Platelet-activating factor modulates pulmonary vasomotor tone in the perinatal lamb

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Ibe, Basil O., Sue Hibler, and J. Usha Raj. Platelet-activating factor modulates pulmonary vasomotor tone in the perinatal lamb. J. Appl. Physiol. 85(3): 1079–1085, 1998.—Eight near-term fetal lambs were studied acutely in utero to determine role of platelet-activating factor (PAF) in the regulation of vasomotor tone in systemic and pulmonary circulations in the immediate perinatal period. Four fetal lambs were studied predelivery and 2 h postdelivery to determine circulating PAF levels. Aortic and pulmonary arterial pressures and cardiac output were measured continuously, and systemic and pulmonary vascular resistances were calculated. Left pulmonary arterial blood flow was also measured in four fetal lambs. After delivery and oxygenation, circulating PAF levels fell significantly. When WEB-2170, a specific PAF-receptor antagonist, was infused to block effect of endogenous PAF in the eight near-term fetal lambs, systemic vascular resistance fell 30% but pulmonary vascular resistance fell dramatically by 68%. Specificity of WEB-2170 was tested in juvenile lambs and was found to be very specific in lowering vasomotor tone only when tone was elevated by action of PAF. Our data show that endogenous PAF levels in the fetus contribute to maintain a high basal systemic and pulmonary vasomotor tone and that a normal fall in circulating PAF levels after birth and oxygenation may facilitate fall in pulmonary vascular resistance at birth.

lung; platelet-activating factor antagonist; hypoxia; pulmonary circulation; pulmonary vascular resistance

PLATELET-ACTIVATING FACTOR (PAF) is a potent phospholipid mediator with a wide spectrum of biological properties (31). It is synthesized by many types of cells, including platelets and lung epithelial and endothelial cells. After a stimulus, 1-0-alkylphospholipids released from membrane are acted on by phospholipase A2 (PLA2) to form lyso-PAF (2, 31). The lyso-PAF so formed may be acted on by acetyltransferase to form PAF. PAF synthesized in vivo may produce any of the physiological effects of PAF, or it may be catabolized by acetyldrolyase, the PAF catabolic enzyme, to form lyso-PAF.

The lung is a major target of the biological activities of PAF (11). Infusion of PAF into the pulmonary circulation increases total pulmonary vascular resistance (16). In spontaneously breathing sheep, administration of PAF induces pulmonary vasoconstriction and increases transvascular fluid filtration, which can be reversed if PAF treatment is discontinued (6, 10). Other reports have demonstrated that PAF plays a significant role in fetal lung development and maturation (4, 19). This suggests that, apart from being an inflammatory mediator, PAF does have some beneficial physiological functions. The interaction of PAF with its receptor involves a series of complex biochemical events that may lead to an increase in intracellular calcium concentration (34), changes in vascular reactivity or vasomotor tone (17), and the stimulation of endothelial cells to release nitric oxide (25, 27), among other effects. In a previous study (1), direct infusion of PAF into the left pulmonary artery of chronically catheterized preterm fetal lambs resulted in pulmonary vasodilation without changes in pulmonary arterial pressure. This suggests that, in the pulmonary circulation, exogenously administered PAF may act as a vasodilator or vasoconstrictor, depending on the level of baseline tone; if baseline tone is high, exogenous PAF acts as a vasodilator and, if baseline tone is low, exogenous PAF acts as a constrictor. Physiological effects of endogenous PAF will depend on its net production and the distribution and/or the numbers of PAF receptors in the vessels, which may be dependent on the age of the animal and on the species. In this study, we have used the specific PAF-receptor antagonist WEB-2170 (18) to study the role of endogenous PAF in maintenance of basal vasomotor tone in both the pulmonary and systemic circulations during the perinatal period. Our hypothesis was that a drop in PAF level after birth may be one method by which a fall in pulmonary vascular resistance postnatally is facilitated.

