Effect of Angiotensin II on Uterine and Systemic Vasculature in Pregnant Sheep

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A B S T R A C T The response of uteroplacental blood flow (UBF) to angiotensin II is controversial. Moreover, the relationship of the uterine and systemic responses to infused angiotensin II is not well understood. Thus, in eight chronically instrumented, near-term pregnant sheep, we have determined the relationships between the dose and duration of constant systemic infusions of angiotensin II ([Val^5] ANG II) and changes in UBF, uterine vascular resistance (UVR), mean arterial pressure (MAP), and systemic vascular resistance (SVR). [Val^5] ANG II caused dose-dependent increases in UVR and MAP at all doses studied ($P < 0.05$). The response in UBF was bidirectional, with increases at doses ≤1.15 μg/min and decreases at ≥2.29 μg/min ($P < 0.05$). Increases in UBF occurred when the relative rise (Δ) in MAP > Δ UVR, whereas UBF was unchanged when Δ MAP = Δ UVR and decreased when Δ MAP < Δ UVR. SVR also rose in a dose-dependent fashion ($P < 0.05$); Δ SVR was >Δ UVR at doses ≤2.29 μg [Val^5] ANG II/min ($P < 0.01$). In studies of the effect of duration of [Val^5] ANG II infusions, UBF increased at all doses during the 1st min, followed by stabilization at 4–5 min, with eventual decreases at doses ≥2.29 μg/min and increases at doses <2.29 μg/min. The relationship between the changes in MAP and UVR to the response of UBF was as noted above. It is evident that (*a*) [Val^5] ANG II is a uterine vasoconstrictor, (*b*) changes in UBF are dependent upon relative changes in perfusion pressure and UVR, which in turn are dependent upon both the dose and duration of a [Val^5] ANG II infusion, and (*c*) the uteroplacental vasculature is relatively refractory to the vasoconstricting effects of low doses of [Val^5] ANG II.

INTRODUCTION

Previous studies of the effects of angiotensin II on the pregnant uterus of various species have resulted in conflicting results. There have been reports of both decreases (1–3) and increases (4–11) in uteroplacental blood flow (UBF).[1] This has resulted in the conflicting conclusions that angiotensin II acts either as a uterine vasoconstrictor, as in other vascular beds, or as if it has a paradoxical and "unique" vasodilator action on the uterine vasculature (5). This is an important issue in view of evidence that renin is produced in the pregnant uterus of several species including the human (12), and that in the pregnant rabbit, uterine renin release is increased under conditions of uterine hypoperfusion (5). If indeed angiotensin II has a selective vasodilator action on the pregnant uterus while increasing constriction of nonuterine vessels of the systemic circulation, a potent mechanism is implied by which a fall in uteroplacental perfusion could result in a selective, compensatory rise in UBF by means of a rise in circulating angiotensin II. Moreover, this would suggest a mechanism for the autoregulation of UBF through the release of uterine renin.

We have recently reported (13) in the chronically instrumented pregnant sheep studied remote from surgery that changes in UBF are related to the dose of infused angiotensin II ([Val^5] ANG II), with increases occurring at low doses and decreases at high doses. We also noted a biphasic response of UBF during the continuous systemic infusion of high doses of [Val^5] ANG II, i.e., an initial rise followed by a fall and subsequent stabilization. Thus it appears that both the dose and duration of the infusion of [Val^5] ANG II are important variables in considering the uteroplacental response to infused doses of [Val^5] ANG II. It also is notable that increases in UBF have been observed only when [Val^5] ANG II has been administered systemically, suggesting that simultaneous systemic responses to [Val^5] ANG II may be directly relevant to observed changes in UBF. In view of these observations, we designed this study to characterize the response of the uterine

[1] *Abbreviations used in this paper*: MAP, mean arterial pressure; SVR, systemic vascular resistance; UBF, uteroplacental blood flow; UVR, uterine vascular resistance.

circulation to infused [Val⁵] ANG II, to determine the influence of the dose and duration of constant infusions of [Val⁵] ANG II on UBF and to evaluate the effect of the concurrent responses in the total systemic vasculature.

METHODS

The effect of anesthesia in modifying the responses to [Val⁵] ANG II and other vasoactive compounds has been amply demonstrated (14–16); consequently, only chronically instrumented animals remote from surgery were involved in this study.

