

Analysis of Responses to Leukotriene D₄ in the Pulmonary Vascular Bed

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SUMMARY. Pulmonary vascular responses to leukotriene D₄ were investigated in the intact-chest animal under conditions of controlled pulmonary blood flow. Intralobar injections of leukotriene D₄ in the sheep caused dose-dependent increases in lobar arterial and small vein pressures without influencing left atrial or systemic arterial pressure. Leukotriene D₄ was very potent in increasing pulmonary vascular resistance in the sheep, with activity similar to that of U-46619, a thromboxane A₂ mimic. Pulmonary vascular responses to leukotriene D₄ in the sheep were similar when the lung was ventilated and when lobar ventilation was arrested. Responses to leukotriene D₄ were similar when the lung was perfused with blood or with dextran. Pulmonary vascular responses to leukotriene D₄ but not U-46619 in the sheep were reduced by inhibitors of cyclooxygenase and thromboxane synthesis. In contrast, leukotriene D₄ had modest pressor activity in the pulmonary vascular bed of the cat whereas U-46619 had marked activity in this species. Responses to leukotriene D₄ in the cat were not altered by cyclooxygenase inhibitors. It is concluded that leukotriene D₄ has marked pulmonary vasoconstrictor activity in the sheep, increasing pulmonary vascular resistance by constricting intrapulmonary veins and upstream segments. In this species, responses to leukotriene D₄ were independent of changes in ventilation or interaction with formed elements but were dependent on the formation of cyclooxygenase products including thromboxane A₂. However, in the cat, leukotriene D₄ had very modest pressor activity, and this activity was not dependent on the integrity of the cyclooxygenase pathway. These data suggest considerable species difference in responses to leukotriene D₄, a major component of the slow-reacting substance of anaphylaxis, in the pulmonary vascular bed. (*Circ Res* 55: 707-717, 1984)

THE leukotrienes are a group of biologically active substances derived from arachidonic acid by the way of the 5-lipoxygenase pathway (Hammarstrom, 1983). It has recently been reported that leukotriene C₄ (LTC₄) and leukotriene D₄ (LTD₄) are major components of the slow-reacting substance of anaphylaxis (Murphy et al., 1979; Morris et al., 1980; Lewis et al., 1980). Since slow-reacting substance of anaphylaxis (SRS-A) is released from the lung by immunological challenge, leukotrienes may be important mediators of symptoms in asthma and other immediate-type hypersensitivity reactions (Kellaway and Trethewie, 1940; Brocklehurst, 1960; Dahlen et al., 1983). The effects of the leukotrienes on the lung are of considerable interest because of their postulated role in asthma (Dahlen et al., 1983). Leukotrienes C₄ and D₄ have potent contractile activity on airway and vascular smooth muscle from the lung (Drazen et al., 1980; Dahlen et al., 1980; Hand et al., 1981; Krell et al., 1981; Jones et al., 1982). These substances have bronchoconstrictor activity in a variety of species (Drazen et al., 1980; Holroyde et al., 1981; Smedegard et al., 1982; Hamel et al., 1982; Graybar et al., unpublished data). However, less is known about the effects of the leukotrienes on the pulmonary vascular bed, although it has been reported that these substances may mediate responses to platelet-activating factor and al-

veolar hypoxia in the pulmonary vascular bed (Voelkel et al., 1982; Morganroth et al., 1984). Moreover, in the monkey, the predominant response to injection of LTC₄ is a fall in pulmonary arterial pressure, whereas aerosol administration of LTC₄ caused a marked rise in pulmonary arterial pressure (Smedegard et al., 1982). In the rat, injections of LTC₄ caused a dose-related fall in pulmonary arterial pressure (Iacopino et al., 1983). In contrast to results obtained in studies with LTC₄ in the rat and monkey, LTD₄ caused a marked increase in pulmonary vascular resistance in the newborn lamb when injected into the pulmonary artery (Yokochi et al., 1982). However, the effects of LTD₄ on the pulmonary vascular bed of the mature animal are uncertain, and little, if anything, is known about the actions of this important constituent of SRS-A on the pulmonary veins in the intact-chest animal. The present study was undertaken to investigate responses to LTD₄ in the intact-chest sheep under conditions of controlled pulmonary blood flow. In addition, responses to LTD₄ were compared in the sheep and cat to determine if responses to LTD₄ vary with species. The present studies in the sheep demonstrate that LTD₄ causes potent cyclooxygenase-dependent vasoconstriction, increasing pulmonary vascular resistance by constricting pulmonary veins and upstream segments. However, in the cat, LTD₄

has very modest pressor activity that is not cyclooxygenase dependent. These studies show that there is marked species variation in the pulmonary vascular response to LTD₄.

Methods

Young sheep of either sex, weighing 19.5 ± 1.4 kg, were anesthetized with pentobarbital sodium, 30 mg/kg, iv, and were strapped in the supine position to a Philips fluoroscopic table. Supplemental doses of anesthetic were administered as needed to maintain a uniform level of anesthesia. The animals spontaneously breathed room air or room air enriched with 100% O₂ through a cuffed endotracheal tube. The left lower lobe was perfused at constant flow using methods similar to those reported earlier in this species (Hyman and Kadowitz, 1975; Kadowitz and Hyman, 1983). In all experiments except those in which responses to LTD₄ were compared during lobar perfusion with blood or dextran, the flow rate averaged 258 ml/min. In experiments in which responses were investigated during perfusion with dextran, the flow rate averaged 138 ml/min since a smaller portion of the left lower lobe was selected for perfusion to facilitate drainage of the perfused dextran using a withdrawal system similar to that previously described (Hyman and Kadowitz, 1975; Kadowitz and Hyman, 1977; 1980). The perfusate was composed of 5% low molecular weight dextran (Sigma) in 0.9% saline.

For studies in the cat, animals of either sex weighing 2.5 ± 0.2 kg were sedated with ketamine hydrochloride (10 mg/kg, im), were anesthetized with pentobarbital sodium (30 mg/kg, iv), and were strapped in the supine position to a Philips fluoroscopic table. Supplemental doses of pentobarbital were given as needed to maintain a uniform level of anesthesia. The trachea was intubated with a cuffed pediatric endotracheal tube, and the animals spontaneously breathed room air or room air enriched with 100% O₂. Systemic arterial pressure was measured from a catheter in the femoral artery, and systemic administration of drugs was made through a catheter in the femoral vein. In this species, the left lower lobe was perfused at constant flow using recently described methods (Hyman and Kadowitz, 1979; Kadowitz and Hyman, 1980). The lobar perfusion rate averaged 41 ml/min in these experiments. All vascular pressures in both species were measured with Statham P23Db transducers zeroed at right atrial level, and mean pressures obtained by electronic averaging were recorded on an Electronics for Medicine recorder model DR-12. In experiments in both species, arterial blood gases and pH were measured with an Instrumentation Labs Micro 13 analyzer and were in the normal range.

For biochemical studies, lung parenchyma was obtained from five sheep. After the administration of pentobarbital sodium (30 mg/kg iv), the chest was opened and the lungs were exposed and perfused with ice-cold 0.1 M potassium phosphate buffer, pH 7.4, via the lobar artery until free of blood. The lungs were removed, rinsed with ice-cold buffer, and transferred to a cold room. The following procedures were done at 0–4°C. Well-perfused areas of parenchyma and adhering pleura, visually free of large airways or vessels, were dissected free and weighed. The tissue from each sheep was separately minced and homogenized in 3 volumes of the phosphate buffer with a Polytron PT20 homogenizer for 10 seconds, 3 times. The

homogenates were then centrifuged at 10,000 g for 15 minutes and the resultant supernatant was strained through cheesecloth and centrifuged at 90,000 g for 70 minutes. The microsomal pellet thus obtained was washed and resuspended in the phosphate buffer and stored at –70°C until used. The protein concentration was determined by the method of Lowry et al. (1951).

