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The Effects of Indomethacin on the Pulmonary Vascular Response to Hypoxia in the Premature and Mature Newborn Goat[1] (39108)

T. TYLER, R. WALLIS, C. LEFFLER, AND S. CASSIN[2]

Department of Physiology, College of Medicine, University of Florida, Gainesville, Florida 32610

Von Euler and Liljestrand (1) demonstrated that hypoxia increases pulmonary arterial pressure. They attributed the pulmonary vasoconstriction to a direct effect of hypoxia on lung vessels. Local regulation of pulmonary vascular resistance (PVR) may be a function of the prostaglandins (2), although the nature of their involvement is not clear. Hypoxia caueses prostaglandin release (3, 4). Experiments in which prostaglandin synthesis has been inhibited have produced conflicting reports concerning the pulmonary vascular response to hypoxia (3, 5).

The increase in PVR due to hypoxia in the perinate (6) is associated with circulatory and ventilatory failure in infants with respiratory distress (7). The present experiments were undertaken to determine the effects of indomethacin, a prostaglandin-synthetase inhibitor (8), on the pulmonary vascular response to hypoxia in the premature and mature newborn goat.

Methods. Premature newborns were delivered by cesarean section (133–135 days gestation, mean wt = 2.7 kg, n = 6) from anesthetized goats (Chloralose, 50 mg/kg iv). The umbilical circulation was left intact, and a saline-filled rubber bag was placed over the fetal head to prevent breathing until a polyethylene cannula filled with saline was introduced into the trachea. Colonic temperature was monitored and maintained between 38 and 39°. The left femoral artery was cannulated for monitoring systemic blood pressure (FAP). A left thoracotomy was performed to give access to the left pulmonary artery. Following anticoagulation (Heparin, 2000 units/kg iv), blood was withdrawn from the inferior vena cava (above the liver) via a cannula introduced through the left femoral vein and was pumped (Cole Parmer Master-Flex Model 7016) into the left pulmonary artery (Fig. 1). Left pulmonary arterial pressure (PAP), left pulmonary blood flow ($\dot{Q}$), and left atrial pressure (LAP) were monitored. $\dot{Q}$ was maintained constant. Lung fluid was aspirated; ventilation was established (25% oxygen in nitrogen, Palmer respirator); and the umbilical circulation was occluded. Supplemental Chloralose was administered to newborns as necessary (10 mg/kg iv).

Mature newborn goats (1–14 days postpartum, 147 days gestation, mean age = 6 days, mean wt = 3.6 kg, n = 9) were treated in a similar manner.

Flows (In Vivo Metric Model FT2C Probes) and pressures (Statham P23AC Transducers) were recorded on a Grass Model 5C six channel polygraph. Blood gases and pH of the newborn were monitored on a Radiometer Blood Gas Analyzer.

All newborn animals were ventilated for 60 sec with 5% oxygen in nitrogen prior to and 30 min following indomethacin administration (preliminary studies demonstrated that the pulmonary vascular response to hypoxia following indomethacin was maximal by 30 min).

Indomethacin (Sigma), prepared daily in aqueous solution (1.8 mg/ml) as a sodium salt using equimolar amounts of sodium carbonate, was infused (Harvard Pump, Fig. 1) directly into the pulmonary arterial circuit over a 10-min period (indomethacin infusion into the femoral artery, n = 5, produced equivalent results). Premature newborns received 1.9 mg/kg indomethacin, and mature newborns received 2.5 mg/kg indomethacin (maximum pulmonary arterial blood concentration was 1.8 and 2.5 μg/ml indomethacin, respectively, during infusion).

Results. The effect of hypoxia on the PAP, LAP, and FAP of the premature and mature

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[2] To whom all correspondence should be addressed.

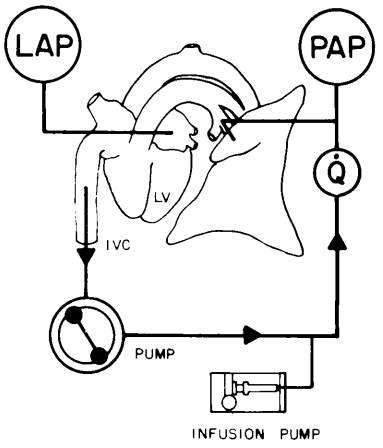

FIG. 1. Schematic representation of *in situ* isolated left lung preparation used to measure left atrial pressure (LAP) and left pulmonary arterial pressure (PAP) at constant flow ($\dot{Q}$).

