5,6-Epoxycosatrienoic acid reduces increases in pulmonary vascular resistance in the dog

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Stephenson, Alan H., Randy S. Sprague, and Andrew J. Lonigro. 5,6-Epoxycosatrienoic acid reduces increases in pulmonary vascular resistance in the dog. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H100–H109, 1998.—We recently reported that canine pulmonary microsomes metabolize arachidonic acid to all four regioisomic epoxycosatrienoic acids (EET). 5,6-EET dilates blood vessels in several nonpulmonary vascular beds, often in a cyclooxygenase-dependent manner. The present study was designed to determine whether 5,6-EET can decrease pulmonary vascular resistance (PVR) in the intact pulmonary circulation. In isolated canine lungs perfused with physiological salt solution, a constant infusion of U-46619 (3.28 μmol/min) increased PVR 62.1 ± 4.5%. Administration of 5,6-EET (10⁻⁵ M) into the perfusate reduced the U-46619-mediated increase in PVR by 23.6 ± 6.1%. These effects of U-46619 and 5,6-EET were limited to changes in resistance solely in the pulmonary venous segment. In contrast, venous as well as arterial segmental resistances were increased in 5-hydroxytryptamine (5-HT)-treated lungs. However, in the latter instance, 5,6-EET reduced arterial but not venous segmental resistance. 5,6-EET increased pulmonary PGI₂ synthesis from 70.5 ± 18.4 to 675.9 ± 125.4 ng/min. In the presence of indomethacin (10⁻⁴ M), 5,6-EET did not increase PGI₂ synthesis nor did it decrease U-46619- or 5-HT-mediated increases in PVR. In canine intrapulmonary vessels, 5,6-EET decreased active tension in veins contracted with U-46619. 5,6-EET decreased active tension in arteries but not veins contracted with 5-HT, consistent with results in the perfused lungs. These results demonstrate that 5,6-EET is a vasodilator in the intact pulmonary circulation. Its dilator activity depends on the constrictor agent present, the segmental resistance, and cyclooxygenase activity. 

thromboxane; prostacyclin; cyclooxygenase; pulmonary vasculature; segmental resistance

Epoxycosatrienoic Acids (EET) are compounds formed by cytochrome P-450 monooxygenase-mediated insertion of an epoxide group across the unsaturated carbons of arachidonic acid (AA) at positions 5,6; 8,9; 11,12; or 14,15 (3, 12). Synthesis of all four EET regioisomers by microsomal preparations of lung tissue has been reported in dogs (40), rabbits (47), and guinea pigs (19). The predominant metabolites of AA metabolism in each of these studies were EET and their hydration products, the dihydroxyicosatrienoic acids (DHET). In addition, endogenously synthesized EET were detected in homogenates of rat and human lung (46). Although the lung is capable of synthesizing EET from AA, the effects of EET on the intact pulmonary circulation have not previously been reported. In other vascular beds, when applied exogenously, EET generally have been reported to act as vasodilators, often requiring cyclooxygenase activity to express the dilator activity (15, 23, 24).

It has been suggested that endogenous vasodilator substances act to moderate pulmonary vasoconstrictor influences such as thromboxane (Tx)A₂ and 5-hydroxytryptamine (5-HT) to maintain low pulmonary vascular resistance (PVR) (1). However, in addition to lowering resistance, these vasodilator substances have the potential to oppose vasoconstriction that occurs in local areas of alveolar hypoxia. When this occurs, perfusion of poorly oxygenated lung units results in systemic hypoxemia due to addition of this poorly oxygenated blood into the systemic arterial circulation, i.e., venous admixture. In a dog model of ethchlorvynol-induced acute lung injury, we previously reported that inhibition of cytochrome P-450 activity prevented the increase in venous admixture associated with the systemic hypoxemia of that injury (40). We therefore propose that if 5,6-EET acts as a dilator in the pulmonary circulation, it may oppose increases in PVR and contribute to the increased venous admixture of acute lung injury. Because inhibition of cyclooxygenase activity also prevents the systemic hypoxemia associated with acute lung injury (21, 22, 38, 44), we propose that 5,6-EET requires cyclooxygenase activity to express its pulmonary vasodilator properties.