Radiometric thin layer chromatography (TLC) previously employed in this laboratory was used to assay the production of TXA₂, PGI₂, PGF_{2α}, PGD₂, and PGE₂ (She et al., 1981; Spannhake et al., 1983). A mixture of 100 μl containing the microsomes, 0.1 M potassium phosphate buffer, pH 7.4, with and without OKY 1581, was preincubated at 37°C for 2 minutes. The reaction was initiated by the addition of the microsomal suspension to a cold (0°C) 1.5-ml Brinkman centrifuge tube containing either 1-¹⁴C-PGH₂ (15,000 cpm) or 1-¹⁴C-arachidonic acid (50–60 μCi/mmol) previously evaporated to dryness under a N₂ stream and was incubated at 37°C for 2 minutes (PGH₂) or 60 minutes for arachidonic acid. The reaction was stopped and the products were extracted by adding 400 μl of ethyl acetate:methanol:0.2 M citric acid, pH 2 (15:2:1). The upper organic layer, containing 85–95% of the radiolabeled PGH₂ or arachidonic acid was spotted for TLC on silica gel plates and developed using the solvent system ethyl acetate:acetic acid:hexane:water (54:12:25:60, organic phase). The products of the reaction were identified by comigration with authentic prostaglandin standards which were located by exposing plates to iodine vapor. Radioactivity on TLC plates was monitored by a radiochromatogram scanner (Packard 7201B) equipped with a disc integrator. Radioactive zones were also scraped off and counted in a Ditol scintillator solvent with a Beckman LS-230 liquid scintillation spectrometer. Good agreement was found by these two methods and incubations were done in duplicate. Data from these experiments are reported in picomoles produced per 2-minute incubation period for PGH₂ and per 1-hour incubation for arachidonic acid.

Synthetic leukotriene D₄ (Merck-Frosst, Canada, and Smith Kline and French) was stored as a stock solution at –80°C in a Revco freezer. On the day of an experiment, the stock solution was diluted with 0.9% saline and injected into the lobar artery in small volumes (0.1–0.5 ml). Aliquots from the same lot of LTD₄ were used in most of the cat experiments and half of the sheep. Arachidonic acid (99% pure) was obtained from NuChek Labs as the sodium salt and was dissolved in 0.9% saline and stored in brown bottles under nitrogen gas at –20°C. Sodium meclofenamate (Warner Lambert-Parke Davis) and OKY 1581 (Ono) were dissolved in 0.9% saline just prior to iv administration. Indomethacin (Merck, Sharp & Dohme) was mixed with an equimolar amount of sodium carbonate and was injected iv. The prostaglandin endoperoxide analog, U-46619 (Upjohn), was dissolved in absolute ethanol and was stored frozen. Working solutions of U-46619 were prepared in 0.9% saline on a frequent basis. U-46619 and arachidonic acid were also injected into the lobar artery in small volumes, and all treatments were randomized. In addition, sufficient time was allowed between injections of LTD₄, U-46619, and arachidonic acid for all vascular pressures to return to baseline value.

Hemodynamic data are expressed as mean \pm SE and were analyzed using paired group comparison and linear regression analysis (Snedecor and Cochran, 1967). The criterion for statistical significance was a P value of less than 0.05.

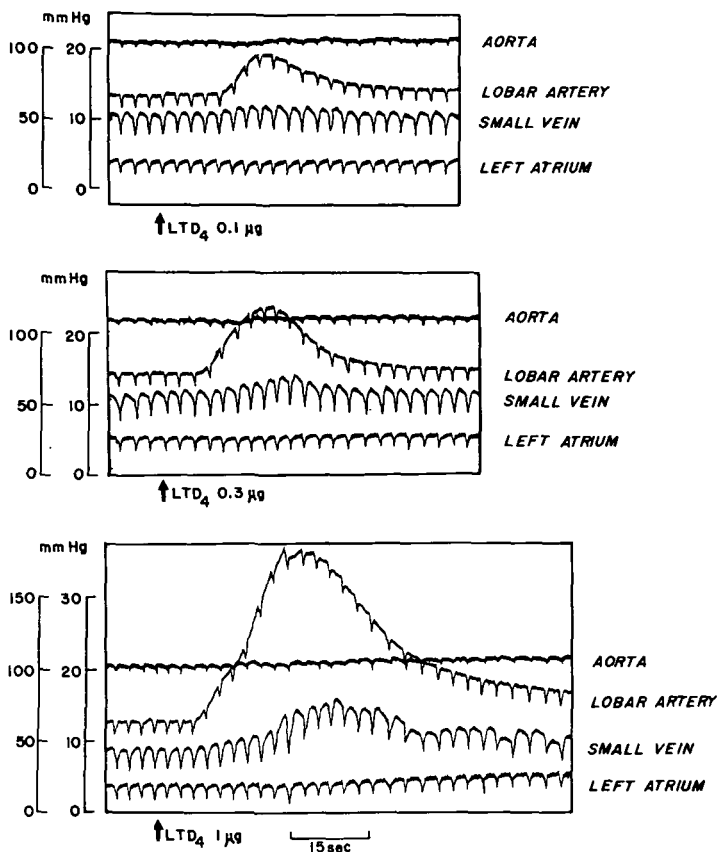


FIGURE 1. Tracings from an experiment illustrating the effects of injections of leukotriene D₄ (LTD₄), 0.1–1 µg, into the perfused lobar artery on mean vascular pressures in the aorta, the perfused lobar artery, the small intrapulmonary vein, and the left atrium in the intact-chest sheep.

Results

Responses to LTD₄

Pulmonary vascular responses to LTD₄ in the intact-chest sheep are illustrated in Figure 1, and data from 11 animals are summarized in Table 1. Under conditions of controlled pulmonary blood flow, intralobar injections of LTD₄ in doses of 0.1–1 µg caused significant dose-related increases in lobar arterial and small vein pressures without changing left atrial pressure (Fig. 2). In the five

animals in which responses to the same doses were repeated, responses to LTD₄ were reproducible. In the doses employed in the present study in the sheep, LTD₄ had no significant effect on systemic arterial pressure (Table 1). The increases in lobar arterial and small vein pressures were rapid in onset, and mean vascular pressures returned to baseline value over a 0.5- to 4-minute period, depending on the dose of the leukotriene. The lobar arterial to small vein pressure gradient and the gradient from small vein to left atrium pressure increased significantly at all doses of LTD₄ studied.

TABLE 1
Influence of Intralobar Injections of Leukotriene D₄ (LTD₄)
on Mean Vascular Pressures in the Sheep

	Pressure (mm Hg)			
	Lobar artery	Small vein	Left atrium	Aorta
Control	15 ± 1	11 ± 1	5 ± 0	102 ± 4
LTD ₄ (0.1 µg)	26 ± 2*	15 ± 1*	5 ± 0	103 ± 4
Control	17 ± 1	12 ± 1	5 ± 1	100 ± 5
LTD ₄ (0.3 µg)	34 ± 2*	20 ± 2*	5 ± 1	100 ± 5
Control	15 ± 1	11 ± 1	5 ± 1	97 ± 5
LTD ₄ (1 µg)	39 ± 2*	22 ± 3*	5 ± 1	101 ± 4

n = 11.

* *P* < 0.05 when compared to corresponding control, paired comparison.

