Microsphere-induced bronchial artery vasodilation: role of adenosine, prostacyclin, and nitric oxide

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Pearse, David B., Thomas E. Dahms, and Elizabeth M. Wagner. Microsphere-induced bronchial artery vasodilation: role of adenosine, prostacyclin, and nitric oxide. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H760–H768, 1998.—We previously found that injection of 15-µm microspheres into the bronchial artery of sheep decreased bronchial artery resistance. This effect was inhibited partially by indomethacin or 8-phenylthephyllyline, suggesting that microspheres caused release of a dilating prostaglandin and adenosine. To identify the prostaglandin and confirm adenosine release, we perfused the bronchial artery in anesthetized sheep. In 12 sheep, bronchial artery blood samples were obtained before and after the infusion of 1 × 10^6 microspheres or microsphere diluent into the bronchial artery. Microspheres, but not diluent, decreased bronchial artery resistance by 40% and increased bronchial artery plasma 6-ketoprostaglandin F1α (194.7 ± 45.0 to 496.5 ± 101.3 pg/ml), the stable metabolite of prostacyclin, and prostaglandin (PG) F2α (28.1 ± 4.4 to 46.2 ± 9.7 pg/ml). There were no changes in PGD2, PGE2, thromboxane B2, adenosine, inosine, or hypoxanthine. Pretreatment with dipyridamole, an adenosine uptake inhibitor, did not affect bronchial artery nucleoside concentrations (n = 7). Microsphere-induced vasodilation was not enhanced by dipyridamole (n = 9) and was not inhibited by either the adenosine receptor antagonist xanthine amine congener (n = 4) or the nitric oxide (NO) synthase inhibitor N(G)-monomethyl-L-arginine (n = 8). These results do not support a role for either adenosine or NO and suggest that microspheres caused bronchial artery vasodilation through release of prostacyclin and an unidentified vasodilator.

xanthine amine congener; N(G)-monomethyl-L-arginine; indomethacin; sheep

FOR MORE THAN 20 years, the intravascular injection of labeled microspheres has been used to measure regional blood flow in experimental animals (29). Carbonized microspheres labeled with radioactive elements, color, or fluorescence can be injected into the heart where they mix with blood, flow through conduit arteries, and lodge in capillaries in proportion to the flow received. The microspheres are quantified in the tissue by measurement of the tracer, and tissue flow is calculated by comparison with a reference flow (8). Alternatively, a known number of microspheres may be injected into a cannulated, perfused artery to study flow within a single organ.

This technique provided the ability to make serial measurements of regional blood flow in studies where other methods were either too invasive or anatomically impractical. For example, the use of microspheres has been considered the standard method of measuring bronchial artery blood flow (1–3, 10, 22) because of the relative inaccessibility of the bronchial circulation and the presence of multiple inflowing bronchial arteries (12). One of the major prerequisites of this technique is that microsphere injections do not significantly alter regional arterial resistance (8).

We recently tested the assumption that microspheres do not alter regional arterial tone by injecting 15-µm polystyrene microspheres into the bronchial artery of anesthetized sheep (27). We found that microspheres caused large, transient, dose-dependent decreases in bronchial artery resistance lasting up to 30 min in duration. This response could be elicited by injecting microspheres either into the cannulated pump-perfused bronchial artery or into the left heart of sheep with uncanualted bronchial arteries in which flow was measured by external flow probe (27). Pretreatment with 8-phenylthephyllyline, an adenosine receptor antagonist, blocked 79% of the microsphere effect, suggesting that microspheres caused release of adenosine. Indomethacin pretreatment attenuated the vasodilating effect of microspheres by 37%, suggesting that microspheres also caused the production of a vasodilating product of cyclooxygenase metabolism (27). The purpose of the present study was to make direct measurements of adenine nucleosides and cyclooxygenase-derived prostanoids in bronchial artery blood after microsphere injection and to determine the role of nitric oxide (NO) in the microsphere-induced vasodilation.

