Blood flow distribution within the airway wall

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Blood flow distribution within the airway wall. J Appl Physiol 92: 1964–1969, 2002. First published January 18, 2002; 10.1152/japplphysiol.00721.2001.—Altered perfusion of the bronchial mucosal plexus relative to the adventitial plexus may contribute to geometric changes in the airway wall and lumen. We studied bronchial perfusion distribution in sheep by using fluorescent microspheres at baseline and during intrabronchial artery challenge with methacholine chloride (MCh; n = 7). Additionally, we measured airway resistance (Raw) during MCh with control or increased perfusion (n = 9). Raw with MCh was significantly greater for high than control flow. Microspheres in histological sections lodged predominantly in the mucosa (60%), and this was not altered by MCh. However, more microspheres lodged in airways >1-mm in diameter during MCh and increased perfusion than MCh and control flow. In airways ≤1 mm in diameter, fewer microspheres lodged during control than increased flow. If the number of microspheres represents regional agonist access to airway smooth muscle, then the differences observed in Raw can be explained by the distribution of agonist. During challenge, there was greater MCh delivery to larger airways during increased flow and less delivery to smaller airways during control flow. The results demonstrate the effects of arterial perfusion distribution on Raw.

Methods

Experimental preparation. The Johns Hopkins Animal Care and Use Committee approved our study protocol. Anesthesia was induced in sheep (25–35 kg) with intramuscular ketamine (30 mg/kg) and subsequently maintained with pentobarbital sodium (20 mg·kg−1·h−1). A tracheotomy was performed, the sheep were paralyzed with pancuronium bromide (2 mg iv), and the lungs were mechanically ventilated (10–12 ml/kg) at a rate (12–15 breaths/min) sufficient to maintain normal blood gases. Positive end-expiratory pressure (5 cmH2O) was applied. The left thorax was opened at the fifth intercostal space, and heparin (20,000 U) was administered. The esophageal and tracheal tracheal branches of the bronchoesophageal artery were ligated as previously described (18). The bronchial branch of the bronchoesophageal artery was isolated, cannulated, and perfused (0.6 ml·min−1, kg−1) with autologous blood withdrawn from the descending aorta and pumped through a variable-speed roller pump (Gilson, Middleton, WI).

Raw. In the first series of nine sheep, the effects of methacholine chloride (MCh; Sigma Chemical, St. Louis, MO) on edema of mucosal vessels have been suggested to encroach on the airway luminal space, thereby causing airway obstruction (12). Additionally, it is not clear whether the responsivity of vessels located in the adventitial plexus differs from that of mucosal vessels. Selective vasodilation of one bed may result in specific airway functional changes. Thus knowledge of the blood flow distribution is important. Fluorescent microspheres are a useful tool to study both spatial and quantitative distribution of blood flow. Glenny et al. (6) have used the technique extensively to chart the spatial distribution of pulmonary blood flow under a variety of experimental conditions. The technique also has been used to measure blood flow to airways (2). In the present study, we used fluorescent microspheres and morphometric techniques to determine the perfusion distribution of the bronchial artery within the normal airway wall and during experimentally controlled changes in bronchial blood flow, vasodilation, and airway smooth muscle constriction. We tested the hypothesis that, in sheep, methacholine, a bronchial vasodilator and airway smooth muscle constrictor, would shift the blood flow distribution within the airway wall to the mucosa and thereby contribute to enhanced Raw.

The complex anatomy of the vasculature supplying the subcarinal airways has been known for many years. The unique arrangement of parallel vascular plexuses of the bronchial circulation was described by Miller (10). However, the precise route for blood flow through this vasculature is still unknown. Although the bronchial artery supplies the vessels of the outer adventitial plexus as well as the inner mucosal plexus, it is not clear whether changes in flow, vasodilation, or airway smooth muscle constriction alter perfusion distribution within the airway wall. The concept that adventitial engorgement and edema might alter airway-parenchymal tethering has been proposed (12). However, our laboratory’s previous work demonstrated that neither a general increase in the airway wall area (4) or specific increase in adventitial area due to edema fluid (17), nor vascular congestion (3), altered airway luminal area or airway resistance (Raw). However, engorgement and edema of mucosal vessels have been suggested to encroach on the airway luminal space, thereby causing airway obstruction (12). Additionally, it is not clear whether the responsivity of vessels located in the adventitial plexus differs from that of mucosal vessels. Selective vasodilation of one bed may result in specific airway functional changes. Thus knowledge of the blood flow distribution is important. Fluorescent microspheres are a useful tool to study both spatial and quantitative distribution of blood flow. Glenny et al. (6) have used the technique extensively to chart the spatial distribution of pulmonary blood flow under a variety of experimental conditions. The technique also has been used to measure blood flow to airways (2). In the present study, we used fluorescent microspheres and morphometric techniques to determine the perfusion distribution of the bronchial artery within the normal airway wall and during experimentally controlled changes in bronchial blood flow, vasodilation, and airway smooth muscle constriction. We tested the hypothesis that, in sheep, methacholine, a bronchial vasodilator and airway smooth muscle constrictor, would shift the blood flow distribution within the airway wall to the mucosa and thereby contribute to enhanced Raw.

