

## Ovine Maternal and Fetal Circulatory Responses to an Endoperoxide Analog (41475)

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**Abstract.** There are extensive data on the circulatory responses to prostaglandins during pregnancy, but little is known about the precursor endoperoxides. The endoperoxide analog (15S)-hydroxy-9 $\alpha$ ,11 $\alpha$ -(epoxymethano)prosta-5Z,13E-dienoic acid (EPA<sub>1</sub>) was used to evaluate the circulatory effects of endoperoxides in pregnant sheep. Ten near-term ewes and their fetuses were chronically catheterized to permit the measurement of regional blood flows by the radioactive microsphere method. In five sheep a fetal IV bolus injection of 12.5  $\mu$ g/kg EPA<sub>1</sub> produced a significant increase in fetal blood pressure from 43 to 51 mm Hg, vascular resistance of the cotyledons from 0.06 to 0.09 PRU  $\cdot$  kg<sup>-1</sup>, membranes from 0.84 to 3.15 PRU  $\cdot$  kg<sup>-1</sup>, and kidneys from 1.18 to 1.94 PRU  $\cdot$  kg<sup>-1</sup>. In five sheep a 10-min maternal infusion of 0.5  $\mu$ g/kg/min EPA<sub>1</sub> produced a significant increase in blood pressure from 89.0 to 115.0 mm Hg, resistance of the uterus from 0.49 to 1.10 PRU, and kidneys from 0.13 to 0.20 PRU. There was no significant change in maternal cotyledonary resistance. It is evident from these results that EPA<sub>1</sub> causes widespread vasoconstriction. In the placenta, however, there is vasoconstriction on the fetal side but the resistance of the maternal vascular bed is unaltered.

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The endoperoxides are intermediaries in the formation of the prostaglandins, are biologically active, and exert strong and independent pharmacologic and physiologic effects on vascular and respiratory smooth muscle (1). For several years we have investigated the role of prostaglandins in regulating and modulating placental blood flows (2-6). The actions of the endoperoxides are not well documented. The half-life of the endoperoxides is 4 to 5 min (7, 8) and some of the circulatory effects attributed to the prostaglandins may well be due to endoperoxide action (1). The endoperoxides derived from arachidonic acid are prostaglandins G<sub>2</sub> (PGG<sub>2</sub>) and H<sub>2</sub> (PGH<sub>2</sub>). These are powerful vasoconstrictors, bronchoconstrictors, and platelet aggregating agents (7). As there is relatively little information on maternal and fetal cotyledonary responses to endoperoxides, this study was designed to evaluate these responses in pregnant sheep. The substance

used in the experiments was the stable endoperoxide analog (15S)-hydroxy-9 $\alpha$ ,11 $\alpha$ -(epoxymethano)prosta-5Z,13E-dienoic acid (EPA<sub>1</sub>) (9).

**Materials and Methods.** Ten cross-bred sheep were used in this study. Surgical preparation was performed between Day 125 and Day 128 of gestation. Anesthesia was induced with an intramuscular injection of ketamine (10 mg/kg) and atropine (0.6 mg). Anesthesia was maintained with a continuous intravenous infusion of ketamine in normal saline (10 mg/min). A maternal left ventricular catheter was placed via the right carotid artery with the use of local xylocaine to supplement the parenteral anesthesia. A polyethylene catheter (PE200), with an inner polyvinyl catheter (i.d. 0.5 mm), was inserted into the common carotid artery and advanced into the left ventricle. The catheter position was confirmed by the characteristic pressure pattern. Polyvinyl catheters (i.d. 0.5 mm) were placed in superficial peripheral arteries of both maternal hindlimbs and advanced for 20 cm into the femoral artery.

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The maternal abdomen was opened in the midline, the uterus palpated, and a fetal hindlimb identified. The fetal hindlimb artery and vein were catheterized as has been previously described (10). The uterus and abdomen were closed in layers and the catheters were led via a subcutaneous tunnel to a flank incision where they were maintained in a pouch. The left ventricular catheter was secured under an elastic bandage placed around the neck.

The experiments were performed 2 days after the surgical procedures to allow for recovery from surgical stress. The ewe was placed in a stanchion in the laboratory and permitted to stabilize for 2 hr. The maternal and fetal mean arterial blood pressures were measured with a Statham P23Db transducer placed at the level of the scapulothoracic joint. These parameters were displayed on a Beckman R411 recorder with an EO-18 oscilloscope. Maternal and fetal arterial blood gas analysis was performed prior to and at the conclusion of each experiment.

The EPA<sub>1</sub> was prepared as a 1 mg/ml stock solution in ethanol and stored at 4°. This solution is stable for 6 months at 4° (9). On the days of the experiments dilutions were made from the stock solution and kept on ice until the time of injection.