METHODS

Perfused Bronchial Artery Preparation

Anesthesia was induced in young sheep (20–30 kg) with intramuscular ketamine (30 mg/kg) and subsequently maintained by intravenous pentobarbital sodium (15 mg/kg loading dose and 20 mg·kg⁻¹·h⁻¹ infusion). Mechanical ventilation was begun via tracheostomy with oxygen-supplemented, warmed, humidified room air at a tidal volume of 12 ml/kg, respiratory rate of 10–15 min⁻¹, and positive end-expiratory pressure of 4 mmHg to maintain an arterial Pco2 of ~40 mmHg and arterial Po2 >80 mmHg. Femoral veins and arteries were cannulated to place Swan-Ganz and systemic arterial catheters and to supply arterial blood for bronchial artery perfusion. After the animals were paralyzed with pancuronium bromide (2 mg iv), a left lateral thoracotomy was performed and heparin sodium (20,000 USP units iv) was administered. A balloon-tipped catheter was placed in the left atrium, the tracheal and esophageal branches of the bronchoesophageal artery were ligated, and the bronchial branch was cannulated with an 18-gauge angiocatheter as previously described (28). The bronchial artery was perfused with a constant flow of blood withdrawn from a femoral artery catheter by a calibrated, variable-speed pump (Gilson...
Microsphere Injections

Colored 15-µm microspheres (Dye-Trak, Triton Technology) were suspended in 0.01% Tween in normal saline (diluent) and vortexed before injection. The absence of microsphere aggregation was confirmed microscopically before injection. For injections into the bronchial artery, all doses of microspheres were suspended in 0.5 ml of diluent before infusion at 1 ml/min through a side port just proximal to the 18-gauge catheter. All injections were followed by a 1-ml flush of normal saline at the same infusion rate.

Bronchial Arterial Blood Collection

Blood samples were obtained from the bronchial artery microcirculation by simultaneously interrupting forward flow, opening a side port of the bronchial artery cannula, and increasing left atrial pressure to 15 mmHg by inflating the balloon-tipped catheter in the left atrium. Under these conditions, retrograde blood flow occurred from the pulmonary to bronchial arteries, which were measured by addition of known concentrations of 2-chloro-4-nitrophenol to block adenosine deamination (12). Bronchial artery blood (4 ml) was collected in a chilled test tube containing 0.4 ml of “stopping solution” consisting of 4 mM 8-azaguanine to inhibit adenosine deaminase, 100 µM dipyridamole to block adenosine uptake, 4 mM EDTA to inactivate adenosine triphosphatase and 5'-nucleotidase, and 5 µg/ml indomethacin to inhibit cyclooxygenase (6). The blood samples were immediately centrifuged and the plasma frozen at −70°C until later analysis.

Adenine Nucleoside, Prostaglandin, and Lactate Measurements

Plasma concentrations of adenosine, inosine, and hypoxanthine were measured by high-pressure liquid chromatography (HPLC) with a System Gold HPLC (model 338, Beckman, Fullerton, CA) as previously described (32). Concentrations of adenosine nucleosides were determined by peak integration and comparison with chromatogram peak areas from known standard concentrations and corrected for recovery that was measured by addition of known concentrations of 2-chloroadenosine to each sample before sample processing. Percent recovery ranged from 50 to 100%.

Plasma concentrations of thromboxane (TX) B₂, the stable metabolite of TXA₂, 6-ketoprostaglandin F₁α, the stable metabolite of prostacyclin, prostaglandin (PG) F₂α, PGD₂, and PGE₂ were measured by combined capillary gas chromatography-mass spectroscopy by a modification of the method described by Liu et al. (24).

Plasma lactate concentrations were measured by standard spectrophotometric assay (Sigma Chemical, St. Louis, MO).

