Adaptive increases in expression and vasodilator activity of estrogen receptor subtypes in a blood vessel-specific pattern during pregnancy

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Mata KM, Li W, Reslan OM, Siddiqui WT, Opsasnick LA, Khalil RA. Adaptive increases in expression and vasodilator activity of estrogen receptor subtypes in a blood vessel-specific pattern during pregnancy. Am J Physiol Heart Circ Physiol 309: H1679–H1696, 2015. First published September 25, 2015; doi:10.1152/ajpheart.00532.2015.—Normal pregnancy is associated with adaptive hemodynamic, hormonal, and vascular changes, and estrogen (E$_2$) may promote vasodilation during pregnancy; however, the specific E$_2$ receptor (ER) subtype, post-ER signaling mechanism, and vascular bed involved are unclear. We tested whether pregnancy-associated vascular adaptations involve changes in the expression/distribution/activity of distinct ER subtypes in a blood vessel-specific manner. Blood pressure (BP) and plasma E$_2$ were measured in virgin and pregnant (day 19) rats, and the thoracic aorta, carotid artery, mesenteric artery, and renal artery were isolated for measurements of ERs, ER$\beta$, and G protein-coupled receptor 30 [G protein-coupled ER (GPER)] expression and tissue distribution in parallel with relaxation responses to E$_2$ (all ERs) and the specific ER agonist 4,4’,4”-(4-propyl-[1H]-pyrazole-1,3,5-triy1)-tris-phenol (PPT; ER$_\alpha$), diarylpropionitrile (DPN; ER$_{\beta}$), and G1 (GPER). BP was slightly lower and plasma E$_2$ was higher in pregnant versus virgin rats. Western blots revealed increased ER$\alpha$ and ER$\beta$ in the aorta and mesenteric artery and GPER in the aorta of pregnant versus virgin rats. Immunohistochemistry revealed that the increases in ERs were mainly in the intima and media. In phenylephrine-precontracted vessels, E$_2$ and PPT caused relaxation that was greater in the aorta and mesenteric artery but similar in the carotid and renal artery of pregnant versus virgin rats. DPN- and G1-induced relaxation was greater in the mesenteric and renal artery than in the aorta and carotid artery, and aortic relaxation to G1 was greater in pregnant versus virgin rats. The nitric oxide synthase inhibitor N’-nitro-l-arginine methyl ester with or without the cyclooxygenase inhibitor indomethacin with or without the EDHF blocker tetraethylammonium or endothelin removal reduced E$_2$, PPT, and G1-induced relaxation in the aorta of pregnant rats, suggesting an endothelium-dependent mechanism, but did not affect E$_2$-, PPT-, DPN-, or G1-induced relaxation in other vessels, suggesting endothelium-independent mechanisms. E$_2$, PPT, DPN, and G1 caused relaxation of Ca$^{2+}$ entry-dependent K$^+$ contraction, and the effect of PPT was greater in the mesenteric artery of pregnant versus virgin rats. Thus, during pregnancy, an increase in ER$\alpha$ expression in endothelial and vascular smooth muscle layers of the aorta and mesenteric artery is associated with increased ER$\alpha$-mediated relaxation via endothelium-derived vasodilators and inhibition of Ca$^{2+}$ entry into vascular smooth muscle, supporting a role of aortic and mesenteric arterial ERs in pregnancy-associated vasodilation. GPER may contribute to aortic relaxation while enhanced ER$\beta$ expression could mediate other genomic vascular effects during pregnancy.

NEW & NOTEWORTHY

The present study describes pregnancy-associated increases in estrogen receptor (ER)-mediated endothelium-dependent relaxation in the aorta and inhibition of Ca$^{2+}$ entry in the mesenteric artery. Increased vascular ERs could promote vasodilation, oppose vasoconstriction, and maintain normotension during normal pregnancy, and ER agonists should be examined for potential usefulness in reducing blood pressure in hypertensive pregnancy.

DURING NORMAL PREGNANCY, several maternal hemodynamic and cardiovascular changes occur to adapt to the metabolic demands of the growing fetus, including an increase in blood volume and cardiac output, systemic vasodilation, and decreased vascular resistance with slight decrease or no change in blood pressure (BP) (70, 84). These hemodynamic and vascular changes involve significant redistribution of blood flow to different maternal tissues and organs. An orchestrated network of vasodilator substances, including nitric oxide (NO), prostacyclin (PGI$_2$), EDHF, kallikrein, angiotensin-(1–7), and VEGF, may participate in the local and systemic hemodynamic adaptations during pregnancy (82, 86). Estrogen (E$_2$) levels also increase during pregnancy (23, 74). E$_2$ induces long-term genomic vascular effects, such as stimulation of endothelial cell growth, upregulation of endothelial NO synthase and NO production, increased cyclooxygenase (COX) activity and PGI$_2$ production (19, 53, 64, 77), inhibition of vascular smooth muscle (VSM) proliferation, and downregulation of VSM Ca$^{2+}$ channels and PKC (33, 61). E$_2$ also causes rapid nongenomic vasodilator effects via activation of endothelium-dependent NO, PGI$_2$, and hyperpolarization pathways (24, 29) and inhibition of Ca$^{2+}$-dependent mechanisms of VSM contraction (13, 31, 64). In addition to its effects on vascular cell growth and proliferation, E$_2$ could also affect extracellular matrix deposition/degradation and, in turn, causes structural changes and remodeling of blood vessels (66).

Many of the vascular effects of E$_2$ are mediated via estrogen receptors (ERs) (78). ERs have been identified in the female reproductive tract, mammary glands, and blood vessels of humans and experimental animals (9, 47, 68, 76, 78, 93). ER$\alpha$ and ER$\beta$ mediate many of the genomic effects of E$_2$ (68, 78, 93), and surface membrane ERs and ER$\beta$ have been implicated in the rapid vasodilator effects of E$_2$ (53). A transmembrane G protein-coupled ER (GPER; G protein-coupled receptor 30) may also bind E$_2$ and mediate some of its nongenomic effects (20, 26, 43, 44, 55, 58, 71, 73). Because different tissues may have different blood flow requirements, the vasodilator
effects of E₂/ERs could vary in different vascular beds. In this respect, a vasodilator effect of E₂ in one vascular bed could be balanced by minimal effect in other vascular beds with no net change in BP. We have recently shown region-specific ERα-, ERβ-, and GPER-mediated vasorelaxation in the celiac, thoracic, and abdominal arteries of nonpregnant female rats (72). Regional differences in vasodilation, blood flow, and E₂/ER activity are particularly important during pregnancy as they could have significant implications on the cardiovascular system and the health of mother and fetus. While E₂ levels increase during pregnancy, whether the amount of vascular ERs increase in parallel is unclear. In addition, whether any pregnancy-associated changes in vascular ERs affect the structure or function of blood vessels is unknown.

