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Angiotensin II Increases Uterine Vascular Resistance in Pregnant and Nonpregnant Rabbits (39726)[1, 2]

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Introduction. That angiotensin II (A-II) produces marked vasoconstriction has been well-documented for most vascular beds including the kidney, intestine, hindlimb, heart, and liver (1–6). In contrast, studies of the effects of A-II on uterine blood flow have generated conflicting findings.

Girard *et al.* (7) using a radiographic technique for detecting changes in distribution of blood flow in pregnant guinea pigs found that intravenous administration of A-II produces virtually no change in uterine blood flow. In contrast, Greiss and van Wilkes (8) reported that A-II significantly increased uterine vascular resistance in pregnant sheep. However, Assali *et al.* (9) and Ladner *et al.* (10) reported that intravenous injections of A-II passively decreased uterine vascular resistance in either pregnant or nonpregnant sheep and dogs. More recently Ferris *et al.* (11), working with pregnant rabbits, and Terragno *et al.* (12, 13), working with pregnant dogs, claimed that A-II decreased uterine vascular resistance and increased uterine blood flow. None of these studies, however, examined the direct effects of A-II on uterine vascular resistance as revealed by injecting A-II into the uterine artery.

To gain insight into this problem we compared the effects of intraarterial injections of A-II on uterine vascular resistance in pregnant and nonpregnant rabbits. Such information is of interest because of the purported role of A-II in local regulation of the uterine vasculature, particularly during pregnancy (11–14).

Methods. Studies were performed with 12 sexually mature New Zealand virgin rabbits (3.2–4.8 kg) and 5 pregnant rabbits (third trimester, 4.0–6.1 kg) anesthetized with sodium pentobarbital (50–100 mg/kg iv), tracheotomized, and artifically ventilated with humidified room air. Supplemental anesthesia was administered through a catheterized jugular vein, and arterial blood pressure (E and M transducer, P-1000) was monitored from the left femoral artery.

The procedure used to study the effects of drugs on uterine vascular resistance was adapted from Ryan *et al.* (15). The uterus and its blood supply was exposed through a midline abdominal incision (Fig. 1). All branches of the right external iliac artery between the iliac bifurcation and the right uterine artery were ligated. The right external iliac artery was ligated immediately distal to the right uterine artery. Ligatures were placed around the right uterine horn where it joined the cervix and around the fallopian tube. The mesometrium was tied in several sections so that blood entered the right uterine horn largely, or solely, through the right uterine artery and drained the horn via the uterine veins which were unaltered. The left uterine horn and its blood supply was kept intact.

Heparin (1500 μ/kg iv) was administered to prevent blood coagulation after all major surgery was completed. Blood from a common carotid artery was diverted through a short (50–60 cm) extracorporeal circuit which contained a precalibrated constant flow peristaltic pump (Sigmamotor, T-8). Blood from the outflow side of the pump

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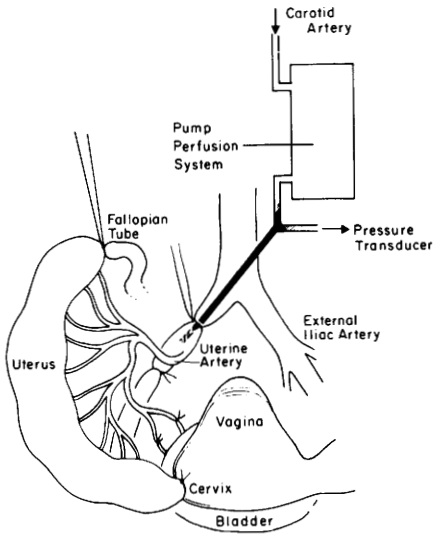

FIG. 1. Schematic representation of the procedure used for isolating and perfusing a segment of the uterine vasculature *in situ*. Technical details are described in Methods.

was used to perfuse the isolated uterine horn via a catheter in the right uterine artery. Uterine perfusion pressure was monitored through a Y-tube placed between the pump and the perfused segment. Flow through the pump, and hence the collateral free uterine horn, was increased gradually until perfusion pressure was within 5% of aortic blood pressure. Preliminary experiments showed that the preparation was stable throughout the experimental period (2–3 hr).