Experimental Protocols

Mediator measurements. In 12 sheep, 1 × 10⁶ microspheres and an equal volume of microsphere diluent alone were injected into the bronchial artery in random order after setting baseline bronchial artery pressure to 140 mmHg. Bronchial blood flow was kept constant after each injection so that changes in bronchial artery pressure reflected changes in bronchial artery resistance. All bronchial pump settings were noted on each record and converted to values of flow based on a four-point pump calibration curve that was generated before each experiment. A retrograde sample of bronchial artery blood was obtained just before and ~6 min after each injection of microspheres or diluent because this time corresponded to the peak microsphere-induced vasodilation in two sheep, multiple microsphere and diluent injections were performed. The data from these injections were averaged to provide a single set of microsphere and diluent data for each sheep.

In seven of these sheep, blood samples were obtained before and after an additional injection of 10⁵ microspheres, which were performed during an intrabronchial artery infusion of dipyridamole (10⁻⁴ M at 1 ml/min) to block the uptake of adenosine into erythrocytes and endothelium (6). Because 1 × 10⁶ microspheres may cause near-maximal bronchial artery vasodilation (27), it was possible that dipyridamole could increase measurable levels of adenosine or its metabolites without causing further enhancement of vasodilation. Therefore, in two other sheep, 1 × 10⁵ microspheres were injected before and during the dipyridamole infusion to better assess the ability of dipyridamole to enhance the microsphere-induced vasodilation. In five of the nine sheep treated with dipyridamole, the efficacy of the dipyridamole infusion was tested by determining the effect of dipyridamole on the bronchial artery vasodilation from an infusion of adenosine. Adenosine was infused into the bronchial artery (10⁻⁵ M at 0.5 ml/min for 3 min) before and 10 min after initiation of dipyridamole. The adenosine (Sigma Chemical) was dissolved in normal saline. The dipyridamole (10⁻³ M solution; the generous gift of Du Pont Pharma, North Billerica, MA) was diluted in normal saline to the desired concentration.

Effect of xanthine amine congener. To examine further the role of adenosine, 5 × 10⁵ microspheres were injected in four sheep during an infusion of xanthine amine congener (XAC), a selective adenosine receptor antagonist (38). Changes in bronchial artery pressure at constant bronchial artery flow were measured under the following conditions in each sheep: 1) 10⁻⁵ M adenosine infused (1 ml/min) into the bronchial artery, 2) XAC vehicle infused (1 ml/min) into the bronchial artery, 3) 10⁻⁵ M adenosine and XAC vehicle, 4) 10⁻⁵ M adenosine and XAC (10⁻⁵ M at 1 ml/min), 5) 5 × 10⁵ microspheres and XAC vehicle, and 6) 5 × 10⁵ microspheres and XAC. XAC or XAC vehicle was always infused for 10 min before administration of either adenosine or microspheres. The XAC (Research Biochemical, Natick, MA) solution was prepared by diluting a stock solution containing 10⁻³ M XAC in 0.1 M NaOH with normal saline.

Effect of N⁶-monomethyl-L-arginine. To determine the role of NO, microspheres (5 × 10⁵ to 1.1 × 10⁶) were injected in eight sheep before and after an infusion of N⁶-monomethyl-L-arginine (L-NMMA, 10⁻³ M at 1 ml/min), a NO synthase inhibitor. Because L-NMMA increased baseline bronchial artery resistance, sodium nitroprusside (SNP), a NO donor, was infused (10⁻⁴ M at ~1 ml/min) with L-NMMA to reverse the L-NMMA-induced increase in resistance before the second microsphere dose was infused. Because NO is capable of inhibiting (5) or stimulating (11) prostacyclin production, it...
was possible that either NO synthase inhibition or SNP could indirectly alter the microsphere-induced vasodilation through changes in prostacyclin concentration. Therefore, four of these sheep were pretreated with indomethacin (5 mg/kg iv over 30 min followed by 3 mg·kg⁻¹·h⁻¹) before the microsphere injections. The indomethacin-treated sheep were injected with larger microsphere doses to produce a comparable level of microsphere-induced vasodilation to that seen in the nonindomethacin-treated animals. We previously showed in this preparation that this dose of indomethacin completely inhibited the transient pulmonary hypertension caused by intrapulmonary artery injection of arachidonic acid (27). L-NMMA and SNP (Sigma Chemical) were dissolved in normal saline. The indomethacin (Sigma Chemical) solution was prepared by dissolving 100 mg in 50 ml normal saline and 10 ml of 1 M NaHCO₃.

