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The Effect of Prostaglandin E_2 and Indomethacin on the Placental Vascular Response to Norepinephrine[1] (40332)

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Various studies provide indirect or direct evidence that prostaglandin E_2 is involved in regulating the maternal placental blood flow (1–5). Terragno *et al.* (1), using anesthetized pregnant dogs, and Venuto *et al.* (2), using anesthetized pregnant rabbits, have demonstrated: (a) that the uteroplacental unit is a rich source of prostaglandin E-like material and (b) that the blockade of prostaglandin synthesis is accompanied by a decrease in the uterine blood flow and a decrease in the concentration of prostaglandin E-like material in the uterine venous blood.

Direct evidence comes from a study previously reported by this laboratory using near-term pregnant sheep (3). In this study the injection of 20 μg/kg prostaglandin E_2 directly into the maternal circulation increased the placental vascular resistance. This increase in placental vascular resistance was due to the uterine contraction induced by prostaglandin E_2 which masked the effect of prostaglandin E_2 on the placental vasculature. When this effect of prostaglandin E_2 on the noncotyledonary uterus was bypassed by administering the drug via the fetal venous catheter, there was a small but significant increase in placental blood flow.

Thus, there appears to be evidence supporting the involvement of prostaglandins in the maintenance of placental blood flow. The mechanisms by which prostaglandins contribute to the control of placental blood flow are not clear. Many investigators have demonstrated that prostaglandins can regulate regional blood flow in a variety of vascular beds by modifying their reactivity to adrenergic stimuli (6–10). However, the modulation of the vascular response to adrenergic stimuli by prostaglandins varies greatly both quantitatively and qualitatively depending upon the species or vascular bed studied. It was therefore the purpose of this study to investigate the possibility that prostaglandins may influence the regulation of blood flow in the near-term ovine placenta by altering the response of the vasculature to catecholamines.

Methods. Eleven pregnant sheep were surgically prepared between Day 125 and Day 135 of gestation. The jugular vein was catheterized and the sheep was sedated with sodium pentobarbital (Nembutal, 10 mg/kg) and a spinal anesthetic (Xylocaine). Xylocaine (3%) was injected subcutaneously in the ventral cervical region to serve as a local anesthetic during the placement of the left ventricular catheter via the carotid artery. The left ventricular catheter consisted of a polyethylene catheter (i.d. 1.6 mm, o.d. 2.0 mm) within which was threaded a polyvinyl catheter (i.d. 0.7 mm, o.d. 1.2 mm) which extended 1 cm from the tip. Correct placement of the left ventricular catheter was confirmed by monitoring the blood pressure recording. A polyvinyl catheter was inserted in a superficial artery of the maternal hindlimb and advanced 20 cm into the femoral artery. In order to monitor amniotic fluid pressure a catheter was secured to the fetal hindlimb via a midline incision in the maternal abdomen. The femoral and amniotic catheters were secured on the side of the abdomen. The left ventricular and jugular catheters were encased in a gauze bandage which was tucked under an elastic bandage wrapped around the neck. The ewes were injected with 200,000 units of penicillin following surgery.

The experiments were performed 2 days after surgery with the ewe standing quietly in a stanchion in the laboratory. At this time the maternal arterial pH of all sheep was not less

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than 7.4. All pressures were monitored with Statham P23Db transducers positioned at the level of the scapulo-humeral joint and recorded by an R411 Beckman recorder. The placental blood flow was measured using the radioactive microsphere technique in which the microspheres were injected into the left ventricle while simultaneously withdrawing an integrated arterial sample from the femoral catheter at the rate of 2.06 ml/min using a Harvard infusion pump as previously described (11). The microspheres (3M Co., New England Nuclear) had a mean diameter of 25 μm and were labeled with one of the following isotopes: ^{125}I, ^{109}Cd, ^{57}Co, ^{46}Sc, or ^{85}Sr. Organ blood flows were measured with the use of microspheres rather than electromagnetic flow probes because the microsphere technique allows the separation of the uteroplacental blood flow, which is measured by the flow meter method, into the individual placental and nonplacental components.

