Mechanisms regulating angiotensin II responsiveness by the uteroplacental circulation

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Rosenfeld, Charles R. Mechanisms regulating angiotensin II responsiveness by the uteroplacental circulation. Am J Physiol Regulatory Integrative Comp Physiol 281: R1025–R1040, 2001.—Pregnancy is associated with increases in cardiac output and uterine blood flow (UBF) and a fall in systemic vascular resistance. In ovine pregnancy, UBF rises from ~3% of cardiac output to ~25% at term gestation, reflecting a >30-fold rise in UBF by term. This increase in UBF supports exponential fetal growth during the last trimester and maintains fetal well-being by providing excess oxygen and nutrient delivery. These hemodynamic changes are associated with numerous hormonal changes, including increases in placental steroid hormones and enhanced activation of the renin-angiotensin and sympathetic nervous systems, all of which are believed to modulate systemic and uterine vascular adaptation and vascular reactivity. Systemic pressor responses to infused ANG II are attenuated in normotensive pregnancies and the uteroplacental vasculature is even less sensitive, suggesting development of mechanisms to maintain basal UBF and permit the rise in UBF necessary for fetal growth and well-being. The effects of ANG II on the uteroplacental vasculature are reviewed, and the mechanisms that may account for attenuated vascular sensitivity are examined, including ANG II metabolism, vascular production of antagonists, ANG II-receptor subtype expression, and the role of indirect mechanisms.

uteroine blood flow; sheep; receptors; angiotensin II metabolism; pregnancy

PREGNANCY IS A UNIQUE physiological state that is responsible for successful propagation of mammalian species. Normal mammalian pregnancy is associated with numerous hemodynamic changes, e.g., in sheep, cardiac output increases >50%, systemic vascular resistance (SVR) falls, arterial blood pressure decreases modestly, and cardiac output is redistributed (36, 91, 114, 116). Unique to pregnancy is a rise in uterine blood flow (UBF), which in sheep is >30-fold and reflects an increase from 3–5% of cardiac output in the nonpregnant state to 20–25% at term gestation (36, 108, 113, 114, 116, 124). This exponential rise in UBF predominantly occurs in the last two-thirds of gestation. It is associated with placentation, i.e., angiogenesis, followed by vasodilation and is essential for normal fetal growth and well-being, providing the oxygen and nutrient delivery required for the exponential fetal growth that parallels the rise in UBF in the last trimester (113, 116). As UBF increases in pregnancy it is redistributed within the uterus, and at term >85% of total UBF is directed toward the maternal placental vascular bed (87, 115). Thus the rise in UBF is predominantly due to exponential increases in placental blood flow (108, 124). These cardiovascular changes are associated with modifications in the endocrine milieu, representing placental- and nonplacental-derived substances, including steroid hormones and products of the renin-angiotensin system (RAS). Although there is an apparent interdigitation between the hemodynamic and endocrine alterations occurring in pregnancy, the precise relationships remain unclear.
In addition to the general cardiovascular modifications described, there are changes in vascular responsiveness or sensitivity to several vasoconstrictors. For example, pregnant women develop attenuated pressor responses to infused ANG II and $\alpha$-adrenergic agents (1, 20, 49). These changes occur early in pregnancy and are of interest because the refractoriness to ANG II is lost as early as the midtrimester in women who later develop pregnancy-induced hypertension (49, 141). It has been proposed that by understanding this particular aspect of pregnancy one may subsequently determine the pathogenesis of this hypertensive disorder, which affects 5–10% of pregnant women (36). The uterine vascular bed also is refractory to ANG II in normotensive pregnancies (44, 95, 125), and, similar to the systemic vasculature, this is lost in the presence of hypertension (45), which is often paralleled by a fall in UBF, as well as uterine oxygen and nutrient delivery, and compromised fetal growth and well-being. Thus it would be important to determine what adaptive mechanisms modulate the systemic and uterine vascular beds during normal pregnancy and if these mechanisms are similarly affected in the presence of hypertensive disease.

The changes in the hormonal milieu in normotensive pregnancies are extensive, and, although they have been reasonably well characterized, their purposes are far from understood. It is believed that ovarian- and placental-derived estrogens and progesterone participate in modifying vascular reactivity and may facilitate the widespread vasodilation observed in pregnancy (116). The increase in the activity of the RAS in pregnancy has received a great deal of attention. It is associated with increases in the synthesis of renin, angiotensinogen of hepatic and nonhepatic origins, and ANG II, resulting in increases in plasma renin activity and circulating levels of ANG II (77, 132, 138). The active agent produced by the RAS, and the uteroplacental circulation. Because much of our knowledge regarding these interactions has been derived from extensive animal studies, in large part from the chronically instrumented ovine species, I will use these data to describe our present state of knowledge, but I will refer to the human when reasonable correlates are available, thereby demonstrating the similarities that exist between the species.