Statistics

The effect of microspheres on bronchial artery resistance and the effects of dipyridamole and L-NMMA on microsphere-induced vasodilation were analyzed by paired t-test. The baseline pressures and flows, the XAC data, and the effect of L-NMMA and SNP on baseline bronchial artery tone were analyzed by one-factor analysis of variance with one repeated measure. The measurements of adenine nucleoside metabolites and prostaglandins were analyzed by a two-factor (microsphere, microsphere diluent) analysis of variance with two repeated measures (34). When significant variance ratios (P ≤ 0.05) were obtained, least significant differences were calculated to allow comparison of individual means. Values presented in the text are means ± SE. Differences were considered significant when P ≤ 0.05.

RESULTS

A total of 22 sheep (27.6 ± 1.0 kg body wt) was studied. Initial bronchial artery blood flow averaged for all animals was 0.98 ± 0.05 ml·min⁻¹·kg⁻¹ and resulted in a bronchial artery pressure of 140.2 ± 0.8 mmHg and a bronchial artery resistance of 151.8 ± 11 mmHg·ml⁻¹·min·kg. Baseline aortic, pulmonary artery, left atrial, and tracheal pressures averaged 107 ± 3.4, 13.5 ± 1.0, 5.9 ± 0.5, and 11.6 ± 0.5 mmHg, respectively.

Effect of Microspheres on Bronchial Artery Adenine Nucleosides and Lactate

Figure 1 is a record from a representative experiment showing the effect of microspheres and the retrograde bronchial artery blood collection technique on vascular pressures. Injection of 1 × 10⁶ microspheres caused a rapid 40% decrease in bronchial artery pressure (140 to 84 mmHg) within the first 6 min of injection without affecting left atrial, systemic artery, or pulmonary artery pressures. Bronchial artery flow was kept constant so that changes in bronchial artery pressure directly reflected changes in resistance. Bronchial artery pressure spontaneously returned to baseline 35 min after injection. To collect bronchial artery blood, the bronchial artery pump was stopped, a side port from the bronchial artery cannula was opened (indicated by bronchial artery pressure of 0 mmHg), and left atrial pressure was increased to 15 mmHg by inflating the left atrial balloon catheter. As shown in Fig. 1, this maneuver had little effect on systemic artery pressure but increased pulmonary artery pressure to 20 mmHg and pulmonary capillary pressure to a value between 15 and 20 mmHg, thereby increasing the gradient for retrograde bronchial blood flow. Interestingly, interruption of antegrade bronchial artery flow after microsphere injection had no apparent effect on the subsequent course of the microsphere-induced vasodilation.

Figure 2 shows the mean decrease in bronchial artery resistance after the injection of either 1 × 10⁶ microspheres or the same volume of diluent into the bronchial artery as well as the level of baseline bronchial artery resistance measured just before injection. Microspheres caused a 36.9 ± 2.6% decrease in bronchial artery resistance, whereas resistance did not change after diluent injection. Baseline values of bron-

![Fig. 1. Experimental record from a single experiment demonstrating microsphere-induced bronchial artery vasodilation and retrograde collection of bronchial artery blood before and after injection (arrow) of 1 × 10⁶ 15-µm microspheres into bronchial artery of an anesthetized sheep. P BA, bronchial artery pressure; P IA, left atrial pressure; P PA, pulmonary artery pressure; P ART, systemic arterial pressure.](http://ajpheart.physiology.org/Downloaded from 10.220.32.246 on October 14, 2017)
chial artery pressure, flow, and resistance measured just before injection of either microspheres or diluent did not differ, averaging 141.5 ± 6.1 mmHg, 1.01 ± 0.6 ml·min⁻¹·kg⁻¹, and 153.4 ± 11 mmHg·ml⁻¹·min⁻¹·kg⁻¹, respectively.