Furthermore, the role of specific ER subtypes and post-ER endothelium-dependent and endothelium-independent signaling mechanisms in mediating the vascular effects of E₂ during pregnancy is unclear. The purpose of the present study was to test the hypothesis that pregnancy-associated vascular adaptations involve regional changes in ER expression, distribution, vasodilator activity, and post-ER signaling mechanisms in a blood vessel-specific manner. We used four blood vessels with specific roles and functions (namely, the thoracic aorta as a conduit vessel, the carotid artery supplying the celiac circulation, the mesenteric artery with a specific role in the splanchic circulation, and the renal artery with a specialized role in the kidney) from virgin and pregnant rats and subtype-specific ER agonists to determine the pregnancy-associated changes in vascular ER expression and distribution in different systemic vessels. We also tested whether pregnancy-associated alterations in vascular ER expression are manifested as changes in blood vessel structure and remodeling. ER-mediated vasodilator activity, post-ER endothelium-dependent relaxation pathways, and/or post-ER endothelium-independent inhibitory effects on VSM contraction mechanisms. The present study addressed two important questions: Does vascular ER expression/activity increase during pregnancy? And is the pregnancy-related increase in vascular ER expression/activity universal or localized to specific vascular beds?

**METHODS**

**Animals.** Time-pregnant (day 11 of gestation) and age-matched virgin female Sprague-Dawley rats (12 wk of age) were purchased from Charles River Laboratories (Wilmington, MA). Rats were housed in the animal facility for at least 1 wk and had access to ad libitum standard rat chow and tap water on a 12:12-h light-dark cycle. All experimental procedures followed the National Institutes of Health (nih) Guide for the Care and Use of Laboratory Animals and the guidelines of the Harvard Medical Area Standing Committee on Animals.

**BP.** On day 19 of pregnancy or the equivalent in virgin rats, rats were anesthetized with isoflurane, and a PE-50 catheter was inserted in the carotid artery and exteriorized at the back of the neck. Rats were allowed to recover from anesthesia for at least 1 h. The carotid arterial catheter was connected to a pressure transducer attached to an amplifier and BP recorder (Living System Instrumentation, Burlington, VT). BP in conscious rats was recorded every 20 min for 1 h, and the average BP was measured (50).

**Plasma E₂.** Blood samples from virgin and pregnant rats were collected in EDTA, and plasma estradiol (E₂) was measured by ELISA (Rat E₂ ELISA Kit, MBS702969, MyBioSource). Virgin rats were studied during estrus to control for reproductive and endocrine confounders. The stage of the estrous cycle was determined by taking a vaginal lavage with a Pasteur pipette (52). An estrus lavage primarily contained amniolized cornified squamous cells (89) and was confirmed before all experiments.

**Materiale.** Pregnant and virgin rats were euthanized by CO₂ inhalation. The thoracic aorta, right and left carotid, mesentery and mesenteric arterial arcade, and kidneys and renal arteries were excised and placed in Krebs solution. We chose the thoracic aorta, carotid artery, and mesenteric artery as representatives of the thoracic, celiac, and abdominal circulation and the renal arteries for their role in the regulation of renal function, plasma volume, and BP. Uteri of virgin and pregnant rats were also examined as known targets and positive controls for ERs. The virgin uterus was cleaned of the surrounding fat and weighed. The pregnant uterus was cut open, the placenta and pups were separated, and the litter size, individual pup weight, and uterine weight were recorded. The kidneys were also cleaned and weighed. With the aid of a dissection microscope, the aorta, carotid artery, mesenteric artery, and renal artery were carefully cleaned of fat and connective tissue and cut into 3- to 4-mm-wide rings for vascular function experiments. For endothelium-intact arteries, extracellular matrix was taken to minimize injury to the endothelium. For endothelium-denuded segments, the endothelium was removed by scraping the vessel interior five times around the tip of forceps (for the aorta) or around thin tungsten wire (for the carotid, mesenteric, and renal artery). Different segments from each vascular bed were used for different experimental protocols and pharmacological manipulations.

The remainder of the vascular tissue was stored at −80°C for Western blots, histology, and immunohistochemistry.

**Western blots.** The aorta, carotid artery, mesenteric artery, and renal artery as well as the uterus were homogenized in homogenization buffer containing 20 mM MOPS, 4% SDS, 10% glycerol, 2.3 mg DTT, 1.2 mM EDTA, 0.02% BSA, 5.5 μM leupeptin, 5.5 μM pepstatin, 2.15 μM aprotinin, and 20 μM (2-aminoethyl)-benzene-sulfonyl fluoride using a 2-ml tight-fitting homogenizer (Kontes Glass, Vineland, NJ). The homogenate was centrifuged at 10,000 g for 10 min, the supernatant was collected, and protein concentration was determined using a protein assay kit (Bio-Rad, Hercules, CA). Protein extracts (20 μg) were combined with an equal volume of 2% Laemmli loading buffer, boiled for 5 min, and size fractionated by electrophoresis on 6% SDS-polyacrylamide gels. Proteins were transferred from the gel to a nitrocellulose membrane by electrophoretic blotting. Membranes were incubated in 5% nonfat dry milk in Tris-buffered saline-Tween 4583 optimal cutting temperature compound (Fisher Scientific) and stored at −80°C. Cross-sectional 6-μm-thick cryosections from the arteries and uterus were placed on glass slides and prepared for staining with hematoxylin and eosin. Stained sections were coded and blinded. Images of arteries were obtained with a Nikon microscope using the same magnification, light intensity, and camera gain and analyzed using ImageJ software. Images of arteries were acquired using bright-field and ×4 and ×10 objectives. Because the rat uterus is large, 20 picture frames of sequential parts of the uterine tissue section were acquired using the ×4 objective, and the composite image of the whole uterine section was reconstituted as previously described.
tension did not cause further contraction to KCl (72). Changes in produced maximal KCl contraction, and further increases in basal incubated with polyclonal ER was blocked in 10% horse serum for 30 min. Tissue sections were =-(4-propyl-[1H]-pyrazole-1,3,5-triyl)-

