Differential Sensitivity to Angiotensin II and Norepinephrine in Human Uterine Arteries

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Background: During pregnancy, uteroplacental responses to norepinephrine (NE) exceed systemic responses. In contrast, uteroplacental responses to angiotensin II (ANG II) are less than systemic. The explanation for these differences in uteroplacental sensitivity remain unclear but may reflect type 2 ANG II receptor (AT2R) predominance in uterine artery (UA) vascular smooth muscle (VSM).

Objective: The objective of the study was to examine VSM sensitivity to KCl, NE, and ANG II in UA from nonpregnant (NP) and pregnant (P) women and determine VSM ANG II receptor subtype expression.

Methods: Responses to KCl, NE, and ANG II were examined in endothelium-denuded UA rings from NP (n = 28) and P (n = 13; 34–40 wk gestation) women, and ANG II receptor subtype, contractile proteins were measured.

Results: KCl and NE dose dependently contracted UA (P < 0.001), P exceeding NP 2-fold or greater; but AT1R receptor expression was unchanged. ANG II did not elicit dose effects in NP or P UA; however, P responses exceeded NP approximately 2-fold (P < 0.001) and were approximately 2.5-fold less than NE (P < 0.001). AT1R and AT1R expression were similar (P > 0.1) in VSM from NP and term P women. AT1R blockade abolished ANG II contractions (P < 0.001); AT2R blockade did not enhance ANG II sensitivity in UA with or without endothelium. Actin contents increased approximately 2-fold in term UA.

Conclusions: Sensitivity to α-stimulation exceeds ANG II in NP and P UA, explaining the differential uteroplacental sensitivity in pregnancy. Because AT2R predominate in UA VSM throughout reproduction, this contributes to the inherent refractoriness to ANG II in the uterine vasculature. The increase in UA contractile proteins at term P suggests remodeling, explaining the enhanced contractility seen. (J Clin Endocrinol Metab 97: 138–147, 2012)

Numerous hemodynamic changes occur during pregnancy, including a greater than 30-fold increase in uteroplacental blood flow (UPBF) in the last two thirds of pregnancy and modifications in cardiac output and blood pressure (1). Talledo et al. (2) observed that normotensive pregnant women develop refractoriness to the pressor effects of infused norepinephrine (NE) and angiotensin II (ANG II). We (3) reported identical findings in intact nonpregnant and pregnant sheep. Furthermore, responses by the uteroplacental vascular bed (UPVB) to these agonists were also attenuated during pregnancy (4, 5). Examination of the simultaneous responses of the UPVB and systemic vasculature to infused NE and ANG II revealed that the UPVB was sensitive to NE, UPBF falling in the absence of systemic pressor responses (5, 6), whereas infusion of physiological doses of ANG II that minimally affected UPBF were associated with increases in systemic blood pressure (4). Erkkola and Pirhonen (7) and Damron et al. (8) reported similar observations in women using Doppler flow technology. The mechanisms responsible for these
changes in pregnancy and differences in the uterine and systemic vascular sensitivity to the NE and ANG II remain unclear.

