Pregnancy and Vascular Regulation

Regulation of Vascular Tone During Pregnancy
A Novel Role for the Pregnane X Receptor

Kathryn A. Hagedorn, Christy-Lynn Cooke, John R. Falck, Bryan F. Mitchell, Sandra T. Davidge

Abstract—During pregnancy, maternal vascular function is altered through mechanisms that remain unclear. Progesterone synthesis and metabolism are also increased. Progesterone metabolites are potent endogenous ligands for the pregnant X receptor (PXR), a nuclear receptor that induces the expression of hepatic cytochrome P450 enzymes. Cytochrome P450 enzymes in the vasculature can metabolize arachidonic acid to produce epoxyeicosatrienoic acids, known vasodilators. We hypothesized that PXR is present in vascular tissue and contributes to vascular adaptations to pregnancy. PXR mRNA was detected in mouse mesenteric arteries by quantitative RT-PCR. Constrictor and relaxation responses in wildtype (PXR+/+) and PXR-deficient (PXR−/−) mice were compared by wire myography. Relative to nonpregnant controls, arteries from pregnant PXR+/+ mice had reduced sensitivity to phenylephrine-induced constriction (EC50: 2.77±0.32 μmol/L versus 5.13±0.36 μmol/L; P=0.009) and enhanced maximal vasorelaxation to bradykinin (26±3% versus 44±16%; P=0.013). However, these pregnancy adaptations were absent in PXR−/− mice. We also hypothesized that PXR is activated by progesterone metabolites. Treatment of PXR+/+ and PXR−/− nonpregnant mice with 5β-dihydroprogesterone for 7 days enhanced endothelium-dependent relaxation in only the PXR+/+ mice, similarly to that seen in pregnancy. In treated mice, inhibition of cytochrome P450 epoxygenase activity with N-methylsulphonyl-6-(2-propargyloxyphenyl)hexanamide attenuated vasorelaxation in arteries from PXR+/+ but not PXR−/− mice. We conclude that PXR contributes to the development of vascular adaptations to pregnancy, likely in response to activation by progesterone metabolites, and that PXR-dependent increases in vasorelaxation may be because of activation of cytochrome P450 epoxygenases. (Hypertension. 2007;49:328-333.)

Key Words: pregnancy ■ vascular tone ■ pregnane X receptor ■ cytochrome P450

Alterations in maternal vascular function, including both increased vasodilation and reduced vasoconstriction, are necessary during pregnancy to compensate for increases in blood volume and cardiac output. Failure of the maternal cardiovascular system to adapt appropriately to the challenge of pregnancy can result in a variety of pathologies, such as pregnancy-induced hypertension and preeclampsia. Unfortunately, however, many gaps still exist in our knowledge of the mechanisms underlying these pregnancy-induced alterations, including the means by which the state of pregnancy triggers these changes in maternal vascular function, and a detailed understanding of the cell signaling pathways involved.

Progesterone synthesis and metabolism are dramatically increased during gestation. Progesterone metabolites, in particular, 5β-pregnane-3,20-dione (also known as 5β-dihydroprogesterone [5β-DHP]), are potent endogenous ligands for the pregnane X receptor (PXR).1 This nuclear receptor is predominately expressed in the liver and intestines,1–5 although PXR mRNA has also been found in the stomach and kidney,1 uterus, ovary and placenta,5 human breast,6 and lung7 and, most importantly to this study, in vascular tissue (rat brain capillaries).7 Functionally, PXR is primarily recognized as a xenobiotic sensor, detecting potentially harmful lipophilic compounds and promoting their elimination by inducing the expression of cytochrome P450 enzymes (CYPs) in the liver.8

