The Differential Effects of Leukotriene C₄ and D₄ on the Pulmonary and Systemic Circulations in Newborn Lambs

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ABSTRACT. Leukotriene (LT) C₄ or D₄ may mediate pulmonary hypertension induced by hypoxia. LT have also been isolated from patients with persistent pulmonary hypertension of the newborn syndrome and the adult respiratory distress syndrome. To compare the effects of LTC₄ and D₄ on the pulmonary and systemic circulations, we performed dose-response studies on spontaneously breathing newborn lambs. To determine whether the hemodynamic effects of LT are mediated through α -adrenergic stimulation, some lambs were pretreated with the α -adrenergic antagonist phentolamine mesylate before LT injection. These results were compared to the effects of pretreatment with the LT receptor antagonist FPL57231. To determine whether the LT-induced decrease in cardiac output was mediated by the decrease in heart rate, other lambs had their heart rate maintained by left atrial pacing. We found that LTC₄ and D₄ increased systemic arterial pressure and decreased cardiac output and heart rate. However, LTD₄, but not LTC₄, increased pulmonary arterial pressure. The hemodynamic effects of LTC₄ and LTD₄ were completely blocked by FPL57231 but not by phentolamine mesylate. Maintenance of heart rate by left atrial pacing did not alter the LT-induced decrease in cardiac output. We conclude that LTC₄ and D₄ have similar effects on the systemic circulation. However, LTD₄ produces more pulmonary vasoconstriction. Because FPL57231 did block the pulmonary vasoconstriction caused by LT, LT antagonists may be useful in treating patients with pulmonary hypertension. (*Pediatr Res* 21: 176–182, 1987)

Abbreviation

LT, leukotrienes

Recent studies have shown that LT cause *in vitro* contraction of smooth muscle in a variety of tissues including trachea (1–4), lung (1, 3–5), uterus (4, 6), gastrointestinal tract (4, 6, 7), and blood vessels (1, 3, 4, 8–11). LT may also play an important role in *in vivo* vascular smooth muscle contraction (12–18). This hypothesis is supported by the finding that the LT receptor antagonist FPL57231 (Fisons, plc, Loughborough, UK) attenuates hypoxia-induced pulmonary vasoconstriction in several

animal species (13, 14, 17). Clinically, LT have been isolated from the lung lavage fluid of patients with either the persistent pulmonary hypertension of the newborn syndrome (19) or the adult respiratory distress syndrome (20, 21).

The specific LT that cause pulmonary vasoconstriction and other types of smooth muscle contraction are LTC₄ and LTD₄, both C-6 peptide derivatives of LTA₄. LTC₄ is converted to LTD₄ through peptidolysis (6). Several previous studies have shown LTD₄ to be a potent *in vivo* pulmonary vasoconstrictor (12, 18, 22, 23). Evidence suggests that LTC₄ is also a pulmonary vasoconstrictor (3, 11, 16, 24). If LT do mediate the pulmonary vasoconstriction seen in some disease states, then LTC₄ and LTD₄ may play equal roles, or one may predominate.

In this study, we first wanted to determine whether LTC₄ and LTD₄ produce equal pulmonary and systemic circulatory effects. We therefore performed dose-response studies of LTC₄ and LTD₄ on spontaneously breathing newborn lambs.

It is possible that the pulmonary and systemic vasoconstriction produced by LT are mediated through other substances. To investigate whether the hemodynamic effects of LT are mediated through LT receptor stimulation or α -adrenergic stimulation (25), we treated some lambs with FPL57231, a LT receptor antagonist, or phentolamine mesylate, an α -adrenergic antagonist, before injecting LT.

Although previous studies had suggested that LT decrease cardiac output without affecting heart rate (9, 26, 27), our preliminary data (28) showed a decrease in heart rate. To determine whether the LT-induced decrease in cardiac output was caused by the decrease in heart rate, we injected LT into lambs whose heart rates were maintained using atrial pacing.

METHODS

Surgical preparation. Sixteen newborn lambs at 1 to 3 days of age were operated on under local anesthesia induced by 1% lidocaine hydrochloride. Polyvinyl catheters were placed in a hind leg artery and vein and advanced to the descending aorta and inferior vena cava, respectively. General anesthesia was induced by having the lamb breathe a mixture of oxygen and isoflurane. The lamb was then intubated with a 4.5 mm OD endotracheal tube and mechanically ventilated with a Harvard animal ventilator.

A left lateral thoracotomy was performed in the fourth or fifth intercostal space. Polyvinyl catheters were placed in the internal thoracic artery and vein and advanced to the ascending aorta and superior vena cava (central venous), respectively. The pericardium was incised along the main pulmonary trunk. Teflon cannulae attached to polyvinyl catheters were inserted into the main pulmonary artery and left atrium. A calibrated electromagnetic flow transducer (CC Instruments, Los Angeles, CA) was placed around the main pulmonary artery to measure cardiac

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output. In four of the 16 newborn lambs, bipolar stainless steel pacing electrodes were sewn onto the epicardial surface of the left atrial appendage. A chest tube was placed in the pleural space. The catheters were filled with heparin, plugged, and, along with the transducer cable and pacing wires, brought to the skin, where they were protected in a pouch sewn to the lamb's flank. The thoracotomy incision was closed in layers. The lamb was weaned from mechanical ventilation, extubated, and, after recovery from anesthesia, returned to its mother. An intramuscular injection of 1 ml of penicillin G procaine and dihydrostreptomycin sulfate suspension was given daily. Three days were allowed for recovery.