Immunohistochemistry. To determine the tissue distribution of ERα, ERβ, and GPER, cryosections of the aorta, carotid artery, mesenteric artery, and renal artery as well as the uterus were thawed and fixed in ice-cold acetone for 30 min. Endogenous peroxidase was quenched in 1.5% H2O2 solution for 30 min, and nonspecific binding was blocked in 10% horse serum for 30 min. Tissue sections were incubated with polyclonal ERα (1:100), ERβ (1:100), and GPER (1:100) antibody (Santa Cruz Biotechnology). After being rinsed with PBS, tissue sections were incubated with biotinylated anti-rabbit secondary antibody, rinsed with PBS, and then incubated with avidin-labeled peroxidase (VectaStain Elite ABC Kit, Vector Laboratories, Burlingame, CA). Positive labeling was visualized using diaminobenzadine and appeared as brown spots. Negative control slides were run simultaneously with no primary antibody. Specimens were counterstained with hematoxylin for 40 s, rinsed with PBS, topped with cytoseal 60, and covered with slide coverslips. Images were acquired on a Nikon microscope using the same microscope magnification, light intensity, and camera gain. Images of the aorta, carotid artery, mesenteric artery, and renal artery were acquired using bright-field and ×4 objectives, and images of the virgin uterus were acquired with a ×4 objective. Because the rat pregnant uterus is large, 20 picture frames of sequential parts of the uterine wall were acquired using the ×4 objective, and the composite image of the whole uterine section was reconstituted as previously described (39). Images were analyzed using ImageJ, the total number of pixels in the tissue section image was defined, and the number of brown spots (pixels) was counted and then presented as a percentage of total pixels. In addition, the number of pixels in the specific tissue layer (intima, media, and adventitia for arteries and endometrium, myometrium, and perime-

trium for the uterus) was defined and transformed into the area (in µm²) using a calibration bar. The relative thickness of the vascular tissue layers (intima, media, and adventitia) was also measured and presented in millimeters or as a percentage of total wall thickness.

RESULTS

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BP, plasma E2, body weight, and tissue weight. Mean arterial pressure was slightly but not significantly reduced (Fig. 1A) and plasma E2 levels were significantly higher (Fig. 1B) in pregnant versus virgin rats. The body weight of intact animals was greater in pregnant versus virgin rats (Fig. 1C). When rats
were euthanized and the uterus, pups, and placentae removed, the body weight without the uterus was still greater in pregnant versus virgin rats (Fig. 1C). The uterus weight without pups and placentae was also dramatically greater in pregnant versus virgin rats (Fig. 1D). The vascular tissue weight as a percentage of body weight was not different in the aorta, mesenteric artery, and renal artery but was reduced in the carotid artery of pregnant versus virgin rats (Fig. 1E). Kidney weight was not statistically different in pregnant versus virgin rats (Fig. 1F).

**ERα expression and tissue distribution.** Western blots for ERα revealed a band at 66 kDa (Fig. 2A). In vessels of virgin rats, the ERα-immunoreactive band relative to actin was greater in the renal artery than in the aorta, carotid artery, and mesenteric artery. In vessels of pregnant rats, ERα was most prominent in the aorta and showed greater levels in the mesenteric and renal artery than in the carotid artery. ERα levels were also higher in the aorta and mesenteric artery of pregnant versus virgin rats (Fig. 2A). Parallel Western blots on the uterus revealed a greater amount of ERα in pregnant versus virgin rats (Fig. 2B). Immunohistochemistry revealed that in vessels of pregnant rats, ERα immunostaining was greater in the renal artery, particularly in the media and adventitia, than in the aorta, carotid artery, and mesenteric artery (Fig. 2C). In vessels of pregnant rats, total ERα was higher in the aorta, mesenteric artery, and renal artery than in the carotid artery, and the relative distribution of ERα in the intima, media and adventitia of the aorta and mesenteric artery and media and adventitia of the renal artery was greater than in the carotid artery. Total ERα and its relative distribution in the intima, media, and adventitia was also greater in the aorta and mesenteric artery of pregnant versus virgin rats (Fig. 2C). Parallel immunohistochemistry experiments revealed a greater total amount and relative distribution of ERα in the endometrium, myometrium, and perimetrium in uterine tissue sections of pregnant versus virgin rats (Fig. 2D).

**ERβ expression and tissue distribution.** Western blots for ERβ in blood vessels revealed a band at 55 kDa (Fig. 3A). In vessels of virgin rats, ERβ relative to actin was greater in the aorta and renal artery than in the mesenteric artery. In vessels of pregnant rats, ERβ was most prominent in the aorta, with
Fig. 2. Protein amount and tissue distribution of estrogen receptor (ERα) in the aorta (A), carotid artery (C), mesenteric artery (M), and renal artery (R) as well as the uterus of virgin versus pregnant rats. Tissue homogenates of blood vessels (A) and the uterus (B) were prepared for Western blots using ERα antibody (1:1,000). ERα and G protein-coupled ER (GPER, shown in Fig. 4) were run on the same gel and therefore have the same actin control. Vascular tissue sections (C) and uterine sections (D) were stained with ERα antibody using immunohistochemistry, and the total amount and relative distribution of ERα brown immunostaining in different layers of the tissue wall were measured using ImageJ. Bar graphs represent means ± SE; n = 4–6 rats/group. *P < 0.05, pregnant vs. virgin rats; ‡significantly different (P < 0.05) from corresponding measurements in the aorta, carotid artery, mesenteric artery, and renal artery of virgin rats; #significantly different (P < 0.05) from corresponding measurements in the aorta, carotid artery, mesenteric artery, and renal artery of pregnant rats.
greater amounts in the aorta, mesenteric artery, and renal artery than in the carotid artery. ERβ was also higher in the aorta and mesenteric artery of pregnant versus virgin rats (Fig. 3A). Parallel Western blots on the uterus showed higher levels of ERβ in pregnant versus virgin rats (Fig. 3B). Immunohistochemistry revealed that in vessels of virgin rats, ERβ was most detectable in the renal artery, with higher levels in the media and adventitia, compared with other vessels. In vessels of

![Fig. 3](http://ajpheart.physiology.org/)

**Fig. 3.** Protein amount and tissue distribution of ERβ in the aorta, carotid artery, mesenteric artery, and renal artery as well as the uterus of virgin versus pregnant rats. Tissue homogenates of blood vessels (A) and the uterus (B) were prepared for Western blots using ERβ antibody (1:1,000). Vascular tissue sections (C) and uterine sections (D) were stained with ERβ antibody using immunohistochemistry, and the total amount and relative distribution of ERβ brown immunostaining in different layers of the tissue wall were measured using ImageJ. Bar graphs represent means ± SE; n = 4–6 rats/group. *P < 0.05, pregnant vs. virgin rats; ‡significantly different (P < 0.05) from corresponding measurements in the aorta, carotid artery, mesenteric artery, and renal artery of virgin rats; #significantly different (P < 0.05) from corresponding measurements in the aorta, carotid artery, mesenteric artery, and renal artery of pregnant rats.
pregnant rats, total ERβ and its relative distribution in the intima and media were more apparent in the aorta, mesenteric artery, and, to some extent, the renal artery than in the carotid artery. ERβ was also higher in the adventitia of the mesenteric artery versus the aorta, carotid artery, and renal artery of pregnant rats. Total ERβ and its relative distribution in the intima, media, and adventitia were also greater in the aorta and mesenteric artery of pregnant versus virgin rats (Fig. 3C). Parallel immunohistochemistry revealed a greater total amount and relative distribution of ERβ in the endometrium, myometrium, and perimetrium in the uterus of pregnant versus virgin rats (Fig. 3D).