In five of the experiments, EPA<sub>1</sub> was infused into the mother and the maternal circulatory responses were measured by the radioactive microsphere method. In the other five experiments a bolus injection of the same substance was administered to the fetus and regional blood flows were measured with the same techniques.

In the maternal series, an infusion of 0.5  $\mu\text{g}/\text{kg}/\text{min}$  was administered via the left ventricular catheter over a 10-min period. Blood flows to individual organs of the mother were measured by injecting radioactive microspheres into the left ventricle while withdrawing an integrated arterial sample from the femoral arterial catheter at a rate of 2.06 ml/min for 1.5 min in a manner previously described (11). A control observation was made of organ blood flows with the initial isotope injection prior to starting the infusion, and a second observation was made just before the conclu-

sion of the 10-min infusion. Microspheres (3M Company and NEN) were 15  $\mu\text{m}$  in diameter and were labeled with one of the following isotopes: <sup>46</sup>Sc, <sup>57</sup>Co, <sup>85</sup>Sr, <sup>109</sup>Cd. Each injection contained approximately 1.25 million spheres.

In the fetal series of experiments a bolus of 12.5  $\mu\text{g}/\text{kg}$  EPA<sub>1</sub> was injected into the fetal hindlimb vein. Regional blood flows were measured in the control condition (C) and at 5 min after the administration of EPA<sub>1</sub> (T).

At the conclusion of the experiment, the ewe was sacrificed and the kidneys, uterus, and contents were removed. The cotyledons were dissected free from the uterus, and cotyledonary and noncotyledonary tissues were separately assayed. The fetal kidneys were also removed. All tissues were weighed and homogenized with a measured volume of water in a Waring blender. Five aliquots were taken of each homogenate. Each aliquot had a volume of approximately 2 ml and was placed in a preweighed, wide-mouth counting vial which was weighed again. The homogenate reached a height of approximately 1 cm in the vial. Details of these techniques are available in an earlier publication (11). A sample progression was established for each experiment in which standard vials, appropriate to each isotope used in the experiment, were followed by vials containing the blood samples obtained from the integrated arterial sample withdrawal. These were followed by the vials containing the homogenized tissue. All measurements of radioactivity were made with a three-channel, well-type automatic gamma counter (Model 1185 Nuclear Chicago). In experiments in which four isotopes were used, the windows of the gamma counting system were reset and a second pass was made to detect the fourth isotope. The output of the gamma counter was on paper tape which was fed into a Univac 1110 computer via an interactive terminal in our laboratories. Data reduction was performed solving the appropriate algorithms by iterative matrix procedures and programs supplied by our laboratories. The spillover of each isotope in the other channels was determined from the information obtained from

the standard vials, as was the number of counts per minute per microsphere at that time. The data were reduced to the number of spheres per vial of homogenized tissue. The number of microspheres per gram of tissue homogenate was considered to be the average of the number of microspheres per gram observed in the five vials. Blood flow was determined with the equations given by Makowski *et al.* (12). The resistance of the maternal organs was defined as the mean maternal arterial blood pressure divided by the organ blood flow. The resistance of subdiaphragmatic fetal organs was defined as mean fetal arterial pressure minus mean fetal venous pressure, divided by the organ blood flow. Comparisons between the control and test observations were made using a paired *t* test. Results were expressed as mean  $\pm$  SEM.

**Results.** Blood gas analysis indicated a mean maternal arterial pH of  $7.50 \pm 0.12$ ,  $PCO_2$  of  $25.8 \pm 3$  mm Hg and  $PO_2$  of  $75.9 \pm 8$  mm Hg. The blood drawn from the fetal hindlimb artery had a mean pH of  $7.43 \pm 0.06$ ,  $PO_2$  of  $19.8 \pm 2$  mm Hg, and  $PCO_2$  of  $34.6 \pm 5$  mm Hg.

**Maternal series.** The dose of EPA<sub>1</sub> chosen,  $0.5 \mu\text{g}/\text{kg}/\text{min}$ , was similar to the dosage regime used by Wasserman (8) in studying bronchoconstrictor effects in dogs. Observations were obtained in four single and one twin pregnancy. The mean fetal weight was  $3.4 \pm 0.2$  kg. The responses to EPA<sub>1</sub> were as follows. The maternal blood pressure changed from  $88 \pm 5$  to  $115 \pm 5$  mm Hg ( $P < 0.002$ ). This change was apparent within 20–40 sec of commencing the infusion and a hypertensive effect was noted to last for 10–15 min after completion of the infusion. The cotyledonary blood flow was  $165 \pm 34$  (ml/min)/kg fetus before the infusion and  $168 \pm 24$  (ml/min)/kg fetus during the infusion (NS). The renal blood flow was  $688 \pm 24$  ml/min before the infusion and  $621 \pm 62$  ml/min during the infusion (NS). During the infusion of EPA<sub>1</sub> the noncotyledonary blood flow changed from  $212 \pm 42$  to  $120 \pm 30$  ml/min ( $P < 0.05$ ). When resistance was calculated it was found that the cotyledonary resistance did not change during the infusion of EPA<sub>1</sub>. The noncotyledonary uterine resis-