Methods

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Raw. In the first series of nine sheep, the effects of methacholine chloride (MCh; Sigma Chemical, St. Louis, MO) on
Raw were studied during control bronchial blood flow and when bronchial blood flow was increased to maintain a constant perfusion pressure despite bronchial vasodilation. This series was performed to determine whether a functional difference would be observed during a constant infusion of MCh delivered at two different blood flows. Conducting Raw was measured by the method of forced oscillation (7). A gas volume of ~30 ml was oscillated for 1.5 s at a frequency of 9 Hz after each tidal breath. Airway pressure was measured at a sidearm of the tracheal cannula, and a flow signal was obtained from a pneumotachograph positioned between the oscillator and the cannula. Oscillatory signals were analyzed with an on-line computer that measured pressures at points of peak flow. An average Raw was obtained over 8–10 oscillatory cycles. Baseline Raw measured in this manner in anesthetized sheep typically results in a value of 1.0 to 2.0 cmH2O.l−1.s−1. Airway reactivity was determined by measuring Raw before and during intrabronchial artery infusions of 0.5, 1, 2, and 5 μg/ml of MCh (2.6 × 10−6, 5.1 × 10−6, 1.0 × 10−5, 2.6 × 10−5 M, respectively) at a constant infusion rate of 1 ml/min (Harvard Instrument, Holliston, MA) among two bronchial blood flow conditions. We obtained concentration-response information while infusing each MCh concentration directly into the bronchial artery at control blood flow (0.6 ml.min−1.kg−1). However, because MCh is known to cause bronchial vascular dilation (19) and, in our system, decreases in bronchial artery perfusion pressure, bronchial blood flow was next increased (high flow) by setting the roller pump to a level that produced a bronchial inflow pressure equal to the bronchial artery pressure recorded at baseline before the first MCh infusion. Steady-state Raw was measured again after an additional 2-min MCh infusion. Then the MCh infusion was stopped, blood flow was set to baseline level, and vascular resistance and Raw returned to prechallenge values. This sequence was repeated for each MCh concentration. In previous experiments, we confirmed that steady-state airway constriction is achieved within 2 min of agonist delivery (19).

Blood flow distribution. To determine the blood flow distribution within subcarinal airways served by the bronchial artery, fluorescent microspheres (15.5 μm; E-Z Trac, Interactive Medical Technologies, North Hollywood, CA) were infused directly into the bronchial artery in another series of seven sheep. After vortexing, 2 × 106 (1 × 106 spheres/ml) fluorescent microspheres suspended in 0.01% Tween in normal saline followed by a 1 ml saline flush were infused (1 ml/min; Harvard Instruments) through a side port of the blood perfusion circuit immediately upstream from the bronchial arterial cannula. MCh (2 μg/ml; 1 ml/min) was infused directly into the bronchial artery through a side port of the blood perfusion circuit upstream from the site of microsphere infusion. Four different fluorescent-colored (fluorescein, 9-aminoacridine, eosin Y, pyronin B) microspheres were randomized for use during four experimental conditions: 1) control perfusion (0.6 ml.min−1.kg−1), 2) MCh infusion with an increased blood flow (to match control perfusion pressure), 3) MCh infusion, and 4) increased blood flow alone. The first three protocols were randomized; however, the increased-flow protocol was performed after the MCh with increased flow, so flow conditions could be matched. Because our laboratory has previously demonstrated that microspheres can have a vasodilatory effect on the bronchial vasculature (13), a 15- to 30-min recovery time was allowed after each microsphere infusion so that bronchial artery pressure could recover to baseline level after each injection. Relative microsphere distribution was determined by examining thick (20-μm) sections of the airway segments prepared histologically. Fluorescent spheres contained in sections of airways were counted and the mucosal and adventitial distribution was determined.