**GPER expression and tissue distribution.** Western blots for GPER revealed a band at ~50 kDa that was not different among vessels of virgin rats and was only significantly greater in the aorta versus other vessels of pregnant rats or in the aorta of pregnant versus virgin rats (Fig. 4A). GPER levels were also greater in the uterus of pregnant versus virgin rats (Fig. 4B). Immunohistochemistry revealed more GPER particularly in the media of mesenteric and renal artery than in the carotid artery of virgin rats. In vessels of pregnant rats, total GPER was more apparent in the aorta, mesenteric artery, and renal than in the carotid artery and, to some extent, pregnant rats (Table 1). PPT-induced relaxation was greater in the mesenteric and renal artery than in the carotid artery (Table 1). The relaxation to E2 and PPT was greater in the aorta (Fig. 6A) and mesenteric artery (Fig. 6C) but not different in the carotid artery (Fig. 6B) or renal artery (Fig. 6D) of pregnant versus virgin rats (Table 1). DPN (ERβ) and G1 (GPER) relaxation was generally less than that induced by E2 or PPT in all vessels tested (Fig. 6 and Table 1). DPN maximum relaxation and EC50 were not different in all vessels of pregnant versus virgin rats. G1-induced maximum relaxation was only greater in the aorta of pregnant versus virgin rats (Fig. 6A and Table 1). In comparison, ACh, a known stimulator of endothelial-dependent relaxation, caused relaxation that was not different in the aorta, carotid artery, mesenteric artery, or renal artery of pregnant versus virgin rats (Fig. 6, A–D).

**Contribution of endothelial NOS, COX, and EDHF to ER-mediated relaxation.** We have previously reported differences in the sensitivity of the ACh relaxation response to the NOS blocker L-NAME, COX inhibitor indomethacin, and the hyperpolarization pathway in different vascular beds of virgin rats (72) and in mesenteric vessels of virgin versus pregnant rats (49). In the present study, we assessed the role of endothelial vasodilators in ER agonist-induced relaxation of blood vessels of pregnant rats. Pretreatment with the NOS inhibitor L-NAME (3 × 10^-4 M) for 15 min reduced E2-, PPT-, DPN-, and G1-induced relaxation of Phe-induced contraction in the aorta (Fig. 7A) but not in the carotid, mesenteric, or renal artery (Fig. 7, B–D, and Table 2). Additional treatment with the COX inhibitor indomethacin (10^-5 M), EDHF inhibitor TEA (30 mM), or endothelium removal did not cause further inhibition of ER agonist-induced relaxation in any of the arteries tested (Fig. 7, A–D, and Table 2). Interestingly, in L-NAME + indomethacin + TEA-treated and endothelium-denuded vessels, maximum relaxation to most ER agonists remained greater in the mesenteric and renal artery than in the aorta and carotid artery, and E2- and PPT-induced relaxation was greater in the carotid artery than in the aorta (Table 2). Pretreatment with blockers of endothelium-derived vasodilators or endothelium removal did not affect ER agonist EC50 in any of the vessels tested (Table 2).

E2-, PPT-, DPN-, and G1-induced relaxation of Phe-induced contraction. We assessed if pregnancy-induced changes in vascular ERs could affect vascular function. In blood vessels isolated from virgin rats and precontracted with Phe, all ER agonists caused greater maximum relaxation with no difference in EC50 in the mesenteric and renal artery versus the aorta and carotid artery (Fig. 6 and Table 1). In vessels of pregnant rats, maximum relaxation to E2 and the ERs agonist PPT was greater in the mesenteric artery and maximum relaxation to the ERβ agonist DPN was greater in the mesenteric and renal artery versus the aorta and carotid artery. In vessels of pregnant rats, PPT was more potent in causing relaxation in the mesenteric artery and G1 was more potent in the aorta and mesenteric artery than in the carotid artery (Table 1). The relaxation to E2 and PPT was greater in the aorta (Fig. 6A and mesenteric artery (Fig. 6C) but not different in the carotid artery (Fig. 6B) or renal artery (Fig. 6D) of pregnant versus virgin rats (Table 1). DPN (ERβ) and G1 (GPER) relaxation was generally less than that induced by E2 or PPT in all vessels tested (Fig. 6 and Table 1). DPN maximum relaxation and EC50 were not different in all vessels of pregnant versus virgin rats. G1-induced maximum relaxation was only greater in the aorta of pregnant versus virgin rats (Fig. 6A and Table 1). In comparison, ACh, a known stimulator of endothelial-dependent relaxation, caused relaxation that was not different in the aorta, carotid artery, mesenteric artery, or renal artery of pregnant versus virgin rats (Fig. 6, A–D).

**Tissue histology and morphometry.** We assessed if pregnancy-induced changes in vascular ERs are associated with changes in vessel structure. The external vessel diameter in pregnant rats (aorta: 1,206 ± 57 μm, carotid artery: 836 ± 88 μm, mesenteric artery: 737 ± 38 μm, and renal artery: 721 ± 39 μm) was not significantly different from that in virgin rats (aorta: 1,357 ± 65 μm, carotid artery: 961 ± 70 μm, mesenteric artery: 802 ± 49 μm, and renal artery: 781 ± 36 μm). Histological analysis of aortic sections showed no significant changes in total tissue area, wall and lumen area, wall-to-lumen ratio (Fig. 5A), and total wall thickness (Fig. 5C) but a significant decrease in aortic media thickness (Fig. 5C) in pregnant versus virgin rats. Carotid artery sections showed decreases in total and wall area (Fig. 5A), total wall thickness, and media thickness (Fig. 5C) in pregnant versus virgin rats. No significant differences in tissue wall, lumen thickness, and relative thickness of the intima, media, and adventitia were observed in the mesenteric and renal artery of pregnant versus virgin rats (Fig. 5, A and C). Parallel measurements in uterine sections revealed increases in total tissue area, wall and lumen area (Fig. 5B), total wall thickness, and endometrium and myometrium layer thickness (Fig. 5D) in pregnant versus virgin rats.
Fig. 4. Protein amount and tissue distribution of GPER in the aorta, carotid artery, mesenteric artery, and renal artery as well as the uterus of virgin versus pregnant rats. Tissue homogenates of blood vessels (A) and the uterus (B) were prepared for Western blots using GPER antibody (1:1,000). GPER and ERα (shown in Fig. 2) were run on the same gel and therefore have the same actin control. Vascular tissue sections (C) and uterine sections (D) were stained with GPER antibody using immunohistochemistry, and the total amount and relative distribution of GPER brown immunostaining in different layers of the tissue wall were measured using ImageJ. Bar graphs represent means ± SE; n = 4–6 rats/group. *P < 0.05, preg vs. virgin rats; ‡significantly different (P < 0.05) from corresponding measurements in the aorta, carotid artery, mesenteric artery, and renal artery of virgin rats; #significantly different (P < 0.05) from corresponding measurements in the aorta, carotid artery, mesenteric artery, and renal artery of pregnant rats.
DISCUSSION

