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The Effects of Indomethacin and Meclofenamate on Estrogen Induced Vasodilation in the Rabbit Uterus[1] (40276)

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Many studies have shown that estrogen increases the blood flow to the pregnant and non-pregnant uterus (1–3), but the mechanism by which this vasodilation occurs has not been determined.

It has been postulated that prostaglandins may mediate this vasodilation. Prostaglandins have been shown to play a role in the regulation of blood flow in the pregnant sheep uterus (4) and some studies have indicated that prostaglandins affect blood flows in non-pregnant uteri (5). Some investigators have also reported finding increased prostaglandin synthesis in uterine tissue following estrogen treatment (6, 7).

The following experiment was designed to study the response of the uterine vasculature to estrogen treatment in the rabbit and to determine if prostaglandins are involved in this response through the use of the known prostaglandin synthetase inhibitors, indomethacin and meclofenamate.

Materials and methods. Non-pregnant female New Zealand white rabbits weighing 1.7–3.5 kg were used in this study. Surgery was performed under Nembutal (Abbott Labs) sedation supplemented by local xylocaine (Astra Pharm.). A left ventricular catheter (i.d. 0.0288 mm) was placed via the left carotid artery and a second polyvinyl catheter was inserted 8–10 cm into the left femoral artery. The femoral catheter was then led to the neck incision via a subcutaneous tunnel. The catheters were secured to a packet made of surgical tape and attached to the rabbit's neck. Experiments were performed the following day with the awake animal resting quietly in a restraining cage.

The mean arterial blood pressure of the rabbit was monitored with a Statham P23Db transducer attached to the femoral catheter. Records were made with an R411 Beckman recorder with an EO-18 oscilloscope display.

Blood flows were determined by the left ventricular injection of 15 micron microspheres (3M Co., New England Nuclear) labelled with either ^{109}Gd, ^{113}Sn, ^{85}Sr or ^{46}Sc. The spheres were prepared as a suspension in 10% Dextran in saline. Each microsphere injection had a volume of 0.1–0.2 ml and contained approximately 0.5 million spheres.

Withdrawal. The microspheres were injected into the left ventricle while simultaneously withdrawing an integrated arterial blood sample from the femoral catheter at a rate of 2.06 ml/min for 1.5 min, starting from the time of sphere injection.

Response to estrogen. In five animals, the control organ blood flows were determined. A solution of 1 mg/ml beta estradiol diacetate (Sigma) in 95% ETOH was then administered at a dosage of 100 μg/kg of body weight via the left ventricle. A second determination of the organ blood flows was made 2 hr after the estrogen treatment.

Effect of indomethacin pretreatment. In this series seven rabbits were pretreated with a 100 mg/ml solution of indomethacin dissolved in dimethyl sulfoxide at a dosage of 20 mg/kg of body wt. Indomethacin was given 30 min prior to the control blood flow measurement, and again 30 min before the final measurement of blood flow. The effect of estrogen on the uterine blood flow was measured as described above.

Effect of meclofenamate pretreatment. In this series meclofenamate was administered to eight rabbits as a 20 mg/ml saline solution in a dosage of 20 mg/kg of body wt. The meclofenamate was given 30 min prior to the control blood flow measurement and again 30 min before the final measurement of blood

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flow. The effect of estrogen on the uterine blood flow was measured as described above.

Assay. Upon completion of the experiment, the animal was sacrificed and the uterus, kidneys and lungs were removed. Care was taken to dissect free any adipose or connective tissue from the organs. The uterus was dissected into five separate samples, and the kidneys into three samples each. Two lung samples were also taken, one sample coming from each main lobe of the lungs. Lung samples were taken for assay to determine that no shunting of microspheres across the vascular bed had occurred. The tissues were weighed and placed in counting vials. No sample vial contained tissue which extended more than 1 cm above the bottom of the vial.