LTC₄ and LTD₄ dose-responses. Lambs were randomly given selected doses (0.01, 0.1, 0.25, 0.5, 1.0, 2.0 µg/kg) of LTC₄ or LTD₄ by bolus injection into the inferior vena cava. Five lambs received all six doses. Seven other lambs received a limited number of the higher doses (Tables 1 and 2). Immediately before each LT injection, while the lamb was breathing spontaneously and resting in a sling, the hemodynamic variables (heart rate, pulmonary and systemic arterial pressures, left atrial and central venous pressures, and cardiac output) and systemic arterial pH and blood gases were measured. After each LT injection the hemodynamic variables were again measured at the maximum response, and systemic arterial pH and blood gases were measured at 1–3 min after the injection. Twenty min were allowed for recovery before the administration of the next dose. Preliminary data showed that the response to LT was reproducible only after waiting 20 min between injections.

In three lambs, LT were injected into the left atrium. Injections of the vehicle caused no hemodynamic changes.

LT injection during LT receptor antagonism. To determine if the LT receptor antagonist FPL57231 blocks all the *in vivo* hemodynamic effects of LTD₄, five lambs were also pretreated with FPL57231 before LTD₄ was injected.

Control measurements were made while the lamb was breathing spontaneously and resting in a sling. LTD₄ (0.5 µg/kg) was then injected and the effects were recorded. After at least 20 min, measurements were repeated. An intravenous infusion of FPL57231, 2 mg/kg/min, a dose previously shown to reverse hypoxia-induced pulmonary vasoconstriction in newborn lambs (17), was started and continued for 10 min. After 5 min of the infusion, 0.5 µg/kg of LTD₄ was again injected and the effects were recorded. The infusion of FPL57231 was then stopped. To determine the effectiveness of FPL57231 at higher doses of LTD₄, the same protocol was also used (in four of the five lambs) for injections of 2.0 µg/kg of LTD₄ after waiting 1–2 h until all hemodynamic variables had returned to control.

LT injection during α-adrenergic antagonism. To determine if the α-adrenergic antagonist phentolamine mesylate blocks the hemodynamic effects of LT, three other lambs were pretreated with phentolamine mesylate before LT were injected.

Control measurements were made while the lamb was breathing spontaneously and resting in a sling. LTC₄ or LTD₄ (0.5 µg/kg) was then injected into the inferior vena cava and the effects were recorded. After 20 min, the other LT was injected. An intravenous injection of phentolamine mesylate, 2 mg, a dose with which we blocked the hemodynamic effects of phenylephrine hydrochloride, 2 µg/kg, was then given. LTC₄ (0.5 µg/kg) or LTD₄ (0.5 µg/kg) was again injected. After 20 min, the other LT was again injected. Prior to and after LT injections, phenylephrine hydrochloride, 2 µg/kg, was injected to confirm adequate α-adrenergic blockade.

LT injection during left atrial pacing. To determine if the LT-induced decrease in cardiac output was caused by the decrease in heart rate, four lambs had their heart rates maintained using left atrial pacing when LT were injected.

Control measurements were made while the lamb was breathing spontaneously and resting in a sling. LTC₄ or LTD₄ (0.5 µg/

Table 1. Hemodynamic effects of LTC₄ in newborn lambs [mean ± SD; (n)]