The main findings of the present study were that 1) expression of ERα and ERβ are greater in the aorta and mesenteric artery and GPER is greater in aorta of pregnant versus virgin rats; 2) pregnancy-associated increases in vascular ERs are mainly in the intima and media, without change in vessel structure; 3) E2- and PPT-induced relaxation of Phe-induced contraction is greater in the aorta and mesenteric artery, DPN-induced relaxation is not different, and G1-induced relaxation is greater in the aorta of pregnant versus virgin rats; 4) NOS blockade or endothelium removal reduce ER agonist-induced relaxation in the aorta, suggesting an endothelial mechanism, but not in other vessels, suggesting other endothelium-independent mechanisms; and 5) E2, PPT, DPN, and G1 cause relaxation of Ca2+-dependent KCl contraction, with PPT-induced relaxation greater in the mesenteric artery of pregnant versus virgin rats.

Pregnancy- and E2/ER-related adaptations in the uterus, BP, and body weight. Sex hormones cause periodic changes in the uterine wall during the ovarian cycle and maintain adaptive structural and functional changes in the uterus during pregnancy. The present study supports pregnancy- and E2/ER-related adaptive changes in the uterus because 1) plasma E2 levels were higher in pregnant versus virgin rats; 2) the uterus weight without pups and placenta was approximately ninefold greater in pregnant versus virgin rats; 3) uterine sections were larger and the thickness of the uterine wall, endometrium, myometrium, and perimetrium were greater in pregnant versus virgin rats, supporting uterine wall remodeling; and 4) Western blots and immunohistochemistry revealed greater levels of ERα, ERβ, and GPER in the uterus of pregnant versus virgin rats.

In addition to its effects on the uterus, E2 could cause other physiological changes during pregnancy. Consistent with other reports (70, 84), BP was slightly lower in pregnant versus virgin rats. The body weight even without the uterus was greater in pregnant versus virgin rats, likely due to salt and water retention and increased blood volume and cardiac output. However, the lack of difference in kidney weight in pregnant versus virgin rats suggests that the changes in body weight during pregnancy may not involve renal tissue structural remodeling, an observation that needs to be further examined.

Pregnancy- and E2/ER-related adaptations in systemic vessels. Previous studies have examined pregnancy-associated changes in the uteroplacental circulation (30, 66, 67) and the effects of E2/ERs on the uterine artery (10, 18, 75). Studies have also shown vasodilator effects of E2 in systemic vessels of nonpregnant animals (15, 24, 31, 53, 76). However, little is known regarding the effects of E2/ERs in the systemic circulation during pregnancy. In addition, while pregnancy-associated changes in the circulation could be partly due to increased E2 levels, the role of vascular ERs is unclear. If vascular ERs play a role in pregnancy-associated changes in the circulation, one would predict changes in the expression and vascular...
distribution of ERs and, consequently, alterations in vascular structure, vasodilator response to E2, and downstream post-ER signaling mechanisms.

**Pregnancy-associated expression of ERα, ERβ, and GPER in specific blood vessels.** With regard to ER expression, previous studies have shown prominent ERα signals in the rat cerebral and coronary artery (16), greater amounts of ERβ than ERα in the baboon carotid artery (1), and GPER immunoreactivity in the carotid artery of Sprague-Dawley rats and the mesenteric artery of female mRen2.Lewis rats (6, 43). Because most of these studies examined one ER subtype in one blood vessel, it has been difficult to relate these findings to each other or to determine the contribution of a specific ER subtype to vasodilation in different vascular beds. Consistent with our recent report (72), we observed differential expression of ERs in the aorta, carotid artery, mesenteric artery, and renal artery of virgin rats, with prominent ERα and ERβ immunoreactivity in the renal artery and increased GPER immunoreactivity in the mesenteric and renal artery of virgin rats. Both Western blots and immunohistochemistry revealed greater amounts of ERα and ERβ in the aorta and mesenteric artery and GPER in the aorta of pregnant versus virgin rats. In addition, among vessels of pregnant rats, ERα and ERβ were greater in the aorta and mesenteric artery and GPER was greater in the aorta than in the carotid artery. These specific increases in vascular ERs may contribute to certain structural or functional adaptations in the vasculature to accommodate the hemodynamic changes during pregnancy, such that the aorta could bear the brunt of the increase in cardiac output, whereas the mesenteric arteries, which harbor a major portion of the systemic circulation, could accommodate the increase in blood volume.

**Pregnancy-associated changes in the tissue distribution of vascular ERs.** In addition to changes in ER expression, pregnancy was associated with changes in the vascular tissue distribution of ERs, with increases in ERα and ERβ in the aorta and mesenteric artery being mainly in the intima and media, suggesting effects on the endothelium and VSM. This is consistent with our previous finding of ERα and ERβ in rat

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**Fig. 6.** Differential effects of E2, 4,4',4''-(4-propyl-[1H]-pyrazole-1,3,5-triyl)-tris-phenol (PPT), diarylpropionitrile (DPN), G1, and ACh on relaxation of the phenylephrine (Phe)-precontracted aorta (A), carotid artery (B), mesenteric artery (C), and renal artery (D) of virgin versus pregnant rats. Endothelium-intact segments of the thoracic aorta, carotid artery, mesenteric artery, and renal artery were precontracted with Phe. Increasing concentrations (10^{-12}–10^{-5} M) of E2 (activator of all ERs), PPT (ERα agonist), DPN (ERβ agonist), or G1 (GPER agonist) were added, and the percent relaxation of Phe-induced contraction was measured. In parallel experiments, the effects of increasing concentrations (10^{-9}–10^{-3} M) of ACh on the relaxation of Phe-precontracted vessels were measured. Data represent means ± SE; n = 8–10 rats/group. *P < 0.05, pregnant vs. virgin rats.
Table 1. Maximum response and EC<sub>50</sub> for ER agonist-induced relaxation of the thoracic aorta, carotid artery, mesenteric artery, and renal artery isolated from virgin and pregnant rats and precontracted with phenylephrine or KCl

