Mesenteric Vascular Responsiveness in a Rat Model of Pregnancy-Induced Hypertension

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Reduced perfusion to the placenta in early pregnancy is believed to be the initiating factor in the development of preeclampsia, triggering local ischemia and systemic vascular hyperresponsiveness. This sequence of events creates a predisposition to the development of altered vascular function and hypertension. This study was designed to determine the influence of placental insufficiency on the responsiveness of mesenteric resistance arteries in an animal model of preeclampsia. Placental insufficiency was induced by reduction in uteroplacental perfusion pressure (RUPP) in experimental Sprague-Dawley rat dams. The uterine branches of the ovarian arteries and the abdominal aortae of pregnant rats were surgically constricted on gestational Day 14. Dams in the control group underwent a sham procedure. Rats were euthanized on gestational Day 20, followed by removal of the small intestine and adjacent mesentery. First-order mesenteric resistance arteries were mounted on a small vessel wire myograph and challenged with incremental concentrations of vasoconstrictors and vasorelaxants. Mesenteric arteries in dams with placental insufficiency demonstrated an increased maximal tension to phenylephrine (7.15 ± 0.15 vs. 5.4 ± 0.27 mN/mm, $P < 0.001$); potassium chloride at 60 m*M* (3.43 ± 0.11 vs. 2.77 ± 0.14 mN/mm, $P < 0.01$) and 120 m*M* (3.92 ± 0.18 vs. 2.97 ± 0.16 mN/mm, $P < 0.01$); and angiotensin II (2.59 ± 0.42 vs. 1.51 ± 0.22 mN/mm, $P < 0.05$). Maximal relaxation to endothelium-dependent relaxants acetylcholine and calcium ionophore (A23187) was not significantly reduced. Data suggest that placental insufficiency leads to hyperresponsiveness to vasoconstrictor stimuli in mesenteric arteries. Exp Biol Med 231:1398–1402, 2006

This work was supported by grant 0215158Z from the American Heart Association Predoctoral Fellowship (C.M.A.) and grant NIDDK-51430 from the National Institute of Diabetes and Digestive and Kidney Diseases (J.N.B.).

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Received February 6, 2006.
Accepted March 27, 2006.

1535-3702/06/2318-1398$15.00

Key words: vascular smooth muscle; mesenteric artery; pregnancy-induced hypertension; placental insufficiency

Reduced perfusion to the placenta in early pregnancy is believed to represent the origin of pathology leading to pregnancy-induced hypertension, or preeclampsia (1, 2). Local factors stimulated by placental hypoxia are released into the systemic circulation, promoting the development of vascular hyperresponsiveness and endothelial dysfunction (3, 4). While the origin of pathology occurs early in pregnancy, the hallmark of preeclampsia, hypertension, is not clinically evident until the latter weeks of pregnancy. Altered vascular tone due to increased arterial resistance is implicated in the development of hypertension, influenced