Histology. After each sheep was exsanguinated, the right middle lobe was removed, inflated with 100–150 ml of 4% formaldehyde in 0.1 M phosphate buffer (pH 7.4), and then submerged in 500 ml of the same fixative overnight. The next day, the fixed lobe was cut transversely into three regions (superior, medial, and inferior). Each region was then cut into twenty to twenty-five 1- to 2-cm cubes, washed overnight in buffer to remove the formaldehyde, and infiltrated overnight in a mixture of 50% distilled H2O-50% OCT followed by an additional 18- to 24-h infiltration in 100% OCT. Tissue blocks were oriented in a cryomold so that large airways would be cut in cross section and then frozen in liquid nitrogen-cooled methylbutane for 15 s. Twenty-six alternating 20-μm cryostat sections (comprising ~1.0-mm airway length) of each block were collected on glass slides. The colors, location (internal or external to airway smooth muscle), and number of microspheres within the airway wall in each complete 1-mm length of airway sample were determined by fluorescent microscopy by using an Olympus BX60 microscope equipped with a U-M61002 D/PR709 multiwavelength fluorescent filter cube. The basement membrane perimeter was measured by using NIH Image software, and the maximum airway diameter was calculated. An effort was made to count ~45 airways/sheep.

Analysis. All data are presented as means ± SE. Concentration-response relationships were analyzed by two-way analysis of variance with the Scheffe’s correction for post hoc comparisons. Microsphere measurements for all airways were pooled for each protocol and also analyzed by two-way analysis of variance with the Scheffe’s correction for post hoc comparisons. Significance was accepted at P < 0.05.

RESULTS

Baseline bronchial artery pressure for the entire group of 16 sheep (body weight 31 ± 1 kg) that were studied was 107 ± 6 mmHg at control bronchial blood flow (19 ± 1 ml/min). Bronchial vascular resistance, estimated as the steady-state inflow pressure/control flow, resulted in an average 5.8 ± 0.4 mmHg·ml−1.min−1. In the first series, baseline Raw averaged 1.0 ± 0.2 cmH2O·l−1·s−1. MCh administration directly into the bronchial artery caused concentration-dependent vasodilation. After the highest MCh concentration, bronchial artery pressure decreased to 60 ± 6 mmHg at control flow, and, to maintain constant pressure, bronchial blood flow was increased to a maximal flow of 46 ± 3 ml/min. The concentration-dependent effects of MCh on Raw during the two blood flow conditions are shown in Fig. 1. Despite a constant infusion of MCh, the increase in Raw was greater for the high-blood flow condition at each MCh concentration (P < 0.0001).

The average total number of fluorescent microspheres lodged in the airway wall during control flow conditions is presented in Fig. 2. Increasing airway size was correlated with an increase in the total number of lodged microspheres (r = 0.45; P = 0.0001), suggesting that vascularity contributes to airway wall mass, which increases in proportion to airway size (11). The actual number of airways counted per airway size category is inserted within each bar. When the four experimental conditions are compared, there was a
significant difference in the average total number of microspheres among the four conditions. The mean number of microspheres was $30 \pm 2, 42 \pm 3, 25 \pm 2,$ and $32 \pm 2$ for the control, MCh + high-flow, MCh, and high-flow alone conditions respectively. Most interestingly, the number of microspheres measured in airway walls during the MCh + high-flow condition was significantly greater than in airways during MCh ($P < 0.0001$). Furthermore, there are significant differences between the MCh + high-flow and the high-flow alone conditions ($P = 0.02$) and between the control and the MCh + high-flow conditions ($P = 0.0008$). Surprisingly, there was no difference in the average number of microspheres in the airways between the control flow condition and during either the MCh treatment ($P = 0.53$) or the high-flow condition ($P = 0.93$).

When we analyzed the airways dichotomously as small ($\leq 1$ mm in diameter) and large ($>1$ mm in diameter) airways, thereby evaluating similar size groups, overall the large airways had a significantly greater number of spheres than small airways ($P = 0.0001$). Figure 3 shows the average total number of microspheres for each treatment. Within airways $\leq 1$ mm in diameter, only MCh treatment caused a significant decrease in the total number of microspheres relative to MCh + high flow ($P = 0.008$) and high flow ($P = 0.01$) but did not achieve statistical significance relative to control ($P = 0.07$). The total number of microspheres was indistinguishable from control after MCh + high flow ($P = 0.41$) and high flow ($P = 0.42$). For airways $>1$ mm in diameter, the total number of microspheres lodged during MCh + high flow were significantly greater than control ($P = 0.0007$), MCh ($P = 0.004$), and high flow alone ($P = 0.0007$). The total number of microspheres counted for control conditions was not different from MCh ($P = 0.99$) or high flow ($P = 0.99$).