Although CYPs are most commonly known for their role in drug metabolism, they are also involved in the regulation of vascular tone.9 Metabolism of arachidonic acid by CYP epoxygenases produces epoxyeicosatrienoic acids, of which there are 4 regioisomers.10 These molecules are considered by some to be endothelial derived hyperpolarizing factors because of their ability to induce vasorelaxation and hyperpolarize vascular smooth muscle cells.11–14 A number of CYP enzymes have epoxygenase activity, primarily those of the CYP2 gene family.15,16 Of these, the 2C, 2E, and 2J isoforms have been shown to be expressed in vascular tissue.17 Moreover, CYP2B, 2C, and 2J have also been detected in endothelial cells.18,19 PXR response elements have been identified in the promoter region of many CYP2C isoforms,20–22 and expression of CYP 2C8/9 has been shown to be upregulated by PXR.20,23

In the present study, we hypothesize that the nuclear receptor PXR contributes to the regulation of vascular tone...
during pregnancy. We have addressed this hypothesis by using a PXR knockout mouse model to determine the role of PXR in the mediation of pregnancy-induced vascular adaptations. Using quantitative RT-PCR, we showed that PXR mRNA is expressed in mouse mesenteric arteries. Isometric wire myography was used to investigate functional changes in both pregnant mice and in mice treated with 5β-DHP, a progesterone metabolite and known PXR ligand.

Methods

Animals and Breeding

Wildtype (PXR+/−) and PXR knockout (PXR−/−) C57Bl/6J mice were generously provided by Dr B. Goodwin (Nuclear Receptor Discovery Centre of GlaxoSmithKline, Research Triangle Park, NC). PXR+/− and PXR−/− females (10 to 14 weeks old) were time-mated with PXR−/− males. The observation of a seminal plug was counted as day 0, and pregnant animals were euthanized on day 17 or day 18 of gestation (term = day 19). Age-matched nonpregnant PXR+/− and PXR−/− mice were used as controls. All of the procedures were approved by the University of Alberta Health Sciences Animal Policy and Welfare Committee.

Analysis of PXR mRNA Expression

Nonpregnant and pregnant PXR−/− mice were euthanized under isoflurane (Bimeda-MTC Animal Health Inc), and the entire mesenteric arcade was harvested and instantly placed on ice in DMEM, supplemented with 5.5 mmol/L glucose, 1 mmol/L sodium pyruvate and 25 mmol/L HEPES, and pH adjusted to 7.4 at 37°C. All of the mesenteric arteries were dissected immediately and snap frozen in liquid nitrogen. Three pools (representing 2 mice each) of arteries were collected from each group, and RNA was extracted using TRIzol (Invitrogen). Glycogen (1 mg) was added to the samples to induce RNA precipitation. A total of 500 ng of RNA was reverse transcribed with Superscript II and random primers (Invitrogen). qPCR was performed using Assays-on-Demand primers and Taqman probes for PXR (catalog No. Mm00439609_m1) and 18S (catalog No. 4333760T) were obtained from Applied Biosystems and run according to manufacturer’s specifications using an iCycler iQ real-time PCR detection system (Bio-Rad). The cycling protocol was as follows: 10 minutes at 95°C, followed by 45 cycles of denaturing for 15 seconds at 95°C, annealing/extension for 1 minute at 60°C. The samples were run in triplicate, and the expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used to normalize expression. The data were analyzed using the 2−ΔΔCt method and presented as fold change relative to nonpregnant controls. Data were analyzed using 2-way ANOVA with Bonferroni post hoc test. All of the data are expressed as mean ± SEM, and statistical significance was set at P < 0.05.

