Cardiovascular Effects of Leukotrienes in Neonatal Piglets
Role in Hypoxic Pulmonary Vasoconstriction?

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SUMMARY. We investigated the effects of exogenous leukotriene D₄, synthesis inhibitors, and a leukotriene receptor antagonist upon chloralose anesthetized, mechanically ventilated, neonatal piglets with constant left pulmonary blood flow and upon piglets with uncontrolled pulmonary blood flow. Leukotriene D₄ (100–10,000 ng, intravenously) caused dose-dependent increases in peak tracheal pressure, pulmonary vascular resistance, and systemic vascular resistance coupled with dose-dependent decreases in cardiac output and systemic arterial pressure. In a limited number of experiments, cardiovascular responses to exogenous leukotriene C₄ were qualitatively similar but quantitatively less than those to leukotriene D₄. Neither treatment with diethylcarbamazine or the lipoxygenase inhibitor nordihydroguaiaretic acid, nor with the leukotriene receptor antagonist, FPL55712, altered any baseline cardiovascular parameter measured, suggesting the absence of any influence of leukotrienes on resting hemodynamics. Hypoxia or hypoxia combined with mild hypercapnia caused pulmonary vasoconstriction. Neither treatment with diethylcarbamazine or the lipoxygenase inhibitor nordihydroguaiaretic acid, nor with the leukotriene receptor antagonist FPL55712, altered the pulmonary vasoconstrictor response to hypoxia or combined hypoxia/hypercapnia. We conclude that endogenous leukotrienes do not appear to have an influence on resting cardiovascular function, neither do they appear to be necessary for hypoxic pulmonary vasoconstriction in the neonatal piglet, although exogenous leukotrienes are capable of producing cardiovascular effects. (Circ Res 55: 780-787, 1984)

INHIBITION of prostaglandin cyclooxygenase augments pulmonary vascular responses to a number of constrictor stimuli, including hypoxia, in newborns and adults of numerous species (Kadowitz et al., 1975; Leffler and Passmore, 1979; Tyler et al., 1975; Vaage et al., 1975; Weir et al., 1976). One possible explanation for this augmentation, supported by the finding that hypoxia increases intrapulmonary prostacyclin synthesis (Voelkel et al., 1981; Green and Leffler, 1982; Hamasaki et al., 1982), is that prostanooids act as modulators of pulmonary vasconstriction, thereby protecting the pulmonary vasculature from the effects of greatly elevated pressures.

With the discovery of leukotrienes (Samuelsson et al., 1980) and the demonstration that they represent an alternate pathway of arachidonic acid metabolism (Samuelsson et al., 1980; Samuelsson, 1983), another possible explanation for the potentiation of pulmonary vasoconstriction by pretreatment with nonsteroidal anti-inflammatory drugs became evident. Since leukotriene C₄ (LTC₄) and leukotriene D₄ (LTD₄) can constrict pulmonary vessels and increase pulmonary vascular resistance (Hand et al., 1981; Smedegard et al., 1982; Voelkel et al., 1982; Yokochi et al., 1982), and since synthesis of leukotrienes might be augmented by inhibiting cyclooxygenase thereby shunting arachidonic acid to the lipoxygenase pathway [although this concept has been disputed (Kuehl et al., 1984)], increased leukotriene synthesis could, at least in part, account for the augmentation of pulmonary vasoconstriction by inhibition of prostaglandin cyclooxygenase. In support of this concept are recent reports that inhibitors of leukotriene synthesis or receptor antagonists attenuate hypoxic pulmonary vasoconstriction in isolated, perfused lungs from adult rats and ferrets (Gottlieb et al., 1984, Morganroth et al., 1984a, 1984b) and in conscious adult sheep (Ahmed and Oliver, 1983).