LTC ₄ dose (µg/kg)	Pulmonary arterial pressure (mm Hg)		Pulmonary vascular resistance (mm Hg/liter/ min/kg)		Systemic arterial pressure (mm Hg)		Systemic vascular resistance (mm Hg/liter/ min/kg)	
	Baseline	LTC ₄	Baseline	LTC ₄	Baseline	LTC ₄	Baseline	LTC ₄
0.01 (5)	12.5 ± 2.5	12.0 ± 2.1	48 ± 8	47 ± 10 (4)	67.0 ± 10.4	69.0 ± 11.9	250 ± 122	265 ± 139
0.10 (5)	12.0 ± 2.1	12.5 ± 1.8	44 ± 8	47 ± 11	66.0 ± 9.6	69.0 ± 10.2	259 ± 115	327 ± 155*
0.25 (5)	12.5 ± 1.8	14.0 ± 2.9	49 ± 12	58 ± 46* (4)	66.0 ± 9.7	77.0 ± 9.9*	259 ± 98	342 ± 143*
0.50 (9)	11.9 ± 2.1	13.3 ± 2.2*	62 ± 36	70 ± 36 (8)	65.8 ± 5.9	79.9 ± 9.9*	320 ± 167	531 ± 269*
1.00 (6)	12.0 ± 1.9	14.2 ± 2.0*	44 ± 12	63 ± 27*	64.2 ± 9.2	82.5 ± 12.9*	251 ± 92	508 ± 190*
2.00 (6)	12.5 ± 1.6	16.3 ± 3.1*	47 ± 17	78 ± 10* (5)	66.7 ± 6.1	85.8 ± 6.6*	259 ± 93	611 ± 278*
	Cardiac output (liter/min/kg)		Left atrial pressure (mm Hg)		Central venous pressure (mm Hg)		Heart rate (beats/min)	
0.01 (5)	0.31 ± 0.12	0.31 ± 0.12	-0.5 ± 1.3	0.0 ± 1.4 (4)	-1.2 ± 0.8	-1.2 ± 0.8	229 ± 28	226 ± 28
0.10 (5)	0.28 ± 0.09	0.24 ± 0.08*	0.2 ± 1.6	0.6 ± 1.9	-0.6 ± 1.5	-0.2 ± 1.9	208 ± 31	185 ± 43*
0.25 (5)	0.29 ± 0.08	0.25 ± 0.08*	0.3 ± 2.1	2.5 ± 3.3* (4)	-1.8 ± 0.4	-0.6 ± 1.3	224 ± 31	187 ± 33*
0.50 (9)	0.25 ± 0.06	0.19 ± 0.05*	-0.7 ± 1.8	2.4 ± 3.1* (8)	-2.1 ± 2.0	-0.7 ± 2.9*	229 ± 18	182 ± 27*
1.00 (6)	0.29 ± 0.10	0.18 ± 0.06*	0.3 ± 1.9	4.2 ± 3.2*	-1.2 ± 0.8	0.8 ± 2.0*	226 ± 18	166 ± 40*
2.00 (6)	0.29 ± 0.10	0.17 ± 0.09*	0.8 ± 2.7	6.8 ± 4.1* (5)	-0.7 ± 1.5	1.8 ± 1.9*	222 ± 31	170 ± 34*

* $p < 0.05$ vs baseline.

Table 2. Hemodynamic effects of LTD₄ in newborn lambs [mean $\pm$ SD; (n)]

LTD ₄ dose (μ g/kg)	Pulmonary arterial pressure (mm Hg)		Pulmonary vascular resistance (mm Hg/liter/ min/kg)		Systemic arterial pressure (mm Hg)		Systemic vascular resistance (mm Hg/liter/ min/kg)	
	Baseline	LTD ₄	Baseline	LTD ₄	Baseline	LTD ₄	Baseline	LTD ₄
0.01 (5)	13.5 $\pm$ 2.2	13.5 $\pm$ 2.2	55 $\pm$ 15	55 $\pm$ 15 (4)	69.0 $\pm$ 5.5	70.0 $\pm$ 5.0	232 $\pm$ 51	236 $\pm$ 53
0.10 (5)	12.5 $\pm$ 1.8	13.3 $\pm$ 2.1	45 $\pm$ 17	51 $\pm$ 17*	70.0 $\pm$ 6.1	73.0 $\pm$ 4.5	229 $\pm$ 65	259 $\pm$ 64*
0.25 (5)	12.0 $\pm$ 1.1	14.0 $\pm$ 1.4*	46 $\pm$ 15	51 $\pm$ 17* (4)	70.0 $\pm$ 9.6	80.5 $\pm$ 8.0*	241 $\pm$ 103	297 $\pm$ 98*
0.50 (12)	12.1 $\pm$ 2.0	22.5 $\pm$ 3.7*	50 $\pm$ 12	140 $\pm$ 65* (8)	69.1 $\pm$ 9.3	81.8 $\pm$ 13.5*	260 $\pm$ 62	483 $\pm$ 214*
1.00 (6)	12.9 $\pm$ 1.9	33.8 $\pm$ 10.7*	44 $\pm$ 9	176 $\pm$ 52* (5)	68.3 $\pm$ 5.2	89.6 $\pm$ 12.3*	242 $\pm$ 63	515 $\pm$ 77*
2.00 (12)	11.8 $\pm$ 2.3	38.0 $\pm$ 8.6*	52 $\pm$ 28	234 $\pm$ 78* (10)	66.1 $\pm$ 6.3	91.8 $\pm$ 10.3*	276 $\pm$ 114	747 $\pm$ 29*
	Cardiac output (liter/min/kg)		Left atrial pressure (mm Hg)		Central venous pressure (mm Hg)		Heart rate (beats/min)	
	Baseline	LTD ₄	Baseline	LTD ₄	Baseline	LTD ₄	Baseline	LTD ₄
0.01 (5)	0.31 $\pm$ 0.05	0.31 $\pm$ 0.06	-1.8 $\pm$ 1.3	-1.5 $\pm$ 1.0 (4)	-1.3 $\pm$ 1.0	1.3 $\pm$ 1.0	221 $\pm$ 33	221 $\pm$ 32
0.10 (5)	0.33 $\pm$ 0.09	0.31 $\pm$ 0.08*	-0.3 $\pm$ 1.3	0.8 $\pm$ 2.2 (4)	-1.8 $\pm$ 0.5	-0.8 $\pm$ 1.0	231 $\pm$ 21	203 $\pm$ 25*
0.25 (5)	0.33 $\pm$ 0.10	0.29 $\pm$ 0.09*	-0.8 $\pm$ 1.0	1.8 $\pm$ 1.7* (4)	-2.3 $\pm$ 1.3	-1.0 $\pm$ 1.1	224 $\pm$ 11	191 $\pm$ 21*
0.50 (12)	0.28 $\pm$ 0.07	0.20 $\pm$ 0.05*	0.9 $\pm$ 2.2	1.1 $\pm$ 3.4* (8)	-2.2 $\pm$ 2.0	1.1 $\pm$ 3.4*	213 $\pm$ 24	177 $\pm$ 34*
1.00 (6)	0.31 $\pm$ 0.09*	0.17 $\pm$ 0.05*	-0.2 $\pm$ 2.4	5.4 $\pm$ 3.2* (5)	-1.0 $\pm$ 1.4	1.4 $\pm$ 2.1*	227 $\pm$ 15	161 $\pm$ 35*
2.00 (12)	0.28 $\pm$ 0.09	0.14 $\pm$ 0.04*	-1.1 $\pm$ 1.9	10.4 $\pm$ 6.9* (10)	-2.3 $\pm$ 2.4	4.4 $\pm$ 5.0*	221 $\pm$ 24	141 $\pm$ 32*