To determine whether exogenously administered 5,6-EET can cause pulmonary vasodilation, we examined its effects in isolated perfused canine lungs in which PVR was increased with 9,11-dideoxy-11α-epoxy-methano-PGF₂α (U-46619), a TxA₂ mimetic, or 5-HT. U-46619 was chosen, since it has been used as a vasoconstrictor in studies examining the effects of EET on other vascular beds (16, 35, 36). However, in the dog, U-46619 selectively increases pulmonary venous segmental resistance. Therefore 5-HT was used because it increases both pulmonary arterial and venous resistance in the dog. The 5,6-EET regioisomer was selected for this study because it is the only EET that can undergo cyclization via cyclooxygenase activity to form endoperoxide intermediates and prostaglandin analogs (28). These metabolites have been suggested to mediate the vasodilator effect of 5,6-EET reported for the perfused rabbit kidney (6), rat tail artery (5), and rat intestinal microvessels (32), preparations in which 5,6-EET required cyclooxygenase activity to produce vasodilation.

We previously reported that 5,6-EET relaxes PGF₂α-contracted isolated canine pulmonary venous rings in a concentration- and cyclooxygenase-dependent manner; i.e., indomethacin prevented the reduction in tension (40). Here, we report in the intact pulmonary circulation of the dog that 1) 5,6-EET can inhibit the increases...
in PVR mediated by U-46619 and 5-HT; 2) the effect of 5,6-EET on PVR is associated with an increase in pulmonary prostaglandin synthesis; and 3) inhibition of cyclooxygenase activity eliminates the effect of 5,6-EET on PVR and prevents the 5,6-EET-mediated increase in endogenous prostaglandin synthesis.

METHODS

Preparation of isolated lungs for perfusion with physiological salt solution. Adult microfilaria-free male mongrel dogs (24–34 kg) were anesthetized with intravenous pentobarbital sodium (30 mg/kg), anticoagulated with intravenous heparin (10,000 U), and exsanguinated. Via a left lateral thoracotomy, the left lower lung lobe was isolated as previously described (45). Cannulas were inserted into the pulmonary artery and pulmonary vein. The lobe was excised, supported on a nylon mesh (Nytex) platform, and suspended from an isometric force transducer (FT03, Grass) within a heated (37°C), humidified box. The lobe was perfused with a physiological salt solution (PSS) containing (in mM) 118.3 NaCl, 4.7 KCl, 2.5 CaCl\(_2\), 1.2 MgSO\(_4\), 1.2 KH\(_2\)PO\(_4\), 25.0 NaHCO\(_3\), 0.026 Na-EDTA, and 11.1 glucose to which 5% dextrose (70,000 mol wt) was added. The perfusate was circulated at 37°C with a Masterflex pump (Cole-Parmer). The lobe was perfused in a nonrecirculating manner until the pulmonary venous effluent was clear. For the remainder of the experiment, the lobe was perfused under constant-flow conditions (550 ml/min, 8.6 ± 0.5 ml·min\(^{-1}·g\) lung wt\(^{-1}\)) with a recirculating volume of 300 ml. Outflow pressure was maintained between 2.0 and 2.5 mmHg (zone 3 lung) by mechanically adjusting resistance in the outflow tubing. Pressure transducers were placed at the level of the hilum for obtaining continuous recording of pulmonary arterial (P\(_{pa}\), inflow), pulmonary venous (P\(_{pv}\), outflow), and airway pressures on a polygraph (model 7, Grass). Total pulmonary pressure gradient (TPPG) represents the pressure drop across the lung (P\(_{pa} - P_{pv}\)). Microvascular pressure (P\(_{mv}\)) was estimated by the double-vascular occlusion method as previously described (10, 45). This technique requires simultaneous occlusion of inflow and outflow catheters. Under these conditions, P\(_{pa}\) and P\(_{pv}\) approach a common pressure. This common value represents the pressure in the most compliant segment of the pulmonary vasculature, the capillaries. The lobe was ventilated for 1 min every 5 min with 15% O\(_2\)-6% CO\(_2\)-balance N\(_2\) at a tidal volume of 100 ml and a rate of 8 breaths/min. In addition, the perfusate was gassed with 15% O\(_2\)-6% CO\(_2\)-balance N\(_2\). Perfusion values for pH, P\(_{co2}\), and P\(_{o2}\) were 7.359 ± 0.025 units, 24.4 ± 1.3 mmHg, and 118.4 ± 4.0 mmHg, respectively (n = 11). Positive end-expiratory pressure was maintained at 0.5–1.0 mmHg.