ANG II mediates its biological effects by activating two primary receptors (ATR) (9, 10). The type 1 ANG II receptor (AT₁R) is the predominant receptor in nearly all adult tissues, including the vascular smooth muscle (VSM), and accounts for most ANG II-mediated biological effects, including smooth muscle contraction. The type 2 ANG II receptor (AT₂R) is derived from a separate gene product on the X-chromosome and is the predominant ATR in the fetus and early postnatal neonate, but its expression decreases after birth (9, 11–13). Thus, AT₂R are minimally expressed in adult systemic VSM (9, 10, 14). However, they are the predominant ATR in myometrium and uterine artery (UA) VSM of women, sheep, and rat, accounting for more than 75–85% of binding in nonpregnant and pregnant UA VSM of women, sheep, and rat, respectively (14, 15). Its role in these tissues remains unclear. Some have suggested it attenuates ANG II-mediated vasoconstriction through vasodilating tissues (14, 15). Others have suggested that AT₁R are expressed in ovine UA endothelium and activate prostacyclin and/or nitric oxide synthesis in pregnancy, which would complicate assessment of VSM function, because we were primarily interested in assessing VSM function, and it is known that AT₁R are expressed in ovine UA endothelium and activate prostacyclin and/or nitric oxide synthesis in pregnancy, which would complicate assessment of VSM function (23, 24). Rings were placed on a stirrup attached to a transducer (Grass FT-03, Grass Instruments, Quincy, MA; and iWorx FT-302, CB Sciences, Dover, NH) to measure force generation in a 25-ml volume chamber with PBS-2 maintained at 37 C (21, 22). After 30 min equilibration, rings were progressively stretched to obtain optimal length (Lₒ), as determined with progressive increments in KCl-induced contractions with 65 mM KCl. To determine the inherent capacity of UA VSM to contract independent of receptor activation, rings from nonpregnant and pregnant women were exposed to KCl (10–120 mM) at Lₒ and dose-response curves generated. To determine differences in UA VSM sensitivity to α-stimulation and ANG II in nonpregnant and pregnant UA VSM and then between α-stimulation and ANG II, rings were exposed to cumulative doses of NE (10⁻⁹ to 10⁻⁴.5 M; Sigma-Aldrich, St. Louis, MO) and single doses of ANG II (10⁻⁸ to 10⁻⁵ M; Sigma-Aldrich, St. Louis, MO) in duplicate to prevent the tachyphylaxis seen in preliminary studies. Dose-response curves were generated at Lₒ and compared. We also examined

Materials and Methods

Tissue preparation

UA were collected from 28 nonpregnant women undergoing elective hysterectomy for benign gynecologic disorders (leiomyomas, 19; pelvic organ prolapse, one; menorrhagia/adenomyosis, four; endometriosis, one; chronic pelvic pain, one; and dysfunctional uterine bleeding, one). Menstrual status was determined by endometrial histology and date of last menstrual period. Of 24 premenopausal women, 18 were in the follicular and six in the secretory phase, whereas two had atrophic endometrium. One sample was obtained from a menopausal woman with menorrhagia due to leiomyomata. None were on hormone replacement therapy at the time of tissue collection. Samples also were obtained from 13 pregnant women, five less than 37 wk gestation and eight 37 wk or longer (39 ± 0.4 wk), who underwent hysterectomy at or after delivery for medical indications. UA were dissected from the parametrium and paracervical tissues and adjacent myometrium, placed in chilled physiological-based saline (PBS-1: 137 mM NaCl and 8 mM Na₂HPO₄), and transported to the laboratory, where they were placed in iced PBS and the adventitia removed. Segments, 3–4 cm long, were placed in sterile fresh PBS-2 containing 120.5 mM NaCl, 4.8 mM KCl, 1.2 mM MgSO₄, 1.2 mM NaH₂PO₄, 20.4 mM NaHCO₃, 1.6 mM CaCl₂, 10.0 mM dextrose, and 1.0 mM pyruvate overnight at 4 C with continuous 95% O₂:5% CO₂ gas bubbling. Additional segments were opened longitudinally, and the endothelium was removed with a soft-tipped cotton swab, frozen in liquid nitrogen, and stored at −80 C for immunoblots and protein measurements. The protocols were approved by the Institutional Review Board for Human Research at the University of Texas Southwestern Medical Center.

