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The Effect of Methylprednisolone on Hypoxic Pulmonary Vasoconstriction in the Newborn Late-Gestation Lamb

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ABSTRACT. Methylprednisolone (30 mg/kg), which inhibits a number of mediators of hypoxic pulmonary vasoconstriction derived from arachidonic acid, has been found to alleviate hypoxic pulmonary vasoconstriction in adult humans and in the isolated rat lung preparation. We studied the effect of 30 mg/kg of methylprednisolone on the pulmonary vascular response to hypoxia in six late-gestation newborn lambs. During hypoxia, pulmonary vascular resistance nearly doubled compared with the baseline hyperoxic state. This was true both before and after administration of methylprednisolone. We conclude that methylprednisolone, when administered at the dosage used in previous studies of adult humans and animals, does not affect the response of the pulmonary vascular bed to hypoxia in newborn lambs. (*Pediatr Res* 27:133–136, 1990)

Abbreviations

MP, methylpredisolone
PVR, pulmonary vascular resistance

Hypoxia plays a role in several pathophysiologic conditions by altering both regional and general pulmonary hemodynamics. Although the exact mechanism underlying hypoxic pulmonary vasoconstriction in unknown, numerous mediators have been thought to play a role in the pulmonary vascular response. Metabolites of arachidonic acid, from both cyclooxygenase (thromboxane A_2, PGF_2, PGD_2) (1–4) and lipoxygenase (leukotrienes C_4 and D_4) (1, 5–8) pathways, have been implicated in mediating hypoxic pulmonary vasoconstriction in adult rats, newborn lambs, and human infants. Corticosteroids have been found to block the synthesis and release of vasoactive metabolites from both pathways of arachidonic acid metabolism (9–14). Glucocorticoids also affect the release and action of many other mediators that may influence pulmonary vascular reactivity (15).

Recently, 30 mg/kg, was found to inhibit hypoxic pulmonary vasoconstriction in healthy adult human volunteers (16). The inhibition was apparent within 5 min after the MP dose was given. MP also was found to reduce pulmonary vascular resistance in hypoxic and atelectatic isolated adult rat lung preparations (17). This inhibition was selective for hypoxic vasoconstriction and was not due to a generalized loss of pulmonary vasoreactivity.

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Because MP (30 mg/kg) has been given to infants in an attempt to alleviate pulmonary vasoconstriction (18), we designed a study to see whether MP has the same effects on the newborn pulmonary circulation as it does on the adult circulation.

MATERIALS AND METHODS

Surgical preparation. Surgery was performed on six time-dated pregnant Western mixed-breed ewes at 136 ± 3 d gestation (mean ± SD; term = 145 d). We used newborn lambs at this gestational age because they develop pulmonary blood pressures equal to systemic pressures when exposed to hypoxia (19, 20).

Epidural anesthesia was induced with 4 mL of 1% tetracaine hydrochloride; ketamine (100 mg) was given intravenously for sedation as required. Local anesthesia with 0.1% lidocaine hydrochloride was used for all incisions. Polyvinyl catheters were inserted into the ewe's pedal artery and vein and a continuous infusion of 0.9% NaCl was given to the ewe throughout surgery.

A hysterotomy was performed through a midabdominal incision and polyvinyl catheters inserted into the fetal pedal vein and artery and advanced to the inferior vena cava and descending aorta. The fetal left chest was identified and a left thoracotomy performed; the left lung was retracted to expose the great vessels and the ductus arteriosus. The ductus was dissected from the surrounding tissue, and a mechanical snare (made from a cardiac catheterization guide wire and a catheter sheath) was looped around the ductus and brought through the fetal chest wall. A catheter was placed in the pulmonary artery, and the fetal thoracotomy was closed. Under local anesthesia, a fetal right upper pedal artery was cannulated. The fetus was intubated through a tracheotomy with a 4.5 Fr cuffed endotracheal tube. Sheep surfactant, prepared from lung lavages of healthy adult sheep as previously described (21), was given to stabilize the immature lungs during the period of the study (21, 22). The surfactant was instilled into the trachea (50 mg/kg body wt), and the fetus maneuvered from side to side to ensure distribution in the lungs.