Intra-arterial (ia) injections of A-II (Beckman) were made through the inflow side of the pump to permit thorough mixing with blood (5, 6, 16). The drug was injected rapidly (1–2 sec) in 0.1 ml of saline (pH 7.3–7.4) and 10–15 min elapsed between injections so that tachyphylaxis did not develop (3–6). The primary response to A-II was recorded as the maximal change in perfusion pressure (expressed as a percentage of control perfusion pressure) which occurred prior to any change in arterial blood pressure (5, 6). Control injections of saline (0.1 ml) were performed in each experiment to insure that responses to A-II were not mechanical artifacts.

In three pregnant and four nonpregnant rabbits, responses to A-II were examined in the presence and absence of 1-sar-8-ala-angiotensin II (saralasin, Norwich, Pharmacal), a specific A-II antagonist. The influence of α-adrenergic blockade with phentolamine (Ciba) on responses to A-II and DL-norepinephrine (Winthrop) was evaluated in four nonpregnant rabbits. The local effects of nitroglycerin (Lilly) were also tested to ensure that the vasculature was able to relax. Since uterine blood flow was maintained constant, agonist-induced changes in uterine perfusion pressure were directly proportional to changes in uterine vascular resistance (16).

Uterine responses were expressed as mean values ± 1 SE and the statistical significance of differences between responses in pregnant and nonpregnant rabbits, and between responses in the presence and absence of antagonists were assessed with Student's t test. In all experiments the schedule of injections was randomized with respect to the agonist and dose of agonist tested.

India ink was injected into the uterine perfusion system shortly before the termination of each experiment to visualize the distribution of ink through the perfused segment and to determine whether the segment was well-isolated. If a diffuse pattern was observed revealing contamination within the vagina, bladder, or contralateral uterine horn, the experiment was discarded.

Results. Mean arterial blood pressure following all surgical procedures was slightly, but significantly ($P < 0.05$), greater in the 12 nonpregnant rabbits (99 ± 6 mm Hg) than in the 5 pregnant rabbits (70 ± 7 mm Hg). Similarly, uterine perfusion pressure was higher in nonpregnant (89 ± 5 mm Hg) than in pregnant (75 ± 4 mm Hg) preparations. However, blood flow through the perfused uterine segments was greater in pregnant (5.0 ± 0.4 ml/min) than in nonpregnant rabbits (3.2 ± 0.3 ml/min; $P < 0.005$). Accordingly, vascular resistance through the perfused segments, calculated from the ratio of uterine perfusion pressure to blood flow through the perfused segment, was markedly and significantly ($P < 0.025$) greater in the nonpregnant population (29 ± 4 mm Hg/ml/min) than in the pregnant population (18 ± 4 mm Hg/ml/min).

Intra-arterial injections of A-II consistently produced marked increases in uterine vascular resistance (increased uterine perfusion pressure) in both pregnant (Table I) and nonpregnant rabbits (Fig. 2, Table I). The influence of time elapsed between termination of the response to a given dose of A-II and initiation of a second response to the same dose was studied in four nonpregnant rabbits. In response to the first test injection of A-II (1 μg ia) uterine perfusion pressure increased to 285 ± 21% of control. However, responses elicited 2 min (115 ± 15% control) or 4 min (180 ± 13% of control) after completion of the initial response were depressed markedly. In contrast, tachyphylaxis was not evident when the interval was lengthened to 8 min (270 ± 18% of control) or 16 min (273 ± 9% control).

In both pregnant and nonpregnant rabbits constrictor responses to A-II were rapid in onset (20–30 sec), attained maximal levels in 45–70 sec after onset, and returned to control levels in 240–300 sec. The responses usually occurred in the absence of changes in systemic arterial pressure. However, when high doses of A-II (> 0.25 μg) were tested, the local responses were often followed by a transient pressor response which was small in magnitude (Fig. 2).

Intravenous injections of A-II (5 μg), like intra-arterial injections, produced marked increases in uterine vascular resistance. Thus, intravenous administration of A-II increased uterine perfusion pressure to 138 ± 8% of control ($P < 0.025$) in nine nonpregnant rabbits, and to 146 ± 12% of control ($P < 0.025$) in five pregnant rabbits.