Measurement of immunoreactive TXB\(_2\) and 6-keto-PGF\(_{1α}\). Samples of the perfusate were obtained at 15-min intervals before and after administration of 5,6-EET to determine the rates of pulmonary PGI\(_2\) and TXA\(_2\) synthesis. Synthetic rates were based on time-dependent increases in the measurements of immunoreactive 6-keto-PGF\(_{1α}\) and TXB\(_2\), the stable degradation products of PGI\(_2\) and TXA\(_2\), respectively. Samples were collected in plastic syringes containing indomethacin (5 µg/ml) and EDTA (1 mg/ml) as previously reported (39). Samples were kept on ice and centrifuged at 1,800 g at 4°C for 20 min. The supernatant was removed and frozen at −30°C until assay. Enzyme immunoassay of 6-keto-PGF\(_{1α}\) and TXB\(_2\) was performed in 96-well microtiter plates precoated with 2 µg/well anti-rabbit IgG, as previously described (31, 39, 43). Before use, the plates were washed with 10\(^{-2}\) M phosphate buffer (pH 7.4) containing 0.05% Tween 20 (wash buffer). The assay was performed in a total volume of 150 µl. In brief, 50 µl of acetylcholinesterase-conjugated eicosanoid tracer, 50 µl of antisera directed against 6-keto-PGF\(_{1α}\) or TXB\(_2\) (PerSeptive Diagnostics), and 50 µl of a standard or sample in assay buffer were combined and incubated at 25°C for 18–20 h. After the plates were washed three times with buffer, DTNB (200 µl) was dispensed into each well. Absorbance was recorded at 412 nm in a microtiter plate spectrophotometer (Biotek) when the absorbance for the well containing the “0” standard exceeded 0.200 absorbance units. Each sample was assayed in duplicate. A standard curve was generated for each assay and sample eicosanoid concentrations were determined by comparison to a log-logit transformation of the standard curve.

Preparation of EET standards. Selective epoxidation of AA to 5,6-EET was achieved using the method of Corey et al. (9). Briefly, AA (10 mg) was incubated with potassium triiodide (8 eq) and potassium bicarbonate (5 eq) in tetrahydrofuran-water (1.5:1) under N\(_2\) gas, for 16 h at 4°C. Excess iodine was removed bydropwise addition of saturated sodium sulfite (500 µl) until the solution cleared. The oily iodolactone was extracted three times with hexane (1 ml). The combined organic extracts were dried by vacuum centrifugation (Savant), dissolved in 1 ml tetrahydrofuran, and incubated with 500 µl lithium hydroxide (0.2 M) with constant stirring for 3 h at 25°C. The reaction mixture, containing 5,6-EET, was acidified with formic acid (pH 4.0), extracted three times with ethyl acetate (2 ml), and washed once with water (1 ml). The extract was purified by reverse-phase HPLC using a Nucleosil C\(_18\) column (5 µm, 4.6 × 250 mm) with a linear gradient from 50% water in acetonitrile-acetic acid (99:1) to 100% acetonitrile-acetic acid (99:1) over 40 min at 1 ml/min. Eluate containing 5,6-EET was collected, evaporated to dryness, and stored under N\(_2\) gas in hexane at −80°C. The identity and quantity of the 5,6-EET synthesized were obtained by comparing the HPLC (ultraviolet absorbance at 192 nm) retention time and peak area, respectively, to an authentic standard (Cayman Chemical). The integrity of the standard was confirmed by gas chromatography-mass spectrometry as previously reported (40). In its free-acid form, 5,6-EET readily decomposes to 5,6-DHET and the corresponding hydroxyeicosatetraenoic acid. Therefore, before use, 5,6-EET was repurified by reverse-phase HPLC.