The umbilical cord was then ligated and the lamb delivered to a warming table, where mechanical ventilation was initiated with a time-cycled, constant-flow, infant ventilator (Sechrist, Anaheim, CA). A polyvinyl catheter was inserted into an umbilical artery. Under local anesthesia, a small cervical cut-down was performed and a catheter threaded into the superior vena cava through the jugular vein. Under continuous pressure monitoring, another catheter was threaded through the carotid artery and advanced into the left ventricle.

At the beginning of the experiment, the lambs were sedated with 0.2 mg/kg of diazepam (in addition to the ketamine that they received during surgery) and were paralyzed throughout the experiment with 0.4 mg of pancuronium bromide. All intravascular catheters were kept patent by infusion of small boluses of 5% dextrose in water (< 1 mL/kg/h). The lambs were dried with a towel and covered with a plastic sheet to reduce evaporative

heat losses. Rectal temperature was maintained at 38–39°C with an Aquamatic K-pad (American Hospital Supply, McGaw Park, IL) and with radiant heat lamps. Heparinized fetal or maternal blood was transfused to replace blood loss from sampling. This protocol was approved by the Committee on Animal Research at the University of California, San Francisco.

Experimental protocol. Immediately after delivery, the ductus arteriosus was closed by tightening the mechanical snare; the ductus remained closed throughout the experiment to prevent right-to-left or left-to-right ductal shunting. The ventilator was set to provide peak inspiratory pressures of 20–25 cm H_2O, inspiratory time of 0.6, and a rate of 35–45 breaths/min with fractional inspired oxygen of 1.0. Peak inspiratory pressures and rates were then adjusted to maintain arterial P_{CO_2} values in a range close to 30 mm Hg. After an initial stabilization period, the peak inspiratory pressures were lowered to 18–23 cm H_2O and the respiratory rates 20–36 breaths/min. Once appropriate settings had been achieved for each individual animal, we found little need for further adjustments; in no case was the coefficient of variation of ventilator settings more than 10% over the course of the experiment.

After a 2-h stabilization period, the lambs were subjected to alternate periods of hypoxia and hyperoxia. Hypoxia was induced by breathing a gas mixture 60–80% N_2 and air to produce an arterial P_{O_2} of about 20 mm Hg. Hypoxia was maintained for 20 min at a time. Hyperoxia was achieved by fractional inspired O_2 of 1.0. Before administration of MP, some animals were subjected to two periods of hypoxia and two of hyperoxia, others to only one period of hypoxia. Before MP administration, the six animals were studied during a total of 11 periods of hyperoxia and nine of hypoxia. At the end of each study period, hemodynamic variables were noted, arterial blood gases recorded, and a microsphere flow study performed.

Four hours after delivery, 30 mg/kg of MP was given intravenously; the animals then were studied during two periods of hyperoxia and one of hypoxia. As before, hemodynamic variables and arterial blood gases were recorded and a microsphere flow study performed at the end of each period. The animals were then killed with 3 mL of euthanasia solution (Anthony Products Co., Arcadia, CA). Figure 1 illustrates a typical experimental sequence.

Cardiovascular function measurements. Vascular and left ventricular pressures were recorded continuously with a Sensormedic Dynograph R611 multichannel recorder and Beckman pressure transducers (Sensormedic, Anaheim, CA). Signals were averaged electronically to obtain mean pressures. Left ventricular output and pulmonary blood flow were determined by injecting two different radiolabeled microspheres, one into the left ventricle and the other into the inferior vena cava, whereas reference blood samples were continuously withdrawn from the ascending and descending aorta (23). At the end of the experiment, the organs and carcass were separated, placed in formalin, incinerated at 325°C for 72h, pulverized, and placed in counting vials.

