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Alpha and Angiotensin Receptor Tone in the Near-Term Sheep Fetus[1] (40163)

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The placental circulation in the near-term sheep has been shown to be sensitive to alpha receptor stimulation (1, 2) indicating the existence of functional alpha receptors. Similarly angiotensin II has been detected in fetal blood in near-term lambs (3, 4). The presence of functioning alpha and angiotensin receptors in the near-term fetus might be expected. The particular role that these receptors play in maintaining the homeostasis of the resting near-term fetus is not so clear. Vapaavouri *et al.* (5) have shown that alpha receptor blockade produces cardiovascular changes in the near-term sheep fetus but it is not clear from this work that the vascular tone of the umbilical circulation is under any alpha receptor control near term. The most powerful known vasoconstrictor is prostaglandin E_2 (6, 7) which is usually thought of as a vasodilating agent. It is possible that the prostglandin E_2 induced umbilical vasoconstriction may be due to the activation of alpha or angiotensin receptors. We therefore have initiated a series of experiments to determine the effects of alpha and angiotensin receptor tone on the placental circulation of near-term sheep fetuses and to test the hypothesis that prostaglandin E_2 activates these receptors.

Methods. Surgical preparation. All experiments were performed in chronically catheterized awake near-term sheep between 120 and 130 days of gestation. The sheep were sedated with intravenous Nembutal (10 mg/kg) supplemented with spinal anesthesia (pontocaine, 6 mg). Catheters were inserted in a maternal jugular vein and femoral artery. The abdomen was opened in a midline incision. A portion of the uterus containing the fetal hindlimb was exteriorized. A hindlimb artery and vein were catheterized using polyvinyl catheters (id 0.7 mm, od 1.2 mm). The catheters were inserted for 20 cm. The incision in the fetal hindlimb was closed and the limb was returned to the uterus. The uterine incision was closed and the catheters exteriorized to the side of the abdomen and filled with 200 units of heparin in normal saline. The midline incision was closed. All experiments were performed 48 hours later with the sheep standing quietly in a stanchion in the laboratory. All procedures were performed in preparations in which the maternal arterial pH was greater than 7.4 and the pH of the blood from the catheter in the fetal hindlimb artery was greater than 7.31.

Measurement of blood flow. Placental blood flows were measured by injecting 25 micron radioactive microspheres (3M Co.) into the fetal venous circulation while withdrawing an integrated arterial sample from the catheter in the fetal hindlimb artery at 2.06 ml/min. The withdrawal commenced before, and was terminated 2 min after, the injection of spheres. Microspheres labelled with ^{85}Sr, ^{125}I, ^{141}Ce or ^{46}Sc were available for use in this study. At the end of the experiment the animal was killed with intravenous Nembutal followed by intracardiac KCl. The placenta was removed and homogenized with known quantities of water. Five 2 ml aliquots of the homogenate were placed in wide mouth glass counting vials and assayed for radioactivity in a three-channel Nuclear Chicago gamma counter. The samples were preceded by the integrated arterial blood samples and standards containing a known number of each of the microspheres for the appropriate isotope. Blood flow was calculated by the method described by Makowski *et al.* (8). The resistance of the umbilical circulation was defined as the mean fetal arterial pressure/the placental umbilical blood flow. The mean fetal arterial pressure was measured using P23Db Statham strain gauges mounted at the level of the maternal scapulo-humeral joint. The

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mean fetal arterial pressure was defined as the mean pressure as measured with the fetal arterial catheter minus the mean fetal venous pressure although it should be realized that umbilical venous pressure will be slightly greater than inferior caval venous pressure. These techniques have been described elsewhere (6, 9).

Alpha receptor blockade. In this series the adult sheep were treated with 5 mg/kg phenoxybenzamine administered via a jugular catheter and the fetus received 3 mg/kg phenoxybenzamine via a catheter in the fetal vein. All infusions were made over a 30-min period and were completed more than 2 hr before the measurements were made. Experiments were performed on five fetuses in five ewes.