On the other hand, seemingly arguing against an important role of leukotrienes in control of pulmonary vascular smooth muscle tone is the observation that, in several species, nonsteroidal anti-inflammatory drugs abolish pulmonary vasoconstrictor effects of exogenous arachidonic acid (Tyler et al., 1977; Leffler and Passmore, 1979; Cassin et al., 1982). One would expect that if lipoxygenases could produce sufficient leukotrienes from endogenously released arachidonic acid to mediate hypoxic pulmonary vasoconstriction, exogenous arachidonic acid would be converted to vasoconstrictor leuko-
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trienes and, thereby, cause an increase in pulmonary vascular resistance following cyclooxygenase blockade. The lack of an arachidonic acid effect following cyclooxygenase inhibition does not necessarily rule out a role for leukotrienes in hypoxic pulmonary vasoconstriction. Leukotrienes could be produced more slowly but stored in mast cells for release in larger quantities (MacGlashan et al., 1982), a specific stimulus might be needed for activation of the lipoxigenase pathway, or the cytosolic nature of 5-lipoxygenase might not permit ready conversion of exogenous arachidonic acid to leukotrienes (Kuehl et al., 1984).

The present investigation was undertaken to determine whether exogenous leukotrienes have cardiovascular effects upon neonatal piglets that include pulmonary vasoconstriction and, if so, to determine whether inhibitors of synthesis and leukotriene antagonist affect pulmonary vasoconstriction induced by hypoxia or hypoxia combined with hypercapnia.

Methods

Animal Preparation

Neonatal piglets 1–3 days postnatal, (2.2 ± 0.1 kg) were anesthetized with ketamine (7 mg/kg) and xylazine (0.2 mg/kg). They were maintained on chloralose (70 mg/kg initially, plus 15 mg/kg per hr). Catheters were placed in the descending aorta through a femoral artery, in the abdominal vena cava through a femoral vein, and in the thoracic vena cava through the other femoral vein. The trachea was cannulated percutaneously and ventilation begun, using approximately 30% O2 in N2, by positive pressure. Ventilation was adjusted to produce a Pco2 of 35–40 mm Hg and not changed for the remainder of the experiment. The heart and pulmonary artery were exposed via a left thoracotomy between the 4th and 5th ribs. A catheter was placed in the left atrium through the left atrial appendage. Arterial blood gases and pH were determined frequently, and intravenous sodium bicarbonate solution was administered as necessary to maintain a base deficit of approximately 0. The arterial gases and pH at the completion of all surgery but prior to experimental protocols were: pH = 7.35 ± 0.02, Pco2 = 37 ± 1 mm Hg, Pao2 = 145 ± 13 mm Hg. The arterial blood gases and pH at the completion of the experiments were: pH = 7.27 ± 0.04, Pco2 = 45 ± 3 mm Hg, Pao2 = 142 ± 17 mm Hg.

Two animal preparations were used to monitor changes in cardiovascular function. In the first, the animal was heparinized (2000 U/kg), and constant left pulmonary blood flow was maintained by cannulating the left pulmonary artery and perfusing the left lung with blood withdrawn from the thoracic vena cava using a roller pump. Pulmonary arterial perfusion pressure was monitored continually from a side arm in the pulmonary arterial perfusion circuit. In the second animal preparation, an electromagnetic flow cuff was placed around the main pulmonary artery for monitoring cardiac output, and pulmonary arterial pressure was measured from a catheter placed directly into the main pulmonary artery and secured with a pursestring suture. In this preparation, the chest was closed by tying the ribs back together. Cardiac output, aortic pressure, pulmonary arterial pressure, and left atrial pressure were monitored continuously. Central venous pressure, which never changed more than 1 mm Hg throughout the day, was checked intermittently. Heart rate was counted from the aortic pressure tracing.

Protocols

Leukotriene Dose-Response Determinations

In experiments using the constant left pulmonary blood flow preparation, LTD4 was injected directly into the pulmonary arterial perfusion circuit at doses of 100, 500, 1000, and 3000 ng. Pulmonary arterial pressure and tracheal pressure were allowed to return to baseline prior to administration of the next higher amount of LTD4. In piglets whose pulmonary arterial blood flow was not maintained constant, LTD4 dose-response curves were obtained by injecting 100–10,000 ng of LTD4 into the abdominal vena cava. Stable cardiac output, aortic pressure, and pulmonary arterial pressure were required before administration of a higher dose.

