PULMONARY CIRCULATION DEMONSTRATION USING AN ISOLATED RAT LUNG MODEL

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We have developed a pulmonary circulation laboratory exercise that effectively illustrates basic concepts typically taught in a graduate physiology curriculum. The demonstration uses an isolated, perfused rat lung model to delineate the mechanisms by which pulmonary vascular resistance can be altered either passively or in an active manner by contraction or relaxation of vascular smooth muscle. The exercise further offers an opportunity to closely observe an experimental preparation commonly used to study the pulmonary circulation and allows students the opportunity to interpret the resulting physiological data. Student evaluations indicate that the demonstration was received with enthusiasm and provides an effective teaching tool for reinforcing concepts in pulmonary vascular physiology.

Key words: pulmonary vascular resistance; distension; recruitment; endothelium-dependent vasodilation; hydrostatic zones of lung

The pulmonary circulation differs from the systemic circulation in many aspects. For example, the entire cardiac output passes through the pulmonary circuit, making it an ideal location for activation or inactivation of biologically active compounds. However, the lung is primarily a gas-exchange organ, and thus the pulmonary circulation serves to maximize exposure of blood to alveolar gas at the capillary level. This anatomic arrangement of capillaries surrounded by air-filled chambers is unique and has mechanical consequences affecting hemodynamics. In addition, the pulmonary circuit is normally a low-pressure, low-resistance circulation that responds passively to a number of physical factors to alter vascular resistance. Pulmonary vascular resistance may also be altered in an active manner by contraction or relaxation of vascular smooth muscle. The laboratory demonstration described here is designed to illustrate many of these properties of this unique vascular bed.

We have conducted this demonstration each of the last eight years as part of a physiology course that is required for all first-year MS and PhD students in biomedical sciences. This laboratory exercise has proven to solidify the students’ understanding of basic concepts and relationships regarding the pulmonary circulation, to provide an opportunity to examine methods by which physiological data are generated, and to allow students to interpret the resulting data. A student group size of four to six is optimal for this demonstration, because it allows students to closely examine the preparation and the various signals displayed on the chart recorder and/or computer monitor and is conducive to questions and discussion. Handouts describing the various experimental protocols outlined here and a brief description of methodology should be provided to the students with sufficient time to carefully study the protocols and predict the results before the laboratory exercise. The demonstra-
tion is most effective when performed after treatment of related topics on the pulmonary circulation in lecture. These topics include 1) passive factors affecting vascular resistance within the pulmonary circulation, such as effects of changes in arterial, venous, and airway pressure and perfusate viscosity (18); 2) active factors affecting pulmonary vascular resistance, including receptor-mediated vasoconstrictors and endothelium-dependent vasodilators (Ref. 2; reviewed in Refs. 8, 10, and 13); 3) Poiseuille’s equation; and 4) hydrostatic zones of the lung (18).