Isolated lung protocols. The relationship between perfusion pressure and flow rate has been used to describe changes in PVR (26). For this form of analysis, pressure-flow curves (PFC) were generated by recording vascular pressures at 100-ml flow increments between 300 and 700 ml/min. At each flow increment, P\(_{pa}\) and P\(_{mv}\) were measured after P\(_{pv}\) was adjusted to 2.0–2.5 mmHg. After the PFC measurements under baseline conditions were completed, a constant infusion of U-46619, a thromboxane-endoperoxide receptor agonist (0.1 µM in 0.9% NaCl), or 5-HT (13 µM in 0.9% NaCl) was added to the perfusate reservoir. Only one of these pulmonary vasconstrictors was used in each perfused lung preparation. Infusion rates were adjusted to achieve an increase in P\(_{pa}\) of 5–6 mmHg. The second PFC was generated during U-46619 or 5-HT infusion. While the increased PVR was maintained with either agent, a third PFC was generated 5 min after addition of 5,6-EET (10 µM final perfusate concn) in 15 µl ethanol. This concentration of 5,6-EET was found to produce a maximal response in isolated rings of canine pulmonary veins in which ring tension was increased with U-46619 (Fig. 1). Administration of the ethanol vehicle (15 µl) alone did not alter PVR. After the third PFC, the perfusate reservoir was filled with fresh PSS and indomethacin (10\(^{-4}\) M) was added to the perfusate. Thirty minutes after the addition...
of indomethacin, PFC measurements were obtained during U-46619 or 5-HT infusion before and 5 min after addition of 5,6-EET to the perfusate. Before and after each PFC, multiple timed samples of perfusate were collected for measurement of U-46619 or 5-HT infusion before and 5 min after addition of indomethacin, PFC measurements were obtained during 5,6-EET-mediated increase in PVR resulted solely from increased resistance in the pulmonary venous segment (Fig. 2, A and B).

Effects of 5,6-EET on PVR in isolated canine lungs perfused with PSS. In five isolated left lower lung lobes, perfused with PSS at a constant flow rate of 550 ml/min (8.6 ± 0.5 ml/g lung tissue), a 0.1 mM solution of U-46619 in 0.9% NaCl was infused into the perfusate reservoir at a rate of 3.28 ± 0.99 nmol/min. The actual infusion rate (range 9–65 µl/min) was adjusted to achieve an increase in $P_{pv}$ of 5–6 mmHg. Within 10–15 min after initiation of the U-46619 infusion, TPPG increased from 7.30 ± 0.61 to 12.42 ± 0.27 mmHg (Fig. 1), with no concurrent change in $P_{pa}$. U-46619 administration resulted in increased TPPG at each of the five flow increments (Fig. 1) chosen for generation of the PFC. During the infusion of U-46619, the increase in PVR remained constant at 550 ml/min perfusate flow. Least-squares linear regression analysis revealed that U-46619 increased the slope of the PFC from 1.8 ± 0.2 × 10⁻² to 2.9 ± 0.1 × 10⁻² mmHg·ml⁻¹·min⁻¹, a 62.1 ± 4.5% increase in PVR. Partitioning the total PVR into arterial and venous segments, using the double-occlusion method, revealed that the U-46619-mediated increase in PVR resulted solely from increased resistance in the pulmonary venous segment (Fig. 2, A and B).

**RESULTS**

Effects of U-46619 on PVR in isolated canine lungs perfused with PSS. In five isolated left lower lung lobes, perfused with PSS at a constant flow rate of 550 ml/min (8.6 ± 0.5 ml/g lung tissue), a 0.1 mM solution of U-46619 in 0.9% NaCl was infused into the perfusate reservoir at a rate of 3.28 ± 0.99 nmol/min. The actual infusion rate (range 9–65 µl/min) was adjusted to achieve an increase in $P_{pv}$ of 5–6 mmHg. Within 10–15 min after initiation of the U-46619 infusion, TPPG increased from 7.30 ± 0.61 to 12.42 ± 0.27 mmHg (Fig. 1), with no concurrent change in $P_{pa}$. U-46619 administration resulted in increased TPPG at each of the five flow increments (Fig. 1) chosen for generation of the PFC. During the infusion of U-46619, the increase in PVR remained constant at 550 ml/min perfusate flow. Least-squares linear regression analysis revealed that U-46619 increased the slope of the PFC from 1.8 ± 0.2 × 10⁻² to 2.9 ± 0.1 × 10⁻² mmHg·ml⁻¹·min⁻¹, a 62.1 ± 4.5% increase in PVR. Partitioning the total PVR into arterial and venous segments, using the double-occlusion method, revealed that the U-46619-mediated increase in PVR resulted solely from increased resistance in the pulmonary venous segment (Fig. 2, A and B).