Analysis of Pregnancy-Induced Changes in Vessel Function

Wire myography was used to assess vessel function. Briefly, 12- to 14-week-old nonpregnant and pregnant PXR−/− and PXR−/− mice were euthanized by cervical dislocation. Second-order mesenteric arteries (150 to 200 μm in diameter) were collected, mounted on an isometric wire myograph apparatus (Kent Scientific) and submerged in 5 mL of warmed DMEM, a medium that has been shown to help maintain vessel viability. After mounting and equilibration, vessels were set to their optimum resting tension, and a phenylephrine (PE) concentration response curve (50 μmol/L to 10 μmol/L) was performed to assess vasoreactivity. Vessels were then washed 3 times for 10 minutes, and the concentration of PE that produced 80% of the maximal response was determined (EC80). This concentration was then used to constrict the vessels, and a second concentration–response curve was performed using the endothelium-dependent relaxing agent, bradykinin (1 nmol/L to 10 μmol/L), to assess vasorelaxation. Vessel constriction in response to the bolus dose of PE resulted in a peak in the response, which eventually reached a stable plateau, except in the pregnant state, where the arteries had a strong tendency to spontaneously relax. Only vessels that maintained a stable constriction plateau for ≥90 seconds were included for analysis to ensure that the response observed was, in fact, the result of bradykinin stimulation and not because of spontaneous relaxation.

In a separate series of experiments, N-methylsulphonyl-6-(2-propargyloxyphenyl)hexanamide (MS-PPOH) 50 μmol/L, an inhibitor of CYP epoxygenase activity,24 was used to assess the contribution of CYP epoxygenases to the vasocostriction response during pregnancy. Arteries were mounted and prepared as described and then randomly treated with either MS-PPOH or vehicle (ethanol) for 15 minutes before the start of the PE curve. The total amount of ethanol in the bath was set at 0.1% of the total bath volume. At the end of the experiment, vessels were exposed to a physiological saline solution containing a high concentration of potassium (123.7 mmol/L of KCl, 1.18 mmol/L of KH2PO4, 1.17 mmol/L of MgSO4, 5.00 mmol of CaCl, and 5.5 mmol/L of glucose) to assess contractility.

Because of difficulties associated with the tendency for vessels from pregnant animals to spontaneously relax, the effect of MS-PPOH on bradykinin-induced relaxation could not be assessed. We, therefore, performed a second series of experiments as described below to directly investigate the role of CYP epoxygenases in PXR-mediated vasorelaxation, in which PXR was activated via the ligand 5β-DHP.

Analysis of the Effect of 5β-DHP Treatment on Vasorelaxation

Virgin PXR−/− and PXR−/− mice (12 weeks of age) were implanted subcutaneously with slow-release pellets (Steraloids) containing either 20 mg of 5β-DHP or a placebo, as we have reported previously.24 One week later, animals were euthanized, and vessels were harvested and prepared for wire myography. After preconstriction with PE, a concentration response curve (10 mmol/L to 10 μmol/L) to the endothelium-dependent relaxing agent, methacholine was performed. Following the methacholine curve, vessels were washed for 30 minutes, preincubated with 10 μmol/L of MS-PPOH for 15 minutes, and then the methacholine concentration response curve was repeated.

Data Analysis and Statistics

The mean EC50 was used to summarize all of the PE concentration response curves, and 2-way ANOVA with least-significant differences post hoc testing was used to compare vasocostriction responses. Relaxation curves were analyzed using 2-way ANOVA with repeated measures. The effect of MS-PPOH on vasorelaxation is reported as the difference between vessel response in the presence and in the absence of MS-PPOH and was compared using a paired t test. All of the data are expressed as mean ± SEM, and statistical significance was set at P < 0.05.