Influence of Inhibitors

In order to determine whether pulmonary vascular responses to LTD₄ in the sheep were dependent on formation of cyclooxygenase products, we investigated the effects of sodium meclofenamate, a cyclooxygenase inhibitor, and of OKY 1581, a thromboxane synthesis inhibitor. The effects of sodium meclofenamate on increases in lobar arterial pressure in response to LTD₄ are shown in the top of Figure 3. After administration of the cyclooxygenase inhibitor in a dose of 2.5 mg/kg, iv, the increases in lobar arterial pressure in response to LTD₄ were reduced significantly at each dose of leukotriene studied. The thromboxane synthesis inhibitor, OKY 1581, in doses of 5–10 mg/kg iv, also significantly

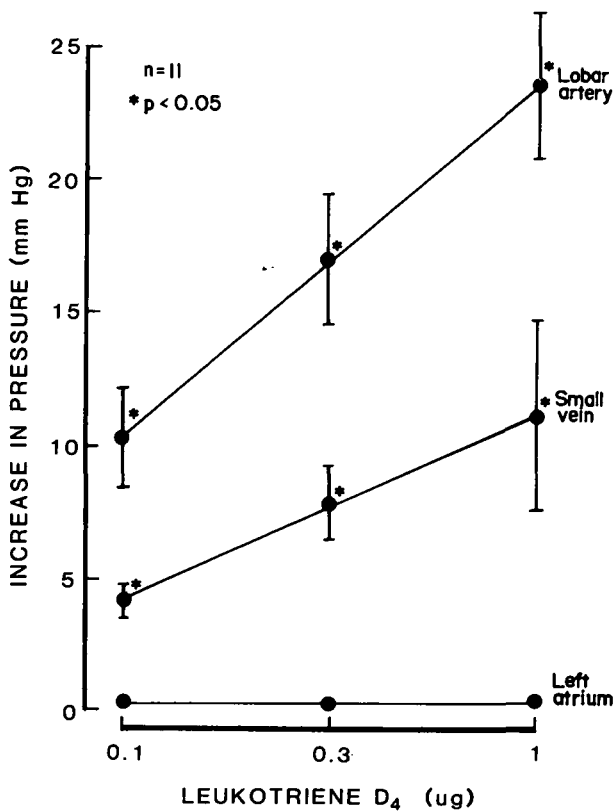


FIGURE 2. Dose-response curves showing the increases in lobar arterial and small intrapulmonary vein pressures in response to intralobar injections of LTD_4 , 0.1–1 μ g. Left atrial pressure was not changed by intralobar injections of LTD_4 in the group of 11 intact-chest sheep.

attenuated the increases in lobar arterial pressure in response to the three doses of LTD_4 (Fig. 3, lower panel). However, the inhibitory effects of the cyclooxygenase inhibitor on responses to LTD_4 were greater than the inhibitory effects of the thromboxane synthesis inhibitor (Fig. 3). Neither OKY 1581 nor sodium meclofenamate had significant effect on pulmonary vascular or systemic arterial pressure in the sheep.

The effects of sodium meclofenamate and OKY 1581 on pulmonary vascular responses to an agent whose actions mimic those of thromboxane A_2 were investigated, and these data are presented in Figure 4. U-46619, an agent whose actions are similar to those of thromboxane A_2 on smooth muscle, caused dose-dependent increases in lobar arterial and small vein pressures without affecting left atrial or systemic arterial pressure (data not shown). The increases in lobar arterial pressure in response to U-46619 were not altered after administration of sodium meclofenamate, 2.5 mg/kg, iv, or OKY 1581, 5–10 mg/kg, iv (Fig. 4).

Biochemical Studies

In biochemical studies, the effects of OKY 1581 on the metabolism of arachidonic acid and of the

prostaglandin endoperoxide, PGH_2 , by microsomal fractions from sheep lung were investigated. The addition of $1\text{-}^{14}\text{C}$ -arachidonic acid (20 μ M) to the microsomal fraction (200 μ g protein) resulted in the formation of 6-keto- $PGF_{1\alpha}$, the stable breakdown product of PGI_2 , 255 ± 21 pmol and TXB_2 , the stable breakdown product of TXA_2 , 230 ± 19 pmol/hr in the absence of the inhibitor (Table 2). Prostaglandins $F_{2\alpha}$, E_2 , and D_2 were also formed (Table 2). Moreover, when OKY 1581 was added to the incubation medium in concentrations of 10^{-9} M or greater, the formation of TXB_2 was reduced to 37% of control at 10^{-7} M and 31% of control at 10^{-6} M (Table 2). However, the synthesis of 6-keto- $PGF_{1\alpha}$ was not decreased at concentrations of OKY 1581 up to 10^{-6} M (Table 2). The formation of $PGF_{2\alpha}$, PGE_2 , and PGD_2 was not decreased by OKY 1581 in concentrations up to 10^{-6} M (Table 2).

The influence of OKY 1581 on endoperoxide metabolism by sheep lung microsomal fraction is shown in Table 3. In the absence of inhibitor, 166

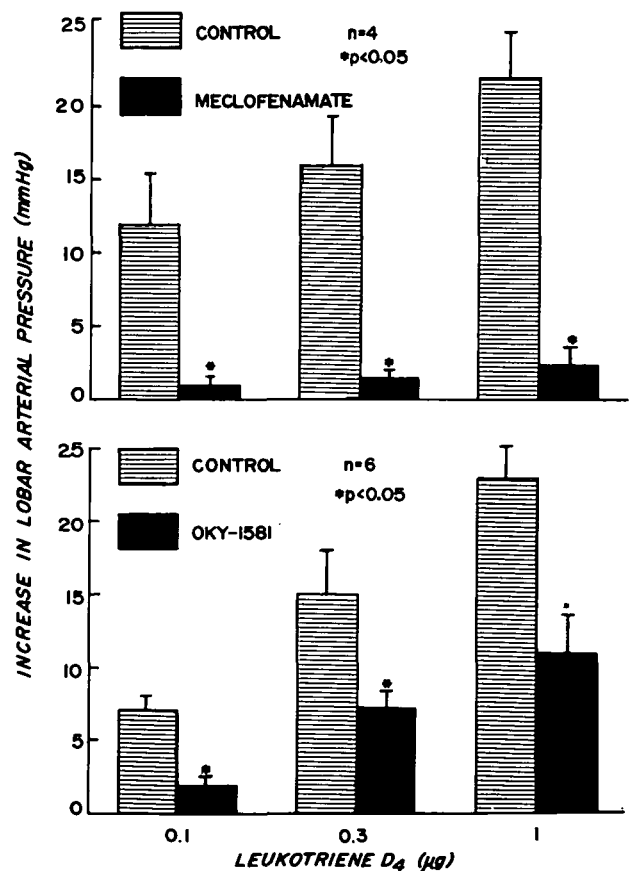


FIGURE 3. Top panel, effect of sodium meclofenamate, a cyclooxygenase inhibitor, on increases in lobar arterial pressure in response to intralobar injections of LTD_4 in the intact-chest sheep. Responses to LTD_4 were compared before and 10–15 minutes after administration of meclofenamate, 2.5 mg/kg, iv. Lower panel, influence of OKY-1581, a thromboxane synthesis inhibitor, on increases in lobar arterial pressure in response to LTD_4 in the sheep. Responses were obtained before and 15–30 minutes after administration of OKY-1581, 5–10 mg/kg, iv. *n* indicates number of animals.