ANG II AND VASCULAR REACTIVITY: SYSTEMIC VS. UTERINE

Normal pregnancy is associated with attenuated pressor responses to systemic infusions of ANG II (1, 20, 49, 141). This is detected as early as the midtrimester and is no longer evident after parturition (1). Several species develop a similar refractoriness during pregnancy (9, 11, 104, 120). In studies from our laboratories we (120) not only observed attenuated pressor responses to continuous systemic infusions of ANG II that were evident early in ovine gestation, but also that the dose-response curves generated from steady-state responses in nonpregnant and pregnant ewes were strikingly similar to those published for nonpregnant and pregnant women (141). Furthermore, the pressor dose, i.e., the dose required to elicit a 20-mmHg rise in MAP, in nonpregnant and pregnant ewes was the same as that reported for women (141). We (81) later observed that pregnant ewes, similar to pregnant women (20), also develop refractoriness to the pressor effects of systemic infusions of $\alpha$-agonists. These observations, therefore, support the value of using chronically instrumented ewes as a model in which to investigate the mechanisms responsible for the adaptive changes in the cardiovascular system associated with normal pregnancy and may permit us to subsequently develop better strategies to investigate and understand hypertensive diseases in pregnant women.

In our initial studies of the systemic responses to ANG II in pregnancy, we implanted electromagnetic flow probes on both main uterine arteries to continuously monitor UBF, having previously demonstrated the reliability of this method (124). We observed a dose-dependent response in UBF, but little or no change occurred until the systemic dose of ANG II exceeded 0.08 $\mu$g·kg$^{-1}$·min$^{-1}$ (Fig. 1), which results in pharmacologic plasma levels of ANG II (93). Furthermore, there was a biphasic response in UBF during systemic ANG II infusions (Fig. 2). That is, during ANG II infusions >0.1 $\mu$g·min$^{-1}$·kg$^{-1}$ there was a rise in UBF that occurred after the more rapid increase in MAP, followed by a progressive fall in UBF although MAP remained stable, achieving a steady state by 3–6 min. In earlier studies using systemic bolus doses of ANG II and/or anesthetized animals, ANG II was considered a uteroplacental vasodilator, because UBF rose soon after ANG II infusions started (5, 48, 69, 89, 134, 142). These studies, however, 1) frequently used bolus doses of the peptide, 2) were often limited to examining the initial phase of the UBF response, 3) did not always consider the simultaneous changes in perfusion pressure, and 4) were frequently performed in acute animal preparations, which modifies vascular responses to several agents (116, 123). When we (95) examined the
change in uterine vascular resistance (UVR) in chronically instrumented animals across a broad range of continuous systemic ANG II doses and only used the steady-state responses, there were only increases in UVR at all doses studied, demonstrating that ANG II was always a uterine vasoconstrictor in pregnant sheep over a broad range of doses (Fig. 3). This was consistent with observations by Cohen et al. (28) in anesthetized rabbits and is now generally accepted (26, 151). On further inspection of the uterine responses to systemic ANG II infusions, we also observed that the rise in UVR was significantly less than the rise in SVR at all doses of ANG II, suggesting that at physiological and even pharmacologic doses (93) the uterine vascular bed was even "less sensitive" to the vasoconstricting effects of ANG II than the systemic vasculature as a whole.

To understand the mechanisms responsible for the biphasic UBF response to ANG II, we (95) examined the simultaneous relative changes (percent change, %Δ) in perfusion pressure or MAP, UVR and UBF. Because each hemodynamic parameter has a different unit of measurement, the raw data cannot be easily compared; but by controlling for baseline values and calculating the percent change, a comparison of the relative responses is obtained, and any interaction between the three variables is easily assessed (95). When this simple rearrangement was performed and the data analyzed using dose and duration of ANG II infusion in the steady state, it became apparent why others had concluded that ANG II was a uterine vasodilator. At doses of ANG II ≤1.15 μg/min, which generally results in physiological plasma concentrations (93), there was a rise in MAP that always exceeded the rise in UVR and was associated with an increase or no change in UBF (Fig. 4). However, when the dose of ANG II exceeded 2.3 μg/min, resulting in high pharmacological plasma levels (93), the relative rise in UVR...
exceeded the increase in MAP and UBF fell. Thus changes in UBF are highly dependent on the difference in the relative responses in MAP or perfusion pressure and local UVR (55, 70, 148). A similar relationship has been observed in studies of UBF and ANG II in pregnant dogs and guinea pigs and confirmed in the ewe (26, 37, 151). When the responses to individual ANG II doses are analyzed across time, a strikingly similar relationship is seen. That is, at “all” doses of ANG II studied, the change in MAP occurs more “rapidly” than the rise in UVR; thus UBF always increases in the initial response to systemic ANG II infusions (Fig. 5). However, if the dose of ANG II ultimately increases UVR greater than MAP in the steady-state response, e.g., 11.5 µg/min (Fig. 5), UBF clearly falls at this time. Until recently, we and others did not appreciate the meaning of this difference in the timing of the systemic and uterine responses to systemic ANG II infusions. This will be addressed later. Nonetheless, the conclusions from these studies are 1) ANG II is only a uterine vasoconstrictor in all species studied under unstressed conditions as reported for other vascular beds, 2) the uterine vasculature is less sensitive to systemic ANG II infusions than the systemic vasculature at physiological plasma levels of the peptide, and 3) UBF responses in pregnancy must be assessed with consideration to

Fig. 3. Comparison of the simultaneous relative changes in systemic and uterine vascular resistance during the continuous systemic infusion of 5 doses of ANG II in pregnant sheep. Means ± SE are presented. [Reprinted from Naden and Rosenfeld (95) with permission.]