Results

We began our investigation by using quantitative RT-PCR to determine whether PXR was expressed in mesenteric arteries from nonpregnant and pregnant PXR−/− mice. All of the samples were positive for PXR expression, indicating that PXR mRNA is present in mouse mesenteric arteries. There was no significant difference between the level of PXR expression in mesenteric arteries from nonpregnant and pregnant mice (nonpregnant PXR−/− 0.595 ± 0.273 relative units versus pregnant PXR−/− 0.343 ± 0.325 relative units). Pregnancy-induced vascular adaptations were evident only in arteries from PXR−/− but not PXR−/− mice. Specifically, vessels from PXR−/− pregnant mice showed a significant reduction in sensitivity to PE relative to that of vessels from PXR−/− nonpregnant controls (nonpregnant EC50: 2.77 ± 0.32 μmol/L versus pregnant EC50: 5.13 ± 0.36 μmol/L; P = 0.009; Figure 1a). However, this pregnancy-induced reduction in sensitivity to PE-mediated vasocostriction was...
absent in vessels from pregnant PXR<sup>+/+</sup> animals (nonpregnant EC<sub>50</sub>: 3.33 ± 0.38 μmol/L versus pregnant EC<sub>50</sub>: 3.65 ± 0.26 μmol/L; P = 0.546; Figure 1b). The contractile capacity of arteries treated with either PE or a high potassium solution was significantly reduced in the pregnant compared with the nonpregnant state but was not affected by genotype (data not shown), indicating that PXR alters sensitivity to PE but not the maximal contractile response of the vascular smooth muscle.

The effect of gestation on vasorelaxation was also assessed in PXR<sup>+/+</sup> and PXR<sup>−/−</sup> mice. Similar to the genotype-specific changes seen in the sensitivity to PE-induced vasoconstriction, vessels from PXR<sup>+/+</sup> pregnant animals demonstrated enhanced relaxation to bradykinin when compared with vessels from PXR<sup>+/+</sup> nonpregnant mice (P = 0.004; Figure 2a), but this pregnancy-induced adaptation was absent in vessels from pregnant PXR<sup>−/−</sup> animals (P = 0.806; Figure 2b).

To investigate the possibility that CYP epoxygenases were contributing to pregnancy-induced alterations in vascular function, we used MS-PPOH to block epoxygenase activity. Treatment with this epoxygenase inhibitor did not alter vasoconstriction in any of the treatment groups (data not shown). Initially, we also intended to assess the contribution of CYP epoxygenases to the vasorelaxation response. However, the strong tendency for vessels from pregnant mice to spontaneously relax effectively precluded this investigation.

We next directly tested the possibility that progesterone metabolites can activate PXR and trigger changes in vascular tone. We implanted virgin PXR<sup>+/+</sup> and PXR<sup>−/−</sup> mice with either 5β-DHP or placebo pellets. After 1 week of exposure, vessels from PXR<sup>+/+</sup> mice treated with 5β-DHP showed a significant increase in methacholine-induced vasorelaxation relative to those animals treated with placebo pellets (P < 0.001; Figure 3a). Conversely, in vessels from PXR<sup>−/−</sup> mice, there was no difference in the vasorelaxation response of vessels from animals treated with 5β-DHP as compared with those who received the placebo (P = 0.635; Figure 3b). As shown in Figure 4, subsequent inhibition of CYP epoxygenase activity with MS-PPOH significantly attenuated the increased vasorelaxation in 5β-DHP-treated PXR<sup>+/+</sup> mice (Δ = −32.8%; P < 0.001), but it did not alter the response to methacholine in any of the other treatment groups (placebo-treated PXR<sup>−/−</sup> = Δ = 0.9%; placebo-treated PXR<sup>+/+</sup> = Δ = −9.6%; 5β-DHP treated PXR<sup>−/−</sup> = Δ = 4.1%).

**Discussion**

These results demonstrate for the first time a role for the nuclear receptor, PXR, in the mediation of vascular adaptations to pregnancy. Alterations in maternal vascular function are necessary during gestation, yet the mechanisms underlying these pregnancy-induced adaptations remain poorly understood. We hypothesized that PXR, activated by progesterone metabolites, provided the means by which changes in vascular function were initiated in response to pregnancy. Small mesenteric arteries were chosen for analysis because
they contribute to the regulation of blood pressure and the contribution of other endothelial factors independent of NO or prostacyclin is proportionally greater in resistance-sized arteries relative to larger vessels. Our results show a decreased sensitivity to phenylephrine-induced vasoconstriction and an enhanced endothelial-dependent relaxation response to bradykinin in arteries from pregnant compared with nonpregnant PXR<sup>+/−</sup> mice. In PXR<sup>−/−</sup> mice, however, the absence of a functional PXR protein prevented these pregnancy-induced alterations in vascular function, indicating that PXR contributes to the regulation of vascular tone during pregnancy. Interestingly, a lack of PXR does not seem to alter vascular resistance in the nonpregnant mouse nor does it alter the fertility or gestational length. We suggest that PXR seems to be necessary for vascular adaptations to pregnancy to occur; however, redundant mechanisms may exist within the mouse that counteract the vascular effects of PXR deficiency and allow for compensation in these animals.

