PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 158, 54–58 (1978)

Effect of Angiotensin II on Ovine Placental Blood Flow[1] (40138)

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Angiotensin II is one of the most potent vasoconstrictors known (1). It is therefore surprising that in the pregnant uterus angiotensin II has been shown to act as a vasodilator (2, 3) or to have no effect on the vascular resistance (4, 5) of that organ. It has been suggested that the angiotensin II induced decrease in uterine vascular resistance is mediated by prostaglandins, particularly those of the E series (5). In contrast to these results, Greiss and VanWilkes (6) and Cohen et al. (7) have observed an increase in uterine vascular resistance in response to angiotensin II. In view of these conflicting reports we have performed a series of experiments designed to determine the effect of angiotensin II on the uterine vascular resistance in near-term sheep and the role that endogenous prostaglandins play in this action.

Methods. Surgery was performed on seven ewes between day 125 and 128 of gestation. The ewes were sedated with intravenous Nembutal (Abbott Labs) supplemented with local xylocaine (Pontocaine, Winthrop Labs). Polyvinyl catheters (id 0.5 mm) were placed in the maternal jugular vein and the femoral artery and the left ventricle via the carotid artery. The position of the left ventricular catheter was verified at the time of surgery and before the experiment. The carotid artery and jugular vein catheters were secured to the animal's neck using an elastic bandage. The femoral artery catheter was led to a flank incision and secured in a pouch. All experiments were performed 2 days after surgery with the animal standing quietly in a restraining cage. Blood pressure was measured using a Statham P23Db transducer positioned at the scapulo–humeral joint and recorded using an R411 Beckman recorder. Regional blood flows were measured by the left ventricular injection of 25 micron spheres (3M Co., New England Nuclear) suspended in 10% Dextran. The spheres were labelled with one of the following isotopes: ^{125}I, ^{57}Co, ^{85}Sr or ^{46}Sc. The microspheres were injected under control conditions and 1.5 min after the bolus injection of 10 μg of angiotensin II into the left ventricle. An integrated arterial blood sample was simultaneously withdrawn from the femoral artery at a rate of 2.1 ml/min starting at the time of the injection of the spheres and continuing for 1.5 min. All animals had returned to control conditions within one hour.

Ten milligrams per kilogram of indomethacin (100 mg indomethacin/ml of dimethyl sulfoxide) was then infused into the jugular vein. Twenty minutes after the end of infusion the response to angiotensin II was measured as above.

At the end of the experiment the animals were sacrificed. The kidneys and uterus were removed. The cotyledons were separated from the rest of the uterine tissue. The cotyledons and noncotyledonary tissues were weighed and homogenized in a known amount of water in a Waring blender. Five 2 ml aliquots of each were taken from the blender and placed in wide mouth counting vials. The vials were placed on a well-type three channel Nuclear Chicago automatic gamma counter (model 1185) and counted for 10 minutes or until 100,000 counts were obtained.

A standard vial was prepared for each isotope used. The standard consisted of a known number of spheres embedded in wax approximately 0.5 cm from the bottom of the vial. The standard vials allowed determination of the spillover between the various channels and the number of counts per minute per sphere. This information, together with the number of counts in each sample and the degree of dilution of each sample were used to determine the blood flow to each organ by using the equations determined

[1] Supported by NICHD Grant No. HD06736.

0037-9727/78/1581-0054$01.00/0

by Makowski *et al.* (8). A resistance ratio was used to study the effects of angiotensin II on each organ. The ratio is defined as the resistance after treatment with angiotensin II (test) divided by the resistance before treatment with angiotensin II (control). All results are expressed as the mean ± the SE of the mean. Student's *t* test for paired data was used to determine the statistical significance of differences between the means.

Results. Seven experiments were performed on five ewes, two of which had twin fetuses (ewes 1 and 5). The arterial blood samples and the samples from each organ contained more than 400 spheres thereby satisfying the criteria set forth by Buckberg *et al.* (9).

The bolus injection of 10 μg angiotensin II caused the maternal blood pressure to change from 93.4 ± 10 to 113.2 ± 10 mmHg ($P < 0.007$). The uterine blood flow decreased from 756.3 ± 80 to 505.8 ± 77 ml/min ($P < 0.003$). The changes in cotyledonary vascular resistance are given in Table I and it can be seen that angiotensin II caused a vasoconstriction in the cotyledonary vascular bed ($P < 0.004$). After pretreatment with indomethacin the injection of angiotensin II caused the maternal blood pressure to change from 109.8 ± 13 to 136.0 ± 14 mm Hg ($P < 0.003$). The uterine blood flow changed from 696.1 ± 64 to 536.1 ± 90 ml/min ($P < 0.003$). The changes in cotyledonary vascular resistance are shown in Table I and after pretreatment with indomethacin we observed that angiotensin II caused significant vasoconstriction of the cotyledons ($P < 0.012$). The resistance ratio of the cotyledons before treatment with indomethacin was 1.905 ± 0.15 and that after treatment with indomethacin was 1.753 ± 0.20. The difference between the values was not significant.

