Leukotriene Inhibition Prevents and Reverses Hypoxic Pulmonary Vasoconstriction in Newborn Lambs

MICHAEL D. SCHREIBER, MICHAEL A. HEYMANN, AND SCOTT J. SOIFER

The Cardiovascular Research Institute and the Departments of Pediatrics, Physiology, and Obstetrics, Gynecology, and Reproductive Sciences, University of California, San Francisco, California 94143

ABSTRACT. The effects of the leukotriene antagonist FPL 57231 on the circulation were studied in spontaneously breathing normoxic and hypoxic newborn lambs in order to evaluate the role of leukotrienes in the perinatal control of hypoxic pulmonary vasoconstriction. Hypoxia produced a 58% increase in pulmonary arterial pressure ($p < 0.05$) and a 37% increase in pulmonary vascular resistance ($p < 0.05$). Hypoxic pulmonary vasoconstriction was abolished by the prior infusion of FPL 57231. Pulmonary arterial pressure increased by only 8% and pulmonary vascular resistance decreased by 10% from normoxia. The infusion of FPL 57231 during hypoxia-induced pulmonary vasoconstriction reversed the increase in pulmonary arterial pressure ($p < 0.05$) and pulmonary vascular resistance ($p < 0.05$). The hypoxia-induced increase in cardiac output was maintained during the infusion of FPL 57231. Leukotrienes may play a significant role in the mediation of hypoxic pulmonary vasoconstriction. Leukotriene inhibition with FPL 57231 may be useful in the management of infants with persistent pulmonary hypertension syndrome. (*Pediatr Res* 19: 437-441, 1985)

Abbreviations

PPHN, persistent pulmonary hypertension of the newborn
PG, prostaglandin
PGI₂, prostacyclin

The exact mechanisms by which hypoxia produces pulmonary vasoconstriction remain unknown. The production and release of the noncyclooxygenase products of arachidonic acid from the lung may play an important role. Leukotrienes, the 5'-lipoxygenase metabolites of arachidonic acid, are produced by mast cells (1, 2), alveolar macrophages (3), and pulmonary vascular tissue (4-6). Leukotrienes induce smooth muscle contraction in a variety of tissues (7-10). Leukotriene D₄ infused into the pulmonary artery of young lambs produces pulmonary arterial hypertension (11). Leukotriene inhibition with FPL 57231 (Fisons, plc, Loughborough, UK), a leukotriene receptor antagonist (12), significantly increases pulmonary blood flow and decreases pulmonary vascular resistance in fetal lambs (13). Leukotrienes

have been isolated from the lung lavage fluid of newborn infants with persistent pulmonary hypertension syndrome but not from newborn infants with other lung diseases (14). These findings suggest that leukotrienes may play a role in maintaining the elevated pulmonary vascular tone in the normal fetus *in utero* and also in the mediation of hypoxic pulmonary vasoconstriction after birth. The effects of the leukotriene antagonist FPL 57231 on the circulation were therefore studied in normoxic and hypoxic spontaneously breathing newborn lambs.

METHODS

Surgical procedure and drug preparation. Six mixed-breed newborn lambs from 3-7 days old were operated on under local anesthesia with 1% lidocaine hydrochloride. Polyvinyl catheters were placed in both hind limb arteries and veins and advanced to the descending aorta and inferior vena cava, respectively. A no. 5 French flow-directed thermodilution catheter, with the proximal injection port 15 cm from the tip, was introduced into the jugular vein and advanced into the pulmonary artery for measuring pulmonary arterial and pulmonary arterial wedge pressures. Right atrial pressure was measured through the proximal port. Cardiac output was determined by the thermodilution technique and calculated by computer (Electro-Catheter model 5000, Electro-Catheter Corp., Rahway, NJ). Three milliliters of iced-saline was injected into the proximal port for each measurement. Each cardiac output value was taken as the average of three determinations. Following surgery, the lambs were placed in a sling under a radiant warmer to maintain body temperature. An intravenous infusion of dextrose in water was started. Two hours were allowed for recovery.