Eight ewes in late pregnancy (130–140 d, term 144±3 d) and of mixed breed were used. The animals' weights ranged from 40 to 67 kg at the time of study, 52±8.6 (mean±SE) kg. The animal preparation has been described in detail (17). Briefly, at operation (~125 d) electromagnetic flow probes (Micron Instruments, Inc., Los Angeles, Calif.) were implanted around both main uterine arteries and polyvinyl catheters (0.75 mm i.d., 1.2 mm o.d.) were inserted into both femoral arteries to the level of the trifurcation of the abdominal aorta. Larger polyvinyl catheters (1.27 mm i.d., 2.29 mm o.d.) were introduced into both femoral veins, with the open ends lying in the inferior vena cava just below the diaphragm. In some animals, catheters (0.75 mm i.d., 1.2 mm o.d.) were introduced into the amniotic sac and into one uterine vein via a peripheral tributary. The flow probes and catheters were brought out to the flank through a subcutaneous tunnel and maintained in a canvas pouch attached to the skin with steel pins. Catheters were flushed daily with heparinized saline (250 U/ml) and closed with sterile pins. Penicillin (600,000 U) and streptomycin (0.5 g) were given on the day of operation and the 2 d following.

The ewes were maintained after operation in individual stalls kept in the laboratory. They were given standard animal chow (Ralston Purina Co., St. Louis, Mo.) and water ad lib. throughout the study period. Body weight remained constant or increased. The animals were studied only after they were considered to have recovered from surgical trauma and anesthesia, as evidenced by a normal response of uterine blood flow to 1 μg/kg of intravenous 17β-estradiol (15, 16). No animal was studied until at least day 6 after surgery. Two studies were carried out on each animal 2–6 d apart.

[Val⁵]ANG II (Ciba-Geigy Corp., Summit, N. J.) was diluted in sterile isotonic saline to a concentration of 3 μg/ml. This solution was infused through one of the venous catheters by a constant-infusion pump. (Harvard Apparatus Co., Inc., S. Natick, Mass.) Five doses of [Val⁵] ANG II were studied (0.115, 0.573, 1.15, 2.29, and 11.5 μg/min). These were administered in random sequence. Each constant infusion was maintained a minimum of 5 min, followed by a rest period of 15–30 min, allowing blood pressure and UBF to return to a stable level for at least 10 min before the next infusion was begun.

Mean arterial pressure (MAP) in the lower abdominal aorta was monitored through an interpolated pressure transducer (Bell & Howell Co., Pasadena, Calif.) and the signal recorded on a model 220 two-channel pen-recorder (Brush Instruments Div., Clevite Corp., Cleveland, Ohio). In a number of experiments, intraamniotic pressure, uterine venous pressure, and systemic venous pressure also were recorded continuously. UBF was monitored with model RC-1000 square-wave electromagnetic flowmeters (Micron Instruments, Inc., Los Angeles, Calif.) and recorded on a second two-channel recorder; the sum of the simultaneous measured flows in both main uterine arteries was used for analysis. Except where noted, observations were made during the steady state before and after 5 min of the infusion of [Val⁵] ANG II.

In five animals cardiac output was measured by the dye-dilution technique. 5 mg of indocyanine green (Hynson, Wescott and Dunning, Inc., Baltimore, Md.) was injected rapidly into the inferior vena cava via one indwelling catheter while blood was withdrawn at 10 ml/min through a densitometer from a femoral arterial catheter. This blood was later reinfused into the animal. Optical density was recorded graphically and cardiac output calculated by electronic integration directly from the signal. The results of cardiac output calculated electronically by this equipment (Waters Instruments, Inc., Rochester, Minn.) were compared with results obtained from the measurement of the area under the curve of the graphic recording. Mean values for repeated measures by the two methods were within 2% of each other, and variability about the mean was the same. Therefore, the value obtained by electronic integration has been used in this report. All measurements were carried out in duplicate and the average taken.