Effects of 5,6-EET on PVR in isolated canine lungs perfused with PSS containing U-46619. During the maintenance of increased PVR with U-46619, administration of 5,6-EET (10⁻⁵ M, final concn) resulted in a sustained decrease in TPPG of 3.42 ± 0.48 mmHg at 550 ml/min perfusate flow (Fig. 1). The PVR remained stable at this reduced level while a PFC was generated. 5,6-EET decreased TPPG at all flow increments (Fig. 1) and reduced the slope of the PFC from 2.9 ± 0.1 × 10⁻² to 2.1 ± 0.1 × 10⁻² mmHg·ml⁻¹·min⁻¹, a value not different from that obtained before the U-46619 infusion. The 5,6-EET-mediated effect on PVR was limited to a reduction in the U-46619-induced increase in pulmonary venous resistance (Fig. 2, A and B). 5,6-EET had no effects on PVR in the arterial segment. This result was supported in separate experiments in which administration of 5,6-EET in the absence of agonist-induced vasoconstriction (n = 2) did not change total,
arterial, or venous segmental resistances (data not shown).

Effects of 5,6-EET on PVR in isolated canine lungs perfused with PSS containing 5-HT. In five additional, identically prepared, isolated left lower lung lobes, a 13 mM solution of 5-HT was added to the perfusate reservoir at 325 ± 36 nmol/min. The infusion of 5-HT increased the TPPG from 7.35 ± 0.48 to 13.75 ± 0.43 mmHg (Fig. 3). Concurrently, 5-HT increased the slope of the PFC from 1.8 ± 0.2 × 10⁻² to 3.3 ± 0.2 × 10⁻² mmHg·ml⁻¹·min⁻¹. In contrast to U-46619, which increased resistance only in the venous segment, 5-HT resulted in a 63.9 ± 19.0% increase in the arterial segmental resistance and a 97.2 ± 19.6% increase in the venous segmental resistance (Fig. 3). During the maintenance of increased PVR with 5-HT, administration of 5,6-EET (10⁻⁵ M) resulted in a sustained decrease in TPPG of 2.93 ± 0.51 mmHg at 550 ml/min perfusate flow. The PVR remained stable at this reduced level while a PFC was generated. 5,6-EET decreased TPPG at some, but not all, flow increments (Fig. 3). However, it reduced Poa at all flow increments and reduced arterial segmental resistance from 1.12 ± 0.09 × 10⁻² to 0.90 ± 0.03 × 10⁻² mmHg·ml⁻¹·min⁻¹ without reducing resistance in the pulmonary venous segment (Fig. 3).

Effects of 5,6-EET on pulmonary synthesis of PGI₂ and TxA₂. The rate of PGI₂ synthesis, estimated by the rate of 6-keto-PGF₁α accumulation in the recirculating perfusate of the isolated lung, increased from 70.5 ± 18.4 to 675.9 ± 125.4 ng/min after administration of 5,6-EET into the perfusate (Fig. 4). Before administration of 5,6-EET, the rate of TxA₂ synthesis was lower (1.4 ± 0.5 ng/min) than that of PGI₂ and did not change.

Fig. 2. Effects of U-46619 (3.3 ± 1.0 nmol/min) and 5,6-EET (10⁻⁵ M) on relationship between flow rate and pressure gradient across arterial segment [pulmonary arterial pressure (Pao, i.e., inflow pressure) – pulmonary microvascular pressure (Pmv); A] and venous segment [Pmv – pulmonary venous pressure (Ppv, i.e., outflow pressure); B] in isolated canine lung perfused with PSS. Control values are those obtained before U-46619 alone and in combination with 5,6-EET; n = 5. *P < 0.05 compared with control values. †P < 0.05 compared with U-46619.

Fig. 3. Effects of 5-hydroxytryptamine (5-HT, 325 ± 36 nmol/min) and 5,6-EET (10⁻⁵ M) on relationship between flow rate and TPPG (Pao – Ppv; A), Pao – Pmv (B), and Pmv – Ppv (C) in isolated canine lung perfused with PSS. Control values are those obtained before 5-HT alone and in combination with 5,6-EET; n = 5. *P < 0.05 compared with control values. †P < 0.05 compared with 5-HT.
reduction in U-46619-mediated resistance to that observed with PSS-perfused lungs (5,6-EET reduced slope of PFC from 2.61 to 2.52 × 10^{-2} mmHg·ml^{-1}·min). These results demonstrate that 5,6-EET, administered into circulating blood, is neither bound so avidly to plasma proteins nor incorporated so rapidly into blood cell lipids as to inhibit its action as a vasodilator within the pulmonary circulation.