FPL 57231 was prepared immediately before each experiment as a 1% solution in sterile water.

Experimental Protocol. FPL 57231 infusion prior to the onset of hypoxia. Following the recovery period, baseline vascular pressures, cardiac output, and arterial blood gas measurements were made in room air (NORMOXIA). Normocarbic hypoxia was then induced by placing a loosely fitting bag over the lamb's head and adding nitrogen with 5% CO₂ to the gas mixture. The FIO₂ was varied to produce a Pao₂ of 30-40 mm Hg in each animal. Measurements were made after 10 min of hypoxia and stable pulmonary hypertension (HYPOXIA). The lamb was returned to room air.

After the lambs resumed baseline values (NORMOXIA), an intravenous infusion of FPL 57231 (1 mg·kg⁻¹·min⁻¹) for 20 min was started. Measurements were made after 10 min of the infusion (NORMOXIA + FPL 57231). Hypoxia was then induced as the drug infusion continued, and measurements were made after another 10 min (HYPOXIA + FPL 57231). The infusion was stopped as hypoxia continued, and measurements

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Requests for reprints should be addressed to Dr. Scott J. Soifer, Cardiovascular Research Institute M-342A, University of California, San Francisco, CA 94143.

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were made after 15 min. The lambs were returned to room air. Measurements were also made with an equivalent infusion of sterile water vehicle during normoxia and hypoxia.

FPL 57231 infusion started during hypoxia. Six to 24 hr were allowed for recovery from the above experiment. In five lambs baseline measurements were made in room air (NORMOXIA). Normocarbic hypoxia was again induced producing hypoxemia (P_{aO_2} 30–40 mm Hg) and stable pulmonary hypertension. After 10 min, measurements were repeated (HYPOXIA). Hypoxia was continued and an infusion of FPL 57231 ($2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) was started. Measurements were made after 10 min (HYPOXIA + FPL 57231). The drug infusion was then stopped, and the lambs were returned to room air.

Measurements and calculations. Pulmonary and systemic arterial and right atrial pressures were measured with Statham P23 Db pressure transducers and recorded continuously on a Beckman multichannel direct-writing recorder. Mean pressures were obtained by electrical integration. Heart rate was measured with a cardiometer triggered from the systemic arterial pressure. Cardiac output, pulmonary arterial wedge pressure, and systemic arterial blood gases were measured during all normoxia and hypoxia periods after a steady-state had been reached. The absence of intracardiac shunting was determined by indicator dilution technique. The validity of the thermodilution method has been reported previously (15, 16). Pulmonary vascular resistance was calculated as the mean pulmonary arterial pressure minus mean pulmonary arterial wedge pressure divided by cardiac output per kilogram. Systemic vascular resistance was calculated as the mean systemic arterial pressure minus mean right atrial pressure divided by cardiac output per kilogram.

The mean $\pm$ SD was computed for the vascular pressures, resistances, cardiac output, heart rate, and arterial blood gases during all normoxic and hypoxic conditions. The differences in these variables were compared by two-way analysis of variance and the Newman-Keuls test for multiple comparisons (17). A $p < 0.05$ was considered statistically significant.

RESULTS

FPL 57231 infusion prior to the onset of hypoxia (Table 1, Fig. 1). Normocarbic hypoxia (HYPOXIA) produced a 58% increase in pulmonary arterial pressure from 18.1 ± 2.7 to 28.6 ± 3.2 mm Hg ($p < 0.05$) and a 37% increase in pulmonary vascular resistance from 44.0 ± 6.6 to 60.4 ± 6.8 mm Hg $\cdot \text{l}^{-1} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ ($p < 0.05$) compared to normoxia. Systemic arterial pressure was unchanged. Systemic vascular resistance decreased by 16% during HYPOXIA ($p < 0.05$), as cardiac output increased by 19%.