**ISOLATED LUNG PREPARATION**

All protocols and surgical procedures used in this study were reviewed and approved by the Institutional Animal Care and Use Committee of the University of New Mexico School of Medicine. The procedure for lung isolation was described previously (6). Male Sprague-Dawley rats (200–350 g; Harlan Industries) are anesthetized with pentobarbital sodium (25 mg ip). After the trachea is cannulated with a 17-gauge needle stub, the lungs are ventilated using a Harvard positive-pressure rodent ventilator (model 683) at a frequency of 55 breaths/min and a tidal volume of 2.5 ml with a gas mixture containing 6% CO₂ in room air. This gas mixture is humidified and warmed by bubbling through deionized water contained within a water-jacketed Plexiglas chamber maintained at 38°C by a recirculating water bath. Peak inspiratory pressure is set at 9 cmH₂O, and positive end-expiratory pressure is maintained at 2 cmH₂O. After a median sternotomy, heparin (100 U in 0.1 ml) is injected directly into the right ventricle. A 13-gauge needle stub is advanced into the pulmonary artery via an incision in the right ventricle and secured into position with 2-0 silk suture. The needle stub is covered with a 2- to 3-mm segment of heat-shrink tubing at its tip, which helps to secure the cannula inside the pulmonary artery. The preparation is immediately perfused at 0.8 ml/min by a Masterflex microprocessor pump drive (model 7524–10) with a physiological saline solution (PSS) containing (in mM) 129.8 NaCl, 5.4 KCl, 0.83 MgSO₄, 19 NaHCO₃, 1.8 CaCl₂, and 5.5 glucose with 4% albumin (wt/vol) (all from Sigma). The left ventricle is cannulated with a plastic tube (4-mm OD) via an incision at the apex and tied in place with a 2-0 silk suture. A 3-mm segment of Tygon tubing is placed on the end of the ventricular cannula, allowing the cannula to be secured in position. The heart and lungs are removed en bloc and suspended directly above a water-jacketed Plexiglas chamber containing PSS maintained at 38°C. A plastic cover is placed around the lung preparation to provide a warmed and humidified chamber. The perfusion rate is gradually increased to 30 ml-min⁻¹-kg body wt⁻¹ and maintained at this rate unless otherwise stated. Twenty milliliters of perfusate are washed through the lungs and discarded before recirculation is initiated with the remaining forty milliliters of PSS. The dead space of this recirculating perfusion system is ~20 ml. The remaining 20 ml of perfusate are contained within a reservoir (50-ml centrifuge tube), which is suspended from a Grass model FT03 force-displacement transducer for continuous measurement of reservoir weight. Because the perfusion system is a closed circuit, monitoring reservoir weight (and thus volume) provides a means of assessing changes in vascular fluid volume (6). The venous line-perfusate reservoir-force transducer assembly is attached with clamps to a vertical rod to provide easy vertical adjustment for those protocols requiring changes in pulmonary venous pressure. Perfusate is pumped through a water-jacketed Radnoti bubble trap maintained at 38°C before entering the pulmonary circulation. Lungs are allowed 30 min to equilibrate before protocols are begun. Pulmonary arterial pressure (Pₐ), venous pressure (Pᵥ), and airway pressure (Pₐ₈) are measured via side ports in the arterial, venous, and airway lines, respectively, with Spectramed model P23 XL pressure transducers and recorded on a Gould RS 3400 chart recorder. Output signals from the Pₐ, Pᵥ, Pₐ₈, and reservoir weight channels of the chart recorder are continuously displayed on a computer screen using a data acquisition and analysis system (AT-CODAS, Dataq Instruments).

**EXPERIMENTAL PROTOCOLS**

**Protocol 1: Effect of Increased Flow**

With Pₐ set between 1 and 3 mmHg and the venous reservoir set below the lung, pump speed is doubled to provide a flow of 60 ml-min⁻¹-kg body wt⁻¹. Figure 1 illustrates an example of data for Pₐ, Pᵥ, and reservoir weight [change (Δ) in volume in µl] from a...
single isolated lung used for one of our demonstrations. Because the venous reservoir is set below the lung, \( P_v \) is \(<0\). Thus the signal for \( P_v \) is not apparent in Fig. 1. Figures 2–7 include data obtained from this same lung.

Question 1: Is the lung in zone 1, 2, or 3 condition?  
Answer: The lung is in zone 2, because \( P_s > P_A > P_v \).

Question 2: From Poiseuille’s equation, and assuming the pulmonary circulation behaves like a series of rigid tubes with laminar flow, what effect would you predict that doubling flow would have on the observed arterial-venous pressure gradient?  
Answer: Poiseuille’s equation states that

\[
R = \frac{\Delta P}{Q} = \frac{8\eta l}{\pi r^4}
\]  

where \( R \) is resistance to flow, \( \Delta P \) is the pressure gradient across the pulmonary vasculature (i.e., \( P_s - P_v \)), \( Q \) is flow, \( \eta \) is perfusate viscosity, \( l \) is vessel length, and \( r \) is vessel radius.

Rearranged, this gives

\[
\Delta P = \frac{8\eta l}{\pi r^4} \times Q
\]
Therefore, because $\Delta P$ is directly proportional to $Q$, a doubling of $Q$ would produce a twofold increase in $\Delta P$ (i.e., the arterial-venous pressure gradient).