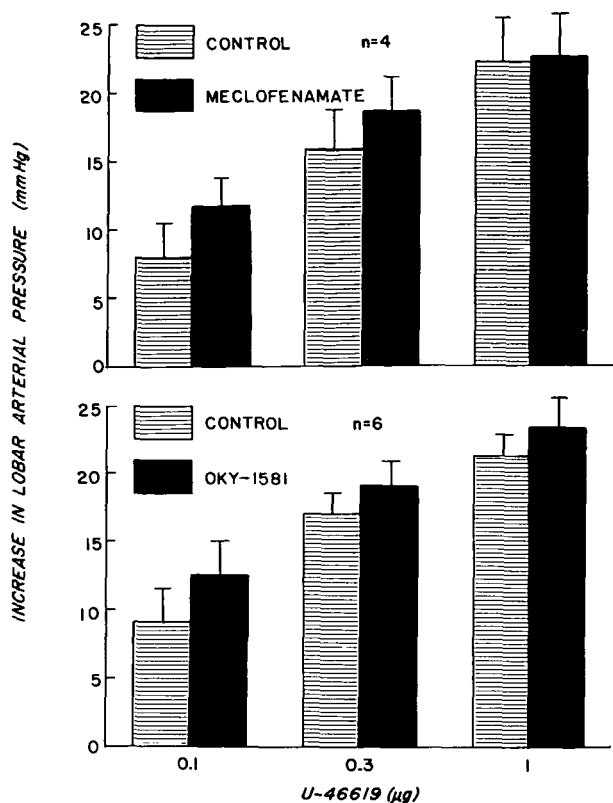


FIGURE 4. Top panel, effect of sodium meclofenamate on increases in lobar arterial pressure in response to the thromboxane A₂ mimic, U-46619. Responses to intralobar injections of U-46619 were obtained before and 10–15 minutes after administration of sodium meclofenamate, 2.5 mg/kg, iv. Lower panel, effect of OKY-1581 on increases in lobar arterial pressure in response to U-46619. Responses to the thromboxane A₂ mimic were compared before and 15–30 minutes after administration of OKY-1581, 5–10 mg/kg, iv. n indicates the number of sheep.

± 15 pmol of 6-keto-PGF_{1 α} and 161 ± 17 picomoles of TXB₂ were formed per 2-minute period when $10 \mu\text{M}$ PGH₂ was added to $200 \mu\text{g}$ microsomal protein (Table 3). PGF_{2 α} , PGE₂, and PGD₂ were also formed from PGH₂ (Table 3). However, addition of OKY 1581 in concentrations of 10^{-9} M or higher

reduced the formation of TXB₂ (Table 3). TXB₂ formation was reduced by more than 80% at the higher concentrations of the inhibitor. The formation of PGF_{2 α} , PGE₂, or PGD₂ was not reduced by OKY 1581 (Table 3). The effects of injection of OKY 1581, 10 mg/kg iv, on thromboxane formation were also studied in lung homogenates from two sheep. In two duplicate samples from each lung, addition of $20 \mu\text{M}$ 1-¹⁴C-arachidonic acid to $10 \mu\text{l}$ homogenate resulted in formation of 62 pmol (3.1%) TXB₂ per hour. In homogenates from two control animals (four samples), addition of $20 \mu\text{l}$ 1-¹⁴C-arachidonic acid to $10 \mu\text{l}$ lung resulted in formation of 239 pmol (11.9%) TXB₂ per hour. These results show that thromboxane formation is greatly reduced in the lung by treatment with OKY 1581, 10 mg/kg, iv.

Inhibitor Effects on Responses to Arachidonic Acid

The effects of the cyclooxygenase and thromboxane synthesis inhibitors on responses to arachidonic acid were also investigated in the sheep and these data are illustrated in Figure 5. Intralobar injections of arachidonic acid in doses of 30 and $100 \mu\text{g}$ caused a dose-dependent increase in lobar arterial pressure without affecting left atrial pressure. The increases in lobar arterial pressure in response to arachidonic acid were markedly decreased after administration of sodium meclofenamate, 2.5 mg/kg, iv (Fig. 5, top panel). The increases in lobar arterial pressure in response to arachidonic acid were also decreased significantly after administration of OKY 1581, 5–10 mg/kg, iv (Fig. 5, lower panel).

Influence of Ventilation

The relationship between the effects of LTD₄ on ventilation and on the pulmonary vascular bed was studied in four sheep. In these experiments, responses to LTD₄ were obtained when the lung was ventilated and when lobar ventilation was arrested at end-expiration by inflating a balloon catheter in the left lower lobe bronchus. In these experiments, the left lower lobe was perfused with arterial blood

TABLE 2
Influence of OKY-1581 on Arachidonic Acid (AA) Metabolism by Sheep Lung Microsomes

Additions	pmol/hr					
	6-Keto-PGF _{1α}	PGF _{2α}	TXB ₂	PGE ₂	PGD ₂	AA
None	255 \pm 21	106 \pm 13	230 \pm 19	175 \pm 39	105 \pm 11	944 \pm 46
OKY-1581						
10 ⁻¹⁰ M	256 \pm 17	121 \pm 23	230 \pm 35	153 \pm 21	92 \pm 21	1046 \pm 80
10 ⁻⁹ M	269 \pm 22	121 \pm 20	153 \pm 14	225 \pm 42	130 \pm 14	1004 \pm 74
10 ⁻⁸ M	321 \pm 26	152 \pm 16	168 \pm 25	223 \pm 31	128 \pm 19	885 \pm 52
10 ⁻⁷ M	300 \pm 27	203 \pm 25	86 \pm 11	259 \pm 34	155 \pm 27	841 \pm 76
10 ⁻⁶ M	281 \pm 33	177 \pm 19	72 \pm 11	269 \pm 44	138 \pm 21	885 \pm 79

Incubation conditions: $200 \mu\text{g}$ microsomal protein and $20 \mu\text{M}$ arachidonic acid. Data are expressed as mean \pm SE for duplicate incubations in five animals.

TABLE 3
Influence of OKY-1581 on Endoperoxide PGH₂ Metabolism by Sheep Lung Microsomes

Additions	pmol/2 min					
	6-Keto-PGF _{1α}	PGF _{2α}	TXB ₂	PGE ₂	PGD ₂	PGH ₂ and HHT
None	166 ± 15	74 ± 11	161 ± 17	124 ± 6	66 ± 16	228 ± 46
OKY-1581						
10 ⁻¹¹ M	198 ± 22	88 ± 15	198 ± 25	132 ± 16	56 ± 10	225 ± 28
10 ⁻⁹ M	150 ± 3	97 ± 16	118 ± 38	181 ± 23	80 ± 14	205 ± 12
10 ⁻⁷ M	180 ± 15	125 ± 8	50 ± 21	226 ± 10	94 ± 11	216 ± 20
10 ⁻⁵ M	128 ± 7	106 ± 20	11 ± 7	258 ± 19	110 ± 11	244 ± 18
10 ⁻³ M	158 ± 16	126 ± 18	22 ± 3	253 ± 17	96 ± 7	199 ± 11

Incubation conditions: 200 μg microsomal protein and 10 μM PGH₂. Data are expressed as mean ± SE for duplicate incubation in 2-3 animals.

to lessen the effects of hypoxia on the lung, and 1-3 ml of a 2% lidocaine viscous solution was instilled into the lobar bronchus to prevent coughing. The correlation between the increases in lobar arterial