The protocol for all experiments was to measure the blood flow before (control) and 1.5 min after (test) the left ventricular injection of 1 μg/kg norepinephrine (Levophed, Winthrop). The response to norepinephrine was measured 1.5 min following norepinephrine injection because of observations made in pilot experiments in which a uterine arterial flow probe was employed. Uteroplacental blood flow was found to be relatively stable and depressed maximally at 1.5 min post-norepinephrine injection. The animal was allowed to return to control conditions and one of two additional procedures was then performed.

(*1*) *Pretreatment with prostaglandin E_2.* In this series of experiments prostaglandin E_2 was infused continuously into the jugular catheter at the rate of 20 μg/min. Ten minutes after the start of prostaglandin E_2 infusion, blood flows were measured before and after the injection of norepinephrine as previously described. Amniotic fluid pressure was monitored throughout the experiment. If the infusion of prostaglandin E_2 caused an increase in amniotic fluid pressure, the infusion rate was decreased to 10 μg/min. This was done in animal number 2. Five sheep were used in this series.

(*2*) *Pretreatment with indomethacin.* Indomethacin was used to inhibit endogenous prostaglandin synthesis. Venuto *et al.* (2) have reported that the intravenous infusion of 2 mg/kg indomethacin significantly decreased uterine venous prostaglandin E_2 concentration in pregnant rabbits. In this series of experiments, 10 mg/kg indomethacin (Sigma) dissolved in dimethyl sulfoxide (100 mg/ml) was infused into the jugular catheter at a rate of 0.5 ml/min. Twenty minutes later the blood flows were measured before and after the injection of norepinephrine as previously described. Six sheep were used in this series.

At the end of the experiments the ewes were sacrificed and the uterus and contents were removed. The fetus and fetal membranes were removed from the uterus. The placental cotyledons were dissected free from the remaining uterine tissue and were prepared and analyzed for radioactivity in a three-channel Nuclear Chicago 1185 gamma counter in a manner previously described (11). Counts per minute obtained from the gamma counter output were reduced to the number of spheres contained in the sample. Placental blood flows were calculated by the following equation from Makowski *et al.* (12):

$$\text{placental flow (ml/min)} = (\text{spheres in organ}/\text{spheres in reference arterial sample})\,(\text{withdrawal rate}).$$

The resistance was calculated by dividing the mean maternal arterial pressure by the blood flow.

The changes in vascular resistance in response to norepinephrine were expressed as resistance ratios. The resistance ratios were defined as the ratio of the resistance seen in the test condition 1.5 min after norepinephrine injection, to the resistance seen in the control condition. The paired *t* test was used to determine the significance of differences between means. Data are reported as means ± standard errors of the mean.

Results. Part 1: The effect of prostaglandin E_2 infusion on the vascular response to norepinephrine. The effect of norepinephrine on the arterial blood pressure, placental flow, and vascular resistance of five sheep before and after pretreatment with prostaglandin E_2 is given in Table I. Twins occurred in sheep 3 and 4. In these cases the placentas serving each fetus were analyzed separately. The in-

jection of norepinephrine increased the arterial blood pressure by 22% ($P < 0.02$). When these animals were pretreated with prostaglandin E_2 the injection of norepinephrine increased the blood pressure by 14% ($P < 0.02$). The injection of norepinephrine decreased the placental blood flow by 35% ($P < 0.003$). After pretreatment with prostaglandin E_2 the injection of norepinephrine decreased the placental flow by 17% ($P < 0.03$). As seen in Table I, norepinephrine injection increased the placental vascular resistance by 126% ($P < 0.03$). With prostaglandin E_2 pretreatment the injection of norepinephrine increased the placental resistance by 48% ($P < 0.04$). Expressing these changes in placental vascular resistance in response to norepinephrine in terms of resistance ratios, we observed a resistance ratio of 2.27 ± 0.52 without prostaglandin E_2 pretreatment. After pretreatment with prostaglandin E_2, we observed a resistance ratio of 1.47 ± 0.21. This depression of the resistance ratio was significant ($P < 0.03$).

In the present study we observed that the continuous infusion of 20 μg/min prostaglandin E_2 for 10 min caused (a) no change in the maternal blood pressure, (b) a decrease in the placental blood flow, and (c) an increase in the placental vascular resistance.