Fig. 4. Comparison of the relative changes in mean arterial pressure (●), uteroplacental blood flow (▲), and UVR (●), expressed as the percent of control values, across a range of continuous systemic infusions of ANG II. Means ± SE are presented. [Reprinted from Naden and Rosenfeld (95) with permission.]

Fig. 5. The simultaneous changes across time in UBF (A), mean arterial pressure (B), and UVR (C) during continuous systemic infusions of ANG II. Data for 3 doses are presented; each point represents the mean of 7 experiments. [Reprinted from Naden and Rosenfeld (95) with permission.]

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simultaneous alterations in perfusion pressure. In other words, UBF is "protected" from the vasoconstrictor effects of elevated circulating ANG II that occurs during pregnancy (77, 132, 138). With the advent of pulse-gated Doppler for measuring UBF or resistance in women, Erkkola et al. (44) observed similar differences in uterine and systemic sensitivity to infused ANG II in normotensive pregnant women. They later reported that this uterine refractoriness, as with systemic refractoriness, was lost in women who developed hypertensive disorders (45). These data, therefore, provide additional evidence that the ewe is an excellent model in which to study normal cardiovascular adaptation in pregnancy and, in particular, the changes in uteroplacental adaptation. Furthermore, although the placental morphology differs between women and sheep (116), changes in uterine vascular reactivity appear to be similar.

Thus far, the data provide insight into changes in total UBF and UVR. The pregnant uterus, however, is comprised of at least three tissues: the myometrium, which makes up the bulk of uterine weight, the endometrium, and the placental cotyledons, the site of gas and nutrient exchange. In nonpregnant ewes these tissues (caruncles in the nonpregnant state are the subsequent sites of implantation and represent the cotyledons) receive a similar proportion of UBF, ~33% (123, 124). However, UBF is gradually redistributed during pregnancy, and the placental portion of UBF increases disproportionately, accounting for ≥85% of total UBF by term (87, 108, 115). Because this portion of UBF is responsible for fetal growth and well-being, it is important to know if ANG II alters placental blood flow. To accomplish this, studies were performed in conscious pregnant ewes using radionuclide-labeled microspheres. To accurately time the microsphere infusions, As anticipated from earlier studies, the relative rise in UVR during systemic ANG II infusions <1 μg/min was less than the rise in SVR, confirming the uterine refractoriness previously noted with flow probe measurements (95). At ANG II doses >5 μg/min, the %ΔUVR exceeded %ΔSVR and MAP, thus UBF fell. Importantly, the lower ANG II dose results in estimated plasma levels of the peptide of ~200 pg/ml (93), which resemble that observed in normotensive pregnant women (77). The higher doses, however, result in plasma levels of ANG II >2,000 pg/ml, which are non-physiological. Placental blood flow and vascular resistance were unchanged with 0.573 and 5.73 μg ANG II/min (Fig. 6), and blood flow fell only 16% with the highest dose studied, 11.5 μg/min. In contrast, endometrial and myometrial blood flows significantly decreased and vascular resistance rose with all three doses (Fig. 6). Thus the placental circulation is refractory to a wide range of systemic ANG II doses. When the distribution of UBF was calculated, the proportion going to the placental cotyledons actually rose from 74% before ANG II infusion to 90% with the pharmacologic dose of the peptide, further demonstrating the protection afforded maternal placental blood flow through increases in perfusion pressure and decreases in blood flow to the other uterine tissues.

It had been suggested that the differences in the responses in total SVR and UVR during systemic ANG II infusions might be due to a greater sensitivity of the nonreproductive compared with reproductive tissues (95). However, data to support this were lacking. This was addressed in the microsphere studies when nearly all of the nonreproductive tissues, including the kidney, adrenal, and adipose, were observed to consistently decrease blood flow and increase vascular resistance at all three doses of ANG II studied (Fig. 7). This provided conclusive evidence of the differences in vascular sensitivity to ANG II that exist between various tissues/organs in normal gestation. Of interest, myometrial and endometrial sensitivities resembled that in peripheral or nonreproductive tissues. Curran-Everett et al. (37) reported similar differences between uteroplacental and nonuteroplacental responses to ANG II in late-gestation guinea pigs. These differences in vascular reactivity are intriguing, because they suggest that ANG II may be a safer pressor agent for treating hypertensive episodes in gravid women than the standard use of α-agonists. Support for this is obtained from observations in women and sheep that ANG II has greater effects on SVR and MAP than UVR, thereby having minimal effects on UBF. More-
over, all α-agonists studied have greater effects on UVR than SVR (53, 56, 81, 99, 110, 117, 126), which may be accentuated in pregnant women with hypertensive disease, but has not been studied.