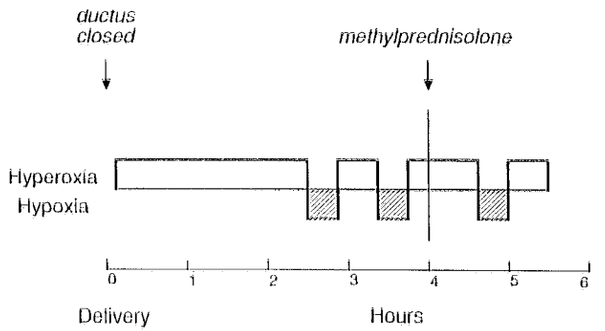

Fig. 1. Typical experiment in which the lamb was studied during two hypoxic periods before MP administration.

The amount of radionuclide in each organ and reference blood sample was measured with a well-type γ-scintillation counter and multiple-channel pulse height analyzer (21, 23, 24). Left ventricular output, pulmonary blood flow, left-to-right shunt, and foramen ovale right-to-left shunt were calculated from the concentration of microspheres in the reference samples, the total number of microspheres recovered from the whole animal, and the total number of microspheres in the lungs (21, 23, 24). Bronchial blood flow was assumed to be 8% of the measured left ventricular output; this assumption was based on measurements made in 35 premature newborn lambs with ligated ductus (Clyman RI, unpublished observations). Pulmonary blood flow was therefore considered equal to the left ventricular output minus the combination of the estimated bronchial blood flow and the right-to-left foramen ovale shunt (24). PVR was calculated as:

$$\frac{\text{mean pulmonary artery pressure} - \text{left ventricular end-diastolic pressure}}{\text{pulmonary blood flow}}$$

Blood gases. Arterial pH, P_{CO_2}, and P_{O_2} were measured using a Corning blood gas analyzer (Corning Medical and Scientific, Medfield, MA).

Statistical analysis. Statistical comparisons were made using the paired t test with the Bonferroni adjustment for multiple comparisons (25, 26). $p < 0.05$ was regarded as evidence of a statistically significant difference.

RESULTS

Six animals were studied during a total of 11 periods of hyperoxia and nine of hypoxia before administration of MP with a total of 14 transitions between hyperoxia and hypoxia. We found that transitions from hypoxia to hyperoxia did not differ in magnitude from the reverse transitions and that the hemodynamic variables during either of the hypoxic episodes were very similar in magnitude (Table 1). Thus, all hypoxic episodes before MP were grouped together for analysis of results, as were all hyperoxic episodes.

After MP administration the animals were studied during 12 periods of hyperoxia and six of hypoxia with a total of 12 transitions between hyperoxia and hypoxia. The values of the hemodynamic variables were similar during the two periods of hyperoxia (Table 1), and again, the order of the transitions did not matter. Thus, all hyperoxic periods after MP were grouped together for analysis.

Table 2 shows values for hemodynamic variables and arterial blood gases before and after MP. Other than higher P_{O_2} values during hyperoxia after MP, the values of hemodynamic variables and arterial blood gases before and after MP were similar. In particular, there was no significant difference in PVR before and after MP.

Another way of demonstrating these results is to use the PVR ratio (the ratio of PVR during hypoxia to the PVR during hyperoxia in each transition) as a measure of the pulmonary pressor response to hypoxia. Hypoxia caused a 2.02-fold increase in PVR before MP, which was similar to the 1.96-fold increase in PVR observed during hypoxia after MP.