Infusing FPL 57231 during normoxia (NORMOXIA + FPL 57231) produced no significant changes in the hemodynamic variables or arterial blood gases compared to NORMOXIA. Hypoxic pulmonary vasoconstriction was prevented by the prior

infusion of FPL 57231 (HYPOXIA + FPL 57231). Pulmonary arterial pressure increased by only 8% and pulmonary vascular resistance decreased by 10% compared to NORMOXIA. Pulmonary arterial pressure and pulmonary vascular resistance during HYPOXIA + FPL 57231 were significantly lower than during HYPOXIA ($p < 0.05$). There were no significant changes in systemic arterial pressure and systemic vascular resistance compared to HYPOXIA. The 24% increase in cardiac output during

Fig. 1. The effects of an infusion of FPL 57231 prior to the onset of hypoxia on (A) pulmonary arterial pressure and (B) pulmonary vascular resistance.

Table 1. Effect of FPL 57231 infusion prior to hypoxia (mean $\pm$ SD)

	Pulmonary arterial pressure (mm Hg)	Pulmonary vascular resistance (U)	Systemic arterial pressure (mm Hg)	Systemic vascular resistance (U)	Cardiac output ($\text{l} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$)	Heart rate (bpm)	Right atrial pressure (mm Hg)	Pulmonary arterial wedge pressure (mm Hg)
NORMOXIA	18.1 $\pm$ 2.7	47.1 $\pm$ 4.8	68.3 $\pm$ 5.8	193.0 $\pm$ 38.3	0.37 $\pm$ 0.07	218 $\pm$ 45	-0.5 $\pm$ 1.6	1.0 $\pm$ 1.7
HYPOXIA	28.6 $\pm$ 3.2*†	60.4 $\pm$ 6.8*†	68.2 $\pm$ 8.3	162.0 $\pm$ 41.0*	0.44 $\pm$ 0.09	266 $\pm$ 53	-0.7 $\pm$ 1.0	0.8 $\pm$ 1.3
NORMOXIA + FPL 57231	17.9 $\pm$ 2.2	44.0 $\pm$ 6.6	65.7 $\pm$ 9.2	172.4 $\pm$ 31.3	0.39 $\pm$ 0.06	246 $\pm$ 35	-1.0 $\pm$ 1.1	0.8 $\pm$ 1.2
HYPOXIA + FPL 57231	19.6 $\pm$ 3.6	42.4 $\pm$ 5.9	63.9 $\pm$ 9.3	141.8 $\pm$ 17.0*	0.46 $\pm$ 0.04	303 $\pm$ 37*	-1.09 $\pm$ 1.3	0.2 $\pm$ 1.2

* $p < 0.05$ vs NORMOXIA.

† $p < 0.05$ vs HYPOXIA + FPL 57231.

$n = 6$; units: mm Hg $\cdot \text{l}^{-1} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$.

HYPOXIA + FPL 57231 compared to NORMOXIA was similar to that during HYPOXIA.

During continued hypoxia, 15 min after the FPL 57231 infusion was stopped, pulmonary arterial pressure had increased significantly by 44% to 28.2 ± 4.4 mm Hg ($p < 0.05$) and pulmonary vascular resistance had increased 36% to 57.8 ± 3.8 mm Hg $\cdot$ l $^{-1}$ $\cdot$ min $^{-1}$ $\cdot$ kg $^{-1}$ ($p < 0.05$). These values were not significantly different from HYPOXIA. Right atrial and pulmonary arterial wedge pressures, heart rate (Table 1), pH, and PCO₂ (Table 2) did not change throughout the study. PaO₂ and arterial saturation were unchanged during normoxic as well as during hypoxic conditions (Table 2). Infusion of vehicle did not affect the hemodynamic variables or arterial blood gases during either normoxia or hypoxia.