As increases in UBF may be produced by either uterine vasodilation or an increase in uterine perfusion pressure, we have calculated uterine vascular resistance (UVR) in these studies. This allows for the effect of increased perfusion pressure in examining the action of [Val⁵] ANG II on the uterine vasculature. Uterine vascular resistance may be calculated as the difference between mean arterial and venous pressures, divided by uterine blood flow. Uterine and systemic venous pressures were measured during the experiments in several animals and both were found to be consistently low: 3–5 and 1–3 mm Hg, respectively. No change was observed during the [Val⁵] ANG II infusions. Because venous pressure is therefore very small compared with MAP, venous pressure was not included in the calculation of UVR. Likewise, intraamniotic pressure in the ewe is also low, 1–3 mm Hg, and was not observed to change significantly during the [Val⁵] ANG II infusions; therefore it, too, was excluded from the calculation of UVR. Thus, UVR was calculated as $UVR_t = MAP_t/UBF_t$, where t is a point in time.

Total systemic vascular resistance (SVR) was calculated as the MAP divided by cardiac output (CO), which represents simultaneous total systemic blood flow. As above, where venous pressure is small and remains constant, $SVR_t = MAP_t/CO_t$.

Analysis of the data involved the comparison of the experimental values obtained during the infusion of [Val⁵] ANG II with the control values obtained immediately before the onset of each infusion. Statistical analysis, except where stated otherwise, was performed using the Wilcoxon Matched Pairs Signed Ranks test (18). Values are presented as the mean and 1 SE.

RESULTS

Effect of dose. We have chosen to use the steady-state responses in these studies because of the changes in UBF observed (13) during the first 2–3 min with infusions of high doses of [Val⁵] ANG II. This is illustrated in Fig. 1. In view of this, the data presented in this section were obtained in every instance after 5 min of a constant systemic infusion of [Val⁵] ANG II, a time when both MAP and UBF were stable. The mean data (±SE) obtained for all animals are given in Table I. It is important to note that although control UBF varied considerably between animals (range, 585–1,720 ml/min), little variation occurred within individual animals during each experiment. UVR varied similarly.
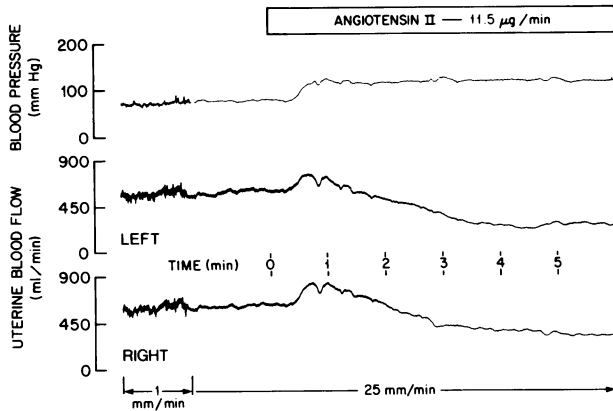
FIGURE 1 Continuous recording of the simultaneous changes in blood pressure and blood flows in the right and left uterine arteries before and during a constant systemic infusion of [Val⁵] ANG II.

FIGURE 2 The change in UVR at 5 doses of [Val⁵] ANG II. Mean±SE are presented, n = 16 experiments. Control UVR = 74.1±6.2 mm Hg × min/liter.

The response to infused [Val⁵] ANG II was consistent for each animal, whereas the absolute level of UBF and UVR attained varied in proportion to the base-line value for each animal. Because we are concerned with the response to infused [Val⁵] ANG II, we have chosen to present the data subsequently in terms of the changes observed. Moreover, where we compare the simultaneous changes in two or more variables (Figs. 3 and 4), the responses are presented as the percent change, as it is not possible to relate the absolute changes directly.

The relationship between the response in UVR and the dose of infused [Val⁵] ANG II is illustrated in Fig. 2; there is a statistically significant increase in UVR (P < 0.005) at all but the lowest dose of [Val⁵] ANG II where no response was apparent. The change in UVR is dose dependent, increases being significantly greater at each successive dose except the two lowest, at which the changes were not statistically different from the changes seen at immediately adjacent doses (P < 0.05
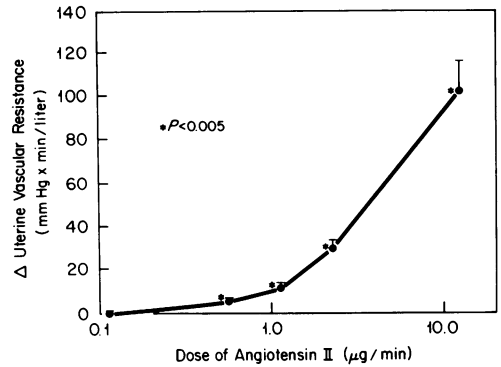
by repeated measures analysis of variance followed by Duncan's Multiple Range Test (19)). At no dose of [Val⁵] ANG II was there a fall in UVR.