Angiotensin receptor blockade. In this series the maternal and fetal circulations were treated with [Sar^1, Ile^8] angiotensin II (Beckman) which is a potent angiotensin inhibitor (8). This agent was infused in the maternal jugular catheter at a rate of 0.40 μg/min/kg and into the catheter in the fetal vein at the rate of 2.4 μg/min/kg. Umbilical vascular resistance was measured 15 min after the initiation of the infusion of the angiotensin receptor inhibitor. The fetal umbilical circulation is close to the maternal placental circulation and it was not clear to us that treatment of the fetal circulation alone would result in complete blockade of the umbilical circulation. For this reason we infused the appropriate blocking agent into the maternal and fetal circulations. This procedure ensured that adequate blockade of the umbilical circulation was produced. Experiments were performed on seven fetuses in seven ewes.

Efficacy of the blockade. The efficacy of the angiotensin and alpha receptor blockade was tested in all animals by observing the fetal pressor responses to the intravenous injection of 3 μg/kg angiotensin II or 3 μg/kg norepinephrine before and after the administration of the appropriate blocking agent.

Effect of the blockade on umbilical vascular responses to prostaglandin E_2. In all animals we examined the effect of the receptor blockade on the umbilical vascular response to the administration of 15 μg/kg prostaglandin E_2 given via the fetal hindlimb vein. The measurements of blood flow were made 1.5 min after the injection of prostaglandin E_2 as previously reported (6).

The effects of receptor blockade were evaluated with the unpaired *t* test. The responses to prostaglandin E_2 were evaluated using the paired *t* test.

Results. A potential source of error in the microsphere method is increased variability due to an inadequate number of microspheres. This error is described by Buckberg *et al.* (10). In the experiments reported here there were more than 400 spheres in all integrated arterial blood samples and more than 400 spheres in all placental samples.

In all preparations we were able to demonstrate that the pharmacological blockade was complete. The administration of phenoxybenzamine abolished the pressor response seen after the injection of norepinephrine and the administration of [Sar^1, Ile^8] angiotensin II abolished the pressor response seen after the injection of angiotensin II.

Effect of alpha receptor blockade. The effect of alpha receptor blockade on mean fetal arterial pressure and umbilical blood flow is given in Table I. It can be seen that the alpha receptor blockade decreased the mean fetal arterial pressure from 46 ± 1 to 44 ± 2 mm Hg (NS). The resistance of the cotyledonary vascular bed decreased from a mean value of 0.291 ± 0.01 mm Hg × min/ml × kg to 0.245 ± 0.04 mm Hg × min/ml × kg. This change was not significant.

Effect of angiotensin receptor blockade. These results are also given in Table I. It can be seen that the mean fetal arterial blood pressure was 43 ± 3 mm Hg in the test condition which is not significantly different from the control value. The cotyledonary vascular resistance was 0.282 ± 0.03 mm Hg × min/ml × kg in the test condition. This value was not significantly different from the control value.

Effect of blockade on responses to prostaglandin E_2. Nine observations of the effects of phenoxybenzamine blockade on the umbilical responses to prostaglandin E_2 were made. Two observations were made on fetuses 10, 11, 13 and 14. In this series the umbilical vascular resistance increased to 0.98 ± 0.28 mm Hg × min/ml × kg ($P < 0.001$) and the blood pressure increased to 49 ± 4 mm Hg ($P < 0.002$). Phenoxybenzamine

TABLE I. EFFECT OF ALPHA AND ANGIOTENSIN RECEPTOR BLOCKADE ON MEAN FETAL ARTERIAL PRESSURE AND UMBILICAL VASCULAR RESISTANCE IN THE NEAR-TERM SHEEP FETUS.