<table>
<thead>
<tr>
<th>Relaxation of phenylephrine-induced contraction maximum, %</th>
<th>Virgin Rats</th>
<th>Pregnant Rats</th>
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<tr>
<td>E&lt;sub&gt;2&lt;/sub&gt;</td>
<td>29.99 ± 3.85</td>
<td>26.55 ± 3.07</td>
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<tr>
<td>pD&lt;sub&gt;2&lt;/sub&gt; (−log M)</td>
<td></td>
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<tr>
<td>E&lt;sub&gt;2&lt;/sub&gt;</td>
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<tr>
<td>PPT</td>
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Data represent means ± SE; n = 7–11 rats/group. E2, estradiol; PPT, 4,4′-(4-propyl-[1H]-pyrazole-1,3,5-triyl)-tris-phenol; DPN, diarylpropionitrile. *P < 0.05, pregnant vs. virgin rats; †significantly different (P < 0.05) from corresponding measurements in the aorta (A), carotid artery (C), and mesenteric artery (M) of virgin rats; ‡significantly different (P < 0.05) from corresponding measurements in the aorta and carotid artery of pregnant rats.

Aortic VSM cells (47). Other studies have identified GPER in endothelial and VSM cells of the carotid artery of Sprague-Dawley rat and the mesenteric artery of mRen2.Lewis female rats (6, 43). In agreement with these reports, we detected GPER in the intima and media of the aorta, carotid artery, mesenteric artery, and renal artery of virgin rats and increased GPER in the intima and media of the aorta of pregnant rats, suggesting pregnancy-related effects on the endothelium and VSM.

Pregnancy and vascular structure and remodeling. Studies in the uterine circulation have shown outward, expansive, and hypertrophic remodeling of uterine vessels during pregnancy (65) in part due to E<sub>2</sub> effects on the VSM and extracellular matrix (65, 66). The present study in systemic vessels showed increases in ERα and ERβ not only in the intima and media of the aorta and mesenteric artery but also in the adventitia, suggesting potential effects on vessel structure. However, measurement of tissue weight and histological analysis of wall thickness showed no difference in the aorta, mesenteric artery, and renal artery of pregnant versus virgin rats, suggesting little pregnancy-related or ER-mediated structural remodeling in these vessels.

In comparison, carotid artery weight and wall thickness were reduced in pregnant versus virgin rats. The carotid artery structural remodeling could be related to a redistribution of blood flow from the cephalic to the uteroplacental circulation to maintain adequate blood supply to the growing fetus. Still, the pregnancy-associated changes in carotid artery structure may not be related to ERs as no changes in ER amount or distribution were observed in this vessel.

Pregnancy- and E<sub>2</sub>/ER-related adaptations in vascular function. To test if the pregnancy-associated changes in ER expression affect ER-mediated vascular activity, we tested the vascular effects of different ER agonists: E<sub>2</sub> (all ER subtypes) (53), PPT (ERα) (79, 81), DPN (ERβ) (27), and G1 (GPER) (21, 37). Consistent with our previous report (72), E<sub>2</sub>- and PPT-induced relaxation was renal artery > mesenteric artery > carotid artery > aorta of virgin rats, supporting a role of ERα in E<sub>2</sub>-induced relaxation. DPN caused less relaxation than PPT but was still renal artery > mesenteric artery > carotid artery > aorta of virgin rats, suggesting a partial role of ERβ in E<sub>2</sub>-induced vascular relaxation. These findings agree with reports that ERα and ERβ mediate rapid vasodilator effects of E<sub>2</sub> (68, 93), E2 causes relaxation of the rat aorta largely via ERα (5), and PPT causes greater relaxation than DPN in the rat mesenteric artery (57, 72). In contrast, G1 caused small relaxation in all vessels of virgin rats, suggesting a little role for GPER in E<sub>2</sub>-induced vasodilation. This is consistent with the above reports showing that G1 caused little relaxation in the aorta of female Sprague-Dawley rats (48, 72). Other studies have shown G1-induced vasodilation similar to E<sub>2</sub> in the carotid and mesenteric artery of Sprague-Dawley rats (6, 25) and in the mesenteric artery of female mRen2.Lewis rats (43).

VASCULAR ESTROGEN RECEPTORS DURING PREGNANCY

Table 1. Maximum response and EC<sub>50</sub> for ER agonist-induced relaxation of the thoracic aorta, carotid artery, mesenteric artery, and renal artery isolated from virgin and pregnant rats and precontracted with phenylephrine or KCl

<table>
<thead>
<tr>
<th>Relaxation of phenylephrine-induced contraction maximum, %</th>
<th>Virgin Rats</th>
<th>Pregnant Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>E&lt;sub&gt;2&lt;/sub&gt;</td>
<td>29.99 ± 3.85</td>
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Although we observed greater relaxation to G1 in the mesenteric and renal artery than in the aorta and carotid artery, it was still less than that of E<sub>2</sub> or PPT. The differences in the vascular effects of G1/GPER may be related to the rat strain or regional differences in the arterial tree (87).

Importantly, the pregnancy-related changes in ER expression were associated with a distinct relaxation response in different vessels. In line with the increased ERα in the aorta and mesenteric artery, PPT caused greater relaxation in the aorta and mesenteric artery of pregnant versus virgin rats that was similar to that induced by E<sub>2</sub>, supporting a role for ERα in mediating the vasodilator effects of E<sub>2</sub> during pregnancy. In addition, in line with the increased GPER levels in the aorta of pregnant rats, G1 caused greater relaxation in the aorta of pregnant versus virgin rats, highlighting a role of GPER in the adaptive aortic response during pregnancy. The pregnancy-associated changes in GPER-mediated aortic relaxation in the
presence of relatively little changes in aortic structure and remodeling provide evidence for important ER-related functional changes rather than simply the mechanical changes that are generally perceived in most conduit arteries due to exposure to the hemodynamic changes during pregnancy.

It could be argued that the observed greater ER agonist-induced relaxation in smaller vessels (mesenteric and renal artery) compared with larger vessels (aorta and carotid artery) is related to more feasible diffusion of the compounds through the fewer cell layers in the small vessels. However, the enhanced PPT-, DPN-, and G1-induced relaxation in small vessels such as the renal artery of virgin rats was associated with specific increases in the protein amount and immunoreactivity of ERα, ERβ, and GPER, respectively. Furthermore, the vasorelaxation effects of ER agonists were enhanced in specific vessels of pregnant compared with virgin rats, and the enhanced relaxation coincided with parallel increases in the expression of specific ER subtypes, making it more likely that the observed differences in the responsiveness to ER agonists among different vessels and in vessels of pregnant versus virgin rats are due to differential expression/activity of vascular ER subtypes.