* $p < 0.05$ vs baseline. Fig. 1. Injection of LTD₄, but not LTC₄, causes a dose-dependent increase in pulmonary arterial pressure in the newborn lamb.

kg) was then injected into the inferior vena cava and the hemodynamic effects recorded. After 20 min, the other LT was injected. Left atrial pacing was then begun using an SD5Z electrical stimulator (Grass Medical Instruments, Quincy, MA), at a heart rate similar to control heart rate. Hemodynamic measurements were again recorded. LTC₄ (0.5 μ g/kg) or LTD₄ (0.5 μ g/kg) was again injected. After 20 min the other LT was again injected.

Drug preparation. In all experiments, LTC₄ and LTD₄ (Merck Frosst Canada, Inc., Dorval, Quebec, Canada), stored under nitrogen, were removed from a -70° C freezer, thawed, and diluted in normal saline immediately before use. FPL57231 was prepared immediately before each experiment as a 1% solution in sterile water.

Measurements and analyses. Pulmonary and systemic arterial,

central venous, and left atrial pressures were measured with Statham Db23 pressure transducers. Mean pressures were calculated by electrical integration. Pulmonary blood flow (cardiac output) was measured on a Statham SP2202 flowmeter. Heart rate was triggered from the systemic arterial pressure pulse wave. All the hemodynamic variables were recorded continuously on a Beckman multichannel direct writing recorder. Systemic arterial pH and blood gases were measured on a Corning 158 pH/blood gas analyzer. Arterial blood hemoglobin concentration and hemoglobin oxygen saturation were measured on a Radiometer OSM2 hemoximeter.

Pulmonary vascular resistance was calculated from mean pulmonary arterial pressure minus mean left atrial pressure divided by cardiac output normalized for body weight (kg). Pulmonary vascular resistance was calculated at the greatest increase in mean pulmonary arterial pressure and only in lambs in which reliable left atrial pressures could be recorded. Systemic vascular resistance was calculated from mean systemic arterial pressure minus mean central venous pressure divided by cardiac output normalized for body weight (kg).

The means and SDs were calculated for hemodynamic variables (including pulmonary and systemic vascular resistances) and systemic arterial pH and blood gases before and after each injection of LTC₄ and LTD₄. The difference for each variable before and after injection was compared by paired *t* test (29). The difference between the effects of LTC₄ and LTD₄ at each dose was compared by unpaired *t* tests and linear regression analysis (29). The effects of FPL57231 infusion and left atrial pacing on responses to LTC₄ and LTD₄ were compared using two-way analysis of variance and the Newman-Kuels test for multiple comparisons. A *p* < 0.05 was considered statistically significant.

RESULTS

Effects of LTD₄ injection. LTD₄ caused a dose-dependent increase in pulmonary arterial pressure, pulmonary vascular resistance, systemic arterial pressure, and systemic vascular resistance. These changes were accompanied by decreases in cardiac output and heart rate (Figs. 1 and 2; Table 1). When plotted on a semilog scale, there was a dose-dependent linear increase in the percent change of mean pulmonary arterial pressure [$y = 70.2(x) + 119.8$, $r = 0.70$, $p < 0.05$], mean systemic arterial pressure [$y = 17.6(x) + 30.2$, $r = 0.73$, $p < 0.05$], and a linear decrease in cardiac output [$y = 23.0(x) - 37.6$, $r = 0.82$, $p < 0.05$]. The maximal changes occurred approximately 30 s after LTD₄ injection. There were also significant increases in left atrial

Fig. 2. Injection of LTD₄ and LTC₄ cause similar dose-dependent increases in systemic arterial pressure and decreases in cardiac output in the newborn lamb.