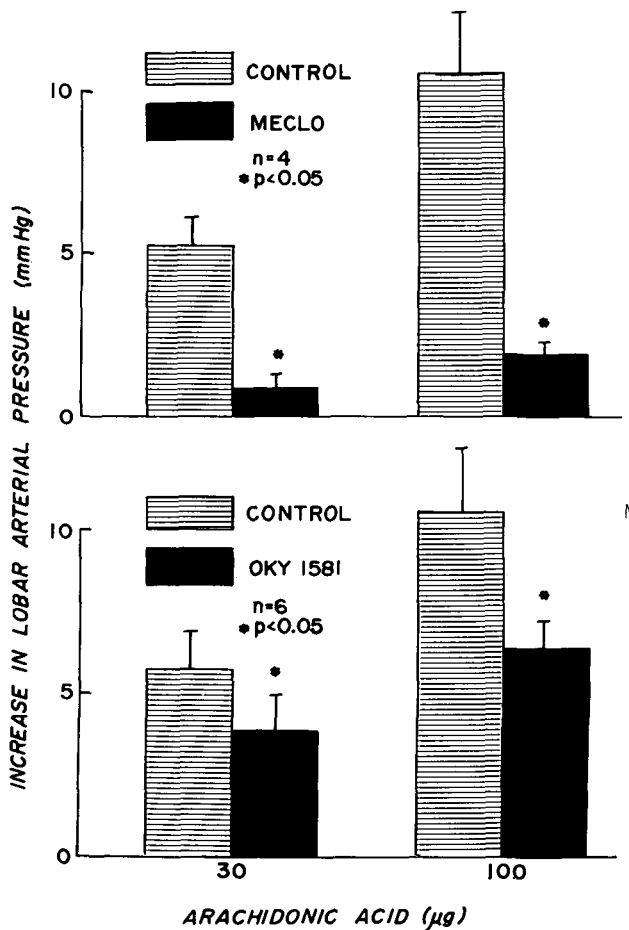


FIGURE 5. Top panel, influence of sodium meclofenamate on increases in lobar arterial pressure in response to intralobar injections of arachidonic acid in the sheep. Responses to the prostaglandin precursor were compared before and 10-15 minutes after administration of meclofenamate, 2.5 mg/kg, *iv*. Lower panel, effect of OKY 1581 on increases in lobar arterial pressure in response to arachidonic acid. Responses to the prostaglandin and thromboxane precursor were compared before and 15-30 minutes after administration of OKY 1581, 5-10 mg/kg, *iv*. *n* indicates number of animals.

pressure in response to intralobar injections of LTD₄, 0.1-1 μg, when the lobe was ventilated and when lobar ventilation was arrested is shown in Figure 6. The correlation coefficient of the regression line was 0.90 (*P* < 0.05) with a slope of 0.83 that was not significantly different from the line of identity. These data show that responses to LTD₄ are similar when the lobe is ventilated and when ventilation is arrested.

Influence of Nonsanguineous Perfusion

The relationship between the effects of LTD₄ on formed elements and on the pulmonary vascular bed was investigated in five sheep. In these animals, responses to LTD₄ in doses of 0.1-1 μg were com-

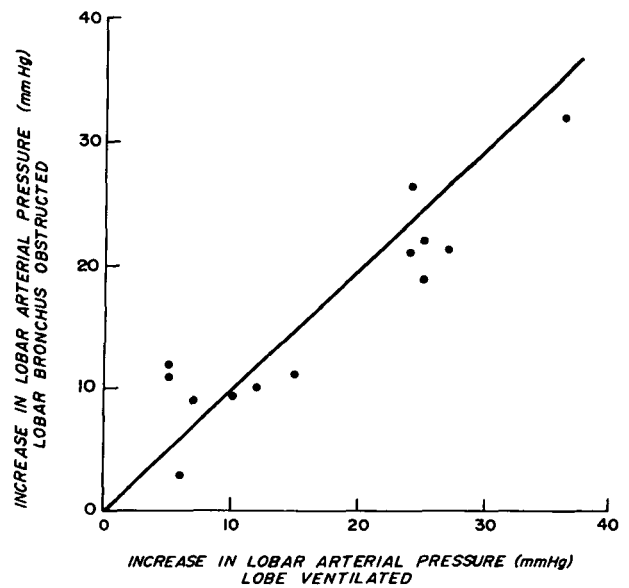


FIGURE 6. Relationship between the increases in lobar arterial pressure in response to LTD₄ during normal lobar ventilation and when lobar ventilation was arrested by obstruction of the lobar bronchus. Responses to intralobar injections of LTD₄, 0.1-1 μg, were determined in four sheep when the lobe was ventilated and when ventilation was arrested. The correlation coefficient of the regression line was 0.90 (*P* < 0.05), and the slope of the line (0.83) was not significantly different from the line of identity shown in the figure.

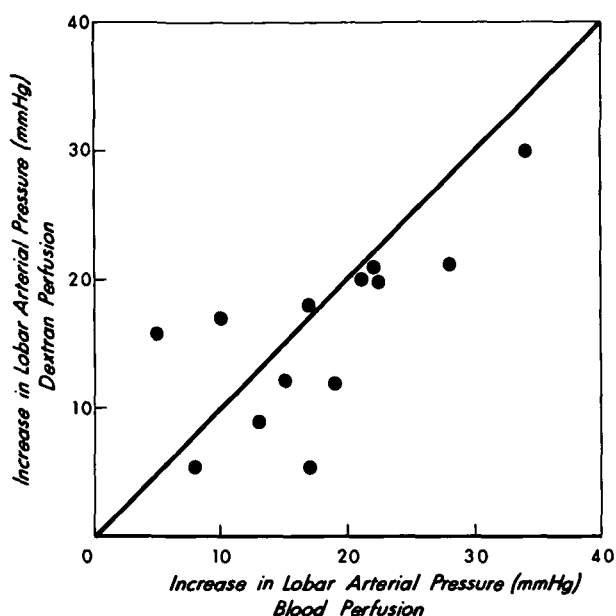


FIGURE 7. Relationship between the increases in lobar arterial pressure in response to LTD₄, 0.1–1 μ g, during normal lobar perfusion with blood and when the lobe was perfused with a 5% dextran solution. Responses obtained during blood perfusion were plotted against responses obtained during dextran perfusion in five sheep. The correlation coefficient of the regression line was 0.70 ($P < 0.05$) with a slope (0.62) that was not significantly different from the line of identity shown in the figure.

pared when the lung was perfused with blood and when it was perfused with a 5% solution of low molecular weight dextran in the same animal. In these experiments, a smaller segment of the left lower lobe was perfused and the results are presented in Figure 7. The correlation coefficient of the regression line for responses obtained during blood perfusion plotted against responses obtained during dextran perfusion was 0.70 ($P < 0.05$) with a slope of 0.62 that was not significantly different from the line of identity shown in the figure. There were no significant changes in left atrial pressure or systemic arterial pressure in these experiments when LTD₄

TABLE 4
Influence of Intralobar Injections of Leukotriene D₄ (LTD₄) on Mean Vascular Pressures in the Cat

	Pressure (mm Hg)		
	Lobar artery	Left atrium	Aorta
Control	14 \pm 1	3 \pm 1	110 \pm 5
LTD ₄ (0.3 μ g)	16 \pm 1*	3 \pm 1	113 \pm 5
Control	13 \pm 1	2 \pm 1	110 \pm 8
LTD ₄ (1 μ g)	16 \pm 1*	2 \pm 1	119 \pm 6*
Control	13 \pm 1	3 \pm 1	115 \pm 10
LTD ₄ (3 μ g)	21 \pm 2*	3 \pm 1	124 \pm 11*

$n = 7$.

* $P < 0.05$ when compared to corresponding control, paired comparison.

was injected. These data suggest that responses to LTD₄ are not different when the lung is perfused with blood or the dextran solution.