As shown in Fig. 3, neither microspheres nor microsphere diluent caused detectable changes in bronchial artery concentrations of adenosine or its metabolites in the presence or absence of dipyridamole. Microspheres also had no effect on bronchial artery lactate concentration, which averaged 1.2 ± 0.1 mM (data not shown).

Effect of Dipyridamole

Dipyridamole pretreatment did not augment the microsphere-induced vasodilation, whereas the decreased bronchial artery resistance caused by exogenous adenosine was greatly enhanced in the presence of dipyridamole from -20.9 ± 3.0 to -40 ± 4.1% of baseline resistance (Fig. 4). Two of these sheep were injected with a lower dose of microspheres (1 × 10⁵) to more closely approximate the vasodilation from 10⁻⁵ M adenosine. As with the larger microsphere dose, dipyridamole failed to enhance the microsphere-induced vasodilation from 1 × 10⁵ microspheres (microspheres alone, -23.2%; microspheres with dipyridamole, -24.4%). Dipyridamole decreased baseline bronchial artery resistance, but the decrease observed before the adenosine injection was not different from the increase seen before the microsphere injection (Fig. 4).

Effect of XAC

As shown in Fig. 5, XAC had no effect on the microsphere-induced decrease in bronchial artery resistance after 5 × 10⁵ microspheres (microspheres plus XAC diluent, -29.3 ± 2.7%; microspheres plus XAC, -28.8 ± 6.2%). As a positive control, the same dose of XAC, but not its vehicle, blocked 81% of the bronchial artery vasodilation caused by infusion of 10⁻⁵ M adenosine into the bronchial artery. XAC increased baseline bronchial artery resistance, but the increase observed before the adenosine injection was not different from the increase seen before the microsphere injection.

Effect of Microspheres on Bronchial Artery Prostaglandins

Figure 6 shows the effect of 1 × 10⁶ microspheres or microsphere diluent on bronchial artery plasma concentrations of 6-keto-PGF₁α, TxB₂, PGF₂α, and PGE₂ (PGD₂ was not detected). Microspheres, but not microsphere diluent, caused a significant, 2.5-fold increase in the concentration of 6-keto-PGF₁α (194.7 ± 45.0 to 496.5 ± 101.3 pg/ml) and a nearly 2-fold increase in the level of PGF₂α (28.1 ± 4.4 to 46.2 ± 9.7 pg/ml). There were no changes in either TxB₂ or PGE₂ concentrations.

Figure 7 shows the average bronchial artery 6-keto-PGF₁α concentrations and the change in bronchial artery resistance associated with three serial injections of 1 × 10⁶ microspheres in the two sheep that received multiple injections. Each injection of microspheres, but...
not diluent, caused a large decrease in bronchial artery resistance associated with an increase in bronchial artery plasma 6-keto-PGF₁α concentration.

**Effect of L-NMMA**

In the absence of indomethacin, infusion of 10⁻³ M L-NMMA at 1 ml/min into the bronchial artery caused a 38% increase in bronchial artery resistance (Fig. 8). The addition of SNP (10⁻⁴ M at 0.1 ± 0.5 ml/min) reversed this increase in resistance, allowing the second microsphere injection to occur at a comparable level of bronchial artery resistance. As shown on Fig. 8, left, L-NMMA did not inhibit microsphere-induced vasodilation; in fact, the combination of L-NMMA and SNP significantly augmented the decrease in resistance by 50% (−32.1 ± 4 to −47.3 ± 4.3%). In the presence of indomethacin, L-NMMA still failed to block the vasodilation from microspheres, but the enhancing effect of L-NMMA plus SNP was lost (Fig. 8, right).