Effects of 5,6-EET on U-46619- and 5-HT-induced tension in isolated canine pulmonary vascular rings. In isolated rings of pulmonary veins, administration of U-46619 resulted in a concentration-dependent increase in active tension (EC_{50} 1.77 ± 1.11 × 10^{-9} M, Fig. 7). U-46619 failed to increase tension in pulmonary arterial rings at 3 × 10^{-8} M, a concentration that resulted in a near-maximal response from the pulmonary veins. In rings of pulmonary veins in which active tension was induced with U-46619 (10^{-9} M), administration of 5,6-EET reduced that tension in a concentration-dependent manner (Fig. 8A). Consistent with the results obtained in the isolated perfused lung, indomethacin (3 × 10^{-5} M) prevented the 5,6-EET-mediated reduction in U-46619-induced active tension in isolated pulmonary venous rings (Fig. 8B). Qualitatively similar results were obtained when ibuprofen (5 × 10^{-5} M), a chemically dissimilar inhibitor of cyclooxygenase activity, was substituted for indomethacin in these experiments (n = 3, data not shown). 5-HT increased the active tension in rings of both pulmonary artery and vein (Fig. 9). Consistent with the results obtained in the isolated perfused lung, 5,6-EET reduced the active tension in pulmonary arterial rings but not pulmonary venous rings (Fig. 9). Either indomethacin or ibuprofen inhibited the 5,6-EET reduction in active tension mediated by 5-HT.

**DISCUSSION**

Previous studies in nonpulmonary vascular beds have established that exogenously administered 5,6-EET most often produces vasodilation (15, 23, 24). We recently reported that 5,6-EET decreased the active tension in isolated rings of canine pulmonary veins contracted with PGF_{2α} (40). In the present study, we extend those observations and report for the first time the effects of an exogenously administered EET on the intact pulmonary circulation. The results demonstrate that 5,6-EET opposes U-46619- and 5-HT-induced increases in PVR in the circulation of the isolated perfused canine lung. The effects of 5,6-EET on pulmonary vascular reactivity were found to be dependent on cyclooxygenase activity; i.e., indomethacin, an inhibitor of cyclooxygenase activity, abolished both the 5,6-EET-mediated reduction in PVR in the isolated perfused lung and the 5,6-EET-mediated reduction in active tension in pulmonary venous rings. These results are consistent with those reported for the effects of 5,6-EET in the isolated perfused rabbit kidney (7) and isolated perfused rat caudal artery (5). In the latter studies, administration of 5,6-EET opposed the increase in vascular resistance induced with phenylephrine. In both the rabbit kidney and rat caudal artery, the
vasodilator responses to 5,6-EET were dependent on cyclooxygenase activity. Similarly, inhibition of cyclooxygenase activity eliminated the vasodilator effect of 5,6-EET in the rat intestinal microcirculation (32) and the rabbit and cat cerebral microcirculation (11).

However, the vasodilator activity of 5,6-EET has been reported to be independent of cyclooxygenase activity in some organ systems studied. In isolated canine (36) and bovine (35) coronary arterial rings contracted with U-46619, 5,6-EET produced a cyclooxygenase-independent relaxation. Administration of 5,6-EET to isolated rat kidneys perfused with a PSS devoid of blood elements also resulted in cyclooxygenase-independent vasodilation (13). However, when perfused with blood, 5,6-EET mediated a vasoconstrictor response in the rat kidney (13, 41). The vasoconstric-

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**Fig. 5.** Effects of U-46619 (3.3 ± 1.0 nmol/min) and 5,6-EET (10⁻⁵ M) on relationship between flow rate and TPPG (A), Pₚₚ - Pₘᵥ (B), and Pₘᵥ - Pₚᵥ (C) in isolated canine lung perfused with PSS containing indomethacin (I, 10⁻⁴ M).

