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Effect of Norepinephrine on the Ovine Umbilical Circulation (39386)

J. H. G. RANKIN AND T. M. PHERNETTON

Departments of Physiology and Gynecology-Obstetrics, and Wisconsin Perinatal Center, University of Wisconsin, Medical School, Madison, Wisconsin 53706

The autonomic nervous system has a role in the regulation of the placental blood flows in the sheep. One of the most distinctive responses of the uterine vascular bed is a vasoconstriction when exposed to norepinephrine (3). Barrett *et al.* (2) showed that the fetal renal and umbilical circulations both reacted to methoxamine with a decreased flow in the face of an increased arterial pressure, thereby indicating that alpha receptors are present in these vascular beds. In contrast to this report Novy *et al.* (9) observed that the umbilical circulation of the fetal sheep does not show a vasoconstriction in response to norepinephrine. This result is difficult to interpret as it is unlikely that norepinephrine would fail to elicit responses from alpha receptors if these receptors are present in the umbilical circulation. In an attempt to clarify this question we have performed a series of experiments in which we examined the effect of norepinephrine on the renal and placental circulations of near-term sheep fetuses.

Methods. Nine pregnant sheep with gestational ages ranging from 120 to 138 days were used in this study (term = ca. 145 days). The sheep were sedated with Nembutal (5 mg/kg) intravenously and given spinal anesthesia (4–8 mg tetracaine hydrochloride).

Polyvinyl catheters (i.d. 0.58 mm) were placed into a superficial artery of the maternal hind limb and advanced 20 cm at which point the tip was in the maternal femoral artery. The abdomen was opened and catheters of the same type were placed in a fetal hind limb artery and vein in the manner that is described by Rankin *et al.* (10). A catheter was attached to the fetal hind limb so that amniotic fluid pressures could be measured. The maternal left ventricle was catheterized with a combination PE200 catheter encompassing a polyvinyl catheter in a manner previously described (12). An electromagnetic flow transducer was placed on one of the middle uterine arteries in a manner which is described by Raye *et al.* (13). The flow transducer leads, maternal femoral catheter, and fetal catheters were brought to the flank through a subcutaneous tunnel and sealed in a pouch. The maternal carotid catheter was sealed in a bandage around the neck. Each ewe received penicillin 300,000 units and streptomycin 0.5 mg im each day following surgery.

Protocol. One to three days postoperatively the ewes were placed in a stanchion in the laboratory and permitted to stabilize for 2 hr. The fetal venous and arterial and maternal arterial blood pressures were monitored by blood pressure transducers (Statham P23Db) with the zero reference at the level of the scapulo humeral joint. The flow in the middle uterine artery was recorded via a Micron RC1000 flowmeter and all signals were recorded on a Beckman R411 recorder. Fetal and maternal bloods were drawn on several occasions for blood gas determinations. All experiments were performed on animals in which the fetal arterial pH was greater than 7.3.

At the end of the stabilization period the distribution of the maternal and fetal blood flows was measured with 25-μm radioactive microspheres (3M Company) using the method of Makowski *et al.* (8). Spheres labeled with separate isotopes were simultaneously injected into the fetal hind limb vein and the maternal left ventricular catheters. The catheters (dead space = 0.2 ml) were then flushed with 3 ml of saline. At the same time arterial blood was drawn from the fetal hind limb artery and the maternal femoral artery into 2 m of PE205 tubing. The withdrawal rate was 2.1 ml/min for fetal blood and 3.8 ml/min for maternal blood. The withdrawal continued for 45 sec after the completion of the microsphere injection. The blood contained in the PE205 tubing

was then forcibly ejected into wide glass counting vials for subsequent assay.

Norepinephrine (Levophed, Winthrop) in a range of dosages was then injected into the hind limb vein catheter and the catheter flushed with 2 ml of saline. One and a half minutes later the distribution of the fetal and maternal blood flows was measured again with spheres labeled with a further two isotopes. Precautions taken during the handling of catecholamines were based on the recommendations of Barton *et al.* (3). Each preparation was given microspheres with four different radioactive labels, these being ^{141}Ce, ^{85}Sr, ^{125}I, and ^{95}Nb.