Part 2: The effect of indomethacin on the placental response to norepinephrine. The effect of pretreatment with indomethacin on the maternal responses to norepinephrine in six sheep is given in Table II. The injection of norepinephrine increased the arterial blood pressure by 9% ($P < 0.007$). When the animals were pretreated with indomethacin the injection of norepinephrine increased the blood pressure by 20% ($P < 0.005$). The injection of norepinephrine decreased the placental blood flow by 44% ($P < 0.02$). Following the pretreatment with indomethacin the injection of norepinephrine decreased

TABLE I. THE EFFECT OF PRETREATMENT WITH 20 μg/min PROSTAGLANDIN E_2 (PGE_2) ON MEAN ARTERIAL PRESSURES, PLACENTAL BLOOD FLOW, AND VASCULAR RESISTANCES BEFORE (C) AND 1.5 min AFTER (T) THE INJECTION OF 1 μg/kg NOREPINEPHRINE IN FIVE NEAR-TERM SHEEP.

	Mean arterial pressures (mm Hg)				Placental blood flow (ml/min)				Placental resistance (mm Hg × min)/ml			
	Before PGE_2		After PGE_2		Before PGE_2		After PGE_2		Before PGE_2		After PGE_2	
Sheep No.	C1	T1	C2	T2	C1	T1	C2	T2	C1	T1	C2	T2
1	95	134	95	115	921	477	627	499	0.103	0.281	0.152	0.230
2	98	102	90	95	1226	1030	873	927	0.080	0.099	0.103	0.102
3a[a]	100	116	92	107	761	549	590	506	0.131	0.211	0.156	0.211
3b					645	446	511	402	0.155	0.260	0.180	0.266
4a[a]	90	110	99	101	411	290	355	312	0.204	0.379	0.279	0.324
4b					530	414	496	433	0.170	0.266	0.200	0.233
5	100	128	105	128	704	174	552	254	0.142	0.736	0.190	0.504
Mean	97	118	96	109	747	483	572	476	0.141	0.319	0.180	0.267
SE	±1.9	±5.8	±2.7	±5.7	±99.2	±106.6	±59.9	±82.9	±0.016	±0.076	±0.020	±0.047
	$P < 0.02$		$P < 0.02$		$P < 0.003$		$P < 0.03$		$P < 0.03$		$P < 0.04$	

[a] Sheep 3 and 4 had twin fetuses, in which case the uterine horns and placentas serving each fetus were analyzed separately.

TABLE II. THE EFFECT OF PRETREATMENT WITH 10 mg/kg INDOMETHACIN (INDO) ON MEAN ARTERIAL PRESSURES, PLACENTAL BLOOD FLOWS, AND VASCULAR RESISTANCES BEFORE (C) AND AFTER (T) INJECTION OF 1 μg/kg NOREPINEPHRINE IN SIX NEAR-TERM SHEEP.

	Mean arterial pressures (mm Hg)				Placental blood flow (ml/min)				Placental resistance (mm Hg × min)/ml			
	Before INDO		After INDO		Before INDO		After INDO		Before INDO		After INDO	
Sheep No.	C1	T1	C2	T2	C1	T1	C2	T2	C1	T1	C2	T2
6	95	100	100	125	954	583	1003	450	0.100	0.172	0.110	0.278
7	106	110	116	124	1642	996	1447	794	0.065	0.110	0.080	0.156
8	90	98	94	104	408	332	312	247	0.221	0.295	0.301	0.421
9	112	119	112	126	461	378	355	305	0.243	0.315	0.315	0.413
10	110	120	118	155	953	604	937	546	0.115	0.199	0.126	0.284
11	80	100	93	125	1179	259	874	173	0.068	0.389	0.106	0.723
Mean	99	108	106	127	933	525	821	419	0.135	0.247	0.173	0.379
SE	±5.2	±4.1	±4.6	±6.7	±188.2	±109.7	±174.8	±93.2	±0.032	±0.042	±0.043	±0.080
	$P < 0.007$		$P < 0.005$		$P < 0.02$		$P < 0.01$		$P < 0.03$		$P < 0.03$	

the placental flow by 49% ($P < 0.01$). As seen in Table II, the injection of norepinephrine caused an increase in the vascular resistance of the placenta by 83% ($P < 0.03$). With indomethacin pretreatment the injection of norepinephrine increased the placental resistance by 119% ($P < 0.03$). When these changes in placental vascular resistance in response to norepinephrine were expressed in terms of resistance ratios, we found a resistance ratio of 2.25 ± 0.70 without indomethacin pretreatment. After pretreatment with indomethacin, the resistance ratio was 2.71 ± 0.84. This increase in the resistance ratio was significant ($P < 0.03$).