Study protocol

UA samples from 15 nonpregnant and nine pregnant women were used for function studies. Samples were cut into consecutive 3- to 4-mm rings. The endothelium was removed by turning the tip of a small forceps in the lumen and verified histologically as reported (21, 22). Endothelium-denuded UA were studied because we were primarily interested in assessing VSM function, and it is known that AT₁R are expressed in ovine UA endothelium and activate prostacyclin and/or nitric oxide synthesis in pregnancy, which would complicate assessment of VSM function (23, 24). Rings were placed on a stirrup attached to a transducer (Grass FT-03, Grass Instruments, Quincy, MA; and iWorx FT-302, CB Sciences, Dover, NH) to measure force generation in a 25-ml volume chamber with PBS-2 maintained at 37 C (21, 22). After 30 min equilibration, rings were progressively stretched to obtain optimal length (Lₒ), as determined with progressive increments in KCl-induced contractions with 65 mM KCl. To determine the inherent capacity of UA VSM to contract independent of receptor activation, rings from nonpregnant and pregnant women were exposed to KCl (10–120 mM) at Lₒ and dose-response curves generated. To determine differences in UA VSM sensitivity to α-stimulation and ANG II in nonpregnant and pregnant UA VSM and then between α-stimulation and ANG II, rings were exposed to cumulative doses of NE (10⁻⁹ to 10⁻⁴.5 M; Sigma-Aldrich, St. Louis, MO) and single doses of ANG II (10⁻⁸ to 10⁻⁵ M; Sigma-Aldrich, St. Louis, MO) in duplicate to prevent the tachyphylaxis seen in preliminary studies. Dose-response curves were generated at Lₒ and compared. We also examined

J Clin Endocrinol Metab, January 2012, 97(1):138–147 jcem.endojournals.org 139
the effects of AT₁R and AT₂R inhibition on ANG II-induced contractions (10⁻⁸ to 10⁻⁵ M) in endothelium-denuded and -intact UA with losartan (10⁻⁵ M; Sigma-Aldrich) and PD123319 (10⁻⁵ to 10⁻⁷ M; Sigma-Aldrich), respectively, to determine which subtype mediates ANG II-induced contractions and whether AT₂R activation modifies these contraction responses. Inhibitors were incubated for 30 min, after which ANG II was added. Studies of endothelium-intact UA allowed us to examine the interaction of VSM and endothelial AT₁R responses (16, 17). Data were recorded on an electronic data-acquisition system (Summit ACQuire and Summit DaStar; Gould Systems, Valley View, OH) in grams of force at Lo. At completion of studies, vessels were fixed in formalin and length and medial width measured. Data are expressed in newtons per square meter generated at L₁₀, which permits comparisons between arteries and agonists.

Protein analysis and content

UA VSM remodeling is associated with increased total and soluble protein and actin/myosin contents (25, 26). Samples of endothelium-denuded UA (50 mg) were weighed and homogenized in 40 volumes of sodium dodecyl sulfate (SDS) buffer containing 2% SDS, 20% sucrose, and 0.4 M Tris (pH 6.8) as reported (21). A sample of the primary homogenate was used to measure total protein, which includes cellular and noncellular proteins. Another portion was centrifuged at 10,000 × g for 2 min, and the supernatant used to measure soluble or cellular protein (21). Total and soluble protein was measured using a bicinchoninic assay protein assay reagent kit (Pierce, Rockford, IL). Bromophenol blue and 2-mercaptoethanol were added to bicinchoninic assay protein assay reagent kit (Pierce, Rockford, IL), respectively, to determine which subtype mediates ANG II-induced contractions and whether AT₂R activation modifies these contraction responses. Inhibitors were incubated for 30 min, after which ANG II was added. Studies of endothelium-intact UA allowed us to examine the interaction of VSM and endothelial AT₁R responses (16, 17). Data were recorded on an electronic data-acquisition system (Summit ACQuire and Summit DaStar; Gould Systems, Valley View, OH) in grams of force at L₁₀. At completion of studies, vessels were fixed in formalin and length and medial width measured. Data are expressed in newtons per square meter generated at L₁₀, which permits comparisons between arteries and agonists.