Little is known about the effect of pregnancy on maternal PXR expression. We found no difference between the levels of expression of PXR mRNA in mesenteric arteries from nonpregnant compared with pregnant mice, although there was a large degree of variability in our samples. In addition, minor changes in endothelial PXR receptors could be masked by changes in vascular smooth muscle expression. Additional studies specifically focused on the vascular distribution of PXR expression and its regulation are warranted. Previously, Masuyama et al<sup>5</sup> reported that, in the liver and ovaries of day-19 pregnant BALB/cA mice, the expression of PXR mRNA was increased 50-fold over nonpregnant controls. Recently, however, Sweeney et al<sup>10</sup> have shown that in C57Bl/6 mice expression of PXR in the liver is decreased on day 19 as compared with nonpregnant controls. Reasons for these differences in expression are as yet undetermined but may reflect differences in methodology (classic versus quantitative RT-PCR) or strain of mouse used. Nevertheless, the presence of PXR mRNA in the mesenteric vascular tissue in our model further supports our hypothesis that PXR can mediate vascular tone.

We also hypothesized that PXR is activated by progesterone metabolites. We, therefore, tested whether treatment with an exogenous progesterone metabolite would also alter vascular function. 5β-DHP was chosen as the tool to activate PXR because of its potency as a PXR ligand and because plasma levels of this steroid are known to rise during pregnancy.<sup>31</sup> This methodology also had the advantage of allowing us to test the effect of PXR activation on vascular function in the absence of any of the other potentially confounding physiological changes that occur during gestation. We found that treatment with this progesterone metabolite enhanced endothelial-dependent vasorelaxation in mesenteric arteries in a manner similar to that occurring in pregnancy. Indeed, 5β-DHP-treatment increased methacholine-induced vasorelaxation in the PXR<sup>+/+</sup> but not PXR<sup>−/−</sup> mice. The observation that exposure to an exogenous progesterone metabolite produced vascular changes similar to those occurring in pregnancy further confirms a role for PXR in the alteration of vascular tone and suggests that the vascular changes that occur during pregnancy may be the result of increases in progesterone metabolites.

CYP enzymes are heme-containing, NAD(P)H-dependent monoxygenases that are responsible for the metabolism of xenobiotics, as well endogenous substances, such as cholesterol, steroids, bile acids, vitamin D, and other lipids including, arachidonic acid. CYP activity has been shown to mediate vasorelaxation in canine, bovine,11–13 and porcine coronary arteries<sup>32</sup>, in hamster gracilis muscle<sup>33</sup>, and in rat mesenteric arteries.<sup>34–36</sup> Most conclusively, Fisslthaler et al<sup>10</sup> have identified CYP2C8 (2C29 in the mouse and 2C34 in the pig) as a putative endothelial-derived hyperpolarizing factor synthase in the porcine coronary artery. Because PXR can regulate CYP expression,<sup>8</sup> we were interested in determining whether PXR-dependent changes in vascular function were mediated by CYPs.

