoconstriction which occurred (rise in inflow pressure) before blood flow was stopped did not disappear in the 30 sec interval during which flow was stopped, and the lungs were frozen.

DISCUSSION

Our data show that the vasoconstrictor response to alveolar hypoxia occurs in the vessels on the arterial side of the capillaries. Serotonin causes vasoconstriction predominantly in the arteries but to a lesser extent in the veins, whereas histamine causes vasoconstriction solely in the veins.

The method is simple and direct and is based on the principle that for any inflow pressure at the pulmonary artery or left atrium during forward or reverse perfusion the capillaries "see" a part of this pressure. It has recently been demonstrated that in this type of animal lung preparation an increase in inflow pressure is accompanied by a greater number of capillary red blood cells, presumably because capillary pressure also increases (6). This increment in the number of capillary red blood cells is due to opening up of new capillaries (recruitment) and distension of those already open. If there is any decrease in caliber of the vessels upstream from the capillaries (e.g. vasoconstriction), there will be a greater pressure drop from the inflow

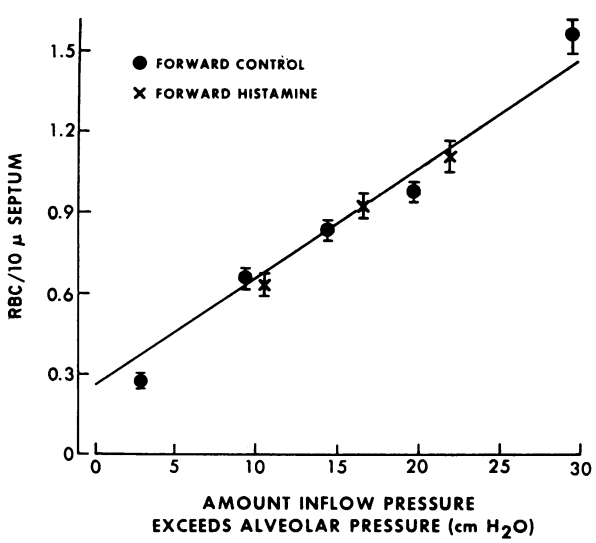

FIGURE 7 Number of capillary red blood cells under control conditions and during histamine infusion into pulmonary artery during forward perfusion. There was no significant difference between the values at any inflow pressure.

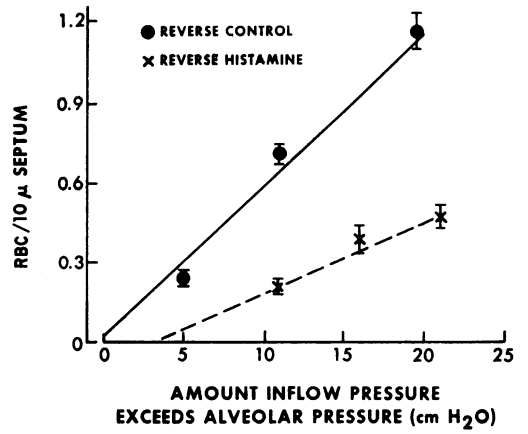

FIGURE 8 Effect of histamine infusion on the number of capillary red blood cells during infusion into pulmonary vein during reverse perfusion.

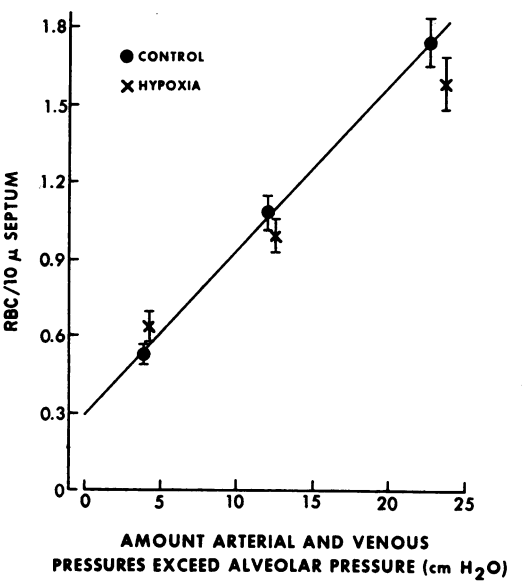

FIGURE 9 Red blood cells per 10 μ septum plotted against amount arterial and venous pressures exceed alveolar pressure. Since blood flow was stopped when the lungs were frozen, the pressures did not differ by more than 1 cm H_2O.

point to the capillaries than under control conditions, and there will be a corresponding decrease in the number of capillary red blood cells.