METHODS

Animal Preparation

Near-term pregnant ewes of 139–146 days gestation, term being 150 days, were premedicated with ketamine hydrochloride (1 g im) and atropine sulfate (1.6 mg im). An endotracheal tube was tied into the trachea, and catheters were placed in the external jugular vein and femoral artery. The ewe was mechanically ventilated with oxygen-enriched air by using a piston-type ventilator (Harvard Instruments). Tidal volume was adjusted to maintain arterial PO2 between 30 and 35 Torr. Arterial PO2 was kept above 80 Torr. Anesthesia was maintained with a continuous infusion of ketamine hydrochloride (500–750 mg/h). Adequacy of anesthesia in the ewe was judged by the presence of a stable heart rate and blood pressure that were unchanged by painful stimuli. The ewe received intravenous fluids (Ringer lactate and 10% dextrose) continuously at a rate of ~500 ml/h. After induction of adequate anesthesia, a hysterotomy was performed.

Fetal Lambs

A total of eight fetal lambs were studied. After delivery of the fetal head and neck through the uterine incision, a glove filled with warm saline was placed over the animal’s head to prevent air breathing. Sedation was provided with intramuscular ketamine (25 mg/kg) and acepromazine (0.5 mg/kg). Subcutaneous lidocaine (2%) was used for local anesthesia. A fetal skin incision was made on the right side of the neck, and a catheter was placed in the carotid artery. A balloon-tipped thermodilution catheter (5-F), floated into the main pulmo-
nary artery via the internal jugular vein, was used to determine cardiac output and monitor core temperature. After a left thoracotomy to expose the heart and great vessels, an ultrasonic flow probe (4 mm) was placed around the left pulmonary artery in four of eight lambs. During the studies, the fetal body remained partially in the uterus and the fetus was covered with warm moist towels.

Five of the fetal lambs were studied after delivery. After delivery of the fetal head and neck through the uterine incision, an endotracheal tube was tied into the trachea. Fetal lung fluid was aspirated via the endotracheal tube (usually 10 ml/kg body wt) and discarded before delivery. Once delivered, the lamb was weighed and dried, and mechanical ventilation (Healthdyne Infant Ventilator) was initiated immediately. Sedation was provided with ketamine (25 mg/kg) and acepromazine (0.5 mg/kg). Subcutaneous lidocaine (2%) was used for local anesthesia. The umbilical artery was cannulated with a 5-F polyvinyl catheter that was advanced into the descending aorta. After a neck incision, a balloon-tipped thermomodilution catheter (5-F) was placed in the internal jugular vein and floated into the pulmonary artery. An additional catheter was placed in the right atrium via the saphenous vein. During mechanical ventilation, inspired oxygen concentration was adjusted to maintain arterial PO2 >90 Torr. Respiratory rate was kept between 20 and 30 breaths/min, and the peak inspiratory pressure was adjusted to keep arterial POC2 between 30 and 40 Torr. End-expiratory pressure was kept constant at 2 cmH2O. Body temperature, monitored by a rectal probe, was maintained between 39 and 40°C with a heating pad and a heat lamp, if necessary. An infusion of 10% dextrose solution (4–5 ml·kg−1·h−1) was given to maintain Dextrostix >45 mg/100 ml, and any blood withdrawn was replaced with autologous whole blood (obtained from the placenta). We waited for a period of 3–6 h, before initiation of the experimental protocol, to allow adequate time for the lamb to undergo successful extrauterine cardiopulmonary transition. During this time, vascular pressures were monitored continuously and arterial blood pH and gas tensions were measured every 30 min.

Juvenile Lambs

Juvenile lambs were used to probe the specificity of WEB-2170 as a PAF-receptor antagonist. Lambs were premedicated with ketamine hydrochloride (25 mg/kg im) and atropine sulfate (40 µg/kg im). Subcutaneous lidocaine (2%) was used for local anesthesia. A catheter was inserted into the carotid artery, and a 7-F balloon-tipped thermomodilution catheter (Baxter Health Care) was floated into the pulmonary artery via the internal jugular vein. An additional catheter was placed in the right atrium via the saphenous vein. The lambs were allowed at least 24 h to recover from operative stress before study protocols were begun. During the studies, lambs were awake, unanesthetized, breathing spontaneously, and resting quietly in slings. Body temperature was maintained at 39–40°C with an overhead warmer, if necessary. An interval of at least 24 h was allowed between studies in each lamb. Between studies, catheters were flushed daily, filled with heparin, and capped. Oxacillin (10 mg/kg im) and gentamicin (2 mg/kg im) were administered twice daily.