Immunoblot analysis

ATR subtype binding density and affinity have been assessed in UA VSM from nonpregnant and term pregnant women (15); there are no data regarding subtype expression in UA VSM from preterm pregnant (<37 wk gestation) or cycling nonpregnant women. Thus, endothelium-denuded UA (see above) were studied because AT₁R are expressed in UA endothelium (14, 23, 24), and we were interested in comparing VSM expression and function. We also examined α₁-receptor expression in denuded UA VSM from nonpregnant and pregnant women. SDS homogenates were prepared from tissue samples as described above. Thirty micrograms of soluble protein were loaded for all samples and subjected to electrophoresis in 10% polyacrylamide gels and transferred to polyvinyl difluoride membrane (Immobilon-P; Millipore, Billerica, MA). Immunoblots were blocked in buffer containing powdered milk [5% (wt/vol)] and incubated overnight at 4°C with antisera against AT₁R (1:500, N-10; Santa Cruz Biotechnology, Inc, Santa Cruz, CA), AT₂R (1:500, ab19134; Abcam Inc., Cambridge, MA), or α₁-adrenergic receptors (1:500, ab54730; Abcam). The membrane was incubated with donkey antirabbit IgG conjugated with affinity-purified horseradish peroxidase diluted at 1:2000 with Tris-buffered saline and 0.2% Tween 20 for ATR and antimouse IgG at 1:1000 for α₁-receptors. Regions containing receptor proteins were visualized by enhanced chemiluminescence. Densitometry was performed, and values were expressed as arbitrary units. We were unable to identify a loading protein unaffected by pregnancy, including smooth muscle actin (see Results). Therefore, comparisons between groups were made on a single gel using the same loading of soluble protein (21).

Immunohistochemistry

Samples of intact UA from nonpregnant and pregnant women were washed in PBS, fixed in 4% paraformaldehyde, and embedded in paraffin (13, 21). Fixed tissues were prepared as reported, mounted on super frost-plus slides, and incubated overnight with 1:25 AT₁ polyclonal antibody (N-10), 1:150 AT₂ antibody (gift of I. Bird, Department of Obstetrics and Gynecology, Perinatal Research Labs, University of Wisconsin, Madison, WI), or nonimmune rabbit serum as negative control (13, 21). Immunostaining was detected with standard streptavidin-biotin-horseradish peroxidase and hematoxylin counterstaining.

Statistics

One-way ANOVA for multiple groups or repeated measures was used to examine dose-response curves for each agonist and two-way ANOVA to compare agonists across doses and between groups, e.g. nonpregnant and pregnant. A Student’s t test was used where noted. When significance by ANOVA was P < 0.05, multiple comparison procedures were used to isolate groups and determine differences between groups. Protein contents were similarly analyzed. Data are presented as means ± SEM.

Results

Vasoconstrictor responses

KCl elicited dose-dependent nonreceptor-mediated contractions in denuded UA from nonpregnant and term pregnant women that were group dependent (Fig.
2; P < 0.001), resulting in significantly greater KCl responses in pregnant vs. nonpregnant UA VSM, e.g., responses were more than 2-fold greater at 65 mM KCl or greater.

NE also elicited dose- and group-dependent contractions in denuded UA from nonpregnant and term pregnant women (Fig. 2B; P < 0.001). Notably, responses in pregnant UA were more than 1.3-fold greater than nonpregnant at 10^{-6} M or less. Additionally, the EC_{50} was shifted to the left in pregnant versus nonpregnant UA, 0.30 ± 0.07 vs. 1.61 ± 0.35 × 10^{-6} M (P = 0.003), respectively.

Although ANG II elicited modest contraction responses at 10^{-5} M in UA from pregnant women (Fig. 2C; P = 0.04), dose effects were not observed in UA from nonpregnant women (P > 0.1). However, comparison of ANG II-mediated contractions in nonpregnant and pregnant UA revealed a dose and pregnancy effect (P < 0.002) but only at 10^{-5} M. Because ANG II had no dose effect in nonpregnant UA rings, the EC_{50} cannot be calculated. At maximum physiological doses, i.e., 10^{-6} M or less, ANG II responses in pregnant UA exceeded nonpregnant greater than 2-fold and were 2-fold less than responses to 65 mM KCl.

A primary objective was to determine whether UA VSM sensitivity to α-stimulation and ANG II differed during pregnancy; thus, we compared contraction responses in UA from nonpregnant (Fig. 3A) and pregnant (Fig. 3B) women. NE elicited maximum contractions at least 2-fold greater than ANG II responses in UA VSM from nonpregnant and pregnant women (Fig. 3; *, P < 0.03).