Control values are those obtained before U-46619 alone and in combination with 5,6-EET; n = 5. *P < 0.05 compared with control values.

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**Fig. 6.** Effects of 5-HT (325 ± 36 nmol/min) and 5,6-EET (10⁻⁵ M) on relationship between flow rate and TPPG (A), Pₚₚ - Pₘᵥ (B), and Pₘᵥ - Pₚᵥ (C) in isolated canine lung perfused with PSS containing indomethacin (10⁻⁴ M).

Control values are those obtained before 5-HT alone and in combination with 5,6-EET; n = 5. *P < 0.05 compared with control values.
tion was proposed to result from rapid uptake of 5,6-EET into platelets resulting in cyclooxygenase-mediated metabolism to a compound with vasoconstrictor properties. In the present study, 5,6-EET produced a dilator response in the canine pulmonary circulation when the perfusate consisted of either PSS (Figs. 1 and 2) or blood. In contrast to results obtained in the isolated blood-perfused rat kidney (13), results of the present study suggest that the vasodilator activity of 5,6-EET in blood-perfused lungs was qualitatively similar to that observed in lungs perfused with PSS.

Oliw (27, 29) reported that of the four regioisomeric EET, only 5,6-EET retains the 8, 11, and 14 cis-double bonds required for cyclooxygenase metabolism to prostaglandin analogs. Cyclooxygenase-dependent, 5,6-EET-mediated vasodilation has been proposed to result from one or a combination of up to three different mechanisms (7). Dilation may result from 1) 5,6-EET stimulated synthesis of endogenous vasodilator prostaglandins, 2) metabolism of 5,6-EET by cyclooxygenase activity to 5,6-epoxy-PGE1 or 5-hydroxy-PGI1, the PGE and PGI analogs of 5,6-EET, respectively (28), or 3) generation of vasodilator reactive oxygen species (20), which act as vasodilators (11).

In the present study, administration of 5,6-EET into the perfusate of the isolated perfused canine lung resulted in a large increase in the rate of PGI2 synthesis, measured by accumulation of 6-keto-PGF1α, the stable degradation product of PGI2 (Fig. 4). Although measurable concentrations of TxB2, the stable degradation product of TxA2, were also present in the perfusate, the rate of thromboxane synthesis did not increase significantly upon administration of 5,6-EET. These results were not unexpected, since PGI2 is the major cyclooxygenase metabolite of AA synthesized by vascular endothelium. 5,6-EET was previously observed to increase PGI2 synthesis in the isolated perfused rabbit kidney and cultured pulmonary artery endothelial cells in culture (4). Administration of 5,6-EET may stimulate an increase in PGI2 synthesis in the vascular endothelium by increasing the availability of AA for cyclooxygenase-mediated metabolism. 5,6-EET has been reported to increase the entry of extracellular calcium into vascular endothelial cells (26). In the vascular endothelium, this increase in free cytosolic calcium may activate the calcium-dependent cytosolic phospholipase A2, the phospholipase purported to mediate most of the agonist-induced AA release from membrane phospholipids for cyclooxygenase-dependent prostaglandin synthesis (8). Although most primary prostaglandins constrict the pulmonary vasculature, PGI2 is a pulmonary vasodilator (17). Because inhibition of cyclooxygenase activity with indomethacin abolished both the increase in PGI2 synthesis (Fig. 4) and the pulmonary vasodilator activity (Fig. 5) mediated by administration of 5,6-EET, the increased PGI2 synthesis may mediate some or all of the 5,6-EET-induced vasodilation in the canine pulmonary circulation.

In the isolated perfused rabbit kidney, addition of 5,6-EET to the renal arterial perfusate resulted in cyclooxygenase-dependent metabolism of 5,6-EET to 5,6-epoxy-PGE1 and 5-hydroxy-PGI1 (4). Carroll et al.

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![Graph](https://via.placeholder.com/150)

**Fig. 7.** Effect of U-46619 on active tension in canine pulmonary arterial (PA) and pulmonary venous (PV) rings; n = 4.

**Fig. 8.** Representative traces of effect of 5,6-EET (10⁻⁸ to 10⁻⁵ M) on active tension in canine PV rings contracted with U-46619 in absence (A) and presence (B) of indomethacin (3 × 10⁻⁵ M). Indomethacin was present at least 30 min before addition of U-46619. Bar (2 g in A) represents active tension.