In our studies, inhibition of CYP epoxygenase activity with MS-PPOH did not alter vasoconstriction in either nonpregnant or pregnant PXR<sup>+/−</sup> or PXR<sup>−/−</sup> mice, implying that CYP epoxygenases do not contribute to differences observed in the mediation of vascular responses to α<sub>1</sub>-adrenergic stimulation. Recently, however, it has been reported that in vivo CYP inhibition by fluconazole alone did not alter the basal radial artery diameter in human male subjects.<sup>37</sup> However, administration of fluconazole combined with an NO synthase inhibitor significantly decreased radial artery diameter to a greater extent than NO synthase inhibition alone. The obser-
vation that the effect of CYP inhibition can be masked by NO may help explain the lack of effect of MS-PPOH on basal tone in our study.

We directly tested whether activation of PXR by a progesterone metabolite (5β-DHP) enhances CYP-mediated vasorelaxation. Indeed, CYP epoxygenase inhibition with MS-PPOH significantly attenuated vasorelaxation in 5β/DHP–treated PXR+/− mice but not in PXR−/− mice. These data indicate that activation of PXR enhances CYP epoxygenase activity leading to enhanced relaxation that may be a mechanism that occurs in pregnancy. This observation is in keeping with the results of Bobadilla et al., who suggest that CYPs partially mediate pregnancy-induced increases in vasorelaxation in the rat abdominal aorta. Gerber et al. also suggest that the endothelium-dependent hyperpolarization seen in mesenteric arteries from pregnant rats may be attributable in part to the action of a cytochrome P450 derivative, although they note that care must be taken in interpreting these results, because some of the original CYP inhibitors can act directly on K+ channels. There are some studies, however, that do not confirm a role for CYP metabolites in mediating the enhanced vasorelaxation observed during pregnancy, although these discrepancies may be a reflection of the differing inhibitors and tissues studied. Interestingly, renal microsomes prepared from pregnant rats show an elevation of CYP expression and activity as gestation progresses. In humans, excretion of the dihydroxy metabolites of epoxyeicosatrienoic acids 8,9-DHET and 11,12-DHET was significantly increased in the urine of healthy pregnant women relative to nonpregnant women. Moreover, the levels of these metabolites were increased even further in women with pregnancy-induced hypertension, possibly indicating a compensatory upregulation of epoxyeicosatrienoic acid synthesis. Further studies on the role of CYP epoxygenases and their products, particularly in vascular adaptations to pregnancy, are warranted.

Perspectives
This work has established a novel role for the nuclear receptor PXR in the regulation of vascular tone. This conclusion is supported by the fact that, unlike PXR+/− mice, PXR−/− mice did not display pregnancy-induced alterations in vascular function either when pregnant or when PXR was directly activated with the progesterone metabolite 5β-DHP. In addition, we have also shown that PXR-dependent enhancement of vasorelaxation is mediated in part by CYP epoxygenases. Together these results demonstrate a unique vasoregulatory pathway whereby PXR, activated by progesterone metabolites, initiates alterations in vascular function via the induction of CYP epoxygenases. Overall, this new role establishes PXR as a link between the state of pregnancy and the vascular changes that occur during gestation, providing a basis for future investigations into this critical area of research.

Acknowledgments
We are grateful to Dr Sheena Fang for her technical expertise and assistance with the development of a protocol to isolate RNA from mouse mesenteric arteries and to the staff of the Health Sciences Laboratory Animal Services for their excellent care of the animals.

Sources of Funding
This work has been supported by Canadian Institutes of Health Research grants MOP-64236 (B.F.M.) and MOP-64288 (S.T.D.), National Institutes of Health grant GM31278 (J.R.F.), and the Heart and Stroke Foundation of Canada. K.A.H. was supported by Canadian Institutes of Health Research. C.-L.C. was supported by the Alberta Heritage Foundation for Medical Research. J.R.F. is supported by the Robert A. Welch Foundation. S.T.D. is the Canada Research Chair in Women’s Cardiovascular Health and is a Scientist of the Alberta Heritage Foundation for Medical Research.
Disclosures

None.

References

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Hypertension. 2007;49:328-333; originally published online December 11, 2006;
doi: 10.1161/01.HYP.0000253478.51950.27

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