DISCUSSION

We have shown that MP (30 mg/kg) does not attenuate the pulmonary vascular response to hypoxia in near-term newborn lambs. We considered the possibility that there was insufficient time for the pharmacologic action of MP to take effect. Although we cannot completely discount this possibility, in those studies that have shown a beneficial action of MP in hypoxic pulmonary vasoconstriction (16, 17), the effects of MP were noticeable within the time range used in our study. Furthermore, our study was designed to investigate the foundations for acute therapy in the context of a relatively short-term insult. Whatever the mech-

Table 1. *Hemodynamic variables and arterial blood gases during separate hypoxic and hyperoxic periods before and after MP*†*

	Hypoxia I	Hypoxia II	*p*	Hyperoxia I	Hyperoxia II	*p*
Before MP						
PaO_2 (mm Hg)	17 ± 3	18 ± 5	NS	251 ± 105	271 ± 116	NS
PVR (torr/L/min/kg)	468 ± 131	436 ± 134	NS	267 ± 82	219 ± 59	NS
$\dot{Q}p$ (mL/min/kg)	105 ± 35	145 ± 73	NS	114 ± 38	149 ± 41	NS
$\bar{P}_{pa}$ (torr)	50 ± 11	61 ± 14	NS	33 ± 5	34 ± 4	NS
LVO (mL/min/kg)	123 ± 35	220 ± 140	NS	131 ± 37	162 ± 44	NS
$\bar{P}_{fa}$ (torr)	57 ± 12	64 ± 10	NS	64 ± 10	68 ± 9	NS
n†	6	3		6	5	
After MP						
PaO_2 (mm Hg)				380 ± 96	425 ± 203	NS
PVR (torr/L/min/kg)				205 ± 92	204 ± 74	NS
$\dot{Q}_p$ (mL/min/kg)				161 ± 54	152 ± 34	NS
$\bar{P}_{pa}$ (torr)				33 ± 5	34 ± 7	NS
LVO (mL/min/kg)				178 ± 58	168 ± 33	NS
$\bar{P}_{fa}$ (torr)				57 ± 12	66 ± 7	NS
n†				6	6	

* PVR, pulmonary vascular resistance; $\dot{Q}_p$, pulmonary blood flow; $\bar{P}_{pa}$, mean pulmonary artery pressure; LVO, left ventricular output; $\bar{P}_{fa}$, mean femoral artery pressure.

† After MP there was only one period of hypoxia for each animal (results in Table 2).

‡ *n* = number of animals subjected to period of study.

Table 2. *Hemodynamic variables and arterial blood gases before and after MP**

	Before MP			After MP		
	Hyperoxia (*n* = 11)	Hypoxia (*n* = 9)	*p*	Hyperoxia (*n* = 12)	Hypoxia (*n* = 6)	*p*
PaO_2 (mm Hg)	260 ± 105	17 ± 3	10^{-7}	402 ± 153†	22 ± 6	10^{-7}
$\bar{P}_{pa}$ (torr)	33 ± 4	54 ± 12	10^{-4}	34 ± 6	56 ± 5	10^{-5}
$\dot{Q}_p$ (mL/min/kg)	130 ± 41	118 ± 50	NS	157 ± 43	155 ± 53	NS
PVR (torr/L/min/kg)	245 ± 73	457 ± 125	6×10^{-4}	205 ± 80	397 ± 164	3×10^{-3}
$PaCO_2$ (torr)	31 ± 3	30 ± 3	NS	31 ± 3	32 ± 2	NS
pH	7.44 ± 0.04	7.45 ± 0.04	NS	7.42 ± 0.06	7.44 ± 0.06	NS
LVO (mL/min/kg)	145 ± 41	155 ± 90	NS	173 ± 45	203 ± 81	NS
$\bar{P}_{fa}$ (torr)	66 ± 9	59 ± 11	NS	62 ± 11	56 ± 9	NS

* Abbreviations as in Table 1.

† $p = 0.04$ compared with PaO_2 during hyperoxia before MP.

anism of acute hypoxic pulmonary hypertension may be, its effects are instantaneous, and we wished to investigate the immediate effects of pharmacologic doses of MP on the particular mechanism(s). Our study was not designed to investigate the effect of MP on chronic hypoxic pulmonary hypertension, nor to study the hypoxic pulmonary pressor response after prolonged administration of corticosteroids. Chronic hypoxic pulmonary hypertension is probably a different pathophysiologic entity. Indeed, in a rat model of chronic hypoxic pulmonary hypertension, prolonged MP administration has been found to ameliorate hemodynamic findings due to its effects on connective tissue remodeling (27).