The infusion of indomethacin caused a significant increase in placental vascular resistance of 28% ($P < 0.02$).

Discussion. Prostaglandins are lipids which are produced by most cells and appear to act locally (13). These characteristics have made their physiologic actions difficult to describe. There are two aspects to prostaglandin action: (i) the substance may act directly and (ii) they may act to modify the action of other agents. Several investigators have attempted to delineate the role of prostaglandins in the maintenance of vascular homeostasis by describing how exogenous prostaglandins modify the action of systemic vasoactive agents and how the blockade of endogenous prostaglandin synthesis modifies the action of systemic vasoactive agents (8, 14). These observations have been made either using nerve stimulation or exogenous norepinephrine as the primary stimulus. The action of the primary stimulus on the organ in question is observed in the control condition, after the infusion of prostaglandin E_2 and after the infusion of indomethacin. Using this rationale Malik and McGiff (8) have shown that indomethacin potentiates the response of the rabbit kidney to norepinephrine and Fink *et al.* (14) have shown that prostaglandin E_2 depresses the response of the rabbit kidney to norepinephrine. These results have led these investigators to postulate that prostaglandins play a role in the maintenenace of vascular homeostasis in this organ. In the work described here we are concerned only with the rationale and logic behind this type of approach. There is considerable controversy over the factors which regulate renal blood flow and the actual results that are obtained vary depending on the species and preparation used. While investigators may differ as to the role that endogenous prostaglandins play in the renal vasculature they appear to be united in their approval of the logical sequence behind the design of the experiments such as those described above.

Previous studies have shown indirectly that endogenous prostaglandins may be important in the regulation of the blood flow to the pregnant uterus (1, 2). In an attempt to obtain direct evidence of this action we have applied the above rationale to the study of the placental vascular bed in near-term sheep. We have used exogenous norepinephrine as the primary stimulus and have attempted to modulate the response of the uterine vascular bed to the stimulus with exogenous prostaglandin E_2 and with indomethacin.

When the placental vascular bed was pretreated with prostaglandin E_2 we observed an increase in vascular resistance which confirms a previous result from this laboratory (3) which was postulated at that time to be due to the ability of prostaglandin E_2 to produce a uterine contraction. In the first series of experiments we observed that pretreatment with prostaglandin E_2 significantly depressed the placental response to norepinephrine. In the second series of experiments we observed that pretreatment with indomethacin caused a significant increase in the uteroplacental vascular resistance. We also observed that indomethacin significantly increased the placental response to norepinephrine. These data support the conclusions that the placental vascular bed synthesizes prostaglandins and that these substances can suppress the response of that vascular bed to exogenous norepinephrine.

Summary. The vascular response to norepinephrine is expressed in terms of a resistance ratio which is defined as the ratio of the vascular resistance seen 1.5 min after norepinephrine administration to that seen before norepinephrine administration. The injection of 1 μg/kg of norepinephrine to near-term sheep significantly increased the vascular resistance of the placenta to a ratio of 2.27 ± 0.52 (mean ± SEM; $N = 7$). Pretreatment with 20 μg of prostaglandin E_2 per minute significantly decreased the placental response

to norepinephrine to a resistance ratio of 1.47 ± 0.21 which was 65% of the untreated response ($N = 7$). Pretreatment with 10 mg/kg indomethacin significantly increased the placental response to norepinephrine from a resistance ratio of 2.25 ± 0.70 to 2.71 ± 0.84, which is 120% of the untreated value ($N = 6$). Prostaglandin E_2 attenuated the placental vascular response to norepinephrine and indomethacin potentiated this response.

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