Hypoxia (Constant Left Pulmonary Arterial Flow Preparation)

Pulmonary vascular responses to hypoxia were determined by ventilating the piglets with 3% O2 for 1 minute. One minute of 3% O2 ventilation uniformly reduced arterial Pao2 to between 25 and 30 mm Hg. Ventilation with 3% O2 was limited to 1-minute periods because longer exposures resulted in severe bradycardia and progressive hypotension. Two hypoxic challenges were administered prior to infusion of synthesis inhibitors and two additional challenges were administered during infusion. Between the two hypoxic challenges, both before and after infusion of the inhibitor, PGF2α was infused directly into the pulmonary arterial perfusion circuit at 50 μg/min for 30 seconds to produce pulmonary vasoconstriction independent of hypoxia.

Hypoxia, Hypercapnia (Unrestrained Cardiac Output Preparation)

Piglets were ventilated with 12% O2, 5% CO2 for 3 minutes two times before infusion of synthesis inhibitors or the receptor antagonist, and twice during the infusions of inhibitors. Three minutes of 12% O2, 5% CO2 were sufficient to change arterial blood gases and pH from pH = 7.41 ± 0.02, Pao2 = 161 ± 15 mm Hg, and Pco2 = 38 ± 1.2 mm Hg to pH = 7.28 ± 0.03, Pao2 = 45 ± 3 mm Hg, and Pco2 = 49 ± 1.5 mm Hg. A combination of very mild hypercapnia and hypoxia was selected in this experiment to enable us to administer a stimulus sufficient to produce pulmonary vasoconstriction over a more prolonged period of time without threatening the life of the piglet. In preliminary experiments, we found that in some piglets administration of 10% O2 coupled with normocapnia (Pco2 = 35–40 mm Hg) produced more modest pulmonary vasoconstriction in 1–2 minutes, and markedly decreased cardiac output prior to 3 minutes. Conversely, 12% O2, 5% CO2 uniformly produced greater pulmonary vasoconstriction which was maintained throughout the 3 minutes of treatment and was tolerated well.
Inhibitors and Agonists

LTC₄ and LTD₄ (gifts from J. Rokach, Merck Frosst Canada, Inc.) initially were dissolved in water. Aliquots were stored at -60°C until use. The aqueous solutions of leukotrienes were diluted in saline for injection of 0.5 ml.

Nordihydroguaiaretic acid (NDG) (Sigma) was dissolved in saline with 20 mEq NaHCO₃/liter at approximately 50°C with constant stirring (1-2 hours). The NDG solution was infused to produce a left pulmonary blood concentration of 5 x 10⁻⁵ mol/liter of left pulmonary arterial blood flow. Vehicle infused during the control period was saline plus 20 mEq of NaHCO₃/liter.

FPL55712 (gift from P. Sheard, Fisons Pharmaceuticals) was dissolved in water (2 mg/ml) and diluted in saline immediately prior to infusion at 100 μg/kg per min. Diethylcarbamazine (DEC) (Sigma) was dissolved in saline and buffered to pH 7.4 with NaHCO₃ for infusion at 2.5 mg/ml of left pulmonary arterial blood flow. Vehicle infusion, which continued throughout the period prior to DEC administration, was the same as used for NDG.

Calculations and Statistical Analysis

Pulmonary vascular resistance was defined as pulmonary arterial pressure minus left atrial pressure divided by flow per unit weight of animal. Systemic vascular resistance was defined as aortic pressure minus central venous pressure divided by cardiac output per unit weight of animal.

Data are presented as means ± SEM.

Least square regressions of response against dose for LTD₄ were performed. Correlation coefficients were calculated. The statistical significance of the dependence of the variable y upon x was determined by testing the hypothesis: B = 0. Comparisons between groups were made using analysis of variance, t-tests, or sign tests, as appropriate. Significance was defined as P < 0.05. (Dixon and Massey, 1969).