**ER-mediated endothelium-dependent and -independent vasodilation during pregnancy.** ER-mediated vasodilation depends not only on ER amount but also on the post-ER signaling mechanisms in the endothelium and VSM. To test the role of the endothelium in ER-mediated relaxation, we first confirmed a functional endothelium and showed that ACh-induced relaxation was not different in vessels of pregnant versus virgin rats. In many vessels, ACh-induced relaxation involves the NO-cGMP pathway. In addition, our previous studies in the aorta, carotid artery, and renal artery of virgin rats confirmed that ACh-induced relaxation was associated with increased NO production and was inhibited by the NOS inhibitor L-NAME (72). In contrast, in the mesenteric artery, a large portion of ACh-induced relaxation remained in the presence of L-NAME.
and the COX inhibitor indomethacin and was abolished by the K+ channel blocker TEA, suggesting a role of a hyperpolarization pathway (49, 51).

E2 induces vasodilation through genomic and nongenomic endothelium-dependent and endothelium-independent pathways (53, 64). E2 activates endothelium-dependent NO-cGMP, PGI2-cAMP, and EDHF relaxation pathways in a blood vessel- and ER-specific manner (35, 78). E2 via ERα increases endothelial NOS expression and NO production in bovine pulmonary artery endothelial cells (38, 48, 80). In addition, G1-induced relaxation in the rat carotid artery is abolished by endothelium removal or endothelial cells (38, 48, 80). The present experiments on the aorta of pregnant rats suggest that ER agonist-induced relaxation may involve endothelium-dependent NO because 1) E2-, PPT-, DPN-, and G1-induced relaxation was inhibited by l-NNAME; 2) blockade of COX and PGI2 production using indomethacin and the hyperpolarization pathway using TEA did not cause further inhibition of ER agonist-induced aortic relaxation; and 3) endothelium removal abolished E2-, PPT-, DPN-, and G1-induced aortic relaxation. The endothelium-dependent relaxation effects of PPT and G1 are in line with the immunohistochemical detection of ERα and GPER in the intima of the aorta of pregnant rats. In comparison, ER-mediated relaxation in the carotid, mesenteric, and renal artery appears to mainly involve an endothelium-independent mechanism in VSM because 1) E2-, PPT-, DPN-, and G1-induced relaxation of the carotid, mesenteric, and renal artery was not blocked by l-NNAME, indomethacin, TEA, or endothelium removal; 2) ER agonist-induced relaxation was still greater in the mesenteric and renal artery and in the carotid artery than in the aorta even after treatment with blockers of endothelium-derived vasodilators or endothelium removal; and 3) immunohistochemistry showed ERα, ERβ, and GPER in the tunica media and VSM of the aorta, the carotid artery, mesenteric artery, and renal artery of pregnant rats. These data are consistent with reports that E2 causes relaxation of the endothelium-denuded aorta and coronary artery (13, 14, 31), and inhibits Phe- and PGI2α-induced contraction in VSM cells from the rat aorta and porcine coronary artery (60, 61).

ER-mediated inhibition of Ca2+−dependent contraction during pregnancy. VSM contraction in response to α-adrenergic agonists such as Phe is triggered by increases in intracellular free Ca2+ concentration ([Ca2+]) due to initial Ca2+ release from the intracellular stores and maintained Ca2+ influx from the extracellular space (4, 36). The observation that ER agonists decreased Phe-induced maintained contraction in endothelium-denuded vessels suggests inhibition of Ca2+ entry into VSM. In addition, high KCl causes vascular contraction mainly by inducing membrane depolarization and Ca2+ entry through voltage-gated channels (61). In the present study, ER agonists inhibited KCl-induced contraction in the endothelium-denuded carotid, mesenteric, and renal artery of virgin and pregnant rats, supporting inhibition of Ca2+ entry into VSM. This is consistent with reports that E2 decreased KCl-induced Ca2+ influx in the endothelium-denuded rat aorta, coronary artery, and rat aortic VSM cells (13, 22, 61) and inhibited Phe-induced capacitative Ca2+ influx in the rat aorta (8) and that E2, PPT, and DPN caused relaxation of KCl-induced contraction in endothelium-denuded cephalic, thoracic, and abdominal vessels (72) and vasodilation and decreased [Ca2+], in mesenteric vessels of female rats (51). Importantly, PPT-induced relaxation of KCl-induced contraction was greater in the mesenteric artery of pregnant versus virgin rats, suggesting specific enhancement of ERα-mediated inhibition of Ca2+ entry mechanisms of vasodilation in the mesenteric circulation during pregnancy.
Mechanism of ER-mediated decrease in Ca^{2+} entry in VSM.

An important question is how ER agonists decrease Ca^{2+} entry into VSM. Some studies have suggested indirect mechanisms whereby ER agonists activate VSM K^+ channels and cause VSM hyperpolarization, which, in turn, inhibits voltage-gated Ca^{2+} channels (2, 46, 85, 88, 91). This is unlikely because ER agonists inhibited KCl contraction, and the high KCl gradient should prevent outward movement of K^+ through K^+ channels. ER-mediated decrease in VSM Ca^{2+} entry could also involve adenylate cyclase/cAMP/PKA or guanylate cyclase/cGMP/PKG pathways (2, 17, 34, 45, 83, 88, 90). However, our recent study showed that the guanylate cyclase inhibitor 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) did not reverse ER agonist-induced relaxation or decreased [Ca^{2+}]_i in mesenteric vessels, suggesting little role for guanylate cyclase/cGMP/PKG (51). Alternatively, the ER-mediated decrease in Ca^{2+} entry may involve direct effects on VSM Ca^{2+} channels. This is consistent with reports that ER agonists decreased [Ca^{2+}]_i in mesenteric vessels of female rats preconstricted with high KCl and the L-type Ca^{2+} channel agonist Bay K 8644 (51) and that E2 caused relaxation of endothelium-denuded aortic rings precontracted with Bay K 8644 or high KCl that was not modified by K^+ channel blockers, inhibited basal and Bay K 8644-stimulated L-type Ca^{2+} current with no effect on

