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The Effect of Several Vasoactive Drugs on Ovarian Blood Flow in the Near-Term Sheep[1] (40150)

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Radioactive microspheres have been used to measure the magnitude and distribution of ovarian blood flow (1, 2). Most of these observations have been made during the estrous cycle or the first few weeks of pregnancy. The effects of some vasoactive agents on ovarian blood flow have been reported incidental to studies of the placental and umbilical circulations in near-term sheep (3, 4). The literature contains little information on the response of the near-term ovary to pharmacological agents. We have therefore performed a series of experiments to test the reactivity of the near-term ovine ovary to various vasoactive agents.

Materials and methods. Twenty-two crossbred ewes of approximately 128 days gestation were used in this study. The sheep were anesthetized with Nembutal (50 mg/ml). The anesthesia was supplemented with 2% xylocaine where needed. The right carotid artery was catheterized using a polyvinyl catheter (id 0.7 mm, od 1.2 mm) placed inside a polyethylene catheter which was advanced into the left ventricle. The catheter position was confirmed by the pressure tracing. A small superficial hind-limb artery was also catheterized with a similar polyvinyl catheter which was advanced such that the tip was in the femoral artery. The catheters were then flushed with heparin (10 units/ml) and secured to the animal.

All experiments were performed 48 hr after surgery with the awake animal standing in a restraining cage. Blood pressure recordings were taken using a pressure transducer (Statham P23Db) and recorded on a Beckman R411 recorder. Twenty-five micron radioactive microspheres labelled with ^{125}I, ^{46}Sc, ^{85}Sr and ^{56}Co (3M Co. and New England Nuclear) were used to measure regional blood flows as previously described (5). The drugs used in this study were norepinephrine (Levophed, Winthrop), indomethacin (Sigma), angiotensin II (Bachem) and prostaglandin E_2 (Upjohn).

Protocols. (a) *The effect of serial injections of microspheres.* The microsphere method was tested in five sheep. The ewe was allowed to stabilize until a steady blood pressure was recorded. A control (C) injection of microspheres (approximately 1.2 million spheres of a randomly selected isotope) was then injected and flushed with 3 ml of normal saline, into the left ventricle while withdrawing an integrated arterial blood sample from the femoral artery. Fifteen minutes later the second injection of spheres labelled with a different isotope was injected. A third isotope was given 15 minutes after the second.

(b) *The effect of norepinephrine, angiotensin II, prostaglandin E_2 and indomethacin.* The ewe was given a control injection of microspheres (C). Approximately 10 min later the appropriate drug was given via the left ventricular catheter. The test (T) injection of microspheres was given 1 min following the injection of the drug.

(c) *The effect of prostaglandin E_2 and indomethacin on the response to norepinephrine and angiotensin II.* The responses to norepinephrine injections were measured as described above. The animal was then allowed to recover for approximately 2 hours and either prostaglandin E_2 (10 μg/min) in 10 ml 40% ethanol was infused, or indomethacin (100 mg) in 5 ml dimethyl sulfoxide (DMSO) was injected into the jugular vein. Fifteen minutes after the start of the infusion or injection the second control (C2) injection of microspheres was given. Approximately 5 min later norepinephrine (50 μg) or angiotensin II (10 μg) was given and the second test (T2) injection of microspheres was given 1 min later. With these procedures we could measure the ovarian vascular resistance be-

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fore (C1) and after (T1) the injection of norepinephrine or angiotensin II. The ratio T1/C1 was used as an index of the ovarian response to the drug. The effect of pretreatment with prostaglandin E_2 or indomethacin on the resistance ratio was described by the ratio (T2/C2)/(T1/C1).

After the experiment the animal was sacrificed with a 50 mg/kg injection of Nembutal followed by an intracardiac injection of saturated KCl. The ovaries were then excised, weighed, placed in wide mouth counting vials and counted on a three channel Nuclear Chicago 1185 γ counter with the appropriate blood samples and standards. The tissue analysis, γ counting and data reduction were performed as previously reported (5). Resistances were calculated as the mean arterial blood pressure divided by the blood flow. Comparisons between means were made using the paired *t* test.

Results. (a) *Effect of serial injections.* These results are given in Table I. It can be seen that the ovarian blood flow and resistance did not change significantly when three serial injections of microspheres were given.

TABLE I. EFFECT OF THREE SERIAL MICROSPHERE INJECTIONS ON OVARIAN BLOOD FLOW AND VASCULAR RESISTANCE IN THE OVINE OVARY.