Measurement of PAF

Extraction of PAF. Plasma samples were prepared from blood drawn from fetal lambs before and after delivery. Plasma samples were extracted for PAF measurement, as recommended by DuPont-New England Nuclear (Boston, MA) on BOND-ELUT C18 extraction columns (Analytichem International, Harbor City, CA) with flow controlled by gravity. Columns were prewashed with 2 ml methanol followed by 5 ml of 10% acetic acid. Plasma samples that were spiked with 10,000 counts/min of [1H]PAF to monitor the recovery of PAF from the plasma were diluted with an equal volume of 20% acetic acid and loaded on the column. The column was then washed sequentially with 2 ml of 10% acetic acid and 3 × 2 ml of ethyl acetate. PAF was eluted from the column with 6 ml of methanol in a polypropylene plastic tube. Further cleanup of the extracted PAF was done by adding 0.1 g of DEAE-cellulose to each sample and then extracting the PAF with a mixture of chloroform-methanol-water (1:1:0.9). Recovery of radioactivity from the spiked samples was between 92 and 95%. Sample extracts were then dried under a stream of nitrogen and taken up in 1 ml of the RIA buffer. The extraction procedure was tested for specificity in alkylphospholipid extraction. For this test, samples dried with a stream of nitrogen were taken up in 200 µl of chloroform and purified by TLC on glass-silica gel plates (J. T. Baker, Phillipsburg, NJ) by using chloroform-methanol-water (65:35:5) as the developing solvent. Purified PAF was tested for retention of biological activity by the serotonin-release method before measurement by 125I RIA.

PAF RIA. PAF was measured by RIA with an equimolar solution of the hexadecyl and octadecyl analogs as standard. Purified PAF was redissolved in the RIA buffer provided with the assay kit (DuPont-New England Nuclear), and the assay was performed as described by the vendor. The assay was tested for linearity and for intra-assay and interassay variabilities. The variabilities were <10%. The cross-reactivity of the antibody with arachidonyl-PAF, carbamyl-PAF, lyso-PAF, phosphatidylcholine, and WEB-2170 were <0.5%. The amount of metabolite measured was corrected for recovery and expressed as nanograms per milliliter of plasma.

Drug Preparation

WEB-2170 (gift of Boehringer Ingelheim) was dissolved in normal saline, and pH was adjusted to 5.6 with NaOH. PAF (Biodol, Plymouth Meeting, PA) was dissolved in ethanol, and then aliquots were mixed with 0.1% bovine serum albumin in distilled water. Angiotensin II (Sigma Chemical, St. Louis, MO) was diluted with normal saline.

Physiological Measurements

Pressures in the aorta and pulmonary artery were measured continuously with Statham P23 transducers and recorded on a polygraph recorder (model R511A, Sensormedics). Heart rate was determined from the phasic pressure tracing and recorded at 15-min intervals. Cardiac output was determined by the thermodilution method (model SP1453, Gould) at ~20-min intervals, in triplicate, and the average value was recorded. Left pulmonary arterial blood flow was measured continuously by using a Transonic flowmeter (model T108) connected to a size 4S perivascular flow probe in four fetal lambs. Arterial pH, gas tensions (model BM53MC2, Radiometer, Copenhagen, Denmark), hematocrit (Readacrit centrifuge), and glucose concentration (Dextrostix) were monitored every 30 min.

Experimental Protocols

Physiological role of endogenous PAF in fetal lambs, before and after birth. Eight fetal lambs, 142–143 days gestation, were studied in utero. After insertion of catheters and placement of flow probes (4 of 8 lambs), baseline hemodynamic variables were monitored for 10–15 min. Arterial pH and blood-gas tensions were measured. If arterial pH, Po2, and
PAF modulation of perinatal pulmonary circulation

PCO₂ were within the physiological range (pH 7.32–7.42, PO₂ 25–32 Torr, PCO₂ 38–45 Torr) and mean arterial pressures remained stable during this time period, the fetus was studied. In four lambs, the PAF receptor-antagonist, WEB-2170, was infused into the pulmonary artery in incremental doses ranging from 20 to 200 mg. An effect was first seen at a total dose of 40 mg. Doses up to 160 mg continued to have an effect. From 160 to 200 mg (47 ± 6 mg/kg) no further effect was seen. On the basis of these results, the remaining four lambs received a 200-mg (55 ± 2 mg/kg) bolus of WEB-2170 into the pulmonary artery. In all lambs hemodynamic measurements were continued for 15–20 min. In two animals, PAF (400 ng) was infused into the pulmonary artery at 10–15 min after WEB-2170. Effectiveness of WEB-2170 was tested by intravenous infusion of PAF (400 ng) in two animals.