Another possible explanation for our inability to observe an effect of MP is that the action of MP may be species specific. As there is considerable variability in the pulmonary pressor response to hypoxia among different animal species (28), the mechanisms underlying hypoxic pulmonary vascular responses also may not be identical. We chose the newborn lamb model for its highly reproducible and brisk pulmonary pressor response to hypoxia, and because previous work in this animal model had suggested that arachidonic acid metabolites, which might have been affected by MP, were possible mediators of hypoxic pulmonary vasoconstriction (1, 2, 6, 7).

Our study does not address the issue of whether endogenous corticosteroids affect the pulmonary pressor response to hypoxia in the fetus or newborn. Endogenous corticosteroids may play a role in pulmonary vascular remodeling in fetal life. Although we did not measure blood levels, there is a reasonable possibility that the stress of surgery and delivery caused an elevation in adrenal output. If there is some saturable mechanism that potentially might be affected by corticosteroids, this might have affected our baseline indices throughout the experiment. However, our study was designed to address the issue of a pharmacologic and perhaps therapeutic effect of MP in similarly stressed newborn infants with idiopathic pulmonary hypertension.

In summary, we conclude that insofar as the newborn lamb model is applicable to humans, methylprednisolone is unlikely to be a useful pharmacologic adjunct to treatment of acute hypoxic pulmonary vasoconstriction.

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Announcements

Seeking Acute Cases of Rheumatic Fever and Sydenham's Chorea

The Child Psychiatry Branch of the National Institute of Mental Health is seeking patients for a study of the course of psychologic symptoms accompanying Sydenham's chorea and rheumatic fever. Eligible patients should have had recent (within 2 mo) onset of rheumatic fever, continue to have symptoms and be at least 6 y of age. This study will rate a variety of psychologic and psychiatric symptoms and link these symptoms to anti-CNS autoantibodies. The results will be important in defining the etiology of childhood-onset psychiatric disorders. Patients and their parents will be asked to travel at NIH expense to the NIMH in Bethesda, MD for an initial interview. Brief follow-up interviews will be conducted by telephone every 2 mo for 1 y. Serum samples (5 cc) will be obtained on four separate occasions. There will be no expense to the patient and no remuneration. *Please call* Dr. Susan Swedo at (301) 496-6081 *or write:* Dr. S. Swedo, Child Psychiatry Branch, NIMH, Bldg. 10, Room 6N240, 9000 Rockville Pike, Bethesda, MD 20892.

Special Program in Nutrition for a Healthy Heart Developed for Grade School Children and Their Parents

The J. David Gladstone Foundation of the University of California, San Francisco, has developed a heart healthy nutrition education curriculum for third grade students and their parents. This curriculum, called the Special Program in Nutrition (SPIN), consists of two manuals and a cookbook collection of low fat, low cholesterol recipes. The manuals contain an in-service program for teachers, 27 lessons for third grade students, seven lessons for fourth grade students, and a parents' program with ten planned meetings.

The cost of the complete set is $35.00; the two-volume curriculum and the cookbook may be purchased separately for $30.00 and $10.00, respectively. Add 6.5% California sales tax where applicable. *For further information contact:* The Gladstone Foundation Laboratories, 2550 23rd Street, P.O. Box 40608, San Francisco, CA 94140, (415) 826-7500.

1990 ANNUAL MEETINGS

The American Pediatric Society, The Society for Pediatric Research and The Ambulatory Pediatric Association will meet May 7–11, 1990; Anaheim Hilton & Towers and Convention Center, Anaheim, CA.

Contact: **APS or SPR:** Association Headquarters, 2650 Yale Blvd., S.E., Suite 104, Albuquerque, NM 87106, (505) 764-9099 or 0068. **APA:** Ambulatory Pediatric Association, 6728 Old McLean Village, McLean, VA 22101, (703) 556-9222.