It was possible to give several injections of norepinephrine to each sheep in order to observe the pressor and flowmeter responses although only two simultaneous observations of the maternal and fetal blood flows could be made with the microspheres.

In five cases the normal response of the uterine vasculature was established by observing the effect of a test injection of 50 μg of norepinephrine given via the left ventricular catheter on the uterine blood flow as observed with the flowmeter. The alpha receptors of the fetal circulation were then blocked with phenoxybenzamine (Dibenzyline) by administering 25 mg of this agent over a period of 10 min through the hind limb vein catheter. After a delay of 45 min an injection of norepinephrine was then given to the fetus, and the fetal and maternal responses were recorded. The uterine vasculature was again tested with 50 μg of norepinephrine administered via the left ventricular catheter to determine whether or not this circulation was affected by the phenoxybenzamine.

At the end of the experiment the animal was sacrificed, and the maternal kidneys, brain, and uterus were removed for radioactive assay. The uterus was opened and the cotyledons, membranes, and fetal kidneys removed as previously described (11). The tissues were homogenized and aliquots of the homogenate were placed in wide, glass counting vials for assay. The integrated arterial blood samples and vials containing the tissue samples were counted on a three-channel Nuclear Chicago 1185 gamma counter and data reduction performed via an interactive terminal in our laboratory. Details of this technique have been described previously (6). The resistance of the maternal organs was calculated by dividing the maternal arterial pressure by the blood flow to that organ. The resistance of fetal organs was calculated by subtracting the fetal venous pressure from the fetal arterial pressure and dividing the result by the organ blood flow. This procedure does not differentiate between changes in the hepatic resistance and changes in the resistance of the placental vascular beds but Barrett *et al.* (2) have shown that methoxamine does not cause a constriction of the hepatic vasculature. We have therefore assumed that changes in the pressure in the cord veins will be closely mirrored by changes in inferior caval pressure. The statistical significance of the differences between control and treatment means were evaluated by the paired Student's *t* test.

Results. Fetal responses. The responses of the fetal circulations of seven sheep are presented in Table I. It can be seen that the dosage of norepinephrine varied widely. We introduced this variation in an attempt to construct a dose response curve relating the amount of norepinephrine injected to the fetus and the maternal response, but were not able to observe a consistent relationship between the amount of norepinephrine and the response of the mother. The fetal arterial pressure always increased secondary to the injection of norepinephrine to the fetus. Mean fetal arterial pressure in the control period was 36 mm Hg which rose to 67 mm Hg, a change which was highly significant ($P < 0.001$). The fetal venous pressure rose secondary to the injection of norepinephrine into the fetus. The control value was 5.5 mm Hg and the test value was 11 mm Hg ($P < 0.001$). We could observe no change in the amnlotic fluid pressure when norepinephrine was given to the fetus. The transplacental pressure drop was calculated as the fetal arterial minus fetal venous pressure. The mean control value for this parameter was 30.4 mm Hg and it rose to 56 mm Hg secondary to the injection of norepinephrine to the fetus. This difference is significant ($P < 0.001$).

The umbilical blood flow changed when

TABLE I. THE FETAL WEIGHT, AMOUNT OF NOREPINEPHRINE INJECTED, FETAL ARTERIAL MINUS VENOUS PRESSURES AND VASCULAR RESISTANCES OF THE FETAL COTYLEDONARY, RENAL, AND PLACENTAL MEMBRANE CIRCULATIONS AS MEASURED WITH RADIOACTIVE MICROSPHERES BEFORE (CONTROL) AND AFTER (TEST) EIGHT INJECTIONS OF NOREPINEPHRINE TO SEVEN FETUSES.