**DISCUSSION**

**Potential Effects of Microspheres on the Circulation**

Theoretically, the injection of microspheres into a vascular bed results in two populations of vessels: occluded and unoccluded. The occlusion of vessels could lead to decreased vascular resistance if significant tissue ischemia causes release of diffusible vasodilator substances. For example, Hori et al. (18) showed that injection of 15-µm microspheres into the coronary circulation of dogs caused sustained coronary vasodilation that was inhibitable by theophylline and associated with increased coronary sinus adenosine and lactate concentrations, suggesting that injection of microspheres caused adenosine-induced vasodilation from ischemic heart injury (18). Although not considered by Hori et al. (18), events taking place in nonoccluded vessels could also be a source of vasodilation after microsphere injection. When a microsphere occludes a small arteriole, adjacent nonoccluded vessels are subjected to increased flow and, therefore, increased shear stress. This would occur either under conditions of constant arterial inflow (as in the current experiments) or constant arterial pressure; the former is because of flow diversion, the latter because of an increased pressure drop across the occluded region secondary to a decrease in downstream pressure (35). As discussed below, increased shear stress can cause release of vasodilating substances from the vessel wall (33).

In addition to the indirect effects of increased shear stress in the nonoccluded vessels, video microscopy studies of in vivo microsphere behavior showed that a direct interaction between microspheres and vascular endothelium may persist in nonoccluded vessels (16, 17). Microspheres moved slowly through the microcirculation frequently lodging at branch points or on the walls of larger arterioles allowing erythrocytes to pass...
by (16, 17). These aberrant microspheres protruded into the vessel lumen causing long microsphere chains to form with only partial flow obstruction (17). Thus many microspheres appear to have prolonged direct contact with endothelial cells in vessels with continued blood flow.

On the basis of these considerations, there are two possible mechanisms to explain how microspheres caused vasodilation in the bronchial vascular bed. First, microspheres may have caused ischemia resulting in the release of adenosine and, presumably, lactate. Second, microspheres may have caused release of vasodilating mediators either from the indirect effects of increased shear stress in the nonoccluded vessels or the direct contact of microspheres with the endothelial surface of partially occluded vessels.

Methodological Considerations

To identify directly the presence of vasodilating substances in the bronchial circulation, we collected bronchial artery blood by transiently allowing retrograde flow from the pulmonary circulation to the bronchoesophageal artery. As shown in Fig. 1, we simultaneously increased left atrial and, therefore, pulmonary capillary pressure to increase the gradient for retrograde bronchial blood flow. We collected 4 ml of retrograde bronchial artery blood because we found that a 3-ml injection of contrast into the unperfused sheep bronchial artery caused enhancement of small airways by high-resolution computed tomography with little or no signal observed in the pulmonary circulation (unpublished data). We obtained a bronchial artery blood sample before each microsphere or diluent injection to provide baseline mediator concentrations and control for the potential effects of the transient increase in left atrial pressure on vasoactive mediators (36).

Role of Adenosine

Based on our previous results with 8-phenyltheophylline (27), we were surprised to find no change in adenosine concentration after injection of microspheres (Fig. 3) despite a similar vasodilatory response (Fig. 2). There are two possible explanations for these contradictory data. First, adenosine could have mediated microsphere-induced vasodilation, but its rapid removal by cellular uptake or metabolism prevented us from detecting increased plasma levels. Adenosine is removed from the circulation by an active nucleoside transporter system present in erythrocytes and endothelial cells (4) and by metabolism via adenosine deaminase to inosine and hypoxanthine (6). This explanation seemed unlikely in our preparation, however, because we did not detect changes in inosine or hypoxanthine after microspheres, and infusion of dipyridamole, an adenosine uptake inhibitor, into the bronchial artery did not alter the concentrations of adenosine or its metabolites after microsphere injection (Fig. 3). Interestingly, Grantham et al. (15) found negligible adenosine uptake and metabolism in the isolated buffer-perfused bronchial circulation of sheep, suggesting that bronchial artery endothelium may lack the nucleoside transporter system.

Second, it was possible that the previously demonstrated attenuation of the microsphere-induced vasodilation by (16, 17). These aberrant microspheres protruded into the vessel lumen causing long microsphere chains to form with only partial flow obstruction (17). Thus many microspheres appear to have prolonged direct contact with endothelial cells in vessels with continued blood flow.