The responses of the noncotyledonary part of the uterine vascular bed are given in Table II. The resistance increased from 0.618 ± 0.03 to 1.422 ± 0.15 (mmHg × min)/ml ($P < 0.0004$) after treatment with angiotensin II. Pretreatment with indomethacin changed the resistance ratio from 1.141 ± 0.11 to 3.683 ± 0.54 ($P < 0.0008$). Indomethacin augmented the response of this tissue to angiotensin II by 141% ($P < 0.03$).

The responses of the renal vascular bed are given in Table III. Angiotensin II increased the resistance from 0.154 ± 0.02 to 0.263 ± 0.04 (mmHg × min)/ml ($P < 0.004$). After pretreatment with indomethacin the resistance increased from 0.240 ± 0.03 to 0.611 ± 0.07 (mmHg × min)/ml ($P < 0.003$). Indomethacin increased the resistance ratio of the renal vasculature from 1.71 ± 0.22 to 2.66 ± 0.75 ($P < 0.02$).

Discussion. Blood flows can be measured with radioactive microspheres or electromagnetic flowmeters. The microsphere method was chosen for these protocols because it permits the measurement of the cotyledonary, noncotyledonary and renal blood flows thereby allowing a comparison of the responses of each tissue. An electromagnetic flowprobe would have provided information

TABLE I. VASCULAR RESISTANCE OF THE COTYLEDONS OF 7 PLACENTAS IN 5 SHEEP BEFORE AND AFTER THE ADMINISTRATION OF 10 μg ANGIOTENSIN II BEFORE (C1, T1) AND AFTER PRETREATMENT WITH 10 mg/kg INDOMETHACIN (C2, T2). THE RESISTANCE RATIOS ARE ALSO GIVEN.

	Resistance (mmHg × min)/ml				Resistance ratios	
Sheep no.	Before Indo.		After Indo.		Before Indo.	After Indo.
	C1	*T1*	*C2*	*T2*	*T1/C1*	*T2/C2*
1a	0.137	0.280	0.199	0.282	2.044	1.417
1b	0.102	0.190	0.143	0.186	1.863	1.301
2	0.160	0.262	0.203	0.357	1.638	1.759
3	0.243	0.516	0.208	0.463	2.123	2.226
4	0.127	0.333	0.183	0.495	2.622	2.705
5a	0.089	0.131	0.107	0.154	1.472	1.439
5b	0.085	0.134	0.109	0.155	1.576	1.422
Mean	0.135	0.264	0.164	0.299	1.905	1.753
SEM	±0.02	±0.05	±0.02	±0.05	±0.15	±0.20
	$P < 0.004$		$P < 0.012$		NS	

TABLE II. VASCULAR RESISTANCE OF THE NONCOTYLEDONARY UTERINE TISSUE SERVING 6 PLACENTAS BEFORE AND AFTER THE ADMINISTRATION OF 10 μg ANGIOTENSIN II BEFORE (C1, T1) AND AFTER PRETREATMENT WITH 10 mg/kg INDOMETHACIN (C2, T2). THE RESISTANCE RATIOS ARE ALSO GIVEN.

	Resistance (mmHg × min)/ml				Resistance ratios	
Sheep No.	Before Indo.		After Indo.		Before Indo.	After Indo.
	C1	*T1*	*C2*	*T2*	*T1/C1*	*T2/C2*
1a	0.701	1.914	1.124	2.857	2.730	2.542
1b	0.640	1.762	0.965	2.143	2.730	2.221
2	0.740	1.786	1.008	3.200	2.414	3.175
3	0.564	1.209	0.676	2.766	2.144	4.092
4	0.547	1.121	1.284	3.628	2.049	2.826
5	0.554	0.943	1.455	5.000	1.702	3.436
Mean	0.618	1.422	1.141	3.683	2.270	3.212
SEM	±0.03	±0.15	±0.11	±0.54	±0.15	±0.28
	$P < 0.001$		$P < 0.001$		$P < 0.03$	

TABLE III. RENAL VASCULAR RESISTANCES OBSERVED IN 5 SHEEP BEFORE AND AFTER THE ADMINISTRATION OF 10 μg ANGIOTENSIN II BEFORE (C1, T1) AND AFTER (C2, T2) PRETREATMENT WITH 10 mg/kg INDOMETHACIN. THE RESISTANCE RATIOS ARE ALSO GIVEN.