Fig. 3. FPL57231, a LT end-organ antagonist, completely blocks the increases in pulmonary arterial (upper) and systemic arterial (middle) pressures and the decrease in cardiac output (bottom) caused by injection of LTD₄ in the newborn lamb.

pressure and central venous pressure (Table 1). The maximal change in left atrial and central venous pressures occurred later, approximately 45–60 s after injection. All hemodynamic variables returned to baseline within 10 min, taking longer at the higher doses. These hemodynamic changes caused no significant change in systemic arterial pH or blood gases. For example, prior to injecting 2.0 $\mu\text{g/kg}$ of LTD₄ (highest dose injected), systemic arterial pH was 7.39 ± 0.06 , PaO₂ was 73.7 ± 10.9 mm Hg, and PaCO₂ was 44.1 ± 3.6 mm Hg. After the injection, systemic arterial pH was 7.38 ± 0.05 , PaO₂ was 75.7 ± 10.6 mm Hg, and PaCO₂ was 44.7 ± 4.6 mm Hg. Left atrial injections produced similar changes in systemic arterial pressure, cardiac output, and heart rate to inferior vena cava injections. However, they produced only minimal changes in pulmonary arterial pressure, likely due to dilution in the systemic circulation.

Effects of LTC₄ injection. Although LTC₄ caused similar dose-dependent increases in systemic arterial [$y = 13.9(x) + 25.9$, $r = 0.80$, $p < 0.05$] left atrial and central venous pressures and dose-dependent decreases in cardiac output [$y = 18.0(x) - 33.4$, $r = 0.73$, $p < 0.05$] and heart rate as LTD₄ (Fig. 2; Table 2), LTC₄ had only a small effect on pulmonary arterial pressure [$y = 10.1(x) + 19.2$, $r = 0.34$] (Fig. 1; Table 2). Although there were significant differences ($p < 0.05$) between the effects of LTD₄ and LTC₄ on pulmonary arterial pressure, there were no significant differences between the slopes or y-intercepts of the linear regression lines for the effects of LTD₄ and LTC₄ on systemic arterial pressure and cardiac output. The time courses of these changes and their return to baseline were also remarkably similar to those caused by LTD₄. These hemodynamic changes caused no significant changes in systemic arterial pH or blood gases. For example, prior to injecting 2.0 $\mu\text{g/kg}$ of LTC₄ (highest dose injected) systemic arterial pH was 7.40 ± 0.06 , PaO₂ was 76.2 ± 11.5 mm Hg, and PaCO₂ was 45.0 ± 3.0 mm Hg. After the injection systemic arterial pH was 7.38 ± 0.05 , PaO₂ was 75.6 ± 13.1 mm Hg, and PaCO₂ was 42.8 ± 3.6 mm Hg. Left atrial injections of LTC₄ produced hemodynamic changes similar to inferior vena cava injections.

LT injection during LT receptor antagonism. FPL57231 caused no change in the baseline hemodynamic variables, systemic arterial pH, or blood gases. It did, however, completely block all of the hemodynamic changes caused by LTD₄ at 0.5 $\mu\text{g/kg}$ (data not shown) and at 2.0 $\mu\text{g/kg}$ (Fig. 3).

LT injection during α -adrenergic antagonism. Phentolamine mesylate did not change baseline pulmonary arterial pressure, systemic arterial pressure, or cardiac output (Table 3). It did increase heart rate. The responses to LTC₄ and LTD₄ injections were not altered by treatment with phentolamine mesylate. The increase in pulmonary and systemic arterial pressures and decrease in cardiac output were similar before and after treatment with phentolamine mesylate. Prior to phentolamine mesylate, LTD₄ caused a 25% decrease and LTC₄ a 27% decrease in heart

rate. Injection of LTD₄ and LTC₄ after phentolamine mesylate still caused a 26 and 25% decrease in heart rate, respectively.

LT injection during left atrial pacing. Left atrial pacing did not change baseline pulmonary and systemic arterial pressures, central venous and left atrial pressures, or cardiac output (Table 4). The responses to LTC₄ and LTD₄ injections were not altered by maintaining the heart rate by left atrial pacing (Table 4). Therefore, the increase in pulmonary and systemic arterial pressures and decrease in cardiac output were similar whether the heart rate decreased or was maintained by left atrial pacing.

DISCUSSION

The results of our study indicate that in newborn lambs, although LTD₄ and LTC₄ have very similar effects on the systemic circulation (increasing systemic arterial pressure and decreasing cardiac output), LTD₄ produces more pulmonary vasoconstriction. This study further suggests that neither the systemic nor the pulmonary hemodynamic effects of LT are mediated through α -adrenergic stimulation. The hemodynamic effects of LT are, however, completely blocked by the LT receptor antagonist FPL57231. The marked decrease in cardiac output produced by LT is not caused by the decrease in heart rate, as maintaining the heart rate by left atrial pacing does not alter the decrease in cardiac output.

Although numerous studies have investigated the *in vitro* effects of LT, few studies have investigated their *in vivo* hemodynamic effects (12, 18, 22, 23). In one study (23) comparing LTC₄ to LTD₄ in anesthetized, mechanically ventilated newborn piglets, a relationship similar to that seen in our study on the pulmonary circulation was found. However, LTD₄ had greater effects than LTC₄ on the systemic circulation as well. These findings suggest that conversion of LTC₄ to LTD₄ provides an overall increase in the degree of vasoconstriction in both the pulmonary and systemic circulations. This difference from our study may be very important. Our study indicates that conversion of LTC₄ to LTD₄ would maintain a similar degree of systemic vasoconstriction and adds potent pulmonary vasoconstriction. The reason for these differences between studies is unclear.