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reported that pulmonary endothelial cells in culture metabolized 5,6-EET, via cyclooxygenase activity, to 5,6-epoxy-PGE1 and 5-hydroxy-PGI1 as well. In the isolated kidney, 5,6-epoxy-PGE1 was a vasodilator. Synthesis of these two metabolites of 5,6-EET and their effects on the pulmonary circulation were not determined in the present study and their significance in 5,6-EET-mediated vasodilation of the pulmonary circulation remains to be determined.

Vasodilation resulting from the cyclooxygenase-mediated production of oxygen radicals has been identified only in the cerebral microvascular circulation (11). No evidence was obtained to evaluate this mechanism of 5,6-EET-mediated vasodilation in the pulmonary circulation.

In the present study, the effects of 5,6-EET on PVR were investigated in the presence of two agents, U-46619 and 5-HT, which increased vascular resistance. The effect of U-46619 on PVR in the isolated perfused canine lung was limited to the venous segment (Fig. 2); i.e., U-46619 did not increase resistance in the arterial segment. Consistent with these findings, rings of canine pulmonary arteries were also insensitive to the application of U-46619 (Fig. 7). Similar results were previously reported for the stable TxA2 analog in the isolated perfused canine lung (37) and isolated rings of canine pulmonary vessels (25). The mechanism for selective pulmonary venous constriction to U-46619 in the dog lung is not known. It has been suggested that functional TxA2 receptors may be more abundant in the dog pulmonary venous segment compared with the arterial segment (37). However, predominance of vasoconstriction in the venous segment is not consistent among species. In the rat, U-46619 produced equal arterial and venous constriction (42). Therefore species-specific differences in the longitudinal distribution of PVR resulting from administration of specific vasoactive agonists must be recognized (2).

Although pulmonary veins are often viewed primarily as conduit vessels, their vasoactivity has been described in several species (14, 25, 33, 37). However, without addition of a constrictor agent to the perfusate, vasomotor effects of 5,6-EET were absent. A requirement for agonist-induced vasoconstriction to observe an EET-mediated response has previously been reported in isolated canine and bovine coronary vessels (35, 36) and in the intact rat intestinal vasculature (32). Pinto et al. (30) reported that administration of AA to isolated rings of rabbit pulmonary artery resulted in contraction in the absence of active tone and dilation only when the vessels were precontracted with phenylephrine. The AA-induced relaxation response was inhibited with SKF-525A, an inhibitor of cytochrome P-450 monooxygenase activity, suggesting the dilator response was mediated by a cytochrome P-450 metabolite of AA.

5-HT has previously been reported to constrict both the arterial and venous segment of the isolated, perfused canine lung (34). At the infusion rates used in this study, similar increases in total PVR were obtained with either U-46619 or 5-HT administration. However, with 5-HT, this increase was divided between the arterial and venous segments (Fig. 3). Unexpectedly,
the reduction in PVR resulting from administration of 5,6-EET was limited to the arterial segment. This finding in the isolated lung was confirmed in isolated rings of canine intrapulmonary veins and arteries; i.e., 5-HT-induced active tension was opposed by 5,6-EET only in rings of intrapulmonary artery. Because 5,6-EET did reduce venous segmental resistance when that resistance was increased with U-46619, these data suggest a difference in the mechanism leading to contraction in the venous segment resulting from administration of U-46619 and 5-HT, the latter being insensitive to the dilator activity of 5,6-EET. Although the nature of this difference was not investigated in the present study, Kaye et al. (18) suggest that differences in the pressor response to U-46619 and 5-HT in the cat may be related to differences in the signal transduction pathway leading to contraction. Therefore the dilator activity of 5,6-EET in the canine pulmonary circulation was not only dependent on cyclooxygenase activity but also on the contractile agent mediating the increased resistance (Fig. 3).

In conclusion, the results of this study demonstrate that exogenously administered 5,6-EET mediates vasodilation in the isolated perfused canine lung. The activity of 5,6-EET was dependent on induction of active vasoconstriction and the activity of cyclooxygenase. Because synthesis of 5,6-EET in canine lung tissue has previously been identified (40), these findings support the hypothesis that endogenously synthesized 5,6-EET may act to oppose agonist-induced increases in vascular resistance in the pulmonary circulation.

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