Results

Direct injections of synthetic LTD₄ into the left pulmonary arterial perfusion circuit of the constant left pulmonary arterial blood flow preparation caused immediate increases in calculated pulmonary vascular resistance and tracheal pressure (Fig. 1). Pulmonary vascular resistance and tracheal pressure returned to baseline within 10 minutes after injection. Intrapulmonary arterial injections of LTD₄ were followed by dose-dependent decreases in systemic arterial pressure (Fig. 1). In the neonatal piglet with unrestrained cardiac output, intravenous injections of LTD₄ caused increases in the pulmonary vascular resistance, but no dose-response relationship could be demonstrated (Fig. 2), probably because rising left atrial pressure when cardiac output was depressed at higher doses prevented a dose-response curve from being performed over a wide range of doses. Rising left atrial pressures precluded meaningful calculation of pulmonary vascular resistance above 1 μg LTD₄ in all but a single piglet. Before the 17 LTD₄ injections shown in Figure 2, pulmonary arterial pressure was 19 ± 2 mm Hg, left atrial pressure was 3.5 ± 1 mm Hg, and cardiac output was 94 ± 11 ml/kg per min. After injection of 671 ± 169 ng LTD₄, pulmonary arterial pressure was 19 ± 2 mm Hg, left atrial pressure was 3.5 ± 1 mm Hg, and cardiac output was 72 ± 9 ml/kg per min. Pulmonary arterial pressure frequently increased, but the large fall in cardiac output in a few piglets, resulting in a fall in pulmonary arterial pressure, causes the increases to be obscured when means are presented and rounded to the nearest whole number. Pulmonary vascular resistance increased from 0.23 ± 0.05 to 0.30 ± 0.07 mm Hg·kg·min/ml (P < 0.05). At the 1-μg dose of LTD₄, pulmonary arterial pressure began to increase 21 ± 9 seconds after injection, reaching a peak at 78 ± 15 seconds after LTD₄ injection. Pulmonary arterial pressure returned to baseline 150 ± 30 seconds after injection. A dose-dependent fall in cardiac output (Fig. 2) began very soon after LTD₄ injection [14 ± 3 seconds (1-μg dose)], reaching a minimum 54 ± 10 seconds after injection.
FIGURE 2. Effects of iv injections (2-5 doses per piglet) of LTD₄ upon the calculated pulmonary vascular resistances (PVR), cardiac output, heart rate, and calculated systemic vascular resistances (SVR) of neonatal piglets (n = 8) with unrestrained cardiac output. Baseline values were: PVR = 0.33 ± 0.05 mm Hg·kg·min/ml, cardiac output = 82 ± 17 ml/kg per min, heart rate = 158 ± 16 beats/min (arterial pressure 61 ± 5 mm Hg), and SVR = 1.03 ± 0.19 mm Hg·kg·min/ml. (Regressions were determined by method of least squares. PVR: r = 0.31; cardiac output: r = 0.74; heart rate: r = 0.51; SVR: r = 0.76. Slope of PVR regression not different from zero at P < 0.05. Slope of other regressions are all different from zero at P < 0.05.)

Injection, and requiring 246 ± 72 seconds to return to a stable baseline. Aortic pressure initially increased 5–10 mm Hg 20 ± 5 seconds after intravenous injection of 1 μg LTD₄, then began declining, reaching a minimum 66 ± 12 seconds after injection. Aortic pressure reached preinjection baseline or highest plateau 210 ± 54 seconds after LTD₄ injection. Although both cardiac output and aortic pressure decreased after LTD₄ injection, cardiac output fell more, revealing dose-dependent increases in systemic vascular resistance (Fig. 2). Accompanying the decreasing cardiac output was a minimal decline in heart rate (Fig. 2).

Cardiovascular responses to LTC₄ were examined by single injections (2.4 ± 0.7 μg) into four piglets. The responses were qualitatively similar but quantitatively less than responses to LTD₄. Thus, 2.4 ± 0.7 μg LTC₄, iv, caused pulmonary vascular resistance to increase from 0.43 ± 0.08 to 0.51 ± 0.06 mm Hg·kg·min/ml (22 ± %), systemic vascular resistance to increase from 1.24 ± 0.21 to 1.76 ± 0.19 mm Hg·kg·min/ml (52 ± 19%), and cardiac output to decrease from 50 ± 5 to 44 ± 8 ml/kg per min (12 ± 12%).

To determine whether repeated leukotriene injection caused desensitization, repeated 300-ng LTD₄ injections were administered to three piglets about 20 minutes apart. There was no indication of desensitization to the leukotriene. Thus, the first injection caused pulmonary vascular resistance to increase 22 ± 9%, and the second caused pulmonary vascular resistance to increase 18 ± 5%. Similarly, systemic vascular resistance increased 25 ± 7% and 37 ± 5% following the first and second injections, respectively.