It is unlikely that the effects of LTC₄ seen in our study were caused by conversion of LTC₄ to LTD₄ in the lung. If conversion of LTC₄ to LTD₄ in the lung was required to cause the observed changes in the systemic circulation, left atrial injections of LTC₄ would cause hemodynamic effects only after circulating through the lung and would thus have a slight delay in the onset of hemodynamic changes. We injected LTD₄ and LTC₄ into the left atrium in three lambs. The systemic hemodynamic response was identical to that seen with inferior vena cava injections. Conversion of LTC₄ to LTD₄ by the lung is, therefore, not required to cause hemodynamic effects.

The circulatory effects of LTD₄ alone have been studied in

Table 3. Hemodynamic effects of LTC₄ and LTD₄ before and during α -adrenergic antagonism [mean $\pm$ SD; ($n = 3$)]

	Baseline	LT	Phentolamine	LT after phentolamine
LTC₄				
(0.5 $\mu\text{g/kg}$)				
Heart rate (beats/min)	204 $\pm$ 22	148 $\pm$ 0	244 $\pm$ 39	182 $\pm$ 33
Pulmonary arterial pressure (mm Hg)	19.2 $\pm$ 3.8	20.8 $\pm$ 3.3	18.3 $\pm$ 3.8	20.8 $\pm$ 1.4
Systemic arterial pressure (mm Hg)	83.3 $\pm$ 5.8	95.8 $\pm$ 10.1	81.7 $\pm$ 10.4	96.7 $\pm$ 15.3
Cardiac output (liter/min/kg)	0.18 $\pm$ 0.02	0.13 $\pm$ 0.01	0.19 $\pm$ 0.03	0.13 $\pm$ 0.02
LTD₄				
(0.5 $\mu\text{g/kg}$)				
Heart rate (beats/min)	205 $\pm$ 29	154 $\pm$ 32	246 $\pm$ 32	182 $\pm$ 27
Pulmonary arterial pressure (mm Hg)	17.5 $\pm$ 2.5	45.0 $\pm$ 6.6	19.2 $\pm$ 3.8	43.3 $\pm$ 8.0
Systemic arterial pressure (mm Hg)	76.7 $\pm$ 10.4	100.0 $\pm$ 27.8	81.7 $\pm$ 10.4	100.8 $\pm$ 26.0
Cardiac output (liter/min/kg)	0.18 $\pm$ 0.02	0.10 $\pm$ 0.02	0.18 $\pm$ 0.02	0.11 $\pm$ 0.01

Table 4. Hemodynamic effects of LTC₄ and LTD₄ before and during left atrial pacing [mean $\pm$ SD; (n = 4)]

	Baseline	LT	Pacing	LT during pacing
LTC₄				
(0.5 μ g/kg)				
Heart rate (beats/min)	215 $\pm$ 27	165 $\pm$ 35*	227 $\pm$ 16	227 $\pm$ 16
Pulmonary arterial pressure (mm Hg)	18.1 $\pm$ 3.8	20.0 $\pm$ 3.5	18.1 $\pm$ 3.1	20.5 $\pm$ 1.1
Systemic arterial pressure	83.8 $\pm$ 4.8	96.3 $\pm$ 8.3*	79.4 $\pm$ 10.5	96.3 $\pm$ 14.9*
Cardiac output (liter/min/kg)	0.19 $\pm$ 0.02	0.13 $\pm$ 0.01*	0.17 $\pm$ 0.02	0.12 $\pm$ 0.02*
LTD₄				
(0.5 μ g/kg)				
Heart rate (beats/min)	212 $\pm$ 28	147 $\pm$ 32*	220 $\pm$ 20	220 $\pm$ 20
Pulmonary arterial pressure (mm Hg)	16.9 $\pm$ 2.4	45.6 $\pm$ 5.5*	18.1 $\pm$ 3.8	47.5 $\pm$ 8.9*
Systemic arterial pressure	78.8 $\pm$ 9.5	103.5 $\pm$ 24.0*	80.0 $\pm$ 8.2	108.8 $\pm$ 26.7*
Cardiac output (liter/min/kg)	0.17 $\pm$ 0.02	0.10 $\pm$ 0.02*	0.17 $\pm$ 0.02	0.10 $\pm$ 0.02*

* $p < 0.05$ vs baseline.

young lambs (18) and adult sheep (12). In these studies, LTD₄ caused similar dose-dependent increases in pulmonary and systemic arterial pressures and decrease in cardiac output. Although some caution must be used when comparing the effects of LTD₄ on pulmonary and systemic vascular resistances reported herein with those reported by Yokachi *et al.* (18), because they did not measure left or right atrial pressures, remarkably similar results were found. After an injection of 1.0 μ g/kg of LTD₄ pulmonary vascular resistance increased 257% in our study and 240% in the Yokachi study. Similarly, systemic vascular resistance increased 113% in our study and 118% in the Yokachi study. Comparison to the results found in the Ahmed study (12) are more difficult as they measured cardiac output (thermodilution technique) and pulmonary artery wedge pressure at fixed times rather than at maximal response. However, both studies (12, 18) describe a biphasic response of systemic vascular resistance: an initial decrease in resistance followed by a marked increase. A biphasic response was not a consistent finding in our study; it occurred in only six of the 16 lambs. The reason for this variability is unclear.