Sheep and injection number	Fetal weight (kg)	Dose injected (μg/kg)	Pressures (mm Hg) Fetal arterial − Fetal venous		Vascular resistances (mm Hg × min/ml × kg)					
					Umbilical		Renal		Membrane	
			Control	Test	Control	Test	Control	Test	Control	Test
1	3.6	27	37	60	0.220	0.583	6.38	9.00	6.07	4.80
3a	2.5	80	27	56	0.165	0.255	3.75	14.93	3.55	14.00
3b[a]	2.5	120	27	55	0.135	0.374	4.22	13.03	2.81	13.75
4	4.0	50	29	50	0.181	0.385	3.41	14.66	1.35	3.62
5	3.5	29	37	56	0.214	0.675	4.81	11.64	2.19	19.31
6	2.6	77	27	55	0.172	1.309	2.60	21.15	1.71	28.95
7	2.3	22	28	54	0.215	1.200	2.92	18.49	2.15	24.55
8	3.9	26	32	60	0.123	0.870	2.22	27.03	2.12	33.33
Mean	3.1	54	30	56	0.178	0.706	3.79	16.24	2.74	17.79
SD ±	0.7		4.3	3.2	0.037	0.390	1.35	5.78	1.50	10.79
			$P < 0.001$		$P < 0.003$		$P < 0.001$		$P < 0.004$	

[a] Two sets of observations were made with sheep 3.

norepinephrine was injected into the fetus. The control value for the umbilical blood flow was 177 ml/min/kg, and the value obtained after the injection of norepinephrine into the fetus was 105 ml/min/kg fetus. This change was significant ($P < 0.001$). The fall in umbilical blood flow was rendered more impressive when it was considered in terms of the rise in arterial minus venous pressure. The umbilical vascular resistances are given in Table I and changed from a control mean of 0.178 mm Hg × min/ml × kg fetus to 0.706 mm Hg × min/ml × kg fetus after norepinephrine ($P < 0.003$). It can be concluded that the administration of norepinephrine to the fetus causes a vasoconstriction in the umbilical circulation. The renal flow changed from a mean value of 8.75 ml/min/kg to 4.43 ml/min/kg fetus ($P < 0.004$). The decrease in flow and the increase in blood pressure indicated vasoconstriction. There was also vasoconstriction of the fetal membranes in which the flow changed from a mean value of 13.2 ml/min/kg to 5.4 ml/min/kg ($P < 0.008$). Data pertaining to the resistance of the fetal renal and placental membrane vascular beds are given in Table I.

An example of the changes in fetal arterial and venous pressure that we observed is given in Fig. 1. The output of the electromagnetic flowmeter and the maternal arterial pressure are also displayed in the figure. At the right of the figure the unmeaned signals are given at a higher paper speed.

In the five animals to which phenoxybenzamine was given to the fetus, we could observe no fetal pressor response nor change in fetal venous pressure secondary to the injection of norepinephrine. The fetal arterial pressure either remained constant or decreased slightly.

Maternal responses. Thirty-five injections of norepinephrine were given to the fetuses of nine sheep. In 29 cases the uterine blood flow was observed to fall subsequent to the injection of norepinephrine to the fetus. In the six cases in which no responses were observed, five were injections given subsequent to phenoxybenzamine blockade and one was an injection given to a fetus which was severely acidotic. An example of the normal response is shown in Fig. 1. It can be seen that the uterine blood flow begins to fall with the onset of the fetal pressor response and remains at lower than normal levels for the duration of the fetal pressor response. In five cases the regional maternal blood flows were quantitated with radioactive microspheres before and during this response. The changes in the cotyledonary and noncotyledonary uterine flows were compared with the responses of the maternal kidney, heart, and brain. These results

are given in Table II. It can be seen that when norepinephrine was given to the fetus there was no significant change in the maternal arterial pressure, maternal kidney flow, maternal coronary flow, nor maternal brain flow. The noncotyledonary uterine flow did not change significantly. The only significant result was that the cotyledonary blood flow changed from a mean value of 778 ml/min to a value of 611 ml/min at the peak of the response. This change is significant ($P = 0.02$).