The major possible source of error in these experiments is that changes in vessel caliber downstream from the collapse point might affect the capillary red blood cell volume. When hemodynamic conditions are such that arterial pressure exceeds alveolar pressure, but alveolar pressure is greater than venous pressure (zone II, vascular waterfall), it has been shown that raising venous pressure will not affect arterial pressure until venous pressure exceeds alveolar pressure. Furthermore, it has also been demonstrated that for any arterial (inflow) pressure, the number of capillary red blood cells will be greater if venous pressure exceeds alveolar pressure (zone III, driving pressure arterial-venous pressure) than if alveolar pressure is greater than venous pressure (6). Therefore, if during forward perfusion with alveolar hypoxia or serotonin infusion the rise in pulmonary artery pressure had been due to venous constriction so marked as to raise venous pressure to a level greater than alveolar pressure and thus passively raise arterial pressure, then the number of capillary red blood cells would have been greater than the control values (capillaries now in zone III) rather than considerably less (Fig. 3). Likewise during histamine infusion during reverse perfusion, if the rise in venous pressure had been due to arterial constriction sufficient to raise arterial pressure greater than alveolar pressure, the number of capillary red cells would have exceeded the control value rather than being less (Fig. 8). Thus it appears quite definite that alveolar hypoxia and serotonin cause arterial constriction, and histamine produces venous constriction. It is also clear from the experiments done under zone III no flow conditions (Fig. 9) that during alveolar hypoxia vasoconstriction does not occur in the pulmonary capillaries.

The more difficult question concerns the findings during reverse perfusion with alveolar hypoxia, that is, inflow (venous) pressure increased comparable with the rise seen during forward perfusion, but there was no change in the number of capillary red blood cells (Fig. 4). This could have been the result of venous (upstream) constriction which would lower the number of capillary red cells for any inflow pressure plus arterial constriction (already demonstrated to have occurred during forward perfusion) sufficient to raise arterial pressure above alveolar pressure and thus increase the number of capillary red cells (i.e., the capillaries would now be in zone III). We think the possibility that changes occurred in both arteries and veins is unlikely for several reasons. First, the outflow (arterial) pressure was set low enough (5 cm below the hilum) that a pressure rise greater than that produced with hypoxia under indentical conditions during forward perfusion (8 cm H_2O) would have been necessary to put most of the lung into zone III. Furthermore, it would seem very fortuitous for the combination of vascular constriction, both upstream and downstream from the capillaries with opposing effects on the number of capillary red blood cells, to balance in such a way as to produce a number of red blood cells just equal to the control values. The same reasoning applies to the experiments of forward perfusion with histamine in which we found a pressure rise equal to that which occurred during reverse perfusion but no change in the number of capillary red cells (Fig. 7).

A possible explanation for the rise in inflow pressure with no change in the number of capillary red blood cells during reverse perfusion with hypoxia (and similarly during forward perfusion with histamine) is that there were changes in muscle tone in the collapsible vessels at the downstream end of the capillaries. These collapsible vessels may be the capillaries themselves and/or the small muscular vessels with tone. Lopez-Muniz, Stephens, Bromberger-Barnea, Permutt, and Riley (10) have suggested that an increase in muscular tone in the collapsible segment alone would raise inflow pressure without a concomitant change in vascular caliber upstream from the capillaries. We propose that during reverse perfusion with alveolar hypoxia arterial constriction (shown to ocur during forward perfusion) occurred within the collapsible segment so as to produce a rise in inflow pressure.

In order to test this hypothesis, a Starling resistor model was constructed with $\frac{3}{8}$ in. diameter Penrose drain 25 cm long. A second model was identical except that the distal 5 cm of drain was narrowed to approximately one-half of the original diameter (Fig. 10*a* and *b*). Pressure within these tubes was measured at 1 cm intervals through a 1 mm I.D. polyethylene catheter with end and side holes attached to a Statham transducer (P23BB). The pressure around the tubing was atmospheric. We found that a narrowing in the collapsible segment (the distal end of the tubing) markedly raised inflow pressure for any flow, so that to achieve a comparable inflow pressure in both models, the flow had to be almost twice as great in the model without a narrowing in the downstream end. However, in each case we found that the entire pressure drop across the collapse point occurred in the 3–4 cm proximal to it. Thus for the same inflow pressure in the two models the mean pressures in the Penrose drain was similar regardless of whether or not there was an increase in tone in the collapsible segment.