The decrease in cardiac output produced by the injection of leukotrienes may be due to: 1) a decrease in preload, 2) a decrease in heart rate, 3) an increase in afterload, or 4) a decrease in myocardial contractility. A decrease in preload did not mediate the decrease in cardiac output as both left and right atrial pressures increased. Our results demonstrated a decrease in heart rate. However, there was no attenuation of the decrease in cardiac output when heart rate was maintained during left atrial pacing. This shows that the decreased heart rate did not cause the decrease in cardiac output produced by LT. The results of our study, using bolus injections of LT, cannot determine whether the decrease in cardiac output is caused by an increase in afterload or a decrease in contractility. In our study, afterload, as measured by calculated systemic vascular resistance, is increased by the injection of LT. A similar increase in afterload produced by the injection of methoxamine in newborn lambs (25) caused a similar decrease in cardiac output. Injection of LTD₄ into the coronary arteries of sheep (9), guinea pigs (26), and dogs (27) causes a decrease in myocardial contractility as demonstrated by a decrease in left ventricular pressure and dP/dt. This effect is in part caused by coronary artery vasoconstriction (9). In our preliminary studies, injection of LTD₄ into the inferior vena cava of newborn lambs also caused a decrease in dP/dt, suggesting a decrease in myocardial contractility. Because the effects of bolus injections of LTD₄ are so transient, further studies on myocardial performance and metabolism during continuous infusions of LTD₄ are needed.

The possible importance of LT in the control of the pulmonary circulation was first suggested in experiments using LT receptor antagonists. FPL57231 has recently been shown to attenuate hypoxia-induced pulmonary vasoconstriction in newborn lambs (17), piglets (14), and adult sheep (13). FPL57231 also inhibits

hypoxic pulmonary vasoconstriction in the perfused rat lung. The concentration used was greater than the concentration required to antagonize LTD₄ on strips of guinea pig ileum (31). In the fetal lamb, both FPL57231 and FPL55712, the parent compound of FPL57231, increase pulmonary blood flow and decrease pulmonary vascular resistance (30). The results of our study clearly demonstrate that FPL57231 also prevents the pulmonary and systemic hemodynamic changes caused by the direct injection of LT *in vivo*.

Previous studies have suggested that LT may mediate the pulmonary vasoconstriction seen in patients with persistent pulmonary hypertension syndrome (19) and with the adult respiratory distress syndrome (20, 21), and in laboratory animals during hypoxia (15). The results of our present study suggest that LTD₄ produces more pulmonary vasoconstriction than LTC₄. Our results also show that the LTD₄ effects are blocked by FPL57231. The ability of this LT receptor antagonist to block the LT-induced pulmonary vasoconstriction may have therapeutic implications in those diseases in which LT may play a significant pathological role.

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REFERENCES

- Burke JF, Eyre P 1977 Effects of bovine SRS-A (SRS-A^{bov}) on bovine respiratory tract and lung vasculature *in vitro*. *Eur J Pharmacol* 44:169-177
- Dahlen SE, Bjork J, Hedqvist P, Arfors KE, Hammarstrom S, Lindgren JA 1980 Leukotrienes are potent constrictors of human bronchi. *Nature* 288:484-486
- Hanna CJ, Bach MK, Pare PD, Schellenberg RR 1981 Slow-reacting substances (leukotrienes) contract human airway and pulmonary vascular smooth muscle *in vitro*. *Nature* 290:343-344
- Hedqvist P, Dahlen SE, Gustafsson L, Hammarstrom S, Samuelsson B 1980 Biological profile of leukotrienes C₄ and D₄. *Acta Physiol Scand* 110:331-333
- Rouzer CA, Scott WA, Hamill AL, Cohn ZA 1982 Synthesis of leukotriene C and other arachidonic acid metabolites by mouse pulmonary macrophages. *J Exp Med* 155:720-733
- Samuelsson B 1982 The leukotrienes: An introduction. In: Samuelsson B, Paoletti R (eds) *Leukotrienes and Other Lipoxygenase Products*. Raven Press, New York, pp 2-17
- Rouzer CA, Scott WH, Cohn ZA, Blackburn P, Manning JM 1980 Mouse peritoneal macrophages release leukotriene C in response to a phagocytotic stimulus. *Proc Natl Acad Sci USA* 77:4928-4932
- Hand JM, Will JA, Buckner CK 1981 Effects of leukotrienes on isolated guinea pig pulmonary arteries. *Eur J Pharmacol* 76:439-442
- Michelassi F, Landa L, Hill RD, Lowenstein E, Watkins WD, Petkau AJ, Zapol WM 1982 Leukotriene D₄: a potent coronary artery vasoconstriction associated with impaired ventricular contraction. *Science* 217:841-843
- Schellenberg RR, Foster M 1984 Differential activity of leukotrienes upon human pulmonary vein and artery. *Prostaglandins* 27:475-481
- Smedegard G, Hedqvist P, Dahlen S-E, Revenas B, Hammarstrom S, Samu-