**Question 3**: However, the pulmonary circulation behaves quite differently than this simple model. What is your prediction for the arterial pressure response to a doubling of flow? Why?

**Answer**: A doubling of flow will decrease pulmonary vascular resistance and result in a less than twofold increase in $P_a$ for two reasons: 1) the resulting recruitment, and possibly some distension as well, will increase the radius term ($r$) in Poiseille's equation for those newly perfused and distended vessels, thereby decreasing pulmonary vascular resistance; and 2) resistances in parallel add as reciprocals

$$R_{total}^{-1} = R_1^{-1} + R_2^{-1} + R_3^{-1} \ldots$$  \hspace{1cm} (3)

Therefore, because recruitment increases the number of parallel resistances at the microvascular level, resistance will decrease in that segment, thereby attenuating the increase in $P_a$.

**Question 4**: Would you expect vascular fluid volume (as detected by a change in reservoir weight) to change in response to this stimulus? Why?

**Answer**: Reservoir weight will decrease in response to a doubling of flow, thus indicating an increase in lung fluid volume. This increased vascular volume occurs secondary to recruitment because a greater vascular.
surface area is being perfused. An additional contribution to the decrease in reservoir weight is increased fluid flux as a result of greater capillary hydrostatic pressure.

**Protocol 2: Effect of Increased Airway Pressure in Zone 2 Conditions**

Flow is returned to 30 ml-min⁻¹·kg body wt⁻¹, and PA is increased by elevating positive end-expiratory pressure (Fig. 2).

**Question 5:** What do you predict will be the arterial pressure response to this stimulus?

**Answer:** Because the lungs remain in zone 2 (i.e., Pₐ > Pₐ > Pᵥ), an increase in Pₐ will compress the pulmonary capillaries, thereby decreasing the radius term in Poiseuille’s equation for those vessels being compressed, leading to an increase in pulmonary vascular resistance. Because

\[ \Delta P = R \times Q \]  

(4)and because Q is constant, an increase in R will result in an increase in \( \Delta P \). Because Pᵥ is held constant, an increase in Pₐ is observed. Note the corresponding decrease in reservoir weight associated with recruitment (Fig. 2).

**Protocol 3: Effect of Increased Venous Pressure in Zone 3 Conditions**

On restoration of Pₐ to control levels (i.e., 1–3 mmHg), the venous reservoir is elevated to achieve a Pᵥ of 5 mmHg (Fig. 3).
Question 6: What will happen to $P_a$ and reservoir weight? Why?

Answer: Because the elevation in $P_v$ places the lungs in zone 3 conditions (i.e., $P_a > P_v > P_{aw}$), the increase in $P_v$ will be transmitted across the pulmonary vasculature, thus increasing $P_a$. Reservoir weight will decrease because of increased lung fluid volume associated primarily with distension of pulmonary veins. Increased fluid flux may additionally contribute to this fall in reservoir weight.

Protocol 4: Effect of Increased Airway Pressure in Zone 3 Conditions

With $P_v$ maintained at $\sim 5$ mmHg, $P_a$ is increased to $\sim 4$ mmHg (Fig. 4).

Question 7: What will be the effect of this maneuver on pulmonary vascular resistance and $P_a$? Why?

Answer: The lungs will remain in zone 3 conditions ($P_a > P_v > P_{aw}$) despite the elevation of $P_a$. Therefore, because microvascular pressure exceeds the surrounding alveolar pressure, the increase in alveolar pressure will not compress the microvasculature and pulmonary vascular resistance and $P_a$ will be unaltered.

Protocol 5: Effect of Increased Perfusate Viscosity

With $P_a$ and $P_v$ returned to control conditions, the saline perfusate is replaced with heparinized whole blood (Fig. 5). This is achieved by emptying the effluent into a waste container until all saline has been drained from the perfusate reservoir, followed by immediate addition of whole blood to the reservoir. Any small bubbles carried from the reservoir will be removed by the bubble trap. A three-way stopcock in the venous line is useful for diverting flow away from the perfusate reservoir and into the waste container.
The effluent is allowed to continue to drain into the waste container until most of the saline has been removed from the system. Finally, flow is diverted back towards the perfusate reservoir using the three-way stopcock in the venous line, thus restoring the system to a closed, recirculating circuit. Whole blood is drawn into heparinized syringes by direct cardiac puncture of donor rats anesthetized with pentobarbital sodium (25 mg ip) as well as from the lung donor immediately before lung isolation.