Model *b* is analagous to the vasomotor changes in the lungs postulated to occur during reverse perfusion with alveolar hypoxia. That is, during alveolar hypoxia, arteriolar constriction in the collapsible vessels caused a rise in the inflow (venous) pressure. However, since

there was no constriction in the vessels between the inflow point and the capillaries, we found the number of capillary red blood cells for any inflow pressure to be the same as for the control. For as we have shown in the models, the mean capillary pressures would be similar in the control and hypoxic conditions, and the number of capillary red cells depends on capillary pressure (6). This conclusion will still be valid even if in the lungs there is some continuous pressure drop from the beginning to end of the capillary (as there is not in the Penrose drain) because the capillary will still be "seeing" the same pressure at its proximal end in both situations.

The correspondence of this model to the hemodynamic situation in the capillaries is, of course, only approximate, but these data combined with the directly opposite effects produced by alveolar hypoxia and histamine infusion strongly suggest that the technique is a valid one to show separately changes occurring in the arteries and veins.

Additional evidence that this model corresponds to the hemodynamic situation in the lungs comes from the experiments in which serotonin was infused (Figs. 5 and 6). Gaddum, Hebb, Silver, and Swan (11) showed that approximately 20% of serotonin is deactivated in one passage through the lungs, and Eiseman, Bryant, and Waltuch (12) showed that about 60% of a test dose is deactivated in 15 min. During reverse perfusion almost twice as much serotonin had to be infused to produce a pressure rise comparable with that during forward perfusion, and this suggests that the vasoactive site was further away from the inflow point during reverse perfusion than during forward perfusion. That is, the major part of the pressure rise during forward and reverse perfusion was caused by vasoconstriction in the same vessels, the arteries. However, the mechanism of the pressure rise was different in each case (see above and Fig. 9).

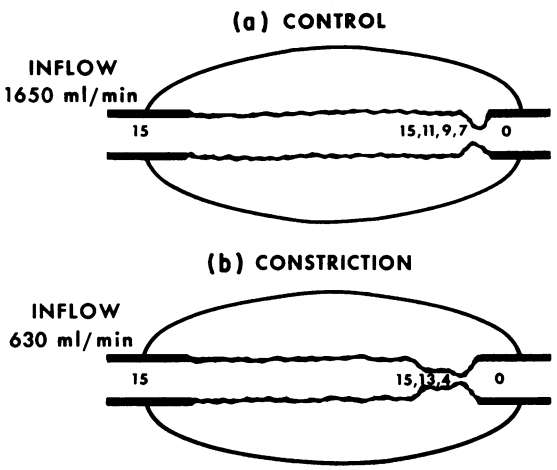

FIGURE 10 Starling resistor models to illustrate the effect on inflow pressure of narrowing of collapsible segment of tubing. Numbers inside the tubing indicate pressures (cm H_2O) measured at 1 cm intervals. When the flow through both models was the same, inflow pressure was markedly elevated in model *b*. Note that for both models the entire pressure drop along the Penrose drain occurs in the downstream few centimeters across the collapsible segment of tubing. Thus, throughout most of the collapsible tubing the pressure is the same for the similar inflow pressure whether or not there is a narrowing in the collapsible segment. If these models are analogous to the lung capillaries in zone II conditions, we would expect the mean capillary pressures and therefore the number of red blood cells to be similar for the same inflow pressure even if the inflow pressure were raised secondary to a change in muscle tone (narrowing) in the collapsible segment.

It is apparent from the most recent reviews (13, 14) that the sites of action for alveolar hypoxia, histamine (15), and serotonin (16, 17) are very much in doubt. A structure-function correlation for alveolar hypoxia is available from the work of Kato and Staub (18) who found constriction of pulmonary arterioles (about 200 μ diameter) accompanying terminal bronchioles in rapidly frozen lungs during hypoxia; they did not evaluate the pulmonary veins. Brody and Stemmler (19) used a plethysmographic method to determine the longitudinal distribution of vascular resistance in the lung and found that serotonin produced constriction in arteries and veins, whereas histamine caused constriction predominantly in veins, but to a lesser extent in arteries.

Recently Hauge (20) has suggested that in the rat the vasoactive response to alveolar hypoxia is mediated through the release of endogenous histamine in the lung. Our data which demonstrate a different site of action for alveolar hypoxia and histamine, make this hypothesis unlikely in the dog. Furthermore, it also seems unlikely that alveolar hypoxia can be implicated in the genesis of pulmonary edema of high altitude through the mechanism of constriction in the pulmonary veins.

Our data clearly indicate that there is differential reactivity in the pulmonary arteries and veins. The experimental results with alveolar hypoxia, in particular, are supported by the findings of Bergofsky and Holtzman (21), who showed that hypoxia caused strips of pulmonary arterial smooth muscle to lose potassium and gain sodium suggesting a depolarization of the muscle cells, whereas these changes did not occur in muscle strips from the pulmonary veins or systemic arteries.

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