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lation by 8-phenyltheophylline represented a nonspecific effect of the drug. To address this possibility, we examined the effects of dipyridamole, an adenosine uptake inhibitor, and the adenosine receptor antagonist XAC (38) on the vasodilation from microspheres and exogenous adenosine. XAC was selected because it has 100-fold greater affinity for adenosine receptors and is 2,500-fold more soluble than 8-phenyltheophylline (38). Bruns and Fergus (7) showed that adenosine receptor antagonists with a solubility to receptor affinity ratio of <100 had poor in vivo activity. The ratios for 8-phenyltheophylline and XAC are 6.3 and 3,300, respectively, for A2 receptor antagonism, suggesting that the in vivo effectiveness of 8-phenyltheophylline was inferior (7).

As shown in Fig. 4, dipyridamole had no effect on the magnitude of the vasodilation to microspheres, whereas it significantly potentiated the effect of exogenous adenosine. The lack of potentiation of the microsphere effect was not because of an inability of further bronchial artery dilation inasmuch as the vasodilation to a smaller dose of microspheres in two of these sheep was also not enhanced by dipyridamole. Dipyridamole alone caused a significant decrease in bronchial artery resistance (Fig. 4), presumably from a combination of adenosine uptake blockade and cGMP phosphodiesterase inhibition (39). This effect was small, however, and did not prevent the potentiation of the vasodilation from exogenous adenosine. It was therefore not an explanation for the inability of dipyridamole to enhance the microsphere effect.

XAC markedly inhibited the vasodilation from exogenous adenosine but had no effect on the decrease in bronchial artery resistance after microsphere injection (Fig. 5). XAC, but not its vehicle, increased baseline bronchial artery resistance, suggesting that endogenous adenosine was a determinate of baseline bronchial artery tone. The small increase in baseline resistance was not an explanation for the inability of XAC to block microsphere-induced vasodilation, however, because the same increase in resistance occurred before XAC successfully blocked the vasodilation from exogenous adenosine (Fig. 5). The dipyridamole, XAC, and lactate data support the negative results shown in Fig. 3 and suggest that ischemia-induced adenosine release does not mediate microsphere-induced vasodilation in the bronchial circulation.

Role of Prostaglandins and NO

It is well established that increased shear force from increased blood velocity causes vasodilation (33). Although some studies suggest that this mechanical stimulus may be capable of directly dilating vascular smooth muscle, the preponderance of evidence indicates that vasodilation occurs in this setting from the release of endothelium-derived vasodilators (33). Depending on the vascular bed and species, vasodilating prostaglandins (21), NO (23), or the combination of prostaglandins and NO (20) has been shown to be responsible for this phenomenon. Our previous results suggested that a dilator prostaglandin was partially responsible for the effect of microspheres on bronchial artery tone, but the identity of the prostanoïd was not determined and the role of NO was not examined (27).

As shown in Fig. 6, microspheres, but not microsphere diluent, caused a large increase in the bronchial artery concentration of 6-keto-PGF1α, the stable metabolite of prostacyclin. Prostacyclin is a potent vasodilator that is produced primarily by endothelial cells (25), suggesting that the interaction of microspheres with the bronchial artery endothelium resulted in the production and release of prostacyclin. These data explain the previously observed inhibitory effect of indomethacin on microsphere-induced vasodilation (27).

There was also a significant increase in PGF2α, a smooth muscle constrictor, but the levels of this prostanoioid were an order of magnitude lower than the 6-keto-PGF1α concentrations. Although the cellular source of the PGF2α increase is uncertain, the ratio of 6-keto-PGF1α to PGF2α observed in this study was similar to the ratios measured from isolated porcine coronary, carotid, and pulmonary arteries (19), suggesting that the increased 6-keto-PGF1α and PGF2α were both generated by the bronchial artery wall rather than inflammatory cells.
As shown in Fig. 7, repetitive injections of microspheres produced superimposable decreases in bronchial artery resistance accompanied by reproducible increases in the concentration of bronchial artery 6-keto-PGF$_{1\alpha}$. Each microsphere injection was also associated with increases in PGF$_{2\alpha}$ (PGF$_{2\alpha}$ = 25, 52, and 32 pg/ml, respectively; data not shown). Although these data are derived from only two sheep, they suggest that the mechanism causing activation of cyclooxygenase was not subject to tachyphylaxis from either feedback inhibition of prostacyclin synthase (25) or endothelial injury.