Injection number	CL Ovary[a] (N = 6)	Ovary without CL[a] (N = 4)
	Blood flow (ml/min)	
1	5.66 ± 1.09	0 318 ± 0.062
2	6.18 ± 1.58	0.325 ± 0.019
3	5.54 ± 1.26	0.288 ± 0.039
	Vascular resistance (mm Hg/ml/min)	
1	24.14 ± 10.20	348.7 ± 85.60
2	21.22 ± 08.18	277.7 ± 30.70
3	26.95 ± 13.33	319.4 ± 18.19

[a] Paired t tests on 1 vs. 2, 1 vs. 3, and 2 vs. 3 for both resistances and flow indicated no significant differences.

(b) *Control observations.* The average number of microspheres found in ovaries containing a corpus luteum (CL) was 878 ± 135. There were 678 total spheres in 12 ovaries without CL's for an average of 56 ± 10 spheres per ovary. The integrated arterial blood samples in the control condition contained an average of 310 spheres per sample.

The CL ovary received 86.6 ± 11.8% of the total ovarian blood flow. The blood flows to the CL ovaries averaged 3.86 ± 0.99 ml/min/g while the blood flows to the ovaries without CL's averaged 0.30 ± 0.07 ml/min/g.

Responses to norepinephrine. These data are provided in Table II. It can be seen that 50 μg norepinephrine caused the blood flow in the CL ovary to change from 7.61 ± 1.44 to 5.27 ± 1.37 ml/min/g ($P < 0.05$). The ovarian vascular resistance changed from 40.63 ± 15.97 to 78.0 ± 26.1 mm Hg/ml/min ($P < 0.05$). The ovary without CL responded with a slight decrease in blood flow and a slight increase in resistance neither of which was significant.

Responses to prostaglandin E_2 and indomethacin. The infusion of 10 mg/min of prostaglandin E_2 or the administration of 100 mg of indomethacin did not produce a significant change in the vascular resistance of the CL ovary. The ovary without CL did not respond to prostaglandin E_2 but responded to indomethacin with an increase in vascular resistance from 334.1 ± 87.9 to 566.3 ± 148.7 mm

TABLE II. THE EFFECT OF NOREPINEPHRINE (NEPI), ANGIOTENSIN II (ANGIO), PROSTAGLANDIN E_2 (PGE_2) AND INDOMETHACIN (INDO) ON OVARIAN BLOOD FLOW AND VASCULAR RESISTANCE IN NEAR-TERM SHEEP.

Drug	Dose	Number of ovaries	Blood flow (ml/min)		Resistance (mm Hg/ml/min)	
			Control	*Test*	*Control*	*Test*
NEPI[a]	50 μg	15	7.61 ± 1.44	5.27 ± 1.37[c]	40.6 ± 15.9	78.0 ± 26.1[c]
ANGIO[a]	10 μg	7	6.81 ± 1.79	3.45 ± 0.80[c]	23.5 ± 6.3	44.8 ± 8.9[c]
PGE_2[a]	10 μg	6	6.73 ± 1.92	8.01 ± 2.37	39.1 ± 21.5	46.4 ± 34.2
INDO[a]	100 mg	13	7.63 ± 1.54	7.10 ± 1.58	54.4 ± 36.2	62.6 ± 35.9
NEPI[b]	50 μg	11	0.96 ± 0.48	0.77 ± 0.33	402.7 ± 92.9	421.4 ± 90.5
ANGIO[b]	10 μg	5	1.28 ± 0.80	0.55 ± 0.31	358.7 ± 170.7	444.7 ± 131.0
PGE_2[b]	10 μg	4	0.58 ± 0.28	0.37 ± 0.22	411.7 ± 155.3	781.5 ± 380.0
INDO[b]	100 mg	11	0.72 ± 0.35	0.69 ± 0.43	334.1 ± 87.9	566.3 ± 148.7

[a] = CL ovary.
[b] = Ovary without CL.
[c] = P = <0.02.

Hg/ml/min ($P < 0.01$). Prostaglandin E_2 decreased the response of the CL ovary to norepinephrine from a T1/C1 resistance ratio of 2.145 ± 0.202 (Table III) to a T2/C2 resistance ratio of 0.887 ± 0.21 ($P < 0.05$). The (T2/C2)/(T1/C1) ratio was 0.414 indicating that prostaglandin E_2 depressed the ovarian vascular response to norepinephrine. Prostaglandin E_2 did not significantly affect the response of the ovary without CL to norepinephrine.

The administration of 100 mg of indomethacin did not change the response of the CL ovary or the ovary without CL to norepinephrine.