Postdelivery study of fetal lambs. Five lambs were delivered and mechanically ventilated. After a postbirth stabilization period of 4 ± 1 h, baseline (pre-WEB-2170) measurements of aortic and pulmonary arterial pressures and cardiac output were obtained. PAF levels were measured before and after birth.

Specificity of the PAF receptor antagonist WEB-2170. Specificity of the WEB-2170 compound was tested in juvenile lambs. Six lambs (age 6 ± 1 wk, body weight 11 ± 2 kg) were studied. After a stable baseline period of at least 45 min, during which time pressures did not vary by >10% of mean values, the experimental protocols were begun.

Group I (n = 4). Specificity of WEB-2170 was tested in this group of lambs. A PAF infusion was begun at 1 µg·kg⁻¹·h⁻¹ and increased as needed to obtain a minimum 30-min period of stable pulmonary arterial pressure approximately double that of baseline. The infusion rate varied from 1 to 10 µg·kg⁻¹·h⁻¹ (mean 4 µg·kg⁻¹·h⁻¹).

Group II (n = 4). Pulmonary vascular resistance was elevated in this group of lambs with hypoxia. Pulmonary arterial pressure was elevated to approximately double that of baseline by allowing the lambs to breathe a hypoxic gas mixture (air and nitrogen) that was blown into a plastic bag loosely fitted over the lamb’s head. Frequent arterial blood gases were monitored to maintain PO₂ < 50 Torr.

Group III (n = 5). Both pulmonary and systemic vascular resistances were elevated in this group of lambs with angiotensin II infusion. Angiotensin II was infused at a dose of 15–50 µg·kg⁻¹·min⁻¹ to elevate aortic pressure at least 50% and pulmonary arterial pressure by at least 30%.

In all lambs, after a steady-state period of ~30 min of elevated aortic and pulmonary arterial pressures, WEB-2170 was infused into the aorta in incremental doses ranging from 5 to 50 mg/kg. Hemodynamic measurements were continued for an additional 10–15 min.

Data Analysis

All data are means ± SE. The data were analyzed statistically using a two-tailed Student’s t-test. For example, a t-test was used to discover the differences in pulmonary arterial pressures during pre- and post-WEB-2170 treatment of lamb lungs. For multiple comparisons, a one-way ANOVA was performed followed by Tukey’s post hoc test. A P < 0.05 was considered significant.

RESULTS

Physiological Role of Endogenous PAF in Fetal Lambs, Before and After Birth

Fetal lambs. The mean arterial pH was 7.35 ± 0.02 and CO₂ and O₂ gas tensions were 28 ± 1 and 40 ± 1 Torr, respectively, at baseline and remained unchanged after infusion of WEB-2170. Hematocrit (Hct) was 42 ± 1%. In the five lambs that received WEB-2170 in incremental doses, the mean dose at which we first saw a hemodynamic effect was 10 ± 1 mg/kg. The maximum effect occurred at a mean dose of 32 ± 5 mg/kg, with additional doses of up to 47 ± 6 mg/kg (200-mg total dose) having no further effect. The mean arterial pH, PCO₂, and PO₂ after infusion of WEB-2170 were 7.34 ± 0.02, 27 ± 2 Torr, and 39 ± 1 Torr, respectively, and they were not different from the values before the infusion of WEB-2170.

Figure 1 summarizes mean values for aortic and pulmonary arterial pressures in fetal lambs at baseline and maximum response after infusion of WEB-2170 in a dose of 48.5 mg/kg (200-mg total dose). Aortic pressure fell 17 ± 3% from 61 ± 4 to 51 ± 3 mmHg, and pulmonary arterial pressure fell 20 ± 3% from 60 ± 5 to 48 ± 4 mmHg. The nadir of the fall in aortic and pulmonary arterial pressures occurred at ~1 min after infusion of WEB-2170, and pressures remained at this lower level. Baseline heart rate and cardiac output were 141 ± 10 beats/min and 267 ± 22 ml·kg⁻¹·min⁻¹, respectively. Cardiac output increased significantly to 340 ± 31 ml·kg⁻¹·min⁻¹ after WEB-2170. Heart rate was unaffected and was 148 ± 10 beats/min. The Hct remained stable at 42 ± 1%. Calculated systemic vascular resistance decreased significantly by 33 ± 4% from 0.241 ± 0.029 to 0.161 ± 0.022 mmHg·min⁻¹·kg⁻¹.