Although UVR rose at all doses of [Val⁵] ANG II, increases in UBF were observed. As shown in Fig. 3, the increases in UBF occurred at doses ≤1.15 μg [Val⁵] ANG II/min, whereas decreases were seen at doses ≥2.29 μg [Val⁵] ANG II/min (all changes significant at P < 0.05), confirming our previous observations (13). To determine the mechanisms responsible for this bi-directional response of UBF, changes in MAP and UVR were compared and related to the changes seen in UBF (Fig. 3). MAP rose at all doses, P < 0.05, in a dose-dependent fashion. At doses ≤1.15 μg [Val⁵] ANG II/min, the rise in MAP exceeded that of UVR, e.g., 13 vs. 9%, respectively, at 0.573 μg [Val⁵] ANG II/min (P < 0.02); at each of these doses UBF was observed to rise. At doses ≥2.29 μg [Val⁵] ANG II/min, the relative increase in UVR exceeded that in MAP, and UBF was observed to fall.

To further describe the difference in the responses found in the uterine and systemic vascular beds during systemic infusions of [Val⁵] ANG II, we measured

TABLE I

Responses to Continuous Systemic Infusions of Angiotensin II in Pregnant Sheep

	Control	Rate of infusion of angiotensin II				
		0.115	0.57	1.15	2.29	11.5
		μg/min				
MAP* mm Hg	75±1	77±1	85±1	90±1	97±2	120±3
UBF* ml/min	1,109±82	1,126±83	1,147±84	1,172±104	1,065±95	826±96
UVR* mm Hg × min/liter	74.1±6.2	75.5±6.5	80.9±6.5	86.7±7.7	103.7±10.0	176.3±20.0
SVR‡ mm Hg × min/liter	10.1±0.6	10.5±0.6	12.5±0.9	14.3±1.1	15.4±1.2	21.1±1.9

Values represent mean±SE.
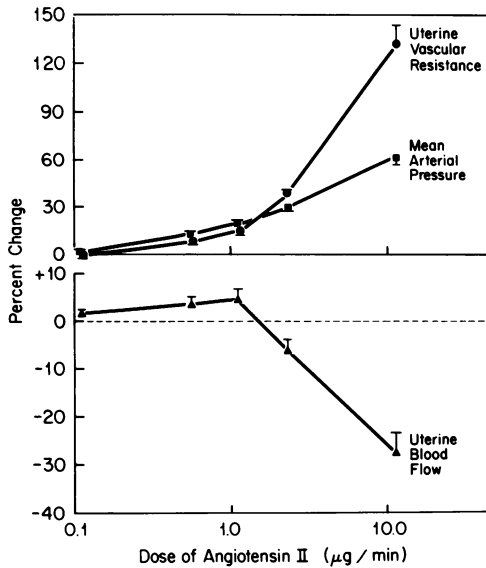* n = 16 experiments.
‡ n = 10 experiments.

FIGURE 3 The relative changes in UBF (▲), MAP (■), and UVR (●), expressed as the percent of control values, at five doses of systemically infused [Val⁵] ANG II. Mean±SE are presented, $n = 16$ experiments. The changes in all parameters are significant ($P < 0.05$) at each dose except the lowest (0.115 μg/min).

cardiac output in 10 experiments. We then calculated total systemic vascular resistance (SVR) and compared the simultaneous changes in UBF, MAP, UVR, and SVR. An increase in SVR occurred at each dose of [Val⁵] ANG II studied, $P < 0.05$, and the responses were dose dependent (Fig. 4). When the changes in SVR were compared with the simultaneous changes in UVR, the response in SVR exceeded that of UVR at 0.573, 1.15, and 2.29 μg [Val⁵] ANG II/min ($P < 0.01$). There was no difference at the lowest dose of [Val⁵] ANG II, whereas at 11.5 μg [Val⁵] ANG II/min, the change in UVR exceeded that of SVR ($P < 0.01$). It should be noted that whereas the profile of the dose-response relationship of the change in SVR is almost
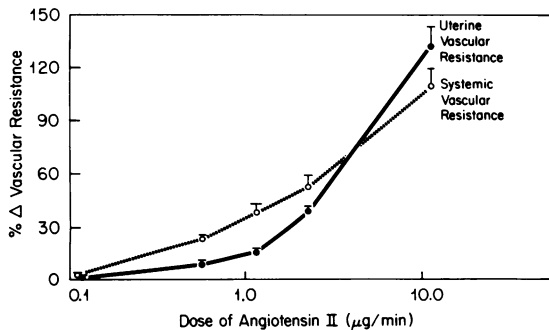