Blockade of A-II receptors with 1-sar-8-ala-angiotensin II (saralasin, 30 μg/kg ia) virtually abolished constrictor responses to local injections of A-II in either pregnant or nonpregnant rabbits. For example, the mean increase in uterine perfusion pressure produced in response to 0.25 μg of A-II ia was 188 ± 12% of control before saralasin, but only 104 ± 4% of control after saralasin ($P < 0.005$). Similarly, the control uterine vascular response to the same dose of A-II in three nonpregnant rabbits was 195 ± 10% of control, but decreased to 106 ± 3% of control ($P < 0.005$) after administration of saralasin. However, treatment with the A-II antagonist did not influence constrictor responses to norepinephrine in any of the three nonpregnant rabbits examined. Thus, norepinephrine (1 μg ia) increased uterine perfusion pressure to 193 ± 5% of control in the absence of saralasin, and to 205 ± 16% of control after blockade of A-II receptors.

In contrast, blockade of α-adrenergic receptors with phentolamine (1.5 mg/kg iv) in four nonpregnant rabbits significantly attenuated local constrictor responses to norepinephrine but did not alter responses to A-II. For example, norepinephrine (1 μg ia) increased uterine perfusion pressure to 192 ± 11% of control before blockade, whereas

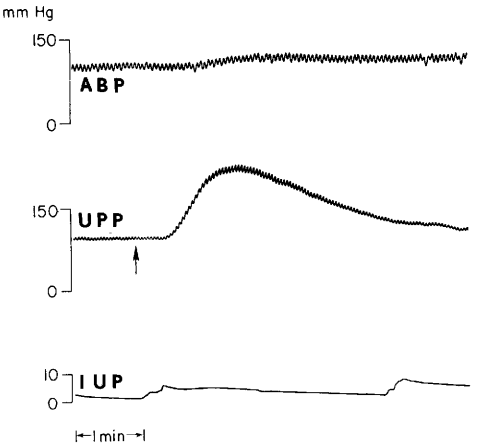

FIG. 2. The effects of A-II (1.0 μg) injected directly into the pump-perfused uterine vasculature of a nonpregnant rabbit at constant flow are shown with respect to arterial blood pressure (ABP), uterine artery perfusion pressure (UPP), and intrauterine pressure (IUP). The time of injection of A-II into the perfusion system is represented by the arrow.

TABLE I. EFFECTS OF GRADED DOSES OF A-II ON UTERINE PERFUSION PRESSURE IN PREGNANT AND NONPREGNANT RABBITS.

	Uterine perfusion pressure (% of control)[a]	
A-II (μg)	Pregnant	Nonpregnant
0.02	109 ± 3 (5)[b]	119 ± 4 (5)
0.06	151 ± 6 (5)	153 ± 9 (9)
0.25	199 ± 13 (5)	228 ± 17 (9)
1.00	275 ± 12 (5)	278 ± 16 (6)

[a] Values represent mean response ± SE.
[b] Number of rabbits tested in parentheses.

the response was decreased to 127 ± 2% of control ($P < 0.005$) after α-blockade. The responses to A-II (0.06 μg ia) before (168 ± 6% of control) and after phentolamine (165 ± 9% of control) were similar.

Nitroglycerin (600 μg ia), as expected, reduced uterine perfusion pressure in three pregnant (62 ± 4% of control; $P < 0.025$) and three nonpregnant rabbits (72 ± 6% of control, $P < 0.025$).

Discussion. The prominent finding in this study was that A-II always increased uterine vascular resistance when injected either intravenously or directly into the uterine arterial blood supply of either pregnant or nonpregnant rabbits (Fig. 2, Table I).