Although existing data supported the thesis that the uterine vasculature in pregnancy was less responsive to systemic ANG II infusions than the systemic vasculature, it was unclear if this was an inherent characteristic of the uterine vascular bed or due to some adaptive change(s) that occurs in pregnancy. Cox et al. (34) addressed this by comparing systemic and uterine responses to systemic ANG II infusions in pregnant and nonpregnant ewes with flow probes on both main uterine arteries. MAP rose dose dependently in both groups, but as expected, the %ΔMAP in nonpregnant ewes greatly exceeded that in pregnant animals at “all” doses, values increasing 40–65% in the former vs. 20–50% in pregnant ewes, P < 0.0001. Although UVR rose dose dependently in both groups, the responses in nonpregnant ewes were substantially greater at all doses studied (Fig. 8), values rising 80–350% vs. 25–90% in pregnant ewes (P < 0.0001). Thus UBF fell only 20% in pregnant ewes with the highest ANG II dose studied compared with 60% in nonpregnant ewes (Fig. 9). When the simultaneous relative changes in systemic and uterine responses were compared, pregnant ewes had greater relative increases in MAP than UVR at physiological doses, confirming prior observations (95, 125). In contrast, nonpregnant animals had greater increases in UVR than MAP at all ANG II doses, resembling responses to α-agonists (81); e.g., with an estimated plasma ANG II level of 0.8 ng/ml the %ΔUVR was ~130% vs. ~50% for MAP. These data (34), therefore, demonstrate that the uterine vasculature “develops” the pregnancy-associated attenuation in ANG II-induced vasoconstriction, which also occurs with α-agonists (81). Furthermore, they also suggest that the differences in systemic and uterine responses to systemic ANG II infusions in normal pregnancy are not inherent to the uterine vasculature but are due to pregnancy-related changes. This could reflect the growth and development of the less responsive placental vasculature, which accounts for >85% of UBF (125). Alternatively, it might be due to modifications in ANG II metabolism, synthesis of local ANG II antagonists, changes in uterine vascular smooth muscle, alterations in ANG II receptor (ATR) expression and/or binding, or any combination of factors.

ANG II AND VASCULAR REACTIVITY: METABOLISM OF ANG II

One potential explanation for the attenuated systemic pressor responses to infused ANG II in pregnancy is that the peptide is cleared from the circulation at a greater rate than that observed in the nonpregnant state. This also would explain the attenuated

Fig. 7. Dose-dependent effects of continuous systemic infusions of ANG II on vascular resistance in kidneys, adrenal glands, and adipose tissue in near-term pregnant sheep. Blood flows were measured with radionuclide-labeled microspheres. SE are presented. *P < 0.05, †P < 0.02, **P < 0.001. [Reprinted with permission (125).]

Fig. 8. Comparison of the effects of comparable systemic and local intra-arterial infusions of ANG II on the relative changes in UVR in pregnant (A) and nonpregnant (B) sheep. Different letters within each group (i.e., systemic and intra-arterial) denote significant differences in responses across concentrations using repeated-measures ANOVA, P < 0.001. [Reprinted with permission (34).]
steady-state infusions. The values for MCR\textsubscript{ANG II} in nonpregnant ewes are consistent with earlier observations in nonpregnant sheep (46, 47) and the human (40, 65, 100). Magness et al. (77) subsequently reported that the MCR\textsubscript{ANG II} was not significantly different in normotensive nonpregnant and pregnant women, 85 ± 10 vs. 68 ± 3 ml·min\textsuperscript{-1}·kg\textsuperscript{-1}, respectively. Moreover, these values resemble those observed in the ewe, demonstrating another similarity between species (93, 121). The half-life for ANG II was ~49 s, which also is consistent with other species and women (40, 42). When the removal rate of ANG II by circulating aminopeptidases was examined, the estimated half-life was ~10 min (47), which is inconsistent with removal rates seen in vivo. Thus increases in circulating aminopeptidase enzymes associated with pregnancy probably play a minor role in ANG II removal. It also was determined that the increased volume of distribution in pregnancy could not account for the attenuated responses.

Subsequently, Rosenfeld et al. (121) examined ANG II clearance across the uteroplacental vascular bed of pregnant sheep. They confirmed the observations of Naden et al. (93) for maternal MCR\textsubscript{ANG II} in pregnant sheep and reported that uteroplacental clearance averaged 20 ± 6% in term animals. This is in sharp contrast to a fetal MCR\textsubscript{ANG II} of 680 ml·min\textsuperscript{-1}·kg\textsuperscript{-1} and >90% clearance of ANG II across the fetal placental vascular bed (121, 135). If the adult rat kidney clears ANG II at 1 μg·min\textsuperscript{-1}·g kidney\textsuperscript{-1} (73) and ovine kidneys weigh ~180 g, renal clearance could account for removal of a predominant portion of ANG II from the maternal circulation, which is consistent with the data for uteroplacental clearance. Therefore, in women and sheep neither the attenuated systemic nor uterine responses to infused ANG II in pregnancy reflect enhanced ANG II removal from the circulation. More recently, Iyer et al. (62) reported that neither ATR subtype is directly involved in ANG II clearance in hypertensive adult male rats. However, the type 2 ATR (AT\textsubscript{2}R) appeared to enhance ANG II clearance. Although the role of the ATR subtypes in MCR\textsubscript{ANG II} has not been examined in pregnancy, the AT\textsubscript{2}R subtype predominates in the uterine vascular bed of women and sheep (see below; 32, 35), and its role in uteroplacental ANG II clearance has not been examined.