In addition, we analyzed the distribution of fluorescent microspheres lodged within the airway wall during the four conditions. The percentage of spheres located within the mucosal region of the airway wall relative to the total number of spheres lodged in a specific airway (%mucosal) was $69 \pm 1, 69 \pm 2, 69 \pm 2,$ and $62 \pm 2\%$ for the control, MCh + high-flow, MCh, and high-flow alone conditions, respectively ($P = 0.01$). The high-flow condition demonstrated a small difference from both control ($P = 0.04$) and MCh + high flow ($P = 0.05$).

When the airways are analyzed dichotomously as small and large airways and the treatment is held constant, the large airways demonstrated a small but significant increase in the percentage of microspheres lodged in the mucosa relative to small airways ($P = 0.004$). As observed in Fig. 4, the distribution of microspheres is predominantly mucosal and does not differ substantially across treatment in either airway size category. In the large airways, there were no significant differences among treatment groups ($P > 0.33$). Furthermore, in the small-airway groups, only the
modulating air obstruction is not clear. Several investigators have demonstrated that increases in airway blood flow contribute to the passive washout of airway smooth muscle agonists (8, 19). However, others have suggested that hyperemia and vascular engorgement contribute to airway narrowing (5). The purpose of the present study was to document the bronchial blood flow distribution within the airway wall during basal conditions as well as during agonist challenge. The overall goal was to determine whether shifts in perfusion distribution could explain changes in airway function. Our results demonstrate that increases in Raw during intravascular MCh challenge were significantly greater when bronchial blood flow was increased. Furthermore, our data demonstrate that changes in bronchial perfusion alone did not change the distribution of microspheres within the airway wall to either the large or small airways. Intra-bronchial artery infusion of MCh alone caused a decrease in the number of microspheres to the small airways, whereas the combination of MCh and high flow increased the number of microspheres to the large airways.

The physical setup of these experiments and the fact that MCh dilates the bronchial vasculature as it constricts airway smooth muscle allowed us to compare the steady-state effects of a constant infusion of MCh at two different bronchial blood flow settings. At each of four MCh concentrations, the increases in Raw were significantly greater during the high flow at constant bronchial perfusion pressure condition than during the control flow with decreased perfusion pressure condition. However, our microsphere data demonstrated that the blood flow distribution within the airway wall always remained unchanged. Although small airways (≤1 mm in diameter) had slightly less mucosal perfusion than larger airways (>1 mm in diameter), in general, distribution of blood flow was predominantly to the mucosa (>60%). Furthermore, during each of the four experimental conditions (control flow, MCh + high flow, MCh + control flow, high flow), an approximately equivalent parallel perfusion distribution (i.e., within the wall at any given axial location) with 15.5-µm microspheres was obtained. We interpret this result as demonstrating that the physical effects of blood flow could not account for the differences observed in Raw during the challenge protocols.

However, the results showing total numbers of microspheres delivered to the different sized airways during the four experimental conditions (Fig. 3) does offer a plausible explanation for the differences in Raw reported. First, the number of microspheres lodged in the larger airways (>1 mm in diameter) was significantly elevated only during the MCh + high-flow condition. This finding may explain the increased Raw in this hemodynamic state because Raw is more dependent on large than small airways (14). Increased delivery to these larger airways might lead to increased airway smooth muscle constriction. Furthermore, increased flow alone did not increase the total number of microspheres lodged in this size of airways, suggesting that increased flow and higher than normal perfusion pressures resulted in shunt flow that bypassed 15.5-µm vessels and did not recruit new vessels as did the vasodilator MCh. Although we did not measure shunt flow, this is an interpretation of our results. In retrospect, it would have been useful to measure the concentration of microspheres reaching the left heart as an index of shunt flow. We did not see fluorescent microspheres lodged in the pulmonary capillaries.