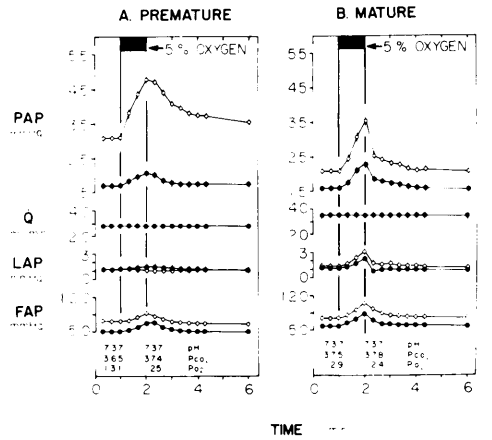

FIG. 2. Effect of 60 sec exposure to 5% oxygen on the left pulmonary arterial pressure (PAP) at constant pulmonary arterial flow ($\dot{Q}$), left atrial pressure (LAP), and femoral arterial pressure (FAP) of premature and mature newborn goats before (●) and after (○) indomethacin infusion. Results are means ± SE.

newborn before and after indomethacin is given in Fig. 2.

The PAP, LAP, and FAP of the premature (Fig. 2A) and mature newborn (Fig. 2B) prior to indomethacin were increased during hypoxia [all differences cited are significant at $P < 0.05$ (t test)]. Indomethacin increased PAP and FAP. There was a greater increase in PAP during hypoxia following indomethacin.

The PVR ([PAP-LAP]kg/$\dot{Q}$) of the newborn during hypoxia prior to (●) and following indomethacin (○) is shown in Fig. 3. The PVR of the premature (Fig. 3A) and mature newborn (Fig. 3B) prior to indomethacin was increased (20% and 39%, respectively) during hypoxia. There was a greater increase in PVR in both groups during hypoxia following indomethacin.

The ratio ○ PVR/● PVR (Fig. 3) describes the increase in PVR resulting from indomethacin administration prior to and during hypoxia. Indomethacin increased the PVR of premature newborns 82% and the PVR of mature newborns 26%. The area between the dotted line and the ○ PVR/● PVR curve represents augmentation of the increase in PVR that results from hypoxia following indomethacin. Whether the effects of hypoxia following indomethacin are expressed as absolute changes in PAP and PVR compared to control, changes in PAP and PVR from the higher baseline following

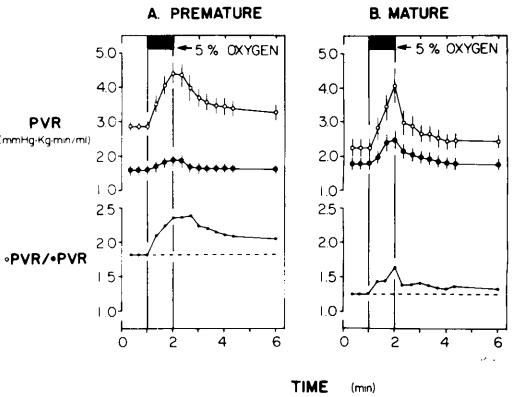

FIG. 3. Effect of 60 sec exposure to 5% oxygen on the pulmonary vascular resistance (PVR) of premature and mature newborn goats before (●) and after (○) indomethacin infusion. The area on the ○PVR/●PVR graph between the dashed line and the solid curve indicates augmentation of the pulmonary vascular response to hypoxia following indomethacin (see text). Results are means ± SE.

indomethacin, or as percent increase over control, they are significantly different than effects of hypoxia prior to indomethacin. We chose to present our actual data (Fig. 2) and a ratio ○ PVR/● PVR (Fig. 3). The ratio ○ PVR/● PVR may be interpreted as percent increase over control if one calculates [(○ PVR/● PVR) − 1.0]100. The

increase in PVR due to hypoxia was 55% greater in premature and 38% greater in mature newborns following indomethacin than prior to indomethacin administration.

Discussion. Hypoxia increases PVR in the fetus and newborn. The pulmonary circulation of perinatal goats is responsive to prostaglandins (9) that may have a role in regulating PVR. The increases in PVR following indomethacin in the newborn under conditions of controlled flow reflects an increase in tone of pulmonary vascular smooth muscle. There is augmentation of the pulmonary vasoconstrictor response to acute hypoxia following indomethacin. The duration of the pressor response subsequent to hypoxia is increased following indomethacin. These effects are more prominent in the premature than in the mature newborn.