tance changed from  $0.49 \pm 0.08$  to  $1.19 \pm 0.22$  PRU ( $P < 0.01$ ), and renal resistance changed from  $0.13 \pm 0.01$  to  $0.20 \pm 0.03$  PRU ( $P < 0.025$ ). These data indicate that EPA<sub>1</sub> causes maternal hypertension and vasoconstriction in the renal and noncotyledonary circulations.

**Fetal series.** The fetal dose of EPA<sub>1</sub> was chosen empirically to produce a consistent and obvious effect on the fetal blood pressure. Observations were obtained on five fetuses in five sheep. The mean fetal weight was  $3.9 \pm 0.3$  kg. The injection of EPA<sub>1</sub> changed the mean arterial blood pressure from  $43 \pm 1$  to  $51 \pm 1$  mm Hg ( $P < 0.01$ ). The cotyledonary blood flow was  $180 \pm 22$  (ml/min)/kg in the control condition and  $143 \pm 14$  (ml/min)/kg after EPA<sub>1</sub> (NS). EPA<sub>1</sub> caused the membrane blood flow to change from  $14 \pm 1$  to  $5 \pm 1$  (ml/min)/kg ( $P < 0.01$ ) and the renal blood flow to change from  $10 \pm 0.6$  to  $7 \pm 0.8$  (ml/min)/kg ( $P < 0.02$ ). From these data the following resistances were calculated. The cotyledonary resistance changed from  $0.06 \pm 0.01$  to  $0.09 \pm 0.01$  PRU/kg fetus ( $P < 0.01$ ), the membrane resistance changed from  $0.84 \pm 0.14$  to  $3.15 \pm 0.70$  PRU/kg fetus ( $P < 0.01$ ), and the renal resistance changed from  $1.18 \pm 0.10$  to  $1.94 \pm 0.32$  PRU/kg fetus ( $P < 0.02$ ). EPA<sub>1</sub> therefore resulted in fetal hypertension and vasoconstriction in the cotyledonary, membranous, and renal vascular beds.

**Discussion.** The radioactive microsphere method for measuring organ blood flow is a technique which has been well established (12–14). Sources of error are described by Buckberg *et al.* (13). The primary error is that of having insufficient microspheres in the assay sample. This problem was avoided by counting several samples of the individual tissues, and all the arterial blood samples and tissue samples contained more than 400 microspheres.

The cyclic endoperoxides PGG<sub>2</sub> and PGH<sub>2</sub> are the initial intermediary metabolites produced by the cyclooxygenase catabolism of arachidonic acid. These are the precursor substrates for the production of PGI<sub>2</sub>, PGE<sub>2</sub>, PGF<sub>2</sub> $\alpha$ , PGD<sub>2</sub>, and the thromboxanes. The biological half-life of the endoperoxides is 4–5 min and they have been demonstrated to have powerful

constrictor effects on the vascular and airway smooth muscle which is independent of the stable prostaglandins (8, 15, 16). The methylene derivatives of PGH<sub>2</sub> (cyclic ethers) are called cyclic endoperoxide analogs 1 and 2 (8, 17). These substances are stable in aqueous solution and have been shown to be more potent smooth muscle stimulants than the prostaglandins (8).

In both series of experiments the profound vasoconstrictor effects of EPA<sub>1</sub> were clearly evident. These effects were noted to be consistent in all tissues tested except for the maternal cotyledonary circulation, in which the blood flow and resistance were unaltered. The fetal placental circulation was sensitive to the vasoconstrictor effects of EPA<sub>1</sub> and demonstrated a significant increase in vascular resistance. It may be argued that these differing responses of the two placental circulations were a function of the dosage and method of administering EPA<sub>1</sub> in the two sets of experiments. As all the other organs that were studied demonstrated significant vasoconstriction, the failure of the maternal cotyledonary circulation to respond in a similar fashion would indicate an absolute or relative insensitivity of this particular circulatory bed to the effects of EPA<sub>1</sub>. The opposing effects on the two placental circulations is not a unique response. It has been observed in similar experiments using prostaglandin I<sub>2</sub> (6). PGI<sub>2</sub> was noted to cause significant changes in maternal cotyledonary blood flow while fetal cotyledonary blood flow was unaltered. As has been suggested for prostaglandin I<sub>2</sub>, the anomalous responses of the placental vasculature to EPA<sub>1</sub> may be the result of an interaction with other vasoactive substances such as circulating catecholamines.

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