Standard vials were used in assaying the samples. Each standard vial contained a known number of spheres of one of the isotopes used in the experiment embedded in wax approximately 0.5 cm from the bottom of the vial. All measurements of radioactivity were made with a three-channel, well-type, automatic γ counting system (Nuclear Chicago, model 1185). A standard pattern of counting the samples was used in which the standard vials were followed by the blood samples, obtained during the integrated arterial withdrawal, followed by the tissue samples. The data were printed on paper tape which was fed into a Univac 1110 computer via an interactive terminal. The data were then processed through programs developed by our laboratory. The spillovers of each isotope into the other channels was determined from the standard vials and the counts per minute per sphere were also calculated at this time. Data were reduced to counts per minute and the number of spheres in each sample. Organ blood flows and vascular resistances per gram of tissue and the ratios of test resistance to control resistance (T/C) for each tissue sample were also calculated. All results are expressed as the mean ± the standard error of the mean. Statistical analysis included paired and un-paired *t* tests (where appropriate) to compare control and test observations.

Dosage and vehicle. The vehicle for the estrogen was ethanol. The dosage administered was small (<.35 ml) and the measurements of blood flow were made 2 hr after the administration of this substance. It is unlikely that the presence of ethanol was a significant factor in these experiments because ethanol was present in both the control (estrogen only) studies and in the studies using prostaglandin synthetase inhibitors. The indomethacin was administered with <1 ml of DMSO (dimethyl sulfoxide) and our observations were made after a delay of 30 min. We have examined the cardiovascular effects of DMSO in the sheep and have observed no significant cardiovascular responses to this agent 30 min after its administration.

The dose levels of indomethacin and meclofenamate were selected to ensure some degree of prostaglandin synthetase inhibition. Ryan *et al.* (5) used 20 mg/kg/day of meclofenamate and 5 mg/kg/day of indomethacin. Venuto *et al.* (8) have shown that 2 mg/kg of indomethacin or meclofenamate both reduce uterine venous prostaglandin E_2 levels in pregnant rabbits.

Results. Part 1. Responses to estrogen. The results obtained in five rabbits are presented in Table I. Organ blood flows were measured before (Control) and 2 hr after (Test) treatment with 0.1 mg/kg estrogen. Mean arterial blood pressures were not affected by the estrogen treatment. In each of the five animals, the vascular resistance of the uterus decreased in response to estrogen. The change in mean resistance of 192.96 ± 32.5 in the control state to 36.92 ± 8.5 mm Hg × min/ml × g after estrogen, was significant ($P < .003$). The renal vascular resistance was not affected by estrogen treatment.

Part 2. Pretreatment with indomethacin. The organ blood flows in seven rabbits which had been pretreated with 20 mg/kg indomethacin were measured both before (Control) and again 2 hr after (Test) estrogen treatment. The results are presented in Tables II and III. Mean arterial blood pressures were not affected by indomethacin pretreatment. Uterine vascular resistance was also not significantly affected by the indomethacin. The renal vascular resistance increased from a mean control value of 24.63 ± 3.0 to 38.51 ± 5.2 mm Hg × min/ml × g (Table III). This was a significant increase ($P < .04$) due to indomethacin pretreatment.

Following indomethacin pretreatment, mean arterial blood pressures were not af-

fected by estrogen treatment. Uterine vascular resistance decreased from a control value of 299.13 ± 69.1 to 137.90 ± 47.3 mm Hg × min/ml × g ($P < .004$) after pretreatment with estrogen (Table II). The renal vasculature was not affected by the estrogen treatment.

Comparisons were made of the resistance ratios (T/C) between normal rabbits and rabbits which had been pretreated with indomethacin to determine any affect which indomethacin might have on the vascular response to estrogen treatment (Table IV). The untreated uterus had a mean T/C value of

TABLE I.[a]

			Resistance (mm Hg × min)/ml × g					
	Blood pressure (mm Hg)		Uterine resistance			Renal resistance		
Animal	C	T	C	T	T/C	C	T	T/C
1	90	92	216.48	50.29	0.233	13.37	25.48	1.905
2	112	102	231.56	53.29	0.231	29.26	24.66	0.843
3	88	86	104.22	11.00	0.106	23.09	15.11	0.654
4	80	86	279.98	47.42	0.169	28.32	27.53	0.972
5	80	92	132.57	22.48	0.170	29.09	30.77	1.058
Mean	90	92	192.96	36.92	0.182	24.62	24.71	1.086
SEM	±7	±3	±32.5	±8.5	±0.02	±3.0	±2.6	±0.24
	NS		$P < .003$			NS		

[a] The uterine and renal vascular resistance per gram of tissue of five rabbits before (C) and 2 h after (T) the administration of 0.1 mg/kg estrogen. Mean arterial blood pressures and resistance ratios (T/C) are also given.