In the four fetal lambs in which left pulmonary arterial blood flow was measured (Fig. 2), pulmonary arterial pressure fell 15 ± 2% from 53 ± 3 to 45 ± 2 mmHg, whereas left pulmonary artery flow increased 157 ± 13% from 23 ± 8 to 60 ± 20 ml·min⁻¹ after infusion of WEB-2170 (49 ± 4 mg/kg, 200-mg total dose) into the pulmonary artery, showing an almost 68% decrease in calculated pulmonary vascular resistance.

In the five fetal lambs that were delivered and ventilated, mean arterial pH after birth was 7.39 ± 0.04 and PO₂ and PCO₂ were 133 ± 13 and 24 ± 3 Torr, respectively. Cardiac output and heart rate were 215 ml·kg⁻¹·min⁻¹ and 138 beats/min, respectively. Figure 3
shows changes in pulmonary arterial and aortic pressures after infusion of WEB-2170 in these five newborn lambs. Aortic pressure fell by 31 ± 3% from 58 ± 4 to 40 ± 2 mmHg. The pulmonary arterial pressure fell by 18 ± 3% from 40 ± 4 to 33 ± 2 mmHg. Calculated systemic and pulmonary vascular resistances (Fig. 4) were 0.284 ± 0.041 and 0.189 ± 0.019 mmHg·min⁻¹·ml⁻¹·kg before infusion of WEB-2170 and changed by 48% to 0.163 ± 0.19 mmHg·min⁻¹·ml⁻¹·kg for systemic resistance and by 30% to 0.132 ± 0.020 mmHg·min⁻¹·ml⁻¹·kg for pulmonary vascular resistance. The postWEB-2170 arterial blood-gas values remained stable at pH 7.32 ± 0.04, PCO₂ 29 ± 4 Torr, and PO₂ 131 ± 18 Torr.

Circulating levels of PAF in fetal lambs, before and after birth. The concentration of PAF in plasma of the lambs 90 min after delivery was 6.43 ± 3.48 ng/ml. The concentration of PAF in plasma of the lambs 90 min after delivery was 1.31 ± 1.03 ng/ml. The concentration of PAF was significantly higher in plasma of fetal lambs than in plasma of the immediate-newborn lambs.

Specificity of WEB-2170

Group I juvenile lambs. In group I juvenile lambs, arterial pH averaged 7.45 ± 0.02 and PO₂ and PCO₂ were 88 ± 12 and 33 ± 3 Torr, respectively, at baseline and did not change significantly throughout the study. The Hct was 32 ± 2%.

Table 1 shows a summary of the mean values for aortic pressure, pulmonary arterial pressure, heart rate, cardiac output, systemic vascular resistance, and pulmonary vascular resistance for baseline, steady-state PAF, and PAF + WEB-2170. Baseline aortic pressure averaged 81 ± 5 mmHg and did not change significantly with PAF infusion or after treatment with WEB-2170. Pulmonary arterial pressure increased significantly with PAF infusion and then fell close to baseline value after infusion of 5 mg/kg WEB-2170. Additional WEB-2170 (50 mg/kg) had no further effect. Baseline heart rate and cardiac output were 122 ± 7 beats/min and 265 ± 20 ml·kg⁻¹·min⁻¹, respectively. There was no significant change in heart rate and cardiac output after the infusion of PAF. However, after infusion of WEB-2170 (5 mg/kg), heart rate and cardiac output increased significantly. Further increases in heartbeat and flow rate were observed with the higher dose of WEB-2170 (50 mg/kg) administered. Heart rate and cardiac output increased with infusion of WEB-2170, especially at the higher dose. Calculated systemic vascular resistance increased by 10% with PAF infusion, whereas calculated pulmonary vascular resistance was nearly doubled. After treatment with WEB-2170 (5 mg/kg) systemic and pulmonary vascular resistances fell to baseline levels. Additional WEB-2170 infusion (50 mg/kg) had no further effect.