Species Variation

In order to determine whether responses to LTD₄ varied with species, we also investigated the effects of LTD₄ on the pulmonary vascular bed in the intact-chest cat, and these data are summarized in Table 4. Intralobar injections of LTD₄ in doses of 0.3, 1, and 3 μ g caused small but significant dose-related increases in lobar arterial pressure without affecting left atrial pressure (Table 4). The time course of these responses was similar to that observed in the sheep with a rapid onset and lobar arterial pressure returning to control level over a 3- to 4-minute period. Systemic arterial pressure was increased significantly in response to intralobar injections of the 1- and 3- μ g doses of LTD₄ (Table 4). Although lobar vascular responses to LTD₄ were modest in the cat, U-46619 had marked vasoconstrictor activity. The increases in lobar arterial pressure in response to LTD₄ and U-46619 in the sheep and cat are compared in Figure 8. Both LTD₄ and U-46619 had marked vasoconstrictor activity in the sheep pulmonary vascular bed, and the dose-response curves

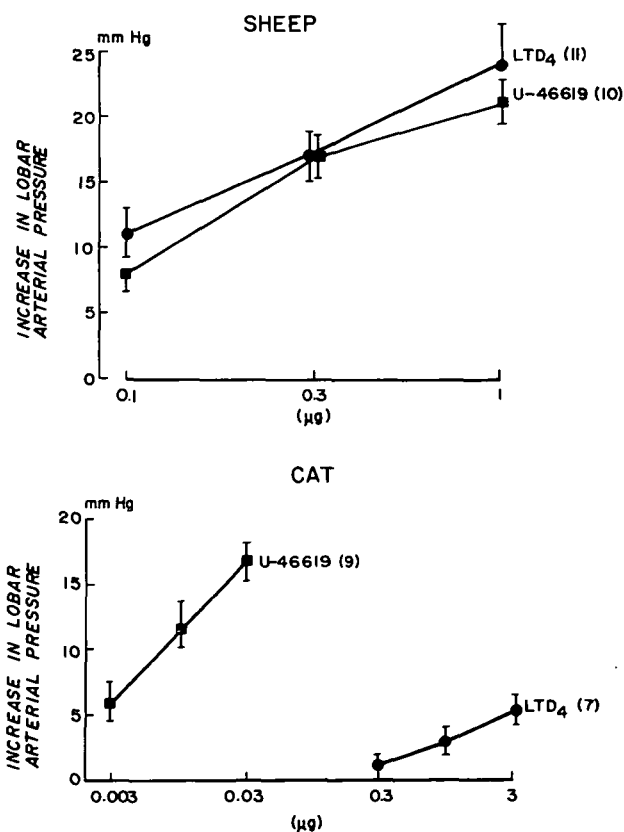


FIGURE 8. Dose-response relationships comparing increases in lobar arterial pressure in response to LTD₄ and U-46619 in the sheep (top panel) and in the cat (lower panel). Responses to the thromboxane mimic and LTD₄ were not significantly different at the 0.1-, 0.3-, and 1- μ g doses in the sheep. Number in parentheses indicates number of animals.

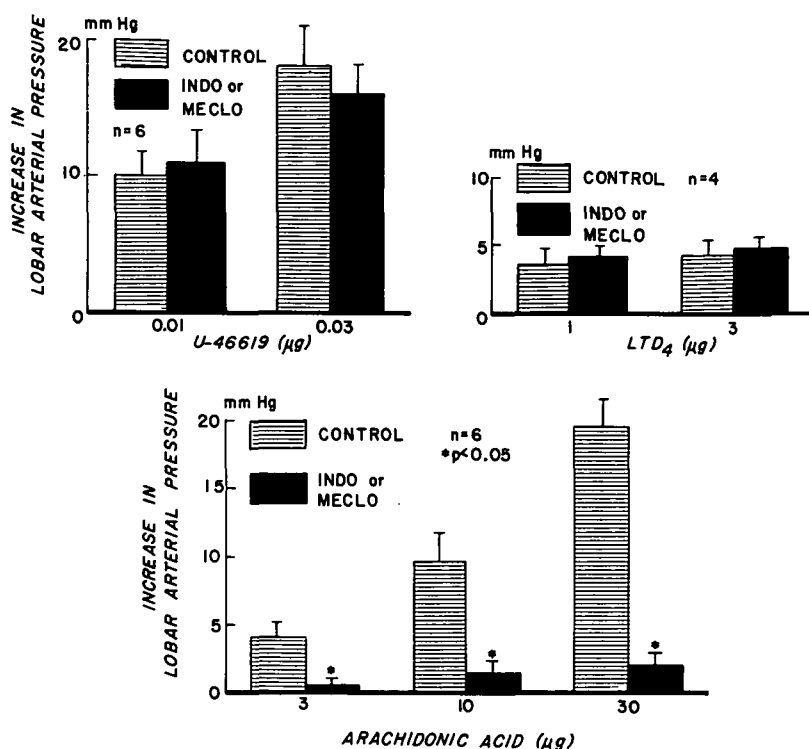


FIGURE 9. Top panels, influence of indomethacin or meclofenamate on increases in lobar arterial pressure in response to U-46619 (left panel) or LTD₄ (right panel) in the cat. Responses were compared before and 10–15 minute after administration of the indomethacin or sodium meclofenamate, 2.5 mg/kg, iv. Bottom panel, influence of the cyclooxygenase inhibitors on increases in lobar arterial pressure in response to intralobar injections of arachidonic acid in the cat. n indicates number of cats.

for both substances in this species did not differ significantly (Fig. 8, top panel). However, in the cat, U-46619 had far greater vasoconstrictor activity than did LTD₄ (Fig. 8, lower panel).

The role of the cyclooxygenase pathway in the mediation of pulmonary vascular responses to LTD₄ was also investigated in the cat. Administration of indomethacin or sodium meclofenamate, 2.5 mg/kg, iv, had no significant effect on pulmonary vasoconstrictor responses to U-46619 or LTD₄ in the cat (Fig. 9, top panels). The increases in systemic arterial pressure in response to the 1- and 3-µg doses of LTD₄ were not altered by the cyclooxygenase inhibitors (data not shown). However, the cyclooxygenase inhibitors, in the doses employed, significantly reduced the increases in lobar arterial pressure in response to intralobar injections of arachidonic acid (Fig. 9, lower panel). The cyclooxygenase inhibitors had no significant effect on pulmonary vascular or systemic arterial pressure in the cat.

Discussion

The present results in the sheep demonstrate that intralobar injections of LTD₄ increase pulmonary lobar arterial pressure in a dose-related manner. Since pulmonary blood flow was maintained constant, and left atrial pressure was unchanged, the increase in pressure gradient across the lung indicates that pulmonary lobar vascular resistance was increased by LTD₄. The increases in lobar arterial pressure in response to LTD₄ were associated with dose-related increases in small intrapulmonary vein pressure and in the pressure gradient from lobar

artery to small vein. These results in the sheep suggest that LTD₄ increases pulmonary vascular resistance by constricting intrapulmonary veins and segments upstream to the small vein believed to be small arteries. These data in yearling sheep are consistent with results in the newborn lamb in which LTD₄ increased pulmonary vascular resistance (Yokochi et al., 1982). It has been reported that LTD₄ has potent coronary vasoconstrictor activity in the sheep that can be associated with impairment of left ventricular function (Michelassi et al., 1982). However, in the present study, LTD₄ had no significant effect on systemic arterial or left atrial pressure in the doses studied. The effects of LTD₄ on left atrial pressure in the newborn lamb were not measured, so that the mechanism of the fall in cardiac output in the neonate is uncertain (Yokochi et al., 1982).

In terms of relative pressor activity in the sheep pulmonary vascular bed, LTD₄ was very potent with activity paralleling that of U-46619, a stable prostaglandin analog whose actions are thought to mimic those of thromboxane A₂ (Coleman et al., 1981). Moreover, when compared to other vasocative hormones whose effects have been studied in the sheep, LTD₄ is more active than other arachidonic acid metabolites, alveolar hypoxia, or histamine which acts over a similar portion of the pulmonary vascular bed and is released along with the leukotrienes in immediate hypersensitivity reactions (Brocklehurst, 1960; Kadowitz et al., 1974; Hyman and Kadowitz, 1975; Kadowitz and Hyman, 1983).