**ANG II AND VASCULAR REACTIVITY: LOCAL ANTAGONISTS**

Pregnancy is associated with enhanced vascular production of several vasoactive substances, including PGs (52, 78, 82), estrogens (36, 116), and nitric oxide (NO; 133), which may increase organ and tissue blood flows and antagonize local vascular responses to ANG II or other vasoconstrictors so that blood flow is maintained. The existing literature on PGs in pregnancy is immense and cannot be completely examined in this review; therefore, only pertinent points relative to ANG II and UBF will be addressed.
During pregnancy there are increases in circulating PGs, particularly vasodilating PGs (52, 82), and in the uterine synthesis of these compounds (78, 142, 149). Furthermore, treatment with cyclooxygenase inhibitors has been shown to increase pressor responses to infused ANG II in some, but not all, species during pregnancy (29, 59, 151, 152). Similarly, infusion of PGs into intact animals has had variable effects on UBF. For example, systemic infusions of prostacyclin (PGI2) in pregnant ewes and guinea pigs are associated with a fall in MAP and UBF (26, 111, 151), whereas local intra-arterial infusions have no effect on MAP but consistently increase UBF (24, 72). In nonpregnant ewes, PGE2 is a uterine vasodilator, but in pregnancy it decreases UBF (25, 72). We now appreciate that many of these contradictory and confusing observations reflect the mode of administration and the simultaneous effects on MAP or perfusion pressure and UVR, whereas in other instances this has been due to increases in myometrial contractility occurring simultaneously with the vascular responses, which obscures vasodilation by increasing intramural pressure (24, 25, 98, 112).

To address these confounding variables, Magness et al. (79) studied in vitro PGI2 synthesis in intact uterine and systemic arteries, the latter represented by omental arteries, at different times in reproduction. Basal PGI2 synthesis by uterine and systemic arteries from near-term pregnant ewes was 10- and 3-fold greater, respectively, than synthesis by vessels from nonpregnant animals, and values fell in the postpartum period, returning to nonpregnant rates by 2 wk postpartum (Fig. 10). Moreover, in pregnancy and the early postpartum period (~1 wk), basal PGI2 synthesis in uterine arteries exceeded that by systemic arteries. This difference was not evident in systemic and uterine arteries collected from nonpregnant and late postpartum ewes, demonstrating a reversible pregnancy-induced rise in basal vascular PGI2 synthesis, which could be involved in the vasodilation and attenuated vasoconstrictor responses seen in both vascular beds. These investigators also observed for the first time that incubation of uterine arteries from pregnant and early postpartum ewes with ANG II resulted in a further dose-dependent increase in PGI2 synthesis, as reported in the renal and splenic vascular beds (41, 88). However, the maximum values achieved with 10^{-5} M ANG II were greatest in arteries from near-term pregnant ewes, 762 ± 145 vs. 229 ± 47 pg·mg^{-1}·h^{-1} (P < 0.001), respectively. This was not seen in systemic vessels from any group of animals and was inhibited by the nonspecific ATR antagonist saralasin. Thus a receptor-mediated mechanism exists in pregnant ovine uterine arteries that has the potential to further attenuate the vasoconstricting effects of infused and endogenous ANG II (or other vasoconstrictors) and account for the differential uterine and systemic responses described (95). This ANG II-induced rise in uterine artery PGI2 is derived solely from the endothelium (83, 85), which accounts for ~60% of basal PGI2 synthesis in pregnant uterine and systemic arteries, is mediated by activating type 1 ATR (AT1R; 32, 86), which are upregulated in pregnancy (10), and is calcium dependent (83). Recently, Janowiak et al. (63) reported that uterine artery endothelium cyclooxygenase-1 is also upregulated in ovine pregnancy, whereas changes in uterine artery smooth muscle expression are less clear. Yoshimura et al. (156) confirmed the stimulatory effects of ANG II on in vitro PGI2 synthesis by uterine arteries from pregnant ewes, but reported that this effect was not evident in the maternal placental vasculature, which had PGI2 and PGE2 synthesis rates that were only 25–30% of that seen in the uterine artery. Glance et al. (50) observed a similar lack of effect of ANG II on PG synthesis in the human placenta. The markedly attenuated placental responses to ANG II, therefore, may not be due to local basal or stimulated PG synthesis. In preliminary studies, ANG II also increased human uterine artery PGI2 synthesis (unpublished observations), but this requires additional study.