TABLE II.[a]

			Resistance (mm Hg × min)/ml × g					
	Blood pressure (mm Hg)		Uterine resistance			Renal resistance		
Animal	C	T	C	T	T/C	C	T	T/C
1	76	108	220.88	218.16	0.988	23.65	77.01	3.256
2	88	80	233.67	33.79	0.145	33.50	22.59	0.674
3	90	88	143.72	37.01	0.257	30.45	30.35	0.997
4	100	94	543.48	302.89	0.557	41.38	44.44	1.074
5	98	106	575.03	283.85	0.494	65.52	85.73	1.308
6	80	76	136.67	59.81	0.437	31.89	21.71	0.681
7	108	100	240.43	29.77	0.124	43.17	38.56	0.893
Mean	91	93	299.13	137.90	0.429	38.51	45.77	1.269
SEM	±4	±5	±69.1	±47.3	±0.11	±5.2	±9.9	±0.34
	NS		$P < .004$			NS		

[a] The uterine and renal vascular resistances per gram of tissue of seven rabbits pretreated with 20 μg/kg indomethacin before (C) and 2 hs after (T) the administration of 0.1 mg/kg estrogen. Mean arterial blood pressures and resistance ratios (T/C) are also given.

TABLE III.[a]

			Resistance (mm Hg × min)/ml × g			
	Blood pressure (mm Hg)		Uterine resistance		Renal resistance	
	N	P	N	P	N	P
Mean	90	91	192.96	299.13	24.63	38.51
SEM	±7	±4	±32.5	±69.1	±3.0	±5.2
N	5	7	5	7	5	7
	NS		NS		$P < .04$	

[a] A comparison of the uterine and renal vascular resistance per gram of tissue during the control period of five normal (N) rabbits and seven rabbits pretreated (P) with 20 mg/kg indomethacin. A comparison of the mean arterial blood pressures is also provided.

0.182 which differed significantly ($P < .05$) from the pretreated T/C value of 0.429. Indomethacin depressed the uterine response to estrogen.

Part 3. Pretreatment with meclofenamate. The organ blood flows in eight animals pretreated with 20 mg/kg meclofenamate were measured both before (Control) and again 2 hr after (Test) estrogen treatment. The results are presented in Tables V and VI. Meclofenamate pretreatment had no effect on the mean arterial blood pressure. The uterine vascular resistance increased from a mean control value of 192.96 ± 32.5 to 416.42 ± 72.6 mm Hg × min/ml × g ($P < .02$) following the meclofenamate treatment. The renal vascular resistance significantly increased from 24.63 ± 3.0 to 40.33 ± 6.1 mm Hg × min/ml × g ($P < .04$) after pretreatment with meclofenamate (Table VI).

Following pretreatment with meclofenamate the mean arterial blood pressure was not affected by estrogen treatment. The uterine vascular resistance significantly decreased from a mean value of 416.42 ± 72.6 before estrogen to 69.58 ± 21.8 mm Hg × min/ml × g ($P < .001$) after estrogen (Table V). The renal vasculature was not affected by the

TABLE IV.[a]

	Resistance ratios			
	Normal T/C	Indomethacin pretreatment T/C	Normal T/C	Meclofenamate pretreatment T/C
Mean	0.182	0.429	0.182	0.158
SEM	±0.02	±0.11	±0.02	±0.03
N	5	7	5	8
	$P < .05$		NS	

[a] The effect of 0.1 mg/kg estrogen on the uterine vasculature of five normal rabbits and rabbits pretreated with either 20 mg/kg indomethacin or 20 mg/kg meclofenamate. The data are expressed as ratios (T/C) of the resistance 2 hr after estrogen treatment (T) to that seen before administration of the estrogen (C).