The cardiovascular responses to exogenous LTD₄ were blocked by continuous infusion of 100 μg/kg per min of the leukotriene receptor blocker FPL55712. In Table 1, note that prior to treatment with FPL55712, 1.1 ± 0.3 μg LTD₄, iv, caused significant increases in pulmonary and systemic vascular resistance and significant decreases in cardiac output. Following treatment with FPL55712, there were no significant changes in pulmonary vascular resistance, systemic vascular resistance, or cardiac output. Similarly, in three piglets, pulmonary and systemic responses to intravenous LTC₄ (2 ± 1 μg) were inhibited by FPL55712 (100 μg/kg per min): the increase in pulmonary vascular resistance was attenuated 88 ± 6%, and the increase in systemic vascular resistance was attenuated 78 ± 15%. To determine whether FPL55712 was having nonspecific depressor effects upon cardiovascular responses of the piglets, we injected 20 mU of lysine vasopressin (n = 2) into piglets before and during infusions of FPL55712. The constrictor effects of lysine vasopressin were not blocked by FPL55712. Thus, prior to infusion of FPL55712, 20 mU of lysine vasopressin increased pulmonary vascular resistance 42% and systemic vascular resistance 80% (means of two). During infusion of FPL55712, lysine vasopressin increased pulmonary and systemic vascular resistances 38% and 97%, respectively.
TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>LTD₄*</th>
<th>% Change†</th>
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<tbody>
<tr>
<td>PVR</td>
<td>Control</td>
<td>0.35 ± 0.09</td>
<td>0.44 ± 0.11</td>
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<tr>
<td></td>
<td>FPL55712</td>
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<td>0.34 ± 0.09</td>
</tr>
<tr>
<td>SVR**</td>
<td>Control</td>
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<td>1.43 ± 0.40</td>
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<tr>
<td></td>
<td>FPL55712</td>
<td>0.83 ± 0.22</td>
<td>0.90 ± 0.25</td>
</tr>
<tr>
<td>Cardiac output‡</td>
<td>Control</td>
<td>83 ± 16</td>
<td>60 ± 12§</td>
</tr>
<tr>
<td></td>
<td>FPL55712</td>
<td>92 ± 21</td>
<td>92 ± 23§</td>
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Values shown are mean ± SEM; n = six piglets. PVR = pulmonary vascular resistance (mm Hg·kg·min/ml).
* 1.1 ± 0.3 µg LTD₄ injected iv. Data presented are maximal deviations from baseline.
† (LTD₄ - Baseline) × Baseline⁻¹ × 100%.
‡ mL/kg per min.
§ P < 0.05 compared to baseline.
|| P < 0.05 compared to control.
†† Data collected during continuous iv infusion of FPL55712 (100 µg/kg per min).
** Systemic vascular resistance (mm Hg·kg·min/ml).

Hypoxia, 3% O₂ for 1 minute, or hypoxia coupled with mild hypercapnia, 12% O₂ with 5% CO₂ for 3 minutes, produced consistent, reproducible increases in pulmonary vascular resistance. There was no evidence that the second, third, or fourth hypoxic challenge in the same animal produced a different pulmonary vascular response from the first. Thus, before treatment with leukotriene inhibitors, 3% O₂ administration for 1 minute increased pulmonary vascular resistance 35 ± 7% the first time it was administered and 25 ± 6% the second time it was administered. Subsequent challenges produced increases in pulmonary vascular resistance of 47 ± 9% for the third challenge, and 39 ± 10% for the fourth.