The response to angiotensin II. These results are given in Table 3. The injection of 10 μg of angiotensin II resulted in a change in the blood flow to the CL ovary from 6.81 ± 1.79 ml/min to 3.45 ± 0.80 ml/min ($P < 0.05$). The ovarian vascular resistance rose from 23.54 ± 6.34 to 44.84 ± 8.94 mm Hg/ml/min ($P < 0.01$). No significant changes in vascular resistance were seen in the ovaries without CL's in response to angiotensin II. No significant changes were seen in the ovarian response to angiotensin II when the animal was pretreated with prostaglandin E_2 or indomethacin.

Discussion. Several investigators have used radioactive microspheres to measure ovarian blood flow (2, 4–6). The number of microspheres that were obtained in the integrated arterial blood samples was slightly less than that required by Buckberg *et al.* (7) and this fact will increase the variance of the data. We obtained more than 400 spheres in the CL ovaries but the ovaries without CL's received fewer than 400 microspheres. The variance of the results were improved by sampling several ovaries without CL's but we must conclude that the microsphere technique had an extremely high variance when used to measure blood flow to ovaries without CL's.

Niswender *et al.* (8) have questioned the use of the microsphere method to measure ovarian blood flow. The objection was based on the anatomy of the ovarian artery and this objection does not invalidate our conclusions because we have compared two or more sets of observations in the same ovary under varying conditions. In addition the blood flows that we report in the control condition to both the CL ovary and the ovary without CL are 7.2 ± 1.67 ml/min/g and 0.83 ± 0.48 ml/min/g respectively. These observations are similar to those reported during the estrous cycle (9) and in near-term sheep (10).

The ovary has been shown to be innervated (11) and reactive to drug stimulation. In the near-term sheep we have shown that the CL ovary is sensitive to norepinephrine and angiotensin II both of which cause vasoconstriction. The CL ovary did not respond significantly to the injection of prostaglandin E_2 as noted. No significant changes were observed with the infusion of indomethacin. Pretreatment with prostaglandin E_2 depressed the response of the CL ovary to norepinephrine.

Prostaglandins other than E_2 may have modulated the responses to norepinephrine. We therefore infused indomethacin, a prostaglandin synthetase blocker and observed that there was no change in the response of the CL ovary to norepinephrine. The responses of this organ to norepinephrine did not appear to be mediated by prostaglandin E_2 release as indomethacin did not change its

TABLE III. EFFECT OF PRETREATMENT WITH PROSTAGLANDIN E_2 (PGE_2) AND INDOMETHACIN (INDO) ON THE EFFECT OF NOREPINEPHRINE (NEPI) AND ANGIOTENSIN II (ANGIO) ON OVARIAN VASCULAR RESISTANCE IN THE NEAR-TERM SHEEP.

Condition	Dose	N	Cl Ovary Resistance ratios[a]	N	Ovary without CL Resistance ratios[a]
NEPI	50 μg	7	2.145 ± 0.202[b]	3	2.260 ± 1.390
PGE_2 + NEPI			0.887 ± 0.210[b]		1.112 ± 0.488
NEPI	50 μg	8	2.301 ± 0.431	8	1.500 ± 0.280
INDO + NEPI			2.570 ± 0.830		1.770 ± 0.450
ANGIO	10 μg	5	2.410 ± 0.220	3	1.190 ± 0.350
INDO + ANGIO			3.020 ± 0.450		1.520 ± 0.190

[a] The resistance ratios are the ratio of vascular resistance seen after the administration of NEPI or ANGIO to that seen before the drug was given.

[b] = $P < 0.001$.

vascular response to norepinephrine. The fact that indomethacin alone caused no change in vascular resistance of the CL ovaries is also evidence that prostaglandins are not responsible for the maintenance of vascular homeostasis of the CL ovary during pregnancy. With the exception of indomethacin, we could not observe any significant changes in the vascular resistance of the ovaries without CL's to any of the drugs used. This may reflect the higher variance of these observations caused by the relatively few spheres found in these organs.

Summary. The effects of various vasoactive agents on ovarian blood flows were measured using radioactive microspheres. Norepinephrine and angiotensin II decreased blood flow and increased vascular resistance significantly in the CL ovary with no significant changes in the ovary without CL. Pretreatment of the animal with prostaglandin E_2 caused a twofold decrease in the response of the CL ovary to norepinephrine with no change in the response of the ovary without CL. Pretreatment with indomethacin did not affect the ovarian responses to norepinephrine. The only significant response of the ovary without CL was an increase in vascular resistance after indomethacin. The data suggest that prostaglandins are not responsible for maintaining vascular homeostasis in the corpus luteum of the near-term ovine ovary.

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