Group II juvenile lambs. In group II juvenile lambs, baseline arterial blood pH was 7.45 ± 0.03 and PO₂ and PCO₂ were 36 ± 3 and 77 ± 2 Torr, respectively. During steady-state hypoxia, arterial blood pH was 7.54 ± 0.02, PO₂ and PCO₂ were 37 ± 3 and 25 ± 2 Torr, respectively, and remained unchanged after infusion of WEB-2170. The Hct was 33 ± 1% during the study. Table 2 summarizes the mean values for aortic pressure, pulmonary arterial pressure, heart rate, cardiac output, systemic and pulmonary vascular resistances for baseline, steady-state hypoxia, and hypoxia + WEB-2170. Baseline aortic and pulmonary arterial pressures were similar to the baseline values for group I lambs.
(81 ± 5 and 13 ± 1 mmHg, respectively). With hypoxia, aortic pressure remained unchanged and pulmonary artery pressure was more than doubled. WEB-2170 (50 mg/kg) had no effect on either aortic or pulmonary arterial pressures during hypoxia. Heart rate and cardiac output remained unchanged from baseline values; however, after treatment with WEB-2170, heart rate increased significantly to 163 ± 10 beats/min. Calculated systemic and pulmonary vascular resistances were 0.419 ± 0.052 and 0.065 ± 0.012 mmHg·min·ml⁻¹·kg at baseline. Systemic vascular resistance was unaffected by hypoxia or WEB-2170; however, pulmonary vascular resistance was doubled with hypoxia and remained elevated after treatment with WEB-2170.

Group III lambs. In group III juvenile lambs, mean arterial pH was 7.47 ± 0.03 and P O₂ and P CO₂ were 80 ± 3 and 36 ± 1 Torr, respectively, at baseline and remained unchanged throughout the study period. The Hct was 29 ± 3%. Table 3 summarizes the mean values for aortic pressure, pulmonary arterial pressure, heart rate, cardiac output, systemic pulmonary vascular resistances for baseline, steady-state angiotensin II, and after WEB-2170 (50 mg/kg). During angiotensin II infusion (25–50 µg·kg⁻¹·min⁻¹), mean aortic and pulmonary arterial pressures increased 58 and 54%, respectively, over baseline values. Infusion of WEB-2170 during angiotensin II infusion had no effect on aortic or pulmonary arterial pressures. Mean cardiac output during the study period was 243 ml·kg⁻¹·min⁻¹. Heart rate increased slightly from 110±13 to 138 ± 18 beats/min, but it did not change significantly with angiotensin II infusion. Calculated systemic and pulmonary vascular resistance increased by 59 and 57%, respectively, with angiotensin II infusion. Infusion of WEB-2170 (50 mg/kg) during angiotensin II infusion had no effect on aortic pressure, pulmonary artery pressure, calculated systemic vascular resistance and calculated pulmonary vascular resistance.

**DISCUSSION**

PAF is an important phospholipid mediator synthesized in response to endotoxemia (5) and other endogenous and exogenous stimuli. PAF also causes decreased lung compliance and increased airway resistance (5, 16). Among its other wide-ranging physiological as well as pathological effects in vivo, PAF is a potent vasoactive mediator in the pulmonary and systemic circulations (7, 9). It has been shown that exogenous PAF induces pulmonary and systemic vasoconstriction, when baseline vasomotor tone is low, which can be abolished by administration of a specific PAF-receptor antagonist (26, 35). However, the role of endogenous PAF in modulation of the pulmonary and systemic circulations in the normal fetus and newborn is not well understood and therefore needs further examination.