Fig. 8. Differential effects of E2, PPT, DPN, and G1 on relaxation of the KCl precontracted aorta (A), carotid artery (B), mesenteric artery (C), and renal artery (D) of virgin versus pregnant rats. Endothelium-denuded segments of the thoracic aorta, carotid artery, mesenteric artery, and renal artery were precontracted with high KCl (96 mM) depolarizing solution to induce a Ca^{2+}-dependent contractile response in vascular smooth muscle. Increasing concentrations (10^{-12}–10^{-5} M) of E2 (activator of all ERs), PPT (ERα agonist), DPN (ERβ agonist), or G1 (GPER agonist) were added, and the percent relaxation of KCl contraction was measured. Data represent means ± SE; n = 7–10 rats/group. *P < 0.05, pregnant vs. virgin rats.
basal K+ current (7), decreased [Ca2+]i in porcine coronary artery and rat aortic VSM cells (60, 61), and inhibited voltage-dependent L-type Ca2+ current in cultured A7r5 rat aortic VSM cells (62, 92). It appears that ER agonist-induced inhibition of Ca2+ entry in blood vessels of pregnant rats involves both indirect and direct action on Ca2+ channels, and the mechanisms of ER-mediated decrease in VSM [Ca2+]i may vary depending on the blood vessel, the vascular region, and the animal species studied.

Limitations. Other considerations include, first, although the ERβ amount and immunoreactivity in the endothelium and VSM of the aorta and mesenteric artery were greater in pregnant rats than in virgin rats, no differences in the vasodilator effects of the ERβ agonist DPN were observed. ERβ mediates both genomic and nongenomic vascular effects (68, 93), and while the lack of difference in the rapid vasodilator effects of DPN suggests no difference in ERβ-mediated nongenomic pathways, it does not rule out possible enhancement of ERβ-mediated genomic effects during pregnancy. Second, although no pregnancy-associated changes in ER amount/distribution were observed in the carotid artery, the E2- and PPT-induced maximum relaxation of Phe-induced contraction was enhanced in the carotid artery versus the aorta of pregnant rats treated with blockers of endothelium-derived vasodilators or endothelium denuded, and maximum relaxation of KCl contraction by all ER agonists was greater in the endothelium-denuded carotid artery versus the aorta of pregnant rats. Whether these findings are related to the observed changes in carotid artery histology and its potential remodeling remain to be examined. Third, maximal effects of E2 were observed at micromolar concentrations, which are higher than physiologically nanomolar plasma E2 levels, and the observed small levels of plasma E2 even in pregnant rats. Because E2 is lipophilic, its plasma levels may not reflect its vascular tissue level, and prolonged exposure to small E2 concentrations in vivo could lead to gradual tissue accumulation that eventually reaches levels similar to those used in the acute studies. In this respect, ex vivo studies may require higher E2 concentrations to bind to plasma membrane lipids and both physiologically active and other ER variants. Fourth, splice variants or isoforms of ER have been described. Most ERα variants differ in their 5′-untranslated region, not in the coding sequence. The biological functions of ERα isoforms are unclear, but they may heterodimerize with full-length ERα and repress activation function (AF)-1-mediated activity. Additionally, surface membrane binding sites for E2 have been identified in cells transfected not only with full-length ER-α66 but also with truncated isoforms ER-α46 and ER-α36 (41). The localization of ERα isoforms in the plasma membrane may help to further elucidate the ERα nongenomic signaling mechanisms and the role of MAPK/ERK and phosphatidylinositol 3-kinase/Akt pathways (42). Multiple ERβ isoforms also exist as a result of either alternative splicing of the last coding exon 8, deletion of one or more coding exons, or alternative usage of untranslated exons in the 5′-region. Among ERβ isoforms, five full-length transcripts (ERβ1-ERβ5) have been reported in humans (28). Future studies should further examine the expression and distribution of different ER isoforms in different blood vessels and the potential changes in the expression of these isoforms during pregnancy. Finally, pregnancy is associated with increases not only in E2 but also progesterone. Progesterone receptors have been identified in the endothelium and VSM (53, 54), and progesterone induces genomic and nongenomic vascular effects (53, 64) and decreases [Ca2+]i (60), PKC (56), and Rho kinase in VSM (63). The pregnancy-associated changes in vascular progesterone receptors should be examined.

Conclusions and perspectives. During pregnancy, vascular ERs exhibit regional changes in their expression, vasodilator activity, and signaling mechanisms with specific increases in ERα and ERβ in the endothelium and VSM of the aorta and mesenteric artery and GPER in the aorta. The pregnancy-associated increases in aortic and mesenteric artery ERα expression coupled with ERα-mediated endothelium-dependent relaxation in the aorta and endothelium-independent inhibition of Ca2+ entry in the mesenteric artery support a role of aortic and mesenteric arterial ERs in pregnancy-associated systemic vasodilation. GPER may contribute to aortic vasodilation via an endothelium-dependent mechanism, while the enhanced ERβ expression could mediate other genomic vascular effects during pregnancy. The observed changes in the expression/activity of ER subtypes in systemic arteries appear to be different from those reported in the local uterine circulation. A review of earlier studies showed that ERα ligands are more potent than ERβ ligands in increasing uterine blood flow in nonpregnant sheep, supporting the premise that E2-mediated uterine vasodilation may be mediated more by ERα than ERβ (69). Similar to the present observations in the aorta and mesenteric artery, ER expression/activity also appears to be enhanced in the uterine artery during gestation, although the dominant ER subtype may be different. While some studies (11, 40) have shown intensive immunoreactive signals for both ERα and ERβ in uterine artery tissue sections from late-pregnant sheep, another study (69) has suggested that ERβ may be the main receptor subtype modulated in the uterine artery during gestation. In addition, a study (12) of the uterine circulation in pregnant women has shown that E2, PPT, and DPN caused significant relaxation of isolated myometrial arteries with relaxation of E2 > DPN > PPT, whereas G1 did not cause significant relaxation. These observations highlight the importance of further examining ER distribution, activity, and downstream signaling mechanisms in the systemic versus uterine circulation. Nevertheless, the data support the contention that pregnancy-associated changes in vascular ER distribution/activity in the systemic versus the uterine circulation could play a role in the regulation of regional blood flow and distribution of blood in the maternal circulation by promoting vasodilation, opposing vasoconstriction, and maintaining normotension during normal pregnancy. Future studies should investigate possible changes in vascular ERs in hypertensive pregnancy and the potential usefulness of ER agonists in promoting vasodilation and improving maternal hemodynamics and BP in preeclamptic pregnancies. Due to their vasodilator effects, specific ER agonists could reduce BP while avoiding the adverse effects of E2 on uterine and breast cancer, inflammation, and plasma lipids. In addition, specific ER agonists could modulate vascular ER activity in specific blood vessels without affecting other vessels in the systemic circulation.

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REFERENCES