FIGURE 4 The relative changes in UVR and SVR, expressed as the percent of control values, at five doses of systemically infused [Val⁵] ANG II. Mean±SE are presented, $n = 10$ experiments.

rectilinear, that of UVR is much more curvilinear. These findings are suggestive of relative uterine refractoriness to the vasoconstricting effects of [Val⁵] ANG II at three of the five doses studied.

Effect of duration of infusion. As pointed out earlier, UBF has been noted to increase shortly after the initiation of a systemic infusion of a high dose of [Val⁵] ANG II, only to fall after the 1st min and to attain a steady state by 5 min, at which time a decrease in UBF has occurred (Fig. 1). In view of this observation we sought to determine the relationship of the changes in UBF, MAP, and UVR during the systemic infusion of [Val⁵] ANG II. Responses, expressed as the change from control values, at each of the five doses of [Val⁵] ANG II were determined at 0.25, 0.5, 0.75, and 1.0 min and then every 30 s thereafter for 5 min in seven consecutive experiments. The means of the responses for three of the doses of [Val⁵] ANG II examined are presented for comparison in Fig. 5. Although the re-
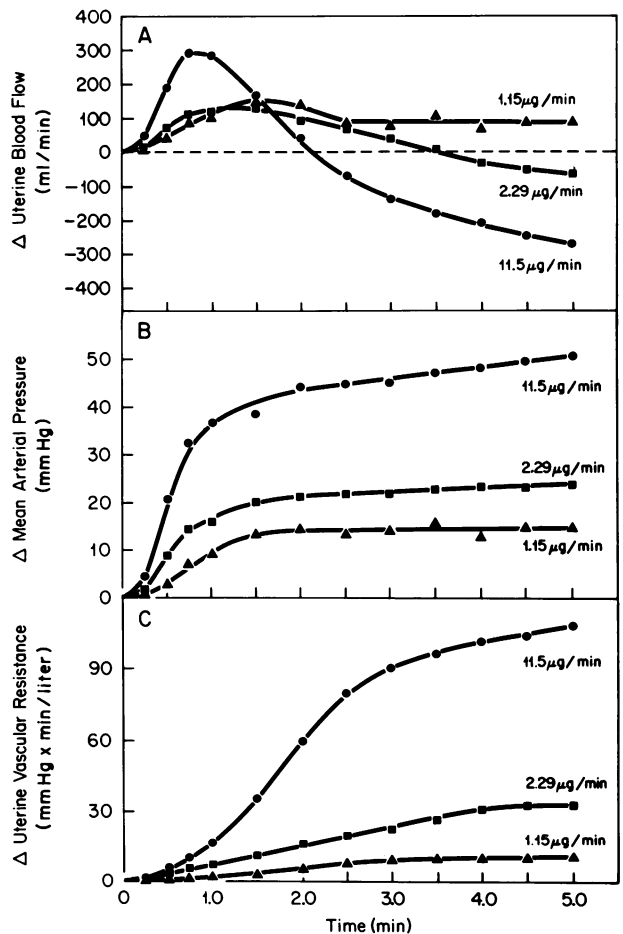

FIGURE 5 The simultaneous changes in UBF (A), MAP (B), and UVR (C) during the continuous systemic infusion of [Val⁵] ANG II. The data for three doses are presented. Each point represents the mean value for seven experiments.

sponses at the two lower doses were similar, the magnitude of the changes was too small to allow satisfactory illustration and thus, are not presented.