In this report, we have investigated the modulation of perinatal ovine pulmonary and systemic vasomotor tone by PAF. To study the role of endogenous PAF in the fetus and newborn, we used WEB-2170, a potent and highly specific PAF-receptor antagonist (14, 18, 22) to unmask the physiological role of PAF. Inhibition of endogenous PAF activity in the fetal lamb resulted in a 30% decrease in systemic vascular resistance and a much greater reduction (68%) in pulmonary vascular resistance. This shows that endogenous PAF acts both as a pulmonary and systemic vasoconstrictor, with a much greater role as a pulmonary vasoconstrictor in the fetus. Inhibition of this potent vasoconstrictor at its receptor resulted in a decrease in vasomotor tone. Because the infusion of WEB-2170 did not result in changes in arterial pH, P CO₂, and P O₂, we can conclude that the decrease in systemic and pulmonary vascular tone was not due to changes in blood pH and blood-gas tensions. The dose of WEB-2170 used in our infusion study is five to six times higher than the dose of inhibitors (e.g., indomethacin) usually used to inhibit

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**Table 1. Effect of WEB-2170 on systemic and pulmonary circulations in lambs during PAF infusion**

<table>
<thead>
<tr>
<th>Steady-State Period</th>
<th>HR, beats/min</th>
<th>Pressure, mmHg</th>
<th>CO₂, ml·kg⁻¹·min⁻¹</th>
<th>Vascular Resistance, mmHg·min·ml⁻¹·kg</th>
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<tr>
<td></td>
<td></td>
<td>Aortic</td>
<td>Pulmonary</td>
<td></td>
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<tr>
<td>Baseline</td>
<td>122 ± 7</td>
<td>81 ± 5</td>
<td>13 ± 1</td>
<td>265 ± 20</td>
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<tr>
<td>PAF infusion</td>
<td>146 ± 20</td>
<td>86 ± 6</td>
<td>27 ± 4*</td>
<td>247 ± 4</td>
</tr>
<tr>
<td>PAF + WEB-2170 (5 mg/kg)</td>
<td>165 ± 10†</td>
<td>84 ± 4</td>
<td>16 ± 1</td>
<td>291 ± 24</td>
</tr>
<tr>
<td>PAF + WEB-2170 (50 mg/kg)</td>
<td>183 ± 11†</td>
<td>82 ± 3</td>
<td>16 ± 1</td>
<td>314 ± 45†</td>
</tr>
</tbody>
</table>

Values are means ± SE for four 5- to 7-wk- old lambs. * Different from baseline and PAF, P < 0.05. † Different from baseline and PAF infusion, P < 0.05.

**Table 2. Effect of WEB-2170 on systemic and pulmonary circulations in lambs during hypoxia**

<table>
<thead>
<tr>
<th>Steady-State Period</th>
<th>HR, beats/min</th>
<th>Pressure, mmHg</th>
<th>CO₂, ml·kg⁻¹·min⁻¹</th>
<th>Vascular Resistance, mmHg·min·ml⁻¹·kg</th>
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<tr>
<td></td>
<td></td>
<td>Aortic</td>
<td>Pulmonary</td>
<td></td>
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<tr>
<td>Baseline</td>
<td>120 ± 15</td>
<td>81 ± 5</td>
<td>13 ± 1</td>
<td>208 ± 19</td>
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<tr>
<td>Hypoxia</td>
<td>128 ± 15</td>
<td>94 ± 6</td>
<td>31 ± 1*</td>
<td>250 ± 13</td>
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<tr>
<td>Hypoxia + WEB-2170 (50 mg/kg)</td>
<td>163 ± 10†</td>
<td>90 ± 6</td>
<td>31 ± 1*</td>
<td>247 ± 27</td>
</tr>
</tbody>
</table>

Values are means ± SE for four 5- to 7-wk- old lambs. * Different from baseline, P < 0.05. † Different from baseline and hypoxia, P < 0.05.
other endogenous vasoactive mediators such as prostacyclin or thromboxane A₂. WEB-2170 is a highly lipophilic compound that may be easily absorbed by lipid membranes, thereby making it less available for binding to the PAF receptor. It is possible that the high concentration of WEB-2170 needed in our study resulted in an optimal amount of the drug being available to bind to the PAF receptor. This explanation is plausible because infusion of more antagonist after the maximum effect had been attained did not produce any further decrease in systemic or pulmonary vascular resistances. This demonstrates that the observed effect was due to inhibition of PAF binding to its receptor in the lung rather than due to a nonspecific interaction of WEB-2170 at the PAF receptor.