If basal uterine artery PGI2 synthesis increases in normal pregnancy as well as uterine synthesis of other PGs, it is logical to assume that PGs may modulate basal UBF and uterine vascular sensitivity to vasoconstrictors. However, indomethacin, a nonspecific cyclooxygenase inhibitor, only transiently alters basal UBF, UVR, and SVR, with values returning to baseline within 15–20 min despite falling PG levels, whereas meclofenamate has no effect (89, 94, 151). Therefore, several investigators have concluded that the rise in basal uterine PG synthesis in pregnancy does not regulate basal UVR and UBF. Alternatively, ANG II-induced increases in uterine artery PGI2 synthesis plus the increase in basal synthesis associated with pregnancy (79) may protect the uteroplacental vascu-
lar bed from the effects of ANG II or other vasoconstrictors. This was addressed in intact pregnant ewes by infusing systemic ANG II in the absence or presence of “local” intra-arterial infusions of indomethacin, a paradigm that would remove any confounding systemic effects (84). In the absence of indomethacin, systemic ANG II infusions increased uterine venous PGI₂ from 192 to 1,044 pg/ml dose dependently ($P < 0.05$) with a modest effect on arterial levels due to the large increase in uterine synthesis (84). Thus in vitro observations (79) were replicated in intact conscious animals. Although local indomethacin did not alter basal UBF, UVR, or MAP, uterine venous and venaarterial concentration differences of PGI₂ fell ~75%, the latter decreasing from $123 \pm 29$ to $28 \pm 14$ pg/ml. Furthermore, ANG II no longer affected uterine venous PGI₂, with values remaining ~50 pg/ml. However, the ANG II-mediated UVR dose-response curve in the treated uterine horn was shifted upward and to the left, and UBF now fell at all systemic doses of ANG II (Fig. 11A). The contralateral uterine horn was unaffected (Fig. 11B) as was the response in MAP. Thus uterine vascular responses to systemic ANG II infusions after local cyclooxygenase inhibition resembled those seen in nontreated nonpregnant ewes (34). Although this is consistent with observations by McLaughlin et al. (89), it differs from that observed by Woods (152) in pregnant dogs. In those studies, utero-placental sensitivity to ANG II was unaffected by PG inhibition with meclofenamate. This might be due to differences in species or the cyclooxygenase inhibitor used.

From these observations it is possible to conclude that total PG synthesis increases during pregnancy in several species, and uterine synthesis increases dramatically. However, existing data do not consistently support the hypothesis that increases in basal PG synthesis account for the systemic or uterine vasodilation characteristic of pregnancy. There is evidence that PGs may modulate uterine and systemic responses to vasoconstrictors, in particular, responses to systemic infusions of ANG II and α-agonists (8, 27). Importantly, these studies and those noted earlier again demonstrate the importance of examining responses to local and systemic infusions of inhibitors, PGs, and maybe ANG II, which will be addressed later.

More recently, there has been accumulating evidence that vascular NO synthase (NOS) activity increases in pregnancy and may play a pivotal role in the vasodilation and attenuated vascular reactivity associated with pregnancy (see review, Ref. 133). For example, systemic and peripheral inhibition of NOS increases responsiveness to infused ANG II in pregnant but not nonpregnant rats (2, 74, 97), suggesting NOS is upregulated in the peripheral vasculature in pregnancy and serves in part to attenuate responses to ANG II and other vasoconstrictors. Uterine artery endothelial NOS also is elevated in pregnancy and is associated with substantial increases in uterine cGMP production (86, 119, 133, 153). However, its role in modulating the >30-fold rise in UBF at term pregnancy is unclear, because short-term intra-arterial infusions of $N^\omega$-nitro-L-arginine methyl ester, a nonspe-

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**Fig. 11.** Effects of continuous systemic ANG II infusions on the simultaneous relative changes in uterine perfusion pressure (MAP), UVR, and UBF before and after local intra-arterial indomethacin (INDO) infusion ipsilateral (A) and contralateral (B) to INDO. [Reprinted with permission (84).]
specific NOS inhibitor, decreased uterine cGMP synthesis without altering basal UBF (133).

Several investigators recently reported that ANG II stimulates local NO synthesis, and pretreatment with an NOS antagonist enhances constrictor responses to infused ANG II (39, 139). Furthermore, this may be mediated through activation of vascular smooth muscle AT2R (130, 144). As discussed later, this may be important in the uterine circulation. In ovine pregnancy, ~80% of uterine artery NOS activity is located in the endothelium (85, 86); in contrast to prostacyclin, this does not differ from that observed in the omental artery. As an indirect measure of NOS activity, uterine artery cGMP synthesis increases approximately twofold in the presence of 50 nM ANG II. Unlike PGI2, this is not unique to the uterine artery of pregnant animals and is observed in systemic arteries from both groups. Nonetheless, total cGMP production by uterine arteries, i.e., basal plus ANG II-stimulated, is 2.5-fold greater than that by omental arteries, ~1,000 vs. 400 fmol/mg of tissue weight, respectively, which could contribute to the attenuated uterine responses to infused ANG II in pregnancy and the differences between the uterine and systemic responses. In fact, the additive effects of enhanced PGI2 and NO synthesis in the uterine and systemic responses. This, however, has not been well studied to date and requires verification and testing. It also is unclear if ANG II directly or indirectly increases uterine artery endothelial NOS activity (130). It is intriguing, however, that estrogen upregulates both endothelial NOS in endothelium and neuronal NOS in smooth muscle of uterine arteries from nonpregnant ewes within 90 min and after daily exposure (119, 127, 146, 147) and is associated with attenuated systemic pressor responses to infused ANG II (80, 104, 122). Furthermore, uterine responses to ANG II after estrogen treatment are strikingly similar to that observed in pregnant animals, i.e., the rise in UVR is less than the rise in MAP (96), which is opposite that seen in untreated nonpregnant animals (34). Thus placental estrogens may be involved in modifying vascular reactivity in pregnancy.