We observed a transient rise in UBF (Fig. 5A) within the first 60 s of initiating the infusion of [Val⁵] ANG II with each dose studied. However, with doses ≥2.29 μg [Val⁵] ANG II/min, this was followed by a gradual fall in UBF and an eventual decrease in blood flow during the steady state at 5 min. This is in contrast to the pattern seen with doses ≤1.15 μg [Val⁵] ANG II/min, where UBF also fell after the initial rise, but during the steady state, rather than decreasing, remained elevated from control values. Thus, it appears that the observation (13) that UBF rises with the initiation of high doses of [Val⁵] ANG II is true for all doses but is less obvious at the lower ones, as illustrated in Fig. 5A. These responses can be explained by examining the simultaneous changes in MAP and UVR. The changes that occurred in MAP and UVR were dose related (Fig. 5B and C). However, when one compares the changes in MAP with those of UVR, there is a distinct difference in the rapidity of the responses, MAP reaching >80% of the final response by 1.4 min, whereas 80% of the final response in UVR is not attained until 2.8 min or later. This difference provides an explanation for the biphasic changes in UBF illustrated in Figs. 1 and 5A. For example, at 11.5 μg [Val⁵] ANG II/min, the peak in UBF occurs at a time, ~0.75 min, when a relatively large increase in MAP, 44%, is offset by only a small increase in UVR, 13%. Subsequently, MAP rises only slightly whereas UVR continues to increase, and UBF is found to fall. Eventually, at ~2 min, the rise in UVR becomes equivalent to the increase in MAP (59%) and no net change in UBF is observed; after this the continuing rise in UVR outweighs the change in MAP, resulting in the net fall in UBF seen during the steady state. This pattern of a delay in the rise in UVR compared with that in MAP was seen at all doses examined.

DISCUSSION

It has not been clear whether the changes in uterine blood flow produced by infused angiotensin II in various species were reflective of uterine vasoconstriction or vasodilation. Part of this dilemma could have resulted from the use of animals studied during the stress of surgery and anesthesia as pointed out specifically with regard to angiotensin II by Assali et al. (14) and other vasoactive compounds by Killam et al. (15) and Rosenfeld et al. (16). However, in studies using chronically instrumented pregnant ewes remote from surgery, we (13) and Lieb et al. (20) have observed that both increases and decreases in UBF could be seen in response to a constant, systemic infusion of [Val⁵] ANG II and that the particular response was dose dependent. In view of these results we sought to clarify the effect of [Val⁵] ANG II on the uteroplacental vascular bed as it relates to the dose and duration of the infusion of [Val⁵] ANG II and to the relationship between the uterine and systemic vascular responses to [Val⁵] ANG II.

In the studies reported herein, we have demonstrated that during the systemic infusion of [Val⁵] ANG II, only increases in UVR occur over a broad range of doses, and that the magnitude of the rise in UVR is dose dependent. Thus, it is apparent that [Val⁵] ANG II acts only as a uterine vasoconstrictor under the experimental conditions used, and there is no evidence for its having a vasodilator action in the gravid ovine uterus. In this respect the uteroplacental vascular bed responds to [Val⁵] ANG II like other vascular beds. This is consistent with the observations of Greiss and Van Wilkes (1), who reported a dose-related fall in uterine conductance during systemic infusions of [Val⁵] ANG II into anesthetized pregnant sheep, and Cohen et al. (2) who reported similar findings with local infusions of [Val⁵] ANG II into the uterine artery of acutely studied pregnant rabbits.

Although the action of [Val⁵] ANG II as a uterine vasoconstrictor would explain the observed decreases in UBF, the increases in UBF seen under various conditions require further elucidation. To explain this bidirectional response of UBF it is necessary to consider the changes in UBF in relation to the simultaneous changes in MAP and UVR. In the present studies UBF rose when the increase in uterine perfusion pressure (MAP) exceeded the relative increase in UVR (Figs. 3 and 5). This occurred during the steady state with the lower doses of [Val⁵] ANG II, 0.115–1.15 μg/min (Fig. 3). At higher doses of [Val⁵] ANG II, ≥2.29 μg/ min, the increase in UVR was relatively greater than the increase in perfusion pressure, resulting in the observed fall in UBF. The same relationship between the relative changes in MAP and UVR to the simultaneous responses in UBF also was seen during the course of the infusion of [Val⁵] ANG II (Fig. 5). That is, whenever the rise in perfusion pressure exceeded that in UVR, there was a net increase in UBF, and when the rise in UVR exceeded that in MAP, a net decrease in UBF resulted. This relationship has been noted recently by others (20). However, in many of the previous studies of the effect of angiotensin II on UBF, in which UVR was not carefully evaluated, it has been concluded that the rise in UBF was a reflection of uterine vasodilation. We would argue that in the situation where perfusion pressure is changing, the existence of vasoconstriction vs. vasodilation in any organ bed is more appropriately determined by considering the changes in vascular resistance rather than those in absolute blood flow. It is likely that if this principle were applied to previously published data, conclusions similar to those made in our study might be drawn. Additionally, it can be seen

from the time-courses of the responses to infused [Val⁵] ANG II (Fig. 5) that observations made after bolus injections or during the first 3–4 min of an infusion of angiotensin II may well have added to the discrepancies between reports on the responses of the uteroplacental circulation to angiotensin II.