**Receptor expression**

To characterize ATR subtype expression in UA VSM throughout human reproduction, we studied four groups of women: nonpregnant during follicular or luteal phase and pregnant preterm and term. AT_{1}R and AT_{2}R protein expression was not different in UA from luteal and secretory phases of the ovarian cycle (P = 0.7; n = 4/group, data not shown) and UA from preterm

![Fig. 2](https://example.com/fig2.png)  
**Fig. 2.** Dose-response curves to KCl (A), NE (B), and ANG II (C) in endothelium-denuded human uterine arteries from nonpregnant (●) and pregnant (□) women. Studies were done in five nonpregnant and six to seven pregnant uterine arteries for KCl and norepinephrine and nine to 12 nonpregnant and six pregnant uterine arteries for each dose of ANG II. *, P < 0.001. Data are means ± SEM.
(<37 wk gestation) and term pregnant women (P = 0.9; n = 4/group, data not shown). Importantly, subtype expression was similar in UA VSM from nonpregnant and term pregnant women (Fig. 4A; n = 7/group, P = 0.4). We performed immunohistochemistry to localize ATR expression within nonpregnant and pregnant UA (Fig. 4B). Immunostaining was seen in the media of both groups, but only AT1R staining occurred in the endothelium (Fig. 4, B1 and B2).

Because NE responses increased in pregnancy, α1-receptor expression was examined in UA VSM from nonpregnant (n = 7) and pregnant (n = 7) women by immunoblot. There was no difference between groups, P > 0.1 (data not shown).

**FIG. 3.** Comparison of dose-response curves to NE (▲, △; n = 5–6) and ANG II (■, □; n = 6–13) in denuded human uterine arteries from nonpregnant (A) and term pregnant (B) women. *, P < 0.02. Data are means ± SEM.

**FIG. 4.** Immunoblot analysis comparing AT1R and AT2R subtype expression in uterine artery from nonpregnant and term pregnant women. A, Immunoblots represent seven nonpregnant and seven term pregnant women. Summary data using densitometry are shown; note the nearly 3-fold difference in the y-axis for AT1R and AT2R. Data are means ± SEM. B, Immunohistochemical localization of AT1R in representative nonpregnant (B1) and pregnant (B2) uterine arteries and AT2R in nonpregnant (B3) and pregnant (B4) shown at ×40 magnification. i, Intima; m, media; l, vessel lumen. Arrows note endothelial immunostaining for AT1R.
Effects of ATR subtype inhibition

Having demonstrated both subtypes in UA VSM, we determined if ATR-specific inhibition modified ANG II-induced contractions. Losartan \(10^{-5}\) M, the AT\(_1\)-R-specific inhibitor, abolished responses to \(10^{-6}\) M ANG II \((P < 0.001)\) in endothelium-denuded UA from non-pregnant women (Fig. 5A). The AT\(_2\)-R specific blocker PD123,319 did not affect ANG II-mediated contractions at doses ranging from \(10^{-7}\) to \(10^{-5}\) M, which was done to examine a dose effect, i.e. there was no increase in contraction responses (Fig. 5A). Similar observations were made in endothelium-intact UA from nonpregnant women (Fig. 5B). Because endothelial AT\(_1\)-R expression and function increase in ovine pregnancy (24, 25, 27, 28) and VSM AT\(_2\)-R activation might attenuate AT\(_1\)-R-mediated contractions in pregnancy via endothelium-dependent mechanisms (16, 28–30), we examined endothelium-intact UA from term pregnant women (Fig. 5C). As in nonpregnant UA, losartan abolished contraction responses to ANG II, and PD123,319 did not enhance ANG II-mediated contractions at any dose studied.

Smooth muscle contractile proteins

VSM contraction responses to each agonist increased approximately 2-fold at term gestation; thus, we examined remodeling in UA from nonpregnant and term pregnant women by measuring contractile and soluble protein contents (25, 26). At term pregnancy, VSM actin increased approximately 2-fold (Fig. 6; \(2.84 \pm 0.7\) to \(5.88 \pm 1.2 \mu g/mg\) wet weight, \(P = 0.02\)) as did total myosin \((2.07 \pm 0.4\) to \(3.61 \pm 0.7 \mu g/mg\) wet weight, \(P = 0.08\)). This was associated with 55\% \((P = 0.06)\) and

![Diagram](https://example.com/diagram.png)
74% (P = 0.03) increases in total and soluble protein contents, respectively.