FPL 57231 infusion started during hypoxia (Table 3, Fig. 2). The hemodynamic variables, arterial blood gases, and the response to hypoxia were similar to the above experiment. During hypoxia-induced pulmonary arterial hypertension, infusing FPL 57231 (HYPOXIA + FPL 57231) decreased pulmonary arterial pressure by 35% ($p < 0.05$) and pulmonary vascular resistance by 37% ($p < 0.05$) from HYPOXIA. There were small, but not statistically significant, decreases in systemic arterial pressure and systemic vascular resistance from HYPOXIA. The 13% increase in cardiac output induced by hypoxia was not lowered by the infusion of FPL 57231. Right atrial and pulmonary arterial wedge pressures, pH, and PCO₂ were unchanged. PaO₂ and arterial saturation were similar during both hypoxic conditions. Whether the infusion of FPL 57231 was started prior to or during hypoxia, pulmonary arterial pressure and pulmonary vascular resistance were similarly affected.

DISCUSSION

The results of this study indicate that an infusion of FPL 57231, a competitive leukotriene receptor antagonist (12), prevents pulmonary vasoconstriction with hypoxia and reverses hypoxia-induced pulmonary hypertension in the newborn lamb. There is no attenuation of the hypoxia-induced increase in cardiac output. Systemic arterial pressure decreased slightly. Whether these effects are caused by leukotriene antagonism or inhibition or activation of other substances, or are direct vascular

effects of the compound is not yet known. When infused during normoxia, FPL 57231 produces no significant hemodynamic changes.

Pulmonary vascular tone is maintained through the apparent balance between vasodilating and vasoconstricting substances (18). Various stimuli, including hypoxia, that increase pulmonary vascular resistance may alter this balance by either increasing or decreasing these substances. Many cells, including mast cells, pulmonary macrophages, and vascular tissue, in the lung can metabolize arachidonic acid via the lipoxygenase pathway to leukotrienes, formerly known as the slow-reacting substance

Fig. 2. The effects of an infusion of FPL 57231 during hypoxia on (A) pulmonary arterial pressure and (B) pulmonary vascular resistance.

Table 2. Arterial blood gas analysis when FPL 57231 was infused prior to hypoxia (mean $\pm$ SD)

	pH	PO ₂ (mm Hg)	PCO ₂ (mm Hg)	Arterial sat (%)
NORMOXIA	7.39 $\pm$ 0.04	82.8 $\pm$ 10.7	38.8 $\pm$ 4.1	95.3 $\pm$ 3.0
HYPOXIA	7.37 $\pm$ 0.06	32.2 $\pm$ 4.1*	38.7 $\pm$ 3.2	54.2 $\pm$ 15.3*
NORMOXIA + FPL 57231	7.40 $\pm$ 0.05	86.7 $\pm$ 10.3	39.2 $\pm$ 3.2	96.3 $\pm$ 2.9
HYPOXIA + FPL 57231	7.41 $\pm$ 0.04	34.7 $\pm$ 5.3*	38.2 $\pm$ 2.5	61.3 $\pm$ 17.0*

* $p < 0.05$ vs NORMOXIA.

$n = 6$.

Table 3. Effect of FPL 57231 infused after hypoxia (mean $\pm$ SD)

	Pulmonary arterial pressure (mm Hg)	Pulmonary vascular resistance (U)	Systemic arterial pressure (mm Hg)	Systemic vascular resistance (U)	Cardiac output (l $\cdot$ min $^{-1}$ $\cdot$ kg $^{-1}$)
NORMOXIA	17.5 $\pm$ 2.9	43.1 $\pm$ 8.8	68.5 $\pm$ 10.5	182.0 $\pm$ 58.2	0.40 $\pm$ 0.08
HYPOXIA	30.3 $\pm$ 4.7*†	70.3 $\pm$ 15.5*†	71.0 $\pm$ 12.4	174.0 $\pm$ 64.4	0.45 $\pm$ 0.11
HYPOXIA + FPL 57231	19.8 $\pm$ 3.6	44.0 $\pm$ 7.0	64.5 $\pm$ 11.1	150.4 $\pm$ 42.1	0.45 $\pm$ 0.08

* $p < 0.05$ vs NORMOXIA.