Indomethacin effectively removes a dilator influence from the pulmonary circulation in the newborn. This removal results in increased pulmonary vascular tone and augmentation of increases in PVR that result from hypoxia. The effects of indomethacin are more pronounced in the premature than in the mature newborn, suggesting that a greater dilator influence existed in the premature pulmonary circulation.

Indomethacin (2 mg/kg) preferentially inhibits prostaglandin-synthetase (8). We selected the dose of indomethacin for our experiments to approximate an effective clinical dose (10, 11) yet preferentially inhibit PG-synthetase (8). We avoided large doses of indomethacin (100 mgs/kg) reported by some authors (12), which would minimize preferential inhibition of the synthetase system (8). In our preparation, there appears to be little difference from 1–5 mg/kg indomethacin or from route of administration (systemic iv or ia, or intrapulmonary). Vane suggests that inhibition of synthetase by indomethacin occurs early in prostaglandin synthesis and that E and F series prostaglandins are affected (13). Inhibition of prostaglandin synthesis in a system in which the dilator effects of E prostaglandins are normally more pronounced than the constrictor effects of F prostaglandins would result in a net pressor effect. Since prostaglandin synthesis involves a complex series of enzymes (8), differential inhibition of the synthesis of E series prostaglandins relative to F series prostaglandins is conceivable. Several factors alter the ratio of products (PGE/PGF) synthesized from exogenous precursors *in vitro* (14). Preferential inhibition of E series prostaglandins would decrease PGE/PGF and thereby increase PVR. Inhibition of prostaglandin synthesis by indomethacin would also remove prostaglandin modulation of sympathetic nervous system in the lung and elsewhere. These experiments do not elucidate the mechanism whereby indomethacin effectively removes a dilator influence on the lung.

The effects of indomethacin on the fetus and newborn require investigation since this agent is used in women to prolong labor (10) and may have a role in ductal shunting in newborns (15). Our results indicate that indomethacin increases PVR in the newborn and augments the increase in PVR that results from hypoxia.

Summary. The effects of indomethacin on the pulmonary circulation and the response of the circulation to hypoxia were investigated in premature and mature newborns using an isolated perfusion technique on otherwise intact left lungs *in situ*. There was an increase in pulmonary vascular resistance and augmentation of the increase in pulmonary vascular resistance during hypoxia following indomethacin. These effects were greater in the premature than in the mature newborn. Indomethacin effectively removes a dilator influence on the pulmonary circulation. The results are consistent with the concept that prostaglandins are important in regulating pulmonary vascular resistance.

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1. von Euler, U. S., and Liljestrand, G., *Acta. Phys. Scand.* **12,** 301 (1947).
2. Liljestrand, G., *in* "Prostaglandins" (S. Berg-

strom and B. Samuelsson, eds.) p. 107. Almqvist and Wiksells, Stockholm (1967).
3. Said, S. I., Yoshida, T., Kitamura, S., and Ureim, C., *Science* **185,** 1181 (1974).
4. Wennmalm, A., Pham-Huu-Chanh, and Junstad, M., *Acta. Physiol. Scand.* **91,** 133 (1974).
5. Weir, E. K., Reeves, J. T., and Grover, R. F., *Physiologist* **17,** 355 (1974).
6. Cassin, S., Dawes, G. S., Mott, J. C., Ross, B. B., and Strang, L. B., *J. Physiol.* **171,** 61 (1964).
7. Strang, L. B., and MacLeish, M. H., *Pediatrics* **28,** 17 (1961).
8. Flower, R. J., *Pharmacol. Rev.* **26,** 33 (1974).
9. Cassin, S., Tyler, T. L., and Wallis, R. G., *Proc. Soc. Exp. Biol. Med.* **148,** 584 (1975).
10. Zuckerman, H., Reiss, U., and Rubinstein, I., *Obstet. Gynecol.* **44,** 787 (1974).
11. Hucker, H. B., Zacchel, A. G., Cox, S. V., Brodie, D. A., and Cantwell, N. H. R., *J. Pharmacol. Exp. Ther.* **153,** 237 (1966).
12. Said, S. I., Hara, N., and Yoshida, T., *Fed. Proc.* **34,** 438 (1975).
13. Vane, J. R., *Hosp. Practice* **7,** 61 (1972).
14. Lands, W., Lee, R., and Smith, W., *Ann. NY Acad. Sci.* **180,** 107 (1971).
15. Elliott, R. B., Starling, M. B., and Neutze, J. M., *Lancet* **1,** 140 (1975).

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