Discussion

Pregnant women develop refractoriness to the pressor effects of infused ANG II and NE (2). They also demonstrate attenuated responses to infused ANG II in the UPVB vs. the systemic vasculature but enhanced uterine responses to NE (7, 8). This differential sensitivity in the UPVB also occurs in sheep (4–6). The mechanisms responsible for these differences within the UPVB remain unclear and poorly studied in women. In the present study, KCl and NE dose dependently contracted UA from nonpregnant and pregnant women, whereas ANG II did not. Moreover, ANG II-mediated contractions were substantially less than NE. However, each agonist elicited greater contractions in term pregnant vs. nonpregnant UA, and the magnitude was strikingly similar, approximately 2-fold. Because the increases in ANG II- and α-mediated contractions in pregnant VSM were not associated with altered receptor expression, this reflects pregnancy-related UA remodeling or changes in ATR signaling. These novel observations clarify the differential sensitivity of ANG II and NE in the uterine vasculature of nonpregnant and pregnant women (7, 8), confirm observations that ATR subtype expression in UA VSM is unchanged during pregnancy (15), as is α₁-receptor, and for the first time suggest AT₂R activation may not modify AT₁R-mediated contractions in human UA.

In studies of pregnant blood pressure regulation, Naden and Rosenfeld (4) discovered that the ovine UPVB was more refractory to ANG II than the systemic vasculature, protecting it from the increased ANG II plasma levels in pregnancy (31). In contrast, the ovine UPVB was exquisitely sensitive to α-agonists, UPBF decreasing at subpressor doses (5, 6). Similar observations are reported in normotensive pregnant and nulligravid women (7, 8), but the mechanisms remain unclear. This is the first study comparing ANG II- and NE-induced contractions in human UA VSM and nonreceptor-mediated contractions with KCl. Notably, ANG II responses were substantially less than those elicited by KCl and NE at physiological doses, demonstrating differences in VSM sensitivity. Importantly, the difference between NE and ANG II occurred in nonpregnant and pregnant UA and was similar, approximately 2-fold, demonstrating for the first time that...
these differences in sensitivity are inherent within the uterine vasculature, unchanged during pregnancy, and not due to alterations in \( \alpha_1 \)-receptor expression and possibly activation (32). These findings are strikingly similar to those in intact sheep and ovine UA rings (20, 33). Thus, the decreased sensitivity to ANG II likely protects maternal placental blood flow from the elevated circulating levels of the peptide in pregnancy. The enhanced sensitivity to NE, however, may facilitate diversion of UPBF, which accounts for 25–30% of cardiac output and greater than 1 liter of blood (1), to vital organs during stress responses. This enhanced sensitivity to \( \alpha \)-stimulation may also contribute to the decrease in UPBF and occurrence of fetal growth restriction associated with excessive sympathetic activity in preeclamptic-hypertensive women (34).

Although the human uterine vasculature is refractory to ANG II (7), the mechanism(s) is unclear. It remains possible that pregnancy-related increases in plasma ANG II down-regulate or modify UA ATR subtype expression. This, however, was not seen in peripheral or UA VSM during ovine pregnancy (35, 36). Baker et al. (31) reported decreased platelet ATR binding in pregnant women but did not study human arteries. Cox et al. (15) reported that both ATR subtypes were present in human UA VSM, but AT2R accounted for greater than 75–85% of binding, total ATR binding density was similar in nonpregnant and pregnant women, and the AT2R to AT1R ratio was unchanged in pregnancy. They reported similar findings in sheep; additionally, they showed that AT1R accounted for greater than 95% of binding in peripheral arterial VSM (14). In studies in intact pregnant sheep, they suggested the attenuated UPVB responses to ANG II reflected AT2R predominance and minimal AT1R expression but not attenuation of AT1R-mediated contractions through AT2R-mediated mechanisms (18, 19). However, the latter studies might be considered incomplete. In the present study, ATR subtype expression was unchanged in human UA VSM throughout reproduction, confirming binding studies by Cox et al. (15) and more recent observations in ovine UA (20). We could not determine the AT2R to AT1R ratio by Western analysis because each subtype requires separate immunoblots and antibodies with different affinities. Nonetheless, immunoblot density was approximately 3-fold greater for AT2R vs. AT1R in the presence of similar quantities of soluble protein. If subtype binding is unchanged (15), as is protein expression, the AT2R to AT1R ratio is unlikely to change during pregnancy. Thus, AT2R predominance and decreased AT1R expression (<20%) appears to explain the decreased ANG II sensitivity in UA (16, 17). Evidence for this is obtained from studies of ovine and human myometrium in which increases and decreases in ANG II-induced stresses paralleled similar changes in AT1R expression during and after pregnancy (15, 37).