† $p < 0.05$ vs HYPOXIA + FPL 57231.

$n = 5$; units: mm Hg $\cdot$ l $^{-1}$ $\cdot$ min $^{-1}$ $\cdot$ kg $^{-1}$.

of anaphylaxis, and/or via the cyclooxygenase pathway to prostaglandins (2–6, 19, 20). PGI₂, PGD₂, and PGE₂ are pulmonary vasodilators in fetal and newborn animals (21, 22). Interestingly, PGD₂ is a pulmonary vasoconstrictor in older animals (22). PGF₂ is a pulmonary vasoconstrictor in fetal and newborn animals (21). The effects of thromboxanes, potent smooth muscle constrictors, on the perinatal pulmonary circulation are not known. Thromboxane levels are elevated, however, during pulmonary hypertension associated with endotoxin infusion in newborn lambs (23). LTC₄ and LTD₄ are not only potent pulmonary vasoconstrictors but also stimulate the synthesis of thromboxane and other prostaglandins (24–27). This leukotriene-induced synthesis of thromboxane and other prostaglandins can be blocked by both indomethacin, a cyclooxygenase inhibitor (26, 27), and FPL 55712 (25, 28), indicating an interrelationship between the leukotriene and prostaglandin synthetic pathways.

FPL 55712, the parent compound of FPL 57231, is one of a series of chromone-2-carboxylic acids (12). FPL 55712 has been shown to inhibit leukotriene constrictor activity in many tissues including pulmonary vascular, lung parenchymal, and tracheal strips in guinea pig and man (8–10, 12). FPL 55712 has other *in vitro* effects. In concentrations greater than 1.0×10^{-6} M, FPL 55712 inhibits thromboxane production from human platelet microsomes (28). It also prevents anaphylaxis-induced thromboxane, PGI₂, and PGE₂ production and release from guinea pig lung (19, 25, 26). PGD₂ production, however, is not inhibited (19). Whether these effects occur *in vivo* is not known.

In the intact animal, FPL 55712 inhibits the coronary artery vasoconstriction and improves the myocardial contractility impairment produced by the intracoronary injection of LTD₄ into sheep (29). FPL 55712 also increases pulmonary blood flow and decreases pulmonary vascular resistance in late gestation fetal lambs (30).

FPL 57231, the compound used in this study, is a propionic acid analog of FPL 55712. FPL 57231 produces a similar degree of leukotriene antagonism with less antiallergic effects (12). Both compounds are rapidly cleared from the plasma, with a serum half-life of <10 min, and have a similar duration of action (12). In adult sheep, FPL 57231 attenuates hypoxic pulmonary vasoconstriction (7). In near term fetal lambs, intrapulmonary infusions of FPL 57231 markedly increase pulmonary blood flow and decrease pulmonary vascular resistance (13). In preliminary studies in four newborn lambs, FPL 57231 (1 mg/kg/min) completely blocked the hemodynamic effects of intravenously injected LTD₄.

Leukotriene receptor stimulation may produce direct cardiovascular changes and induce thromboxane and PG synthesis (24–27, 31). These formed substances may add to the overall hemodynamic effects. FPL 55712 blocks both the direct leukotriene effects and the effects produced by PG and thromboxane synthesis (7–9, 26, 28). FPL 57231 also blocks the direct effects of leukotriene (12) but its effect on PG production is not known. In newborn lambs, the intrapulmonary injection of $1 \mu\text{g} \cdot \text{kg}^{-1}$ of LTD₄ increases pulmonary vascular resistance by more than 300%. There is also a biphasic effect on the systemic circulation with an initial fall in systemic vascular resistance followed by a 200% increase. Pretreatment with indomethacin partially blunts the increase in pulmonary vascular resistance and abolishes the initial decrease in systemic vascular resistance (11). In adult sheep, FPL 57231 but not indomethacin blocks hypoxia-induced pulmonary hypertension, while both alter the pulmonary vascular response to infused LTD₄ (7, 32). This interaction between leukotrienes and PG may be important in controlling the pulmonary circulation.