Question 8: What will be the effect of perfusion with whole blood on pulmonary vascular resistance? Explain.

Answer: Because blood is more viscous than saline, perfusion with blood will increase the viscosity parameter \( h \) in Poiseuille's equation, thereby increasing pulmonary vascular resistance. This increase in resistance is detected by an increase in \( P_a \). Note the patches of red on the lung surface that may develop as blood enters the lung. Within several seconds, the surface of the lung exhibits a more homogeneous color as a consequence of recruitment secondary to the increase in \( P_a \). Reservoir weight decreases because of recruitment and increased lung fluid flux.

Protocol 6: Response to Pulmonary Vasoconstrictor U-46619

The synthetic thromboxane analog U-46619 (9,11-dideoxy-9\(\alpha\),11\(\alpha\)-methanoepoxy prostaglandin F\(2\alpha\); Cayman) is added to the perfusate reservoir in cumulative doses until a stable arterial pressor response of \(~10\) mmHg is achieved (Fig. 6). This agent stimulates thromboxane \( A_2 \) receptors on pulmonary vascular smooth muscle cells, resulting in an increase in the concentration of intracellular calcium \([Ca^{2+}]_i\) \(\) (3).
U-46619 is prepared in 95% ethanol at a stock concentration of 10 µg/ml and stored at -80°C. A recirculating concentration of 100–200 nM is usually sufficient to produce a stable 10-mmHg pressor response. Because U-46619 constricts both arterial and venous segments of the rat pulmonary vasculature (5, 12), larger constrictions will elevate microvascular pressure resulting in edema. In the demonstration depicted by Fig. 6, 143 nM U-46619 was required to achieve a stable pressor response of 11 mmHg. This was achieved by adding two successive doses of 1 µg of U-46619 to the perfusate reservoir. Note the artifactual increase in reservoir weight with each dose.

Question 9: What will be the effect of U-46619 on $P_a$ and reservoir weight? Explain.

Answer: The increase in [$Ca^{2+}$], causes contraction of pulmonary vascular smooth muscle, thus constricting

the muscular pulmonary vasculature. The resultant decrease in vessel radius will increase resistance as demonstrated by Poiseuille’s equation, leading to an increase in $P_a$. The increased vascular fluid volume and flux that occurs with increased $P_a$ is evidenced by a greater rate of fluid loss from the perfusate reservoir.

Protocol 7: Response to Endothelium-Dependent Pulmonary Vasodilator Arginine Vasopressin

At the plateau of the pressor response to U-46619, the hormone arginine vasopressin (AVP) is added to the perfusate reservoir to achieve a circulating concentration of 2.5 nM (Fig. 7). In the pulmonary circulation, receptors for AVP appear to be localized to the vascular endothelium (not on vascular smooth muscle), which likely elicit an increase in [$Ca^{2+}$], on activation (14, 15).
Question 10: What do you predict will be the response to AVP? What is the likely mechanism of this response?

Answer: An increase in endothelial \([\text{Ca}^{2+}]_i\) will stimulate the enzyme endothelial nitric oxide (NO) synthase (eNOS), thus increasing NO synthesis (reviewed in Ref. 8). This NO diffuses to the underlying vascular smooth muscle where it elicits relaxation. The resulting vasodilatory response leads to a decrease in vascular resistance according to Poiseuille’s equation. The decrease in P_a in response to AVP will initiate translocation of fluid from the vascular compartment to the perfusate reservoir as a consequence of derecruitment, thus increasing reservoir weight. The administration of AVP to the perfusate reservoir is responsible for the artifactual increase in reservoir weight at time 0 in Fig. 7. Although an increase in endothelial \([\text{Ca}^{2+}]_i\) in response to AVP may be predicted to stimulate the production of prostacyclin and endothelium-derived hyperpolarizing factors (EDHF) as well, previous studies from our laboratory do not support a role for either prostaglandins or EDHF in mediating the pulmonary vasodilatory response to AVP (4, 16). Although we have demonstrated AVP to be an extremely potent and efficacious vasodilator in the isolated perfused rat lung (5), large vasodilatory responses to AVP are not always observed at this point in the demonstration, probably as a result of decreased endothelial viability after a lengthy series of experimental protocols.