The output of the electromagnetic flowmeter and the pressure recordings were used to calculate the uterine vascular resistance before and after the postblockade injection of norepinephrine in the fetuses of sheep 2, 3, 4, 5, and 7. The ratios of uterine resistance before and the uterine resistance after giving norepinephrine to these fetuses were 1.00, 1.17, 0.94, 1.00, and 0.83, respectively, with a mean value of 1.0. These data indicate that there was no change in the uterine vascular resistance subsequent to the injection of norepinephrine to the fetus after the fetal circulation was blocked with phenoxybenzamine.

The response of the maternal placental blood flow to norepinephrine administered to the mother was observed in the same five

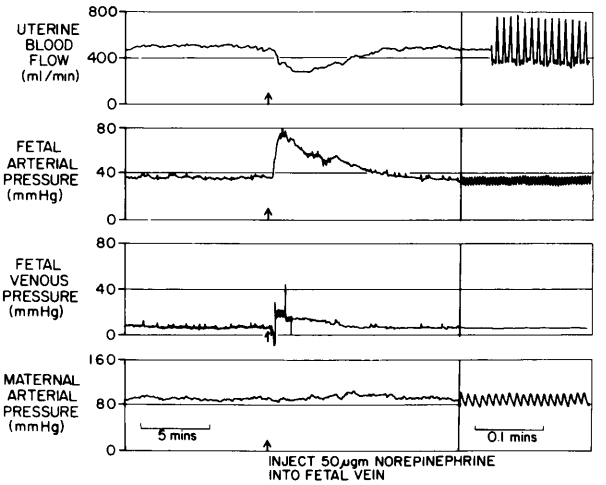

FIG. 1. Effect of administering 50 μg of norepinephrine to the fetus. The uterine blood flow, fetal arterial pressure, fetal venous pressure, and maternal arterial pressure are shown as they were observed in sheep number 7 after the injection of 50 μg of norepinephrine to the fetal vein. The undamped signals are shown in an expanded time frame at the right-hand side.

TABLE II. MEASUREMENT OF RENAL, CORONARY, BRAIN, COTYLEDONARY, AND NONCOTYLEDONARY BLOOD FLOWS MADE WITH RADIOACTIVE MICROSPHERES IN FIVE PREGNANT EWES BEFORE (CONTROL) AND AFTER (TEST) THE ADMINISTRATION OF NOREPINEPHRINE TO THE FETUS.

	Arterial pressure (mm Hg)		Kidney flow (ml/min)		Coronary flow (ml/min)		Brain flow (ml/min)		Cotyledonary flow (ml/min)		Noncotyledonary uterine flow (ml/min)	
Sheep number	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
1	88	88	454	465	318	117	62	53	786	695	148	139
2	81	81	394	580	47	58	27	39	658	355	119	183
5	74	72	930	1010	154	167	51	60	908	886	251	260
6	100	100	540	527	225	232	62	57	522	399	137	178
9	78	72	798	781	–	–	–	–	1017	721	324	425
Mean	84	83	623	673	186	143	51	52	778	611	196	237
SD ±	10	12	250	223	114	74	17	9.3	196	227	88	114
	NS		NS		NS		NS		$P < 0.023$		NS	

sheep. When 50 μg of norepinephrine was given to the maternal left ventricle of these animals before fetal blockade, the uterine blood flow decreased an average of 67%. After the fetal circulation was blocked with phenoxybenzamine, the administration of norepinephrine to the mother caused the uterine flow to decrease an average of 64%. The difference between these two responses was not significant.

Discussion. Archie *et al.* (1) have discussed in depth the validity of the radioactive microsphere technique in the measurement of organ blood flows. Buckberg *et al.* (5) have described the possible errors in the technique and point out that at least 400 microspheres should be in each sample if statistical errors are to be acceptably low. We had at least 400 microspheres in each of our integrated arterial samples and the use of five aliquots of the organ homogenates meant that we had at least 400 microspheres in the total tissue mass assayed from each organ.