During infusion of FPL55712, when cardiovascular response to exogenous LTC₄ and LTD₄ were inhibited, pulmonary vasoconstriction caused by hypoxia/hypercapnia was not attenuated compared to the vehicle control period before FPL55712 infusion. Figure 3 shows the responses of six piglets to alveolar hypoxia/hypercapnia before and during iv infusions of FPL55712 that inhibited responses to exogenous LTC₄ and LTD₄. FPL55712 infusion did not alter pulmonary arterial pressure, left atrial pressure, cardiac output, or calculated pulmonary vascular resistance during ventilation with 30% O₂ in N₂. Ventilation with 12% O₂, 5% CO₂, 83% N₂ resulted in significant increases in pulmonary arterial pressure and significant decreases in cardiac output in both groups. The increases in calculated pulmonary vascular resistance caused by ventilation with 12% O₂/5% CO₂ before and during FPL55712 infusion were not significantly different: 126 ± 37% increase before infusion and 172 ± 72% increase during infusions of FPL55712.

Similarly, using the constant left pulmonary blood flow preparation in order that the inhibitor concentration in the pulmonary blood flow could be maintained accurately at the specified level, neither NDG (5 × 10⁻⁵ mol/liter of pulmonary blood flow) nor DEC (2.5 mg/ml of left pulmonary arterial blood flow) had any effect upon hypoxic pulmonary vasoconstriction (Figs. 4 and 5). Thus, both before and during infusions of NDG, 3% O₂ administration for 1 minute produced consistent increases in pulmonary arterial pressure with little change in left atrial pressure at constant flow, thereby resulting in increased calculated pulmonary vascular resistance (Fig. 4). Results from experiments employing DEC were virtually identical to those using NDG, as can be seen from the data in Figure 5. Figure 5 clearly shows that treatment with either NDG or DEC did not consistently alter pulmonary vascular responses to 3% O₂ administration or exogenous PGF₂α.

Discussion

Our results in neonatal piglets indicate that intravenous LTD₄ constrists the pulmonary vasculature,
increases systemic vascular resistance, and decreases cardiac output. Pulmonary vascular effects could be related, in part, to changes in bronchomotor tone, since LTD\textsubscript{4} increased peak tracheal pressure. Responses to LTC\textsubscript{4} are qualitatively similar but quantitatively less than responses to LTD\textsubscript{4}. In general, these findings in newborns are consistent with reports of others who used newborn and adult animals. LTD\textsubscript{4} constricts adult airway smooth muscle (Krell et al., 1981; Piper and Samhoun, 1981; Smedegard et al., 1982; Jones et al., 1982; Dahlen et al., 1983) and adult and newborn pulmonary vasculature (Hand et al., 1981; Smedegard et al., 1982; Voelkel et al., 1982; Yokochi et al., 1982). When compared to prostanoids, pulmonary vascular responses to leukotrienes are quite modest. For example, comparing responses to LTD\textsubscript{4} in Figure 1 with responses to PGF\textsubscript{2\alpha}, in Figure 5, it can be seen that, on an equal molar basis, the pulmonary vasculature is roughly 20 times more sensitive to PGF\textsubscript{2\alpha} than to LTD\textsubscript{4}. Also, similar effects of LTD\textsubscript{4} upon cardiac output and arterial pressure of adults to those we have observed in newborns have been reported (Feuerstein et al., 1981; Burke et al., 1982; Smedegard et al., 1982). The decrease in cardiac output appears to be the result of a decrease in cardiac contractility, since heart rate changes very little, and venous pressure was not altered. Many of the effects of leukotrienes on both the respiratory and cardiovascular systems appear to be related to their ability to stimulate prostaglandin synthesis (Mathe et al., 1977; Folco et al., 1981; Omini et al., 1981; Piper and Samhoun, 1981; Siros et al., 1981).

In both adults and neonates of numerous species, inhibition of fatty acid cyclooxygenase augments hypoxic pulmonary vasoconstriction and pulmonary vasoconstriction caused by other stimuli (Kadowitz et al., 1975; Tyler et al., 1975; Vaage et al., 1975; Weir et al., 1976; Leffler and Passmore, 1979). This augmentation appears to be, at least in part, the result of inhibition of synthesis of prostacyclin, which can be stimulated by some pulmonary vasoconstrictor stimuli (Cryglewski et al., 1980; Voelkel et al., 1981; Green and Leffler, 1982).
of synthesis of a mediator leukotriene by inhibiting cyclooxygenase, thereby shunting arachidonic acid toward the lipoxygenase pathway, would be another possible explanation for augmentation of hypoxic pulmonary vasoconstriction by nonsteroidal anti-inflammatory drugs. However, the concept of shunting of arachidonic acid from the cyclooxygenase pathway to the 5-lipoxygenase pathway is controversial (Kuehl et al., 1984). In piglets, hypoxia increases pulmonary vascular resistance. Treatment with two inhibitors of leukotriene synthesis did not affect the pulmonary vascular response to hypoxia. Further, treatment with a leukotriene receptor antagonist had no effect upon pulmonary vasoconstriction caused by hypoxia combined with hypercapnia.