Leukotriene antagonism through both receptor blockade with FPL 55712 and synthesis inhibition with diethylcarbazine citrate attenuates hypoxic pulmonary vasoconstriction in isolated perfused rat lungs (33). The results of this and the above studies (7, 33) indicate a role for leukotrienes in the mediation of hypoxic pulmonary vasoconstriction. Similarly, FPL 55712 blocks exog-

enous LTC₄-induced pulmonary vasoconstriction, with no effect on angiotension II-induced vasoconstriction (33). These results suggest a specific capacity for leukotriene antagonists to block leukotriene-mediated vasoconstriction.

Although FPL 57231 prevented and reversed hypoxic pulmonary vasoconstriction in this study, it had little effect on other hemodynamic variables during hypoxia. Cardiac output is either increased, decreased, or unchanged during hypoxia (18). The hypoxia-induced increase in both cardiac output and heart rate observed in this study were maintained during the infusion of FPL 57231. These results would suggest that the mechanism of the hypoxia-induced increase in pulmonary arterial pressure is different from that which induces the change in cardiac output. The former mechanism is possibly mediated through leukotrienes, the latter possibly through autonomic pathways.

Various doses of FPL 57231 were infused prior to the onset of hypoxia to determine the lowest dose that would prevent hypoxic pulmonary vasoconstriction: $1\text{--}2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ of FPL 57231 completely inhibited the hypoxia-induced increase in pulmonary arterial pressure and pulmonary vascular resistance; $0.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ was less effective in preventing hypoxic pulmonary vasoconstriction. The effect of exogenous LTD₄ in adult sheep can be inhibited by $0.66 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ of FPL 57231 (32). Therefore, we used a $1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ infusion during the preinfusion study. To reverse pulmonary hypertension induced by hypoxia, $2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ was used because higher doses of FPL 57231 may be necessary to block existing pulmonary vasoconstriction. This dose of FPL 57231 reverses hypoxic pulmonary vasoconstriction in adult sheep (7).

In the clinical syndrome of PPHN, pulmonary vascular resistance does not fall normally with the initiation of respiration. Pulmonary hypertension is seen in a variety of clinical situations, including meconium aspiration, sepsis, and hyaline membrane disease (34, 35). There may be a common pathway mediating syndromes that produce pulmonary hypertension. Leukotrienes have been isolated in lung lavage fluids from infants with PPHN, but not in others with pulmonary disease without pulmonary hypertension (14), suggesting a role for leukotrienes in the pathogenesis of PPHN. Despite the use of supplemental oxygen, mechanical hyperventilation, and nonspecific vasodilators in treating infants with PPHN, the mortality rate remains unacceptably high (35). Leukotriene inhibition with FPL 57231 may be useful in the management of infants with PPHN and other patients with pulmonary hypertension. The results of this and other studies suggest a significant role for leukotrienes in the perinatal control of pulmonary blood flow and pulmonary vascular resistance and in the mediation of hypoxic pulmonary vasoconstriction.

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REFERENCES

1. Haas F, Bergofsky EH 1972 Role of the mast cell in the pulmonary pressor response to hypoxia. *J Clin Invest* 51:3154–3162
2. MacGlashan DW Jr, Schleimer RP, Peters SP, Schulman ES, Adams GK III, Newball HH, Lichtenstein LM 1982 Generation of leukotrienes by purified human lung mast cells. *J Clin Invest* 70:747–751
3. 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
4. Fleisch JH, Haisch KD, Spaethe SM 1982 Slow-reacting substance of anaphylaxis (SRS-A) release from guinea pig lung parenchyma during antigen- or ionophore-induced contraction. *J Pharmacol Exp Ther* 221:146–151
5. Piper PJ, Letts LG, Galton SA 1983 Generation of a leukotriene-like substance from porcine vascular and other tissues. *Prostaglandins* 25:591–599
6. Piper PJ, Seale JP 1978 Release of slow-reacting substance from guinea pig and human lung by calcium ionophore A23187. *Br J Pharmacol* 63:364P (abstr)
7. Ahmed T, Oliver W Jr 1983 Does slow-reacting substance of anaphylaxis mediate hypoxic pulmonary vasoconstriction? *Am Rev Respir Dis* 127:566–571