It is probable that the lowest dose of norepinephrine used was maximal or supramaximal because all fetuses responded with strong vasoconstriction in the umbilical and renal vascular beds. The dose dependent nature of the response would therefore not be apparent in the range used in this study. The phenoxybenzamine blockade of the fetal circulation was apparently effective at 45 min after the administration of this drug, because norepinephrine did not elicit a pressor response at that time even though complete alpha receptor blockade in the adult requires up to 2 hr.

The fetal pressor response to norepinephrine is in accordance with the results obtained by Novy *et al.* (9). The changes in peripheral resistance that we observed in the fetal circulation are not in agreement with the established literature. We know of no previous description of the effect of norepinephrine on the fetal kidney but we observed a vasoconstriction in this organ when norepinephrine was given to the fetus. This observation confirms the presence of alpha receptors in the fetal kidney as postulated by Barrett *et al.* (2). We found that the umbilical vascular bed responded to norepinephrine with vasoconstriction which is also in agreement with the work of Barrett *et al.* (2) but not in agreement with the work of Novy *et al.* (9).

There are three possible reasons for this disagreement. In the first case the dose levels were different. Novy *et al.* used lower dose levels than were used in our studies and the responses may not be observable in the lower range. In the second case, Novy *et al.* worked with acute preparations which may have already been under maximum catecholamine stimulation. A third possible reason for the discrepancy is that prostaglandins seem to act as modulators of autonomic effector sites. Prostaglandins of the E series tend to diminish the cardiovascular responses to norepinephrine (4, 7). In a previous series we found that in adult sheep the norepinephrine-induced uterine placental vasoconstriction was severely depressed for over an hour after the administration of prostaglandin E_2. This depression of alpha adrenergic activity persisted long after the blood pressure and heart rate had returned to normal. Novy *et al.* gave PGE_2 and norepinephrine to each animal and the responses to norepinephrine would have been abnormally small in those cases where PGE_2 administration preceded the norepinephrine administration.

The changes in the uterine blood flow that we observed secondary to the injection of norepinephrine to the fetus are purely local phenomena. Concomitant changes are not seen in the maternal blood pressure or in the maternal brain, kidney, coronary, or even noncotyledonary uterine flow.

The mechanism of this change in uterine blood flow is not as obvious as would first appear. The first conclusion upon examining the data is to ascribe the change in the uterine flow to the transplacental flux of small amounts of norepinephrine which locally affected alpha recep tors in the maternal uterine circulation. If this had happened then giving phenoxybenzamine to the fetus would have either had no effect on the uterine response to fetal norepinephrine or blocked part of the uterine vasculature. We observed that phenoxybenzamine abolished the uterine response to fetal norepinephrine but caused no change in the uterine response to maternal norepinephrine. We conclude that the response of the uterine vascular bed to the injection of norepineph-

rine to the fetus may not be due to the direct action of norepinephrine on the uterine vasculature.

Summary. The administration of norepinephrine (50 μg/kg) to the fetuses of nine sheep caused an increase in fetal arterial pressure (Control = 30 mm Hg; Test = 56 mm Hg; $P < 0.001$) and vasoconstriction in the fetal kidney (Control = 3.79 mm Hg × min/ml × kg; Test = 16.24 mm Hg × min/ml × kg; $P < 0.001$), placental membranes (Control = 2.74 mm Hg × min/ml × kg; Test = 17.79 mm Hg × min/ml × kg; $P < 0.004$), and fetal cotyledons (Control = 0.178 mm Hg × min/ml × kg; Test = 0.706 mm Hg × min/ml × kg; $P < 0.003$). These results indicate the presence of alpha receptors in these organs. The uterine blood flow fell subsequent to the injection of norepinephrine to the fetus and this response was abolished by phenoxybenzamine blockade of the circulation of four fetuses even though we could demonstrate no phenoxybenzamine blockade in the uterine circulation of these animals.

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