The present findings contrast with studies reported from other laboratories showing that A-II had no effect on the uterine vasculature of pregnant guinea pigs (7), or that it decreased uterine vascular resistance in pregnant rabbits (11) and dogs (12, 13). Although the reasons for these disparate findings are obscure, contributing factors probably include species differences with respect to guinea pigs, rabbits, and dogs (17, 18) and procedural differences including anesthesia, surgical preparative techniques, and methods used for assessing uterine vascular responses to A-II. For example, Ferris *et al.* (11) used microspheres to assess uterine blood flow and found that prolonged intravenous infusions (30 min) of A-II decreased uterine vascular resistance in near-term pregnant rabbits which had been nephrectomized 24 hr prior to study. This decrease in resistance was reportedly indirect since blockade of β-adrenergic receptors with propranolol abolished the response. In this context, we found that rapid (2–4 sec) intravenous injections of A-II, which reveal the immediate effects of A-II, increased uterine vascular resistance in either pregnant or nonpregnant rabbits. However, because of the procedural differences between these studies a strict comparison of our findings with those reported by Ferris *et al.* (11) is not strictly appropriate.

In the present study, a segment of the uterine vasculature was isolated *in situ* so that it was collateral free and pump perfused with the animal's own arterial blood under conditions of constant flow. In contrast to the procedures used by other investigators, we focused primarily on responses elicited when A-II was injected directly into the uterine blood supply. Changes in uterine vascular resistance were directly proportional to changes in uterine perfusion pressure because flow was constant (5, 6, 16). Local uterine responses occurred rapidly and were probably independent of extrauterine reflexogenic mechanisms which could have been elicited when A-II was administered intravenously (19). Our finding that uterine vascular resistance was significantly lower in pregnant than in nonpregnant rabbits is consistent with findings reported in other laboratories (17, 18) and suggests that the preparations were not seriously compromised by the surgical procedures used. In addition, nitroglycerin significantly reduced uterine perfusion pressure in pregnant and nonpregnant preparations showing that the vascular beds could relax when stimulated appropriately. Therefore, the consistent observation that A-II increased uterine perfusion pressure must be due to active vasoconstriction of the uterine vasculature.

Uterine vasoconstriction produced with A-II was dose dependent and similar in magnitude for both pregnant and nonpregnant rabbits (Table I). This finding suggests that pregnancy was not associated with a change in the responsiveness of the uterine vasculature to A-II. However, this conclusion should be viewed with caution because uterine vascular resistance was lower in pregnant animals and because precise information on the E_{50} for uterine vascular responses to A-II in either pregnant or nonpregnant rabbits is lacking.

The vasoconstrictor responses to A-II were probably ascribable largely to direct interactions between the agonist and specific receptors for A-II in uterine vascular resistance vessels and were independent of interactions between A-II and α-adrenergic mechanisms in the uterine vasculature. This conclusion is supported by two observations. First, responses to A-II were virtually abolished during blockade of A-II receptors with 1-sar-8-ala-angiotensin-II whereas responses to norepinephrine were unaltered. Second, responses to A-II were unaltered during α-adrenergic blockade with phentolamine, whereas responses to norepinephrine were significantly attenuated.

Although this study does not permit an evaluation of the role of A-II in regulating uterine blood flow since the drugs were administered at levels higher than those encountered under physiological conditions, it clearly shows that A-II receptors exist in the uterine vasculature of both pregnant and nonpregnant rabbits and that direct stimulation of these receptors produces active increases in resistance to blood flow.

Summary. Uterine vascular responses to graded doses of angiotensin-II (A-II) injection directly into the uterine blood supply were studied in pregnant and nonpregnant rabbits anesthetized with pentobarbital. A segment of the uterus was isolated *in situ* and pump perfused at a constant rate of flow with the animal's own arterial blood. Uterine perfusion pressure, and hence uterine vascular resistance, always increased when A-II was injected into the arterial perfusion system. Responses to A-II were dose dependent and similar in magnitude in both pregnant and nonpregnant rabbits. These responses were virtually abolished in the presence of 1-sar-8-ala-angiotensin-II, a specific A-II antagonist. In contrast, responses to A-II were unaltered after α-adrenergic blockade with phentolamine. The present findings, in contrast to earlier studies in which A-II was injected systemically, suggest that uterine vasoconstriction induced with A-II was active, ascribable to direct interactions between A-II and its specific receptor, and that such interactions were independent of α-adrenergic mechanisms.

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