It has been reported that LTD₄ has potent contractile activity on isolated airway smooth muscle

and lung parenchyma and that it increases bronchomotor tone (Dahlen et al., 1980; Drazen et al., 1980; Krell et al., 1981; Holroyde et al., 1981; Jones et al., 1982; Graybar et al., unpublished data). However, in the present study, the effects of LTD₄ on the pulmonary vascular bed appear to be independent of alterations in ventilation or those that occur as a consequence of changes in bronchomotor tone or lung volume, since similar responses were obtained when the lobe was ventilated or when lobar ventilation was arrested by obstruction of bronchial airflow. In previous studies, responses to a number of vasoactive substances, including cyclooxygenase metabolites of arachidonic acid and histamine, were similar when the lobe was ventilated or lobar ventilation was arrested, suggesting that the actions of many vasoactive hormones on pulmonary vascular resistance appear to be independent of alterations in bronchomotor tone (Hyman et al., 1978; Kadowitz and Hyman, 1983).

In the sheep, pulmonary vasoconstrictor responses to LTD₄ were markedly attenuated after treatment with sodium meclofenamate, suggesting that responses to this lipoxygenase product are dependent on the formation of products in the cyclooxygenase pathway. In addition, vasoconstrictor responses to LTD₄ were decreased by OKY 1581, a thromboxane synthesis inhibitor, suggesting that a portion of the pressor response to LTD₄ is due to the release of thromboxane A₂. The observation that meclofenamate had greater inhibitory effect on responses to LTD₄ than did OKY 1581 suggests that pulmonary responses to this lipoxygenase metabolite are dependent on the formation of thromboxane A₂ and other cyclooxygenase products such as prostaglandins (PG) D₂ and F_{2α}, which also have substantial pressor activity in the pulmonary vascular bed (Kadowitz et al., 1974; Kadowitz and Hyman, 1980). It has been shown that SRS-A or synthetic LTC₄ and LTD₄ release prostaglandins and TXA₂ from isolated guinea pig lung (Engineer et al., 1978; Piper and Samhoun, 1981). Cyclooxygenase inhibitors reduce the contractile effects of LTD₄ on guinea pig lung parenchymal strips (Piper and Samhoun, 1981). The results of the present experiments in the sheep are consistent with data obtained with isolated guinea pig parenchyma and on bronchoconstrictor responses in the guinea pig, indicating that responses to LTD₄ are dependent on the release of TXA₂ and prostaglandins (Piper and Samhoun, 1981; Weichman et al., 1982).

A similar relationship between these inhibitors and responses to arachidonic acid was also observed, in that there was a greater reduction in response after treatment with meclofenamate than after OKY 1581. These data confirm previous studies showing that pulmonary vasoconstrictor responses to arachidonic acid are due to formation of cyclooxygenase products (Hyman et al., 1978, 1980; Spannake et al., 1980) and extend these findings by showing that

a portion of the response to the precursor is due to TXA₂ formation.

Although responses to LTD₄ and arachidonic acid were markedly reduced by meclofenamate, this cyclooxygenase inhibitor had no significant effect on pulmonary vasoconstrictor responses to U-46619, an analog whose actions are thought to mimic those of thromboxane A₂ (Coleman et al., 1981). These data indicate that sodium meclofenamate inhibited cyclooxygenase activity in the lung, and that the inhibitor did not influence vascular responses to the thromboxane mimic. In addition, vasoconstrictor responses to U-46619 were not altered by OKY 1581 in doses that inhibited responses to LTD₄ and arachidonic acid, suggesting that the thromboxane synthesis inhibitor did not alter thromboxane receptor-mediated responses, and that the effects of the inhibitor were due to inhibition of thromboxane formation. The results of microsomal studies show that OKY 1581 inhibited the formation of TXA₂ as measured by formation of its stable breakdown product TXB₂. TXB₂ formation was inhibited over a wide range of concentration of OKY 1581 when either arachidonic acid or the endoperoxide, PGH₂, was employed as substrate. Although TXB₂ formation was decreased by OKY 1581, PGI₂ formation as measured by the production of 6-keto-PGF_{1α} was not inhibited, even at very high concentrations of OKY 1581. Prostaglandins E₂, F_{2α}, and D₂ were formed when PGH₂ or arachidonic acid was used as substrate; however, it is not known if this prostaglandin synthesis was enzymatic. The amount of these prostaglandins formed was not decreased by OKY 1581, and in the case of PGE₂, was enhanced by the inhibitor. Since the total amount of cyclooxygenase product formed from arachidonic acid was not decreased, although TXB₂ formation was reduced, it is unlikely that OKY 1581 inhibited sheep lung cyclooxygenase activity. These experiments suggest that effects of OKY 1581 were specific in the sheep lung. It is possible that the pulmonary hypertensive effect of LTD₄ may result in part from an interaction with formed elements. In order to investigate this possibility, responses to LTD₄ were compared when the lung was perfused with blood or with dextran. These experiments show that responses to LTD₄ were not different when the lung was perfused with blood or a cell-free solution. These data indicate that the response to LTD₄ is independent of an interaction with white cells or platelets, and suggest that the cyclooxygenase products were derived from lung cells.

The results of studies in the sheep demonstrate that LTD₄ has very potent vasoconstrictor activity in the pulmonary vascular bed, and that this activity is due for the most part to release of cyclooxygenase products. However, the effects of LTD₄ in the pulmonary vascular bed of the sheep and the cat are different. In the cat, LTD₄ had only modest pressor activity equal to that of arachidonic acid, and far

less than that of $\text{PGF}_{2\alpha}$, PGD_2 , or PGE_2 in that species (Kadowitz and Hyman, 1980). Furthermore, in this species, cyclooxygenase blockers did not modify responses to this lipoxygenase product. Although the relative magnitude of responses to LTD_4 , as well as the mechanism of action, differ in the sheep and the cat, both species were extremely responsive to the effects of U-46619. Thus, there appears to be true species variation in the pulmonary vascular response to LTD_4 . This variation was not observed with U-46619, which may operate via TXA_2 receptors in the pulmonary vascular bed. In addition to demonstrating marked species variation in the response to LTD_4 , the present data may be interpreted to suggest that LTD_4 itself does not have potent vasoconstrictor activity in the lung when the cyclooxygenase system is blocked. Moreover, the remaining pressor activity of LTD_4 in the sheep after cyclooxygenase blockade and the pressor activity in the cat, which were very similar, suggests that the activity of this lipoxygenase metabolite is far less than that of cyclooxygenase products such as TXA_2 , $\text{PGF}_{2\alpha}$, or PGD_2 (Kadowitz and Hyman, 1977, 1980). The data from the present study suggest that it would be difficult to formulate a unified hypothesis on the role of LTD_4 , a major component of SRS-A, on the pulmonary circulation, since species variation is so marked.