To examine the role of NO, we performed microsphere injections before and during an infusion of L-NMMA into the bronchial artery. As shown in Fig. 8, L-NMMA alone caused a 38% increase in bronchial artery resistance, suggesting a significant role for constitutive NO in the maintenance of normal bronchial artery tone. This result is similar to that of Sasaki et al. (31) who showed that NO synthase inhibition decreased baseline bronchial artery blood flow by ~60% in anesthetized sheep. This increased bronchial artery resistance could be reversed by L- but not D-arginine, supporting the specificity of the NO synthase inhibitor (31).

To allow the two microsphere injections to be made from the same level of baseline tone, we reversed the increased bronchial artery resistance caused by L-NMMA with SNP. We did not use an arterial constrictor to match the increased tone caused by L-NMMA because of the potential differences in the loci of vasoconstriction under the two conditions. We reasoned that vasodilating the constricted vascular bed with SNP would more accurately mimic the vascular hemodynamics that were present before administration of either drug. Moreover, NO is known to cause feedback inhibition of NO synthase (9), thus providing additional NO synthase blockade.

We were surprised to find that the combination of L-NMMA and SNP enhanced the vasodilation from microspheres (Fig. 8). Given the complex relationship between NO and cyclooxygenase (5, 11, 13, 30), we hypothesized that either NO synthase inhibition or SNP resulted in an upregulation of microsphere-induced prostacyclin generation. For example, endogenous NO inhibited prostacyclin synthesis in rat diaphragmatic arterioles (5), and exogenous NO caused a dose-dependent inhibition of bradykinin-induced generation of prostacyclin from endothelial cells (13). Alternatively, both endogenous (11) and exogenous NO (11, 30) have been shown to stimulate in vivo (30) and in vitro (11, 30) prostacyclin production, possibly through activation of prostaglandin H synthase (11). We therefore repeated the protocol in four additional sheep that were pretreated with indomethacin to block prostacyclin production. As shown in Fig. 8, indomethacin pretreatment prevented the enhanced vasodilation associated with L-NMMA and SNP, suggesting that additional stimulation of prostacyclin production was probably responsible. More importantly, microsphere-induced vasodilation was not inhibited by NO synthase blockade, suggesting that NO was not involved.

Although our results clearly indicate that prostacyclin plays a role in microsphere-induced vasodilation in the bronchial circulation, indomethacin blocked less than half of the response (27); thus the major source of the vasodilation remains unknown. A growing amount of literature has shown the existence of endothelium-derived vasodilating substances other than NO and prostacyclin in several vascular systems including sheep bronchial artery (31), rat basilar artery (14), and rat cremaster muscle artery (37). One possible candidate vasodilator that may explain these data is endothelium-derived hyperpolarizing factor (EDHF), an as yet uncharacterized substance (26) that is released from endothelium by muscarinic agonists, increased intracellular calcium concentration, and, possibly, increased shear stress (26). Interestingly, EDHF may be more important than NO in the vasomotor regulation of small peripheral arterioles (26), a finding which could explain why NO did not appear to contribute to microsphere-induced vasodilation in the bronchial circulation. Alternatively, much of the vasodilation from microspheres could result from a mediator-independent process through direct cell-to-cell communication between the endothelium and smooth muscle (33). Further studies to elucidate the effect of microspheres on bronchial artery tone may provide insight into mechanisms of microcirculatory control. On a more practical level, microsphere-induced vasodilation has the potential to cause an overestimation of true blood flow if large frequent injections of microspheres are utilized to estimate regional perfusion (27).

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