8. Hand JM, Will JA, Buckner CK 1981 Effects of leukotrienes on isolated guinea-pig pulmonary arteries. *Eur J Pharmacol* 76:439-442
9. 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
10. Schellenberg RR, Foster A 1984 Differential activity of leukotrienes upon human pulmonary vein and artery. *Prostaglandins* 27:475-481
11. Yokochi K, Olley PM, Sideris E, Hamilton F, Huhtanen D, Coccani 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
12. Sheard P, Holroyde MC, Ghelani AM, Bantick JR, Lee TB 1982 Antagonists of SRS-A and leukotrienes. In: Samuelsson B, Paoletti R (eds) *Leukotrienes and Other Lipoxygenase Products*. Raven Press, New York, pp 229-235
13. Soifer SJ, Schreiber MD, Loitz RD, Roman C, Heymann MA 1984 The effects of leukotriene inhibition on the perinatal pulmonary circulation in the lamb. In: Jones CT (ed) *Physiologic Development of the Fetus and Newborn*. Academic Press, London (in press)
14. 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
15. Kuipers JRG, Sidi D, Heymann MA, Rudolph AM 1982 Comparison of methods of measuring cardiac output in newborn lambs. *Pediatr Res* 16:594-598
16. Schreiber MD, Heymann MA, Soifer SJ 1984 pH, not PCO₂, decreases hypoxic pulmonary vasoconstriction (HPV) in the newborn lamb. *Pediatr Res* 18:347A (abstr)
17. Zar JH 1974 *Biostatistical Analysis*. Prentice-Hall, Inc, Englewood Cliffs, NJ, pp 158-173
18. Fishman AP 1976 Hypoxia on the pulmonary circulation: how and where it acts. *Circ Res* 38:221-231
19. Blair IA, Dollery CT, Ennis M, Hault JRS, Robinson C, Waddell KA 1983 Prostaglandin release in pulmonary anaphylaxis: PGD₂ a marker of mast cell activation in situ? *Br J Pharmacol* 78:49P (abstr)
20. Friedman Z, Lunyong VE, Courtney J, Smith H, Berkowitz P, Sun F 1984 Prostaglandin formation in the isolated human ductus arteriosus, aorta, pulmonary and umbilical arteries. *Prostaglandins Leukotrienes Med* 14:279-286
21. Cassin S 1980 Role of prostaglandins and thromboxanes in the control of the pulmonary circulation in the fetus and newborn. *Semin Perinatol* 4:101-107
22. Soifer SJ, Morin FC, Kaslow DC, Heymann MA 1983 The developmental effects of prostaglandin D₂ on the pulmonary and systemic circulations in the newborn lamb. *J Dev Physiol* 5:237-250
23. Cartwright D, Soifer S, Murray F, Clyman R 1983 Endotoxin produces acute pulmonary hypertension and thromboxane elevation in the newborn lamb. *Pediatr Res* 17:306A (abstr)
24. Feuerstein N, Foegh M, Ramwell PW 1981 Leukotrienes C₄ and D₄ induce prostaglandin and thromboxane release from rat peritoneal macrophages. *Br J Pharmacol* 72:389-391
25. Folco G, Hansson G, Grastrom E 1981 Leukotriene C₄ stimulates TXA₂ formation in isolated guinea pig lungs. *Biochem Pharmacol* 30:2491-2493
26. Piper PJ, Samhoun MN 1982 Stimulation of arachidonic acid metabolism and generation of thromboxane A₂ by leukotrienes B₄, C₄, and D₄ in guinea-pig lung in vitro. *Br J Pharmacol* 77:267-275
27. Seale JP, Piper PJ 1978 Stimulation of arachidonic acid metabolism by human slow-reacting substances. *Eur J Pharmacol* 52:125-128
28. Welton AF, Hope WC, Tobias LD, Hamilton JG 1981 Inhibition of antigen-induced histamine release and thromboxane synthetase by FPL 55712, a specific SRA-A antagonist? *Biochem Pharmacol* 30:1378-1382
29. Michelassi F, Landa L, Hill RD, Lowenstein E, Watkins WD, Petkau AJ, Zapol WM 1982 Leukotriene D₄: a potent coronary artery vasoconstrictor associated with impaired ventricular contraction. *Science* 217:841-844
30. Soifer SJ, Loitz R, Roman C, Heymann MA 1984 Do leukotrienes control pulmonary blood flow in the fetal lamb? *Pediatr Res* 18:347A (abstr)
31. Feddersen OC, Murphy RC, Voelkel NF 1983 Leukotriene E₄ causes pulmonary vasodilation which is transformed into vasoconstriction with cyclooxygenase blockade. *Fed Proc* 42:302 (abstr)
32. Ahmed T, Yerger L, Wanner A, Marchette B 1983 Pulmonary and systemic hemodynamic effects of leukotriene D₄ (LTD₄): role of leukotriene receptor stimulation and cyclo-oxygenase metabolites. *Fed Proc* 42:302 (abstr)
33. Morganroth ML, Reeves JT, Murphy RC, Voelkel NF 1984 Leukotriene synthesis and receptor blockers block hypoxic pulmonary vasoconstriction. *J Appl Physiol* 56:1340-1346
34. Drummond WH, Gregory GA, Heymann MA, Phibbs RH 1981 The independent effects of hyperventilation, tolazoline and dopamine on infants with persistent pulmonary hypertension. *J Pediatr* 98:603-611
35. Fox WW, Duara S 1983 Persistent pulmonary hypertension in the neonate: diagnosis and management. *J Pediatr* 103:505-514