In small mammals, AT2R activation or their absence in VSM modifies contraction responses to ANG II. Blood pressure is elevated in AT2R-/- mice, and AT2R inhibition in rats enhances pressor responses to infused ANG II (16, 17). Additionally, blood pressure does not fall in the middle third of pregnancy in AT2R-/- mice (30). There are rare studies of AT2R-AT1R interactions in human arteries (16). In the present study, AT1R blockade abolished ANG II-mediated contractions in all UA studied. In contrast, AT2R inhibition did not modify AT1R contractions in denuded or intact UA from nonpregnant or pregnant women, i.e. contractions were not enhanced. Importantly, endothelium-denuded UA from nonpregnant women dose dependently vasodilate during exposure to sodium nitroprusside (22). Thus, unlike small mammals, there was no AT2R-AT1R interaction in human UA. Notably, there also is no interaction in UA from nonpregnant and pregnant sheep (20), suggesting species specificity (38).

Ovine and rat UA remodel during pregnancy (25, 26). This is associated with enhanced contraction responses to KCl and NE and increased actin/myosin contents at term (25, 26). Contraction responses by UA VSM from term pregnant women increased approximately 2-fold with each agonist, including ANG II. Because ATR and \( \alpha_1 \)-receptor expression was unchanged, this cannot explain the enhanced responses. However, VSM cellular protein increased 74% at term and was associated with approximately 2-fold increases in actin and total myosin contents, suggesting vascular remodeling resembling changes in sheep (20, 25, 26). This likely explains the increased stresses at term gestation with each agonist; further studies are needed to define the extent of remodeling. These findings also suggest the UA endothelium contributes to the modulation of contraction responses during pregnancy and warrants further study (23–25).

We have shown that the differential sensitivity to ANG II and NE in the UPVB of pregnant women reflects inherent differences in vascular sensitivity to both agonists. We have confirmed that ATR subtype expression in human UA VSM is unchanged in the menstrual cycle and pregnancy (15), as is the \( \alpha_1 \)-receptor, suggesting that AT2R predominance contributes to the attenuated responses to ANG II in UA from nonpregnant and pregnant women; however, this is not due to AT2R-mediated vasodilatation. \( \alpha \)-Agonists decrease UPBF at subpressor doses (5, 6) and easily cross the placental barrier (39), whereas ANG II does not (40); thus, the use of \( \alpha \)-agonists to treat hypertension in pregnancy should be revisited, especially in view of reports showing adverse effects of \( \alpha \)-therapy on fetal well-being (39). Although unstudied, the inherent sensi-
tivity to α-stimulation may also contribute to reduced UPBF in preeclamptic-hypertensive women if sympathetic overactivity exists (34). Finally, we report that observations in women and sheep are similar, demonstrating the value of this animal model.

Acknowledgments

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This work was supported by Grants R01HD008783 (to C.R.R.) and P01HD11149 (to R.A.W.) from the Eunice Kennedy Shriver National Institute of Child Health and Human Development and George L. MacGregor Professorship in Pediatrics. The content is the responsibility of the authors and does not represent views of the Eunice Kennedy Shriver National Institute of Child Health and Human Development and the National Institutes of Health.

Disclosure Summary: The authors have nothing to disclose.

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