- elsson B 1984 Leukotriene C₄ affects pulmonary and cardiovascular dynamics in monkey. *Nature* 295:327-328
12. Ahmed T, Marchette B, Wanner A, Yerger L 1985 Direct and indirect effects of leukotriene D₄ on the pulmonary and systemic circulations. *Am Rev Respir Dis* 131:554-558
13. Ahmed T, Oliver W Jr 1983 Does slow-reacting substance of anaphylaxis mediate hypoxic pulmonary vasoconstriction? *Am Rev Respir Dis* 127:566-571
14. Goldberg RN, Sugihara C, Ahmed T, DeCudemus BD, Barrios P, Setzer ES, Bancalari E 1985 Influence of an antagonist of slow-reacting substance of anaphylaxis on the cardiovascular manifestations of hypoxia in piglets. *Pediatr Res* 19:1201-1205
15. Morganroth ML, Stenmark KR, Zirrolli JA, Mauldin R, Mathias M, Reeves JT, Murphy RC, Voelkel NF 1984 Leukotriene C₄ production during hypoxic pulmonary vasoconstriction in isolated rat lung. *Prostaglandins* 28:867-875
16. Omini C, Folco GC, Vigano T, Rossoni G, Brunelli G, Berti F 1981 Leukotriene C₄ induces generation of PGI₂ and TXA₂ in guinea-pig *in vivo*. *Pharmacol Res Commun* 13:633-640
17. Schreiber MD, Heymann MA, Soifer SJ 1985 Leukotriene inhibition prevents and reverses hypoxic pulmonary vasoconstriction in newborn lambs. *Pediatr Res* 19:437-441
18. Yokachi K, Olley PM, Sideris E, Hamilton F, Huhtanen D, Cocci F 1982 Leukotriene D₄: a potent vasoconstrictor of the pulmonary and systemic circulations in the newborn lamb. In: Samuelsson B, Paoletti R (eds), *Leukotrienes and other Lipoxygenase Products*. Raven Press, New York, pp 211-214
19. Stenmark KR, James SL, Voelkel NF, Toews WH, Reeves JT, Murphy RC 1983 Leukotriene C₄ and D₄ in neonates with hypoxemia and pulmonary hypertension. *N Engl J Med* 309:77-80
20. Lloyd JE, Tagari P, Chapel H, Hussein A, Boullin DJ, Lee GJ 1984 Slow-reacting substances are present in bronchoalveolar lavage of patients with adult respiratory distress syndrome. *Am Rev Respir Dis* 129:A118 (abstr).
21. Matthay MA, Eschenbacher WC, Goetzl EJ 1984 Elevated concentration of leukotriene D₄ in the pulmonary edema fluid of patients with the adult respiratory distress syndrome. *J Clin Immunol* 4:479-483
22. Kadowitz PJ, Hyman AC 1984 Analysis of responses to leukotriene D₄ in the pulmonary vascular bed. *Circ Res* 55:707-717
23. Leffler CW, Mitchell JA, Green RS 1984 Cardiovascular effects of leukotrienes in neonatal piglets: role in hypoxic pulmonary vasoconstriction. *Circ Res* 55:780-787
24. Morganroth ML, Reeves JT, Murphy RC, Voelkel NF 1984 Leukotriene synthesis and receptor blockers block hypoxic pulmonary vasoconstriction. *J Appl Physiol* 56:1340-1346
25. Manders WT, Pagani M, Vatner SF 1979 Depressed responsiveness to vasoconstrictor and dilator agents and baroreceptor sensitivity in conscious newborn lambs. *Circulation* 60:945-955
26. Letts LG, Newman DL, Greenwald SE, Piper PJ 1983 Effects of intra-coronary administration of leukotriene D₄ in the anesthetized dog. *Prostaglandins* 26:563-572
27. Letts LG, Piper PJ 1982 The actions of leukotriene C₄ and D₄ on guinea pig isolated hearts. *Br J Pharmacol* 76:169-176
28. Schreiber MD, Heymann MA, Soifer SJ 1984 Differential effects of leukotriene (LT) C₄ and D₄ on the circulation in newborn lambs. *Pediatr Res* 19:365A(abstr)
29. Zar JH 1974 *Biostatistical Analysis*. Prentice-Hall, Inc., Englewood Cliffs, NJ, pp 121-173
30. Soifer SJ, Loitz RD, Roman C, Heymann MA 1985 Leukotriene end-organ antagonists increase pulmonary blood flow in fetal lambs. *Am J Physiol* 249:H570-H576
31. Holyroyde MC, Murphy JJ 1986 Inhibition of hypoxic pulmonary vasoconstriction (HPV) in rats. *Br J Pharmacol* 88:396P(abstr)