	Resistance (mmHg × min)/ml				Resistance ratios	
Sheep No.	Before Indo.		After Indo.		Before Indo.	After Indo.
	C1	*T1*	*C2*	*T2*	*T1/C1*	*T2/C2*
1	0.142	0.247	0.292	0.538	1.739	1.842
2	0.227	0.403	0.319	0.658	1.775	2.063
3	0.133	0.259	0.227	0.839	1.947	3.696
4	0.170	0.229	0.210	0.565	1.347	2.690
5	0.101	0.178	0.152	0.458	1.762	3.013
Mean	0.154	0.263	0.240	0.611	1.714	2.661
SEM	±0.02	±0.04	±0.03	±0.06	±0.10	±0.33
	$P < 0.004$		$P < 0.003$		$P < 0.02$	

on flow changes to the combined cotyledonary and non-cotyledonary tissues only. The microsphere method suffers from the disadvantage that time dependent changes cannot be followed. Our results should be interpreted with this limitation in mind. We measured the responses to angiotensin II 90 sec after the administration of that drug because preliminary experiments had shown that the blood pressure and uterine blood flow were relatively stable at that time. Variance between animals was minimized by making observations at 90 sec because the angiotensin II induced depression of the uterine blood flow was at its maximum 90 sec after the injection of the angiotensin II.

The administration of indomethacin increased the cotyledonary vascular resistance, a result which confirms the earlier work of Terragno *et al.* (5). These authors assumed that the vasoconstricting effect of indomethacin was due to its ability to suppress prostaglandin synthesis. In the interpretation of our results we have also assumed that the effects of indomethacin on the responses to angiotensin II are due to the blockade of endogenous prostaglandin synthesis.

The effects of angiotensin II on the vascular resistance of the pregnant uterus are unclear at this time. Terragno *et al.* (5) have reported that angiotensin II produces no significant change in the uterine vascular resistance in pregnant dogs and this result has been supported by Ladner *et al.* (4) in anesthetized near-term sheep. In contrast to these results Ferris observed a decrease in the uter-

ine vascular resistance in pregnant nephrectomized rabbits after the injection of angiotensin II and Assali and Westerstein reported similar results in sheep (2). Opposite results are reported by Greiss and VanWilkes who found that angiotensin II increased the resistance of the uterine vasculature in the near-term anesthetized sheep (6). These results were confirmed by Cohen *et al.* (7) in both pregnant and non-pregnant rabbits, and Tulenko *et al.* (10) have shown that angiotensin II can cause vasoconstriction of human placental blood vessels.

In the experiments reported here we have shown that 10 μg angiotensin II increased the resistance of the cotyledonary and noncotyledonary uterine vascular beds of near-term sheep. We conclude that angiotensin II acts as a vasoconstricting agent in the vasculature of the pregnant ovine uterus.

Some investigators who did not observe vasoconstriction in the placental vascular bed in response to angiotensin II attributed this anomalous response to the action of endogenous prostaglandins of the E series (5). These authors postulated that angiotensin II caused the release of E prostaglandins which masked the effect of angiotensin II in this vascular bed. Prostaglandins of the E series have been shown to have a dilating action on the uterine vasculature (11) and in fact Gimbrone and Alexander (12) and Ferris *et al.* (3) have both shown that angiotensin II causes the secondary release of E prostaglandins. If this interpretation of the literature is correct then we would expect that the blockade of endogenous prostaglandin synthesis would potentiate the effects of angiotensin II in the placental vascular bed. In the studies reported here we used indomethacin to block endogenous prostaglandin synthesis and we examined the change in vascular resistance induced by 10 μg of angiotensin II before and after pretreatment with indomethacin. We found that pretreatment with indomethacin did not affect the response of the cotyledonary vascular bed to angiotensin II. Pretreatment with indomethacin potentiated the response of the renal and non-cotyledonary uterine vasculature to angiotensin II.

The potentiation of the renal vascular response to angiotensin II by indomethacin is established in the literature (13) and was observed in our preparation. We also observed an equivalent response in the noncotyledonary uterine circulation. This concordance with the literature is evidence that if such a response had occurred in the cotyledonary vasculature then we would have observed it under the conditions of this experiment.

In our experiments indomethacin did not increase the response of the cotyledonary vascular bed to angiotensin II and we conclude that such a potentiation did not occur. The results of the work of Terragno *et al.* (5) indicate that we should have seen an increased vasoconstriction after angiotensin in the indomethacin treated animals. Our inability to observe this potentiation may have been due to the particular conditions of our experimental series. It is possible that indomethacin may modulate the responses to concentrations of angiotensin II which were not represented in our protocols.

Summary. The injection of 10 μg angiotensin II to 5 near-term sheep caused significant increases in the renal, uterine and placental vascular resistances. After pretreatment with 10 mg/kg indomethacin the injection of 10 μg angiotensin II caused a significantly greater change in the vascular resistance of the kidneys and uterus. The response of the placental vasculature to angiotensin II was not affected by the pretreatment. Endogenous prostaglandins may play a role in modulating the response of the renal and uterine vascular beds to angiotensin II but this study provides no evidence to support the hypothesis that prostaglandins modulate the placental response to angiotensin II.

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Received October 21, 1977. P.S.E.B.M. 1978, Vol. 158.