In addition to establishing the mechanisms responsible for the bidirectional and biphasic responses of UBF to systemic [Val⁵] ANG II, we also noted another phenomenon of considerable interest, namely, the relative refractoriness of the uteroplacental vascular bed to the vasoconstricting effects of [Val⁵] ANG II. These findings appear to be in conflict with those of Greiss and Van Wilkes (1), who concluded that the degree of vasoconstriction induced in the uteroplacental vascular bed by [Val⁵] ANG II is as great or greater than that seen in other peripheral beds. However, these investigators used doses of [Val⁵] ANG II equivalent to the highest used in this study, >5 μg/min. At these doses, we too observed that the change in UVR exceeded that of MAP and SVR, whereas at all doses <4–5 μg/min uterine refractoriness to [Val⁵] ANG II was quite evident (Fig. 4). Also, it is likely that infusion rates of [Val⁵] ANG II > 1 μg/min produce pharmacological rather than physiological levels of [Val⁵] ANG II in the circulation.

The explanation for this relative refractoriness of the uteroplacental circulation to [Val⁵] ANG II is not clear. Because, in this study, [Val⁵] ANG II was infused into the vena cava, it is reasonable to assume that all systemic arterial beds received an approximately equal concentration of [Val⁵] ANG II. However, the refractoriness of the uteroplacental vessels could reflect increased metabolism of [Val⁵] ANG II at the vessel wall, a difference in the number or affinity of the [Val⁵] ANG II receptors, or the antagonizing influence of a separate, locally produced vasodilator substance.

This refractoriness of the uterine vasculature during pregnancy cannot be generalized to all vasoconstrictor substances. As we have reported previously, continuous infusions of norepinephrine (21) and epinephrine (22) into pregnant sheep result in a greater reduction in blood flow to the uterus than to any of the other organs or tissues examined. In these studies, SVR rose ~12% in the case of norepinephrine and fell ~20% with epinephrine, a striking contrast to the simultaneous 60% increase in UVR seen with both catecholamines. It is important to note that these data were obtained with a low dose of catecholamine at which no change in systemic arterial pressure occurred.

The implications of the uterine refractoriness to [Val⁵] ANG II are of clinical and physiological interest. This would confer a significant advantage on the uteroplacental circulation, in that [Val⁵]ANG II, and possibly some other vasoconstrictor substances, at plasma concentrations sufficient to produce modest increases in arterial pressure, can result in increased uteroplacental perfusion, suggesting a protective mechanism whereby fetal well-being is maintained. A situation analogous to this may exist in women with mild pregnancy-induced hypertension in whom the estimated UBF has been reported to be either normal (23) or increased (24). In this instance fetal jeopardy and growth retardation are uncommon. However, more severe degrees of systemic vasoconstriction associated with marked pregnancy-induced hypertension are generally considered to be associated with impaired UBF (25). This may be analogous to the loss of relative refractoriness of the uteroplacental vasculature to the effect of a circulating vasoconstrictor, as observed at higher doses of [Val⁵] ANG II. This phenomenon may have additional clinical significance as to whether mild to moderate increases in blood pressure during pregnancy should be reduced by antihypertensive agents, especially in the absence of any readily available measure of uteroplacental perfusion. Antihypertensive drugs, even those whose primary action appears to be that of vasodilation, may cause a greater reduction in SVR than UVR, resulting in a disproportionate fall in uterine perfusion pressure compared with the degree of uterine vasodilation. In this regard Gant and co-workers (26–28) have provided data from human pregnancies in which it appears that the use of antihypertensive agents could have deleterious effects on uteroplacental perfusion. The implications of these observations as well as the mechanism responsible for the uterine refractoriness to [Val⁵] ANG II observed in our studies require further investigation.

ACKNOWLEDGMENTS

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