TABLE V.[a]

	Blood pressure (mm Hg)		Resistance (mm Hg × min)/ml × g					
			Uterine resistance			Renal resistance		
Animal	C	T	C	T	T/C	C	T	T/C
1	84	82	685.06	28.30	0.041	38.99	33.75	0.866
2	80	92	298.94	60.84	0.204	25.94	26.20	1.010
3	86	86	315.82	47.82	0.151	37.40	36.78	0.984
4	86	80	674.91	198.78	0.295	73.20	94.04	1.280
5	108	100	611.76	122.55	0.200	57.13	61.48	1.070
6	82	93	172.58	16.89	0.098	25.01	26.66	1.066
7	88	90	268.15	25.74	0.096	41.13	51.47	1.251
8	90	102	304.17	55.69	0.183	23.81	29.71	1.248
Mean	88	91	416.42	69.58	0.158	40.33	45.01	1.097
SEM	±3	±3	±72.6	±21.8	±0.03	±6.1	±8.3	±0.05
	NS		$P < .0005$			NS		

[a] The uterine and renal vascular resistances per gram of tissue of eight rabbits pretreated with 20 μg/kg meclofenamate before (C) and 2 hr after (T) the administration of 0.1 mg/kg estrogen. Mean arterial blood pressures and resistance ratios (T/C) are also given.

TABLE VI.[a]

	Blood pressure (mm Hg)		Resistance (mm Hg × min)/ml × g			
			Uterine resistance		Renal Resistance	
	N	P	N	P	N	P
Mean	90	88	192.96	416.42	24.63	40.33
SEM	±7	±3	±32.5	±72.6	±3.0	±6.1
N	5	8	5	8	5	8
	NS		$P < .02$		$P < .04$	

[a] A comparison of the uterine and renal vascular resistance per gram of tissue during the control period of five normal (N) rabbits and eight rabbits pretreated (P) with 20 mg/kg meclofenamate. A comparison of the mean arterial blood pressures is also provided.

estrogen treatment. Meclofenamate did not affect the uterine vascular response to estrogen.

Discussion. It has been postulated that estrogen induced uterine vasodilation is mediated via a biochemical chain of events initiated by estrogen receptor binding and continuing to the synthesis of new mRNA and, subsequently, the synthesis of new protein (9). Killam *et al.* (10) have described the effects of estrogen on the sheep uterus and have indicated a possible release of acetylcholine or histamine as the intermediate step in this chain of events. Clark *et al.*, however, have determined that the administration of histamine receptor antagonists has no effect on estrogen induced increases in uterine blood volume (11). Resnik *et al.* (12) also have concluded that acetylcholine, isoproterenol and histamine are not mediators of the response to estrogen and proposed the release of a small polypeptide such as bradykinin or of an enzyme which has a role in the biosynthesis of adenosine and its release (2). Several studies have indicated an increase in the synthesis of uterine prostaglandins after estrogen treatment (5–7). Ryan et al. have shown that prostaglandins exhibit properties concurrent with the hypothesis that prostaglandins mediate estrogen induced hyperemia (5). They also show that blocking prostaglandin synthesis with both indomethacin and meclofenamate depressed the uterine response to estrogen in rats. Castracane and Jordan, however, have found that inhibiting protein synthesis, and thereby blocking the biological chain of events leading to the hyperemic response, had no effect on the estrogen induced formation of prostaglandins (13). They conclude that the production of prostaglandins due to estrogen may be a function of estrogen unrelated to its function as an initiator of estrogen induced hyperemia.

This study was designed to determine whether prostaglandin synthesis is a necessary step in the mediation of the estrogenic response. The experimental data presented in this paper indicate that estrogen induced vasodilation is not mediated by prostaglandin formation. The vasoconstriction shown to be occurring in the kidneys following treatment with either indomethacin or meclofenamate indicates that prostaglandin synthesis blockade occurred in concordance with the study by Malik and McGiff on prostaglandin modulation of vascular resistance in rabbit kidneys (14). The uterus showed no vasoconstriction due to indomethacin so that the vasoconstriction seen after meclofenamate may have been due to a side effect of the drug. The fact that indomethacin depressed the uterine response to estrogen is in concordance with the literature, but must be examined in view of the fact that meclofenamate did not produce a similar response. It is our conclusion that the indomethacin induced depression of the response to estrogen was not due to the blockade of prostaglandin synthesis, but due to a side effect of indomethacin or its vehicle. Therefore, prostaglandin synthesis does not appear to be essential to estrogen induced vasodilation in the rabbit uterus.

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