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References

- Brocklehurst WE (1960) The release of histamine and formation of a slow-reacting substance during anaphylactic shock. *J Physiol (Lond)* 151: 416-435
- Coleman RA, Humphrey PPA, Kennedy I, Levy GP, Lumley P (1981) Comparison of the actions of U-46619, a prostaglandin H_2 -analogue, with those of prostaglandin H_2 and thromboxane A_2 on some isolated smooth muscle preparations. *Br J Pharmacol* 73: 773-778
- Dahlen SE, Hedqvist P, Hammarström S, Samuelsson B (1980) Leukotrienes are potent constrictors of human bronchi. *Nature* 288: 484-486
- Dahlen SE, Hansson G, Hedqvist P, Björck T, Granström E, Dahlen B (1983) Allergic challenge of lung tissue from asthmatics elicits bronchial contraction that correlates with the release of leukotriene C_4 , D_4 and E_4 . *Proc Natl Acad Sci USA* 80: 1712-1716
- Drazen JM, Austen KF, Lewis RA, Clark DA, Goto G, Marfat A, Corey EJ (1980) Comparative airway and vascular activities of leukotrienes C-1 and D in vivo and in vitro. *Proc Natl Acad Sci USA* 77: 4354-4358
- Engineer DM, Morris HR, Piper PJ, Sirois P (1978) The release of prostaglandins and thromboxanes from guinea-pig lung by slow-reacting substances of anaphylaxis and its inhibition. *Br J Pharmacol* 64: 211-218
- Hamel R, Masson P, Ford-Hutchinson AW, Jones TR, Brunet G, Piechuta H (1982) Differing mechanisms for leukotriene D_4 -inducing bronchoconstriction in guinea pigs following intravenous and aerosol administration. *Prostaglandins* 24: 419-432
- Hammarström S (1983) Leukotrienes. *Annu Rev Biochem* 52: 355-377
- Hand JM, Will JA, Buckner CK (1981) Effects of leukotrienes on isolated guinea-pig pulmonary arteries. *Eur J Pharmacol* 76: 439-442
- Holroyde MD, Altounyan REC, Cole M, Dixon M, Elliot EV (1981) Bronchoconstriction produced in man by leukotrienes C and D. *Lancet* 2: 17-18
- Hyman AL, Kadowitz PJ (1975) Effect of alveolar and perfusion hypoxia and hypercapnia on pulmonary vascular resistance in the lamb. *Am J Physiol* 228: 397-403
- Hyman AL, Kadowitz PJ (1979) Pulmonary vasodilator activity of prostacyclin (PGI_2) in the cat. *Circ Res* 45: 404-409
- Hyman AL, Mathe AA, Leslie CA, Matthews CC, Bennett JT, Spannhake EW, Kadowitz PJ (1978) Modification of pulmonary vascular responses to arachidonic acid by alterations in physiologic state. *J Pharmacol Exp Ther* 207: 388-401
- Hyman AL, Spannhake EW, Kadowitz PJ (1980) Divergent responses to arachidonic acid in the feline pulmonary vascular bed. *Am J Physiol* 239: H40-H46
- Iacopino VJ, Fitzpatrick TM, Ramwell-PW, Rose JC, Kot PA (1983) Cardiovascular responses to leukotriene C_4 in the rat. *J Pharmacol Exp Ther* 227: 244-247
- Jones TR, Davis C, Daniel EE (1982) Pharmacological study of the contractile activity of leukotriene C_4 and D_4 on isolated human airway smooth muscle. *Can J Physiol Pharmacol* 60: 638-643
- Kadowitz PJ, Hyman AL (1977) Influence of a prostaglandin endoperoxide analogue on the canine pulmonary vascular bed. *Circ Res* 40: 282-287
- Kadowitz PJ, Hyman AL (1980) Comparative effects of thromboxane B_2 on the canine and feline pulmonary vascular bed. *J Pharmacol Exp Ther* 213: 300-305
- Kadowitz PJ, Hyman AL (1983) Pulmonary vascular responses to histamine in sheep. *Am J Physiol* 244: H423-H428
- Kadowitz PJ, Joiner PD, Hyman AL (1974) Influence of prostaglandins E_1 and $\text{F}_{2\alpha}$ on pulmonary vascular resistance in the sheep. *Proc Soc Exp Biol Med* 145: 1258-1261
- Kellaway CH, Trethewie EF (1940) The liberation of slow reacting smooth muscle-stimulating substance in anaphylaxis. *Q J Exp Physiol* 30: 121-145
- Krell RD, Osborn R, Vickery L, Falcone K, O'Donnell M, Gleason J, Kinzig C, Bryan D (1981) Contraction of isolated airway smooth muscle by synthetic leukotrienes C_4 and D_4 . *Prostaglandins* 22: 387-409
- Lewis RA, Austen KF, Drazen JM, Clark DA, Marfat A, Corey EJ (1980) Slow reacting substances of anaphylaxis: Identification of leukotrienes C and D from human and rat sources. *Proc Natl Acad Sci USA* 77: 3710-3714
- Lowry OH, Rosebrough NJ, Farr AL, Randall RS (1951) Protein measurement with the Folin phenol reagents. *J Biol Chem* 193: 265-275
- Michelassi F, Landa L, Hill RD, Lowenstein E, Watkins WD, Petkau AJ, Zapol WM (1982) Leukotriene D_4 : A potent coronary artery vasoconstrictor associated with impaired ventricular contraction. *Science* 217: 841-843
- Morganroth ML, Reeves JT, Murphy RC, Voelkel NF (1984) Leukotriene synthesis and receptor blockers block hypoxic pulmonary vasoconstriction. *J Appl Physiol* 56: 1340-1346
- Morris HR, Taylor GW, Piper PJ, Tippins JR (1980) Structure of slow-reacting substance of anaphylaxis from guinea-pig lung. *Nature* 285: 104-108
- Murphy RC, Hammarström S, Samuelsson B (1979) Leukotriene C: A slow reacting substance from murine mastocytoma cells. *Proc Natl Acad Sci USA* 76: 4275-4279
- Piper PJ, Samhoum MN (1981) The mechanism of action of leukotriene C_4 and D_4 in guinea-pig isolated perfused lung and

- parenchymal strips of guinea pig. *Prostaglandins* 21: 793-803
- She HS, McNamara DB, Spannhake EW, Hyman AL, Kadowitz PJ (1981) Metabolism of prostaglandin endoperoxide by microsomes from cat lung. *Prostaglandins* 21: 531-541
- Smedegard G, Hedqvist P, Dahlen SE, Revenas B, Hammarstrom S, Samuelsson B (1982) Leukotriene C₄ affects pulmonary and cardiovascular dynamics in monkey. *Nature* 295: 327-329
- Snedecor CW, Cochran WG (1967) *Statistical Methods*, ed 6. Ames, Iowa, Iowa State University Press
- Spannhake EW, Hyman AL, Kadowitz PJ (1980) Dependency of the airway and pulmonary vascular effects of arachidonic acid upon route and rate of administration. *J Pharmacol Exp Ther* 212: 584-590
- Spannhake EW, Colombo JL, Craigo PA, McNamara DB, Hyman AL, Kadowitz PJ (1983) Evidence for modification of pulmonary cyclooxygenase activity by endotoxin in the dog. *J Appl Physiol* 54: 191-198
- Voelkel NF, Worthen S, Reeves JT, Henson PM, Murphy RC (1982) Nonimmunological production of leukotrienes induced by platelet-activating factor. *Science* 218: 286-288
- Weichman BM, Muccitelli RM, Osborn RR, Holden DA, Gleason JG, Wasserman WA (1982) In vitro and in vivo mechanisms of leukotriene-mediated bronchoconstriction in the guinea pig. *J Pharmacol Exp Ther* 222: 202-208
- Yokochi K, Olley PM, Sideris E, Hamilton F, Huhtanen D, Coceani F (1982) Leukotriene D₄: A potent vasoconstrictor of the pulmonary and systemic circulations in the newborn lamb. In *Leukotrienes and Other Lipoxygenase Products*, edited by B Samuelsson, R Paoletti. New York, Raven Press, pp 211-214

INDEX TERMS: Leukotrienes • Pulmonary vascular bed • Cyclooxygenase pathway • Species variation • Dextran perfusion

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