ANG II AND RECEPTOR EXPRESSION

It is now clear that at least two subtypes of the ATR are expressed in large mammals, AT1R and AT2R, whereas there are three in the rodent, AT1AR, AT1BR, and AT2R (7, 13, 60, 61). They are considered members of the seven transmembrane-spanning receptor superfamily, are derived from separate gene products, and have only 40% homology. Of particular note, the AT2R is located on the X chromosome, whereas the AT1R is on chromosome 3 (60). The AT1R is the predominant subtype in the adult, is inhibited by the specific antagonist losartan, is G protein coupled, activates phosphoinoside metabolism and phospholipase C, mobilizes intracellular calcium, and mediates vascular smooth muscle contraction as well as most other biologic actions of ANG II (7, 13, 60). In contrast, the AT2R predominates in the developing fetus (30, 54, 150), is the major subtype in fetal and early neonatal vascular smooth muscle (31), does not appear to interact with G proteins (12, 31), and, although its function is relatively unclear, it appears to modify AT1R effects and participate in vascular remodeling (13, 30, 43, 60, 130).

Inasmuch as plasma ANG II is elevated in pregnant women and sheep (76, 77, 93), it stands to reason that the vascular ATR would be downregulated in normotensive pregnancy, which would explain the attenuated pressor responses to infused ANG II. However, in contrast to the myometrium (30, 131), we (30, 76, 118) and others (15, 105) observed that total vascular smooth muscle ATR binding density (Bmax) and affinity were similar in pregnant and nonpregnant animals, and this was true in both the systemic and uterine vasculature. Although Burrell and Lumbers (17) also observed no change in ovine aortic ATR Bmax in pregnancy, uterine artery ATR Bmax rose from ~20 to ~40 fmol/mg protein, the opposite of that anticipated. Nonetheless, the absence of ATR downregulation in pregnancy raises questions regarding the mechanisms regulating ATR expression and turnover in pregnancy, which remain unanswered. While this is not the subject of this review, placental steroids may be involved (68, 128). When Cox et al. (32) determined ATR subtype expression in vascular smooth muscle throughout ovine reproduction, the AT1R was the predominate receptor in all vascular beds examined except the uterus, where the AT2R accounted for 75–90% of total binding in uterine artery smooth muscle from nulliparous, pregnant, postpartum, and nonpregnant ewes. Similar observations were made in uterine arteries from nonpregnant and pregnant women (35), again demonstrating the striking similarity between the two species. The only other adult vascular bed with AT2R predominance is the rat cerebral vasculature (145). This lack of change in vascular ATR subtype expression in pregnancy differs from that in the myometrium of women and sheep, where total Bmax not only falls but is associated with AT2R downregulation, resulting in AT1R predominance (30, 35, 131). Burrell and Lumbers (17), however, reported that AT2R Bmax rose from <5 fmol/mg protein to ~38 fmol/mg protein in ovine uterine arteries by term gestation, and although AT2R accounted for ~60% of total binding at term, only 5% was seen in nonpregnant arteries. AT1R binding density was unaltered. Their study differed in that they used uterine arteries that were frozen with intact endothelium and analyzed in the absence of protease inhibitors. It is unclear, however, what accounts for the discrepancy in the two studies. Furthermore, it is unclear why they did not see the rise in AT1R expression reported in uterine artery endothelium in ovine pregnancy (10).

Since AT2R do not mediate smooth muscle contraction responses (30, 32, 35), yet they account for nearly 85% of ATR binding in uterine artery smooth muscle, we sought to determine if this might explain the uterine vascular refractoriness to ANG II in pregnant women and sheep. If so, it would raise important questions regarding the mechanism(s) whereby systemic
ANG II infusions increase UVR. Although the majority of studies examining UBF responses to ANG II used systemic infusions, Clark et al. (26) studied the effects of both systemic and local intra-arterial ANG II infusions on UBF and UVR. However, only high doses of the peptide were locally infused, and there were no data given regarding the effects on MAP. We (34), therefore, performed studies in nonpregnant and pregnant ewes comparing uterine and systemic responses to a wide range of ANG II doses infused either systematically or locally into the uterine artery to exclude systemic effects. These doses resulted in arterial plasma concentrations ranging from physiological, i.e., ~400 pg/ml, to pharmacologic values, ~2,000 pg/ml. To compare responses, intra-arterial doses were calculated to attain arterial plasma concentrations achieved during systemic infusions (93). As anticipated, systemic ANG II infusions recapitulated previous observations; i.e., uterine and systemic responses were greater in nonpregnant vs. pregnant ewes, and in pregnant ewes, uterine responses were less than systemic. In contrast, local intra-arterial ANG II infusions in nonpregnant and pregnant ewes did not elicit a significant rise in UVR (Fig. 8) or a fall in UBF (Fig. 9) in the absence of a systemic pressor response. However, whenever UVR rose and UBF fell during local ANG II infusions, responses were always delayed and always followed a rise in MAP (Fig. 12), suggesting that ANG II had to reach the systemic circulation before eliciting a uterine response. Furthermore, the rise in MAP was consistently delayed compared with that seen during systemic ANG II infusions. This pattern of response resembles that observed in the initial studies of ANG II, i.e., the fall in UVR always followed the rise in SVR and MAP (Figs. 2–5). More recently, Lambers et al. (71) reported vasoconstrictive responses to intra-arterial ANG II infusions in estrogenized nonpregnant ewes. However, the bolus dose used to show specificity of the response was pharmacologic and is estimated to result in arterial concentrations >5,000 pg/ml. Furthermore, rises in MAP occurred with all other local doses of ANG II studied, which are estimated to range from >600 to >6,000 pg/ml.