Control condition			Alpha receptor blockade			Angiotensin receptor blockade		
Fetus	Pressure (mm Hg)	Resistance (mm Hg × min/ml × kg)	Fetus	Pressure (mm Hg)	Resistance (mm Hg × min/ml × kg)	Fetus	Pressure (mm Hg)	Resistance (mm Hg × min/ml × kg)
1	40	0.271	8	44	0.299	15	40	0.412
2	51	0.273	9	47	0.293	16	40	0.263
3	49	0.328	10	51	0.290	17	36	0.237
4	53	0.369	11	43	0.255	18	41	0.209
5	42	0.241	12	46	0.301	19	38	0.197
6	46	0.231	13	38	0.106	20	53	0.340
7	45	0.317	14	42	0.275	21	55	0.313
Mean	46	0.291		44	0.245		43	0.282
SEM	±1	±0.01		±2	±0.04		±3	±0.03

blockade did not change the umbilical vasoconstriction seen after prostaglandin E_2 (cf 6). Nine observations were made of the effect of angiotensin II receptor blockade on the umbilical response to prostaglandin E_2. Two observations were made on fetuses 5 and 12. In this series the placental resistance increased to 1.10 ± 0.24 mm Hg × min/ml × kg ($P < 0.001$) and the blood pressure increased to 47 ± 3 mm Hg ($P < 0.001$). The blockade of angiotensin II receptors did not change the umbilical vasoconstriction seen after the injection of prostaglandin E_2 (cf 6).

Discussion. It is apparent from the literature that the near-term sheep fetus possesses functioning alpha and angiotensin II receptors in the umbilical circulation and it is probable that these receptors play a role in the response of the near-term fetus to stress (1–3, 5). Under normal circumstances the near-term fetus is relatively unstressed and the experiments described here provide information as to the role that alpha and angiotensin receptor tone plays in the maintenance of the umbilical circulation in that condition. The results pertaining to the responses of the umbilical circulation to alpha receptor blockade indicate that alpha receptors do not play a significant role in maintaining the tone of the umbilical vascular circulation of the unstressed near-term fetus.

We are not aware of previous descriptions of the effect of angiotensin receptor blockade on the fetal circulation. The agent used in this study may have had some agonist activity (11) but it is still probable that the absence of a significant change in umbilical vascular resistance after angiotensin receptor blockade indicates that angiotensin II does not play a major role in maintaining the tone of the umbilical circulation in the near-term fetus.

The placenta is known to produce relatively large quantities of prostaglandins of the E series (12, 13) and it has previously been shown (6, 7) that prostaglandin E_2 produces strong umbilical vasoconstriction. It was possible that this effect was caused by the secondary release of catecholamines or angiotensin II but the experiments reported here indicated that the umbilical vasoconstriction seen after the injection of prostaglandin E_2 was not abolished by alpha receptor blockade as previously suggested (7), nor by angiotensin II receptor blockade.

It is apparent that alpha and angiotensin receptor tone do not play a major role in the control of the umbilical vascular bed in the unstressed fetus and that umbilical circulation is capable of responding to prostaglandin E_2 in the presence of alpha and angiotensin receptor blockade.

Summary. We have described the effects of alpha and angiotensin receptor blockade on the mean arterial blood pressure and umbilical vascular resistance of the near-term sheep fetus. In nine control fetuses the mean arterial pressure was 46 ± 1 mm Hg and the umbilical vascular resistance was 0.291 ± 0.01 mm Hg × min/ml × kg. Alpha receptor blockade decreased the mean fetal arterial pressure to 44 ± 2 mm Hg (NS). The umbilical vascular resistance decreased to 0.245 ± 0.04 mm Hg × min/ml × kg ($N = 5$). This change was not significant. The effect of angiotensin re-

ceptor blockade on the umbilical vascular resistance was tested in seven near-term fetuses. The mean fetal arterial pressure dropped to 43 ± 3 mm Hg (NS). The umbilical vascular resistance was 0.285 ± 0.03 mm Hg × min/ml × kg. This change was not significant. Alpha and angiotensin receptor blockade did not abolish the umbilical vasoconstriction that was seen after the injection of prostaglandin E_2.

We conclude that in the near-term sheep fetus neither alpha receptors nor angiotensin II receptors play a significant role in maintaining the tone of the umbilical vascular bed and that the prostaglandin E_2 induced umbilical vasoconstriction is not due to the activation of alpha or angiotensin receptors.

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