We did not measure leukotriene production and, therefore, we cannot be sure that NDG and DEC inhibited leukotriene production in our preparations. The concentrations of NDG and DEC we used were greater than those which have been shown to inhibit by 90% or greater the production of slow reacting substance of anaphylaxis by lung or lung tissue (Armour et al., 1981; Engineer et al., 1978). In addition, the concentration of DEC we used was similar (2.5 vs. 3 mg/ml) to that used by Morganroth et al. (1984b) in blood-perfused rat lungs which prevented the increase in lavage LTC4 during hypoxia. Further, due to recirculation, the true concentrations of NDG and DEC in the present experiments were much higher than the reported values. The efficacy of receptor blockade with FPL55712 could be tested readily by injecting synthetic leukotrienes. The amount of FPL55712 we used was sufficient to inhibit by 88 and 97%, respectively, the effects of doses of leukotriene C4 and D4 that increased pulmonary vascular resistance 22 and 35%, respectively. Nevertheless, in the same piglets, FPL55712 did not affect pulmonary vasoconstriction caused by combined hypoxia/hypercapnia. Thus, receptor activation by leukotrienes does not appear to be involved in the vasoconstriction; but, as we did not demonstrate inhibition of 5-lipoxygenase, an intracellular action of a lipoxygenase product remains a possible contributor to hypoxic pulmonary vasoconstriction.

These results clearly are in contrast to those of Morganroth et al. (1984a) and Gottlieb et al., (1984) in isolated, blood- or Ficoll-perfused adult rat and ferret lungs, and Ahmed and Oliver (1983) in adult sheep. Morganroth and colleagues found that diethylcarbamazine (2 mg/ml) and FPL55712 (1 µg/ml) inhibited hypoxic pulmonary vasoconstriction but did not greatly decrease the response to exogenous angiotensin II. Further, they have detected LTC4 in lavage fluid collected from rat lungs during hypoxia that was not present during normoxia (Morganroth et al., 1984b). They concluded that, in the rat lung, leukotrienes appear to be important mediators of hypoxic pulmonary vasoconstriction. Gottlieb and colleagues found that NDG (1-5 µM) reduced hypoxic pulmonary vasoconstriction in isolated, blood-perfused, adult ferret lungs without decreasing the constrictor response to KCl and only minimally decreasing the pulmonary vasoconstriction caused by exogenous PGE2. Similarly, Ahmed and Oliver found that FPL57231, a propionic acid analog of FPL55712, prevented hypoxic pulmonary vasoconstriction in conscious adult sheep. They concluded that slow-reacting substances of anaphylaxis mediate hypoxic pulmonary vasoconstriction directly or indirectly in sheep. We do not know the reasons for our differing results, but would speculate that species differences and/or age may be involved.

Stenmark et al. (1983) have detected LTC4 and LTD4 in lung lavage from neonates with persistent pulmonary hypertension of the newborn. They were unable to detect leukotrienes in lavage from neonates without this diagnosis. Their investigation did not determine the cause of the elevation of leukotrienes in lavage fluid or whether the elevated leukotrienes were the cause or result of the syndrome. Our findings that LTD4 and LTC4 can cause pulmonary vasoconstriction in newborn piglets is consistent with the speculation of Stenmark et al. that leukotrienes could contribute to the clinical features of persistent pulmonary hypertension of the newborn. Our investigation of healthy newborn pigs, however, does not address the question directly.

In conclusion, LTC4 and LTD4 have cardiovascular effects upon neonatal piglets that are blocked by the putative leukotriene receptor antagonist FPL55712. However, our results do not suggest that leukotrienes are involved in hypoxic pulmonary vasoconstriction in the neonatal piglet.

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References


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