STUDENT EVALUATIONS

Students were asked to anonymously fill out a questionnaire at the end of the course that was designed to assess the perceived value of this exercise (Table 1). Our findings indicate that the demonstration was
generally well received by the students. The overwhelming response to question 7 was an appreciation for observing methods by which physiological data are generated, as opposed to only reading about general results and conclusions in a textbook. Furthermore, the students felt that reviewing concepts and equations previously covered in class in the context of an experimental situation was extremely valuable in reinforcing those topics. The major criticism (question 8) of this exercise was that it required too much time (1.5 h) to complete, although other students suggested that more time be allotted for this exercise. Another criticism was the lack of hands-on participation by students, although such participation would be very difficult to incorporate into the demonstration.

DISCUSSION

Additional topics in pulmonary physiology could be included in this demonstration depending on the areas covered in preceding lectures. For example, our students have usually received lectures in cellular mechanisms of vascular smooth muscle contraction and relaxation as well as lectures on endothelium-dependent responses before the demonstration, providing us with the opportunity to review in detail signal transduction mechanisms associated with receptor-mediated pulmonary vasoconstrictors such as the thromboxane mimetic U-46619 and with endothelium-derived NO (EDNO)-dependent pulmonary vasodilators such as AVP. The signal transduction pathways associated with vascular smooth muscle thromboxane-receptor activation are similar to other receptor-mediated vasoconstrictors and have been described previously (3). EDNO-dependent pulmonary vasodilators stimulate eNOS by increasing endothelial \([\text{Ca}^{2+}]\) as described in protocol 7. The NO produced elicits relaxation of vascular smooth muscle through various mechanisms, which include stimulation of soluble guanylyl cyclase and direct activation of potassium channels on vascular smooth muscle. The signal transduction pathways associated with these responses have also been previously described (2, 10, 13).

Other pulmonary vasoconstrictor stimuli or endothelium-dependent dilators may alternatively be used for this demonstration. For example, the lung may be ventilated with a hypoxic gas mixture to demonstrate hypoxic vasoconstriction (11), allowing review of the current evidence regarding the cellular mechanisms associated with this response (7; reviewed in Ref. 17). Furthermore, many other receptor-mediated endothelium-dependent vasodilators may be used in this preparation, including serotonin, endothelin-1, and histamine (9, 12).

A further concept that could be incorporated into the present demonstration is that of vascular permeability and the Starling equation (18). Measurement of reservoir weight not only provides an index of acute changes in vascular fluid volume but is useful in determining lung fluid flux under steady-state conditions (6). For example, the doubling of flow and associated increase in \(P_{\text{a}}\) in protocol 1 results in a rapid drop in reservoir weight as recruitment causes lung fluid volume to increase. However, the rate of change in reservoir weight under steady-state conditions is greater after the pressure increase than before. This
increased lung fluid flux can be attributed to greater capillary hydrostatic pressure as described by the Starling equation. The rationale for adding albumin to the perfusion buffer to maintain proper oncotic pressure can also be explained by this relationship. Finally, simple protocols may be incorporated to demonstrate methods for calculating the capillary filtration coefficient in the isolated lung as previously described (1).

In summary, we have described a pulmonary circulation laboratory demonstration that reinforces concepts in pulmonary physiology typically taught in a first-year graduate curriculum. This demonstration allows students to observe methods by which physiological data are generated using an isolated, perfused rat lung model, a preparation frequently used in studies of the pulmonary circulation, and provides students an opportunity to interpret the results. This preparation uses a commonly used laboratory animal and can be set up with standard equipment found in most cardiovascular physiology laboratories. Student evaluations revealed that this laboratory exercise was generally perceived by the students as an effective and enjoyable learning experience.

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References