In a previous report, infusion of exogenous PAF into preterm lambs led to vasodilation of the fetal pulmonary circulation (1). This apparent discrepancy is explainable by the differences in experimental conditions. When we block the effect of endogenous PAF, we are truly unmasking its physiological role. However, infusion of exogenous PAF will merely demonstrate the effect of exogenous PAF on a pulmonary vascular bed that is constricted. There were also differences in gestational age, 122- to 130-day-gestation fetuses employed in the study infusing exogenous PAF (1) as opposed to 139- to 146-day-gestation fetuses used in our study. Furthermore, it is possible that exogenous PAF, by acting directly on the PAF receptor or indirectly by stimulating release of other vasoactive mediators, such as eicosanoids (3, 13, 20, 33) and/or nitric oxide (27), may result in different hemodynamic effects in the pulmonary circulation of the fetus and newborn. For example, in the ferret, PAF induces relaxation of pulmonary arteries but contraction of pulmonary veins (15). We found that circulating levels of endogenous PAF are high in the fetal lamb, whether preterm or near term, and that this level drops significantly after birth. For instance, when we measured plasma PAF concentration in very-preterm lambs (122–123 days gestation; n = 5) that were delivered and mechanically ventilated for <2 h, the pre- and postdelivery plasma PAF levels were 2.09 ± 0.04 SE and 0.32 ± 0.41 ng/ml plasma, respectively. The pre- and postdelivery plasma PAF concentrations in near-term fetal lambs that we studied and have reported here are over three times higher than the plasma PAF concentrations in very-preterm fetal lambs. The higher plasma PAF concentration in near-term fetal lambs compared with that in very-preterm fetal lambs may be one other reason for the difference in our findings from those of Accurso et al. (1). The high circulating plasma level of PAF may be indicative of a high tissue production in the lung of the fetus, which may serve to maintain high pulmonary vascular resistance in utero. A successful transition to the immediate neonatal period is facilitated by a decrease in the endogenous production of this potent vasoconstrictor in the system.

Pathological conditions such as persistent pulmonary hypertension of the newborn (PPHN) are associated with high circulating levels of PAF (7), which fall to the normal range with an improvement in the clinical condition. The concentration of PAF in plasma of PPHN patients was ~10 times more than that in normal controls. However, concentration of PAF in plasma of non-PPHN controls was 1.6 ± 0.07 ng/ml plasma (7) and is similar to the values (1.31 ± 1.03 ng/ml plasma) we obtained in the immediate newborn ovine plasma. Our findings regarding a high circulating level of PAF in the fetus with a significant fall at birth suggest that PAF plays a crucial role in maintaining a high pulmonary vascular resistance in utero.

Pulmonary vascular resistance in utero is high, and pulmonary blood flow is 8–10% of total combined ventricular cardiac output (28, 30). At birth, with the onset of ventilation, pulmonary vascular resistance falls dramatically and pulmonary blood flow increases such that the entire cardiac output passes through the pulmonary circulation. The exact mechanisms controlling these changes are not well understood. Certainly, the change in oxygen tension to which the lung vessels are exposed after birth most likely does play some role. However, changes in production and release of some endogenous vasoactive mediators in the lung may be equally important. For instance, eicosanoids, the metabolites of arachidonic acid, have been shown to play significant roles in the control of pulmonary vasomotion in the fetus and newborn (28). There is an increase in the production of prostacyclin by the lung at the onset of breathing (21), and an increase in nitric oxide, a potent vasodilator, has also been shown to play a significant role in postnatal changes in the pulmonary circulation (12, 24).

Regulation of the fetal and immediate postnatal pulmonary circulation portends a preference for factors producing active vasoconstriction and those producing vasodilation, respectively. In the fetus, it seems that the balance is tipped toward factors producing vasoconstriction. PAF is a potent vasoactive mediator in the pulmonary circulation (5, 8, 16, 23, 32). Our data suggest that PAF is also one of the active vasoconstrictors operating in the fetal pulmonary circulation. Fall...
in pulmonary vascular resistance at birth could result from decreased synthesis of vasoconstrictors and/or increased synthesis of other vasodilators, such as prostacyclin (21) and endogenous nitric oxide (12), or from enhanced removal by catabolism of the vasoconstrictor PAF (29). We have previously reported that oxygenation results in an increase in catabolism of PAF, which may be one additional mechanism by which there is decrease in amount of vasoconstrictors at birth.

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