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Effect of Infant Age on Aminopyrine Breath Test Results

ROBERT J. SHULMAN, CHARLES S. IRVING, THOMAS W. BOUTTON, WILLIAM W. WONG, BUFORD L. NICHOLS, AND PETER D. KLEIN

USDA/ARS Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Texas Children's Hospital, Houston, Texas 77030

ABSTRACT. The aminopyrine breath test has been used in adults as a measure of hepatic N-demethylase activity. In order to study maturational changes in enzyme function, ¹³C aminopyrine (2 mg/kg) was administered orally to infants (*n* = 16) between the ages of 1 and 38 wk. Breath samples were collected for 6 h after administration of the labeled aminopyrine for the measurement of ¹³CO₂ enrichment. Using a number of different scoring methods to

quantitate ¹³CO₂ elimination of breath, demethylation of aminopyrine was found to be positively correlated to age. By 20 wk of age, some infants had rates of elimination similar to those measured in adults. Absorption was excluded as a limiting variable, because no improvement in oxidation rates was found when the aminopyrine was readministered as an intravenous bolus. Changes in nutritional status and route of feeding (enteral *versus* parenteral) did not prevent the effect of maturation on aminopyrine elimination. **Conclusions:** 1) maturational differences are seen in the metabolism of aminopyrine; 2) these differences may reflect immaturity of N-demethylase activity or diversion of the liberated formaldehyde into biosynthetic rather than oxidative pathways. (*Pediatr Res* 19: 441-445, 1985)

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Address correspondence to Dr. Robert J. Shulman, Section of Nutrition and Gastroenterology, Texas Children's Hospital, 6621 Fannin Street, Houston, TX 77030.

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