We interpreted our results to mean that the effects of ANG II on the uterine vascular bed may be mediated by the systemic release of another more potent vasoconstrictor. This is supported by the predominance of AT2R binding in uterine vascular smooth muscle and studies demonstrating that ANG II may enhance catecholamine release (16, 106), delay catecholamine reuptake at the neuromuscular junction (18), and/or stimulate synthesis and release of smooth muscle endothelin (19). While studies are underway to examine this, preliminary evidence supports involvement of another agent (33). Alternatively, simultaneous AT2R activation may attenuate or inhibit AT1R-mediated increases in UVR. This is supported by recent studies in nonpregnant estrogenized ewes and in vitro studies with uterine arteries from pregnant sheep and rats (71, 90, 157). We also observed ANG II-mediated constriction of uterine artery rings, which is inhibited by the AT1R antagonist losartan; however, the responses were quite small compared with KCl and α-stimulation. We did not see potentiation by AT2R inhibition or stimulation. Additional studies are warranted to address this aspect of ANG II-mediated effects.

ANG II AND VASCULAR REACTIVITY: SMOOTH MUSCLE GROWTH

Although space does not permit a detailed review of this aspect of the effects of ANG II on the uteroplacental circulation, ANG II is known to mediate vascular smooth muscle hypertrophy via the AT1R (21, 102). In vitro studies of denuded uterine artery strips from nonpregnant, pregnant, and postpartum sheep, Annibale et al. (3) were unable to elicit reproducible responses to ANG II, which is consistent with the observation of AT1R predominance in these vessels (32). However, responses to KCl and α-agonist stimulation were enhanced in uterine arteries from pregnant vs. nonpregnant sheep, which was no longer evident in the postpartum period. In contrast, renal and carotid artery responses were unaffected by pregnancy. This is consistent with other studies examining the difference...
between uterine artery responses to ANG II and α-agonists (53, 81, 99, 117, 126). Annibale et al. (4) subsequently reported that the uterine artery was hypertrophied in pregnancy and contained increased myosin and actin contents, consistent with observations by Griendling et al. (57). St. Louis et al. (136) reported similar increases in responsiveness to agonists by arcuate arteries from pregnant rats. They concluded that the mechanical properties of these arteries had changed. Growth and hypertrophy also occur in more distal uterine arteries from pregnant rabbits and guinea pigs (23, 66, 67, 101). Thus the uterine vascular bed undergoes substantial growth and remodeling, resulting in uterine artery hypertrophy, but the mechanism for this is unclear. It could be due to the rise in UBF and increase in shear stress, increases in circulating ANG II via AT1R activation, or the increase in local synthesis of placental estrogens. Obviously, this is an area that deserves further attention.

SUMMARY

Maintenance and growth of the uteroplacental circulation is essential for the normal growth and well-being of the developing fetus, and prolonged decreases in UBF result in fetal growth restriction, which may also impair fetal tolerance of labor. The RAS is believed to play an important role in modulating cardiovascular adaptation during pregnancy. The development of refactoriness to the vasoconstrictor effects of ANG II is considered an important aspect of this adaptation, because its absence is associated with maternal cardiovascular disease and increases in fetal growth restriction and fetal and neonatal morbidity. In this review I have examined the mechanisms whereby the effects of ANG II on the uterine circulation are normally modulated. Existing data suggest that the predominance of AT2R binding in uterine vascular smooth muscle may be the predominant mechanism responsible for the attenuated uterine responses to infused ANG II in women and sheep. They also suggest that systemic ANG II infusions may mediate their effects on the uterine circulation through the release of other vasoconstricting agents, such as catecholamines. Furthermore, the combined effects of increases in uterine artery basal and stimulated PGI2 and NO synthesis in pregnancy may serve to modify responses to ANG II and these secondary vasoconstrictors. Thus women with pregnancy-induced hypertension and increased uterine sensitivity to infused ANG II may have abnormalities in vascular synthesis of PGs and/or NO, alterations in AT2R function or expression, or marked increases in these secondary vasoconstrictors, such as catecholamines. Evidence for the latter is obtained from studies in women with pregnancy-induced hypertension who appear to have increases in sympathetic outflow (129, 155). Studies are now underway to examine this hypothesis. With the recent advent of genetically engineered models that delete or overexpress various components of the RAS, e.g., angiotensinogen and ATR subtypes, it may be possible to further delineate the importance of each component in normal and abnormal pregnancy adaptation.

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