In the smaller airways (≤1 mm in diameter), the only condition that resulted in a significant change in the number of microspheres lodged was during MCh alone. Thus there was a significant decrease in the delivery of the airway smooth muscle agonist to the smaller airways only during MCh delivery at control flow. The decreased perfusion pressure likely was responsible for the decreased numbers of microspheres during MCh at control flow. However, it appears that the vasculature of the smaller airways remained fully recruited during the other three conditions because the number of microspheres did not differ among control, MCh + high flow, and high flow alone. Thus the results showing Raw greater during MCh challenge and high flow than MCh challenge and control flow were due to the combined effects of increased MCh delivery to larger airways during high flow and decreased delivery of MCh to smaller airways during control flow. This statement assumes that MCh delivery to airway smooth muscle can be deduced from the lodging of 15.5-µm microspheres in precapillary arterioles of the
airway vasculature as well as that the measure of $R_{aw}$ includes these sizes of airways. Although our hypothesis suggested that parallel redistribution within the airway wall would be associated with differences in $R_{aw}$, only the axial redistribution manifested itself with these experimental protocols.

Consistent with these observations, Scuiri et al. (16) showed proportionate changes in blood flow to mucosal and deep wall vessels treated with intravenous acetylcholine. However, in their study, blood flow measurements were made 10 min after an intravenous bolus injection of acetylcholine. Thus it is not clear that changes in regional blood flow observed with lodged microspheres represented steady-state or recovery measurements. Interestingly, these authors reported that mucosal flow comprised 72% of total airway blood flow to large extraparenchymal (mainstem bronchi) airways. This value is close to that observed in the larger airways (>1 mm in diameter) in the present study (Fig. 4).

Although 15-μm radiolabeled microspheres have long been used to measure regional organ blood flow (15), the availability of fluorescent microspheres has allowed for precise determination of regional distribution when combined with quantitative morphometry (6). Luchtel et al. (9) explored and reported on a variety of tissue fixation techniques to maximize visualization of microspheres. In our preliminary studies, 20-μm-thick sections were the maximum thickness that allowed clear visualization of microspheres lodged within subcarinal airways and their location (internal or external to airway smooth muscle). The standard 15-μm-sized microspheres lodge in precapillary arterioles and have been used extensively to measure airway blood flow (1).

Although our study design involved intrabronchial vascular delivery of agonist, we believe the results have implications for aerosol challenge also. Whereas inhaled contractile agonists may reach airway smooth muscle by direct diffusion from airway lumen to smooth muscle, it is likely that a substantial fraction is taken up in transit by the airway mucosal vasculature as well as the pulmonary capillaries of the peripheral lung. Thus some fraction of the inhaled agonist can be carried in the blood to other sites. We showed previously that the increase in airways resistance induced with an intravenous agonist challenge can be almost completely prevented if the bronchial artery is obstructed (20). These results were interpreted to suggest that agonist in the pulmonary capillaries recirculates to the systemic bronchial circulation where it more directly reaches airway smooth muscle. Consequently, some of aerosolized agonist reaching the lung periphery recirculates to affect both bronchial smooth muscle and bronchial blood flow. From the perspective of study design, we wanted to examine the possibility that blood flow distribution within the airway wall could affect airway function. To study whether this mechanism is operative, we delivered the agonist directly to the bronchial artery where we could control the delivery precisely. Although the study of blood flow distribution within the airway wall after aerosol challenge is of interest, given the lack of precision of aerosol delivery, we opted to study the plausibility of our hypothesis in a preparation that could be controlled more tightly. Our results show that, in a controlled preparation, both vascular resistance and driving pressure can affect the overall airway smooth muscle effect. On the basis of the results of this study, both of these factors could affect intravascular axial delivery of agonist but ultimately could also affect clearance of agonist from airway smooth muscle. Each of these factors contribute to the complexity of the measurement of airways reactivity.

In summary, we have challenged subcarinal airways with an agent that constricts airway smooth muscle and dilates vascular smooth muscle. These dual properties of MCh enabled us to measure $R_{aw}$ at two physiological bronchial blood flow levels that bracket the normal in vivo response. Because of the enhanced $R_{aw}$ observed during the high-flow condition, we evaluated perfusion distribution by examining histological sections after injecting fluorescent microspheres. The radial perfusion distribution within the airway wall was unaltered by any of the experimental conditions. However, larger airways (>1 mm) demonstrated increased numbers of fluorescent microspheres during MCh challenge and high flow, whereas smaller airways (<1 mm in diameter) showed decreased numbers of microspheres during MCh challenge alone compared with control. We assumed that the quantification of fluorescent microspheres represented the availability of agonist to smooth muscle within the airways measured and that the determination of $R_{aw}$ included these airways. Thus the difference in airway reactivity was related to the delivery of both more agonist to larger airways during high flow and less agonist to smaller airways during control flow. The results demonstrate the influence of perfusion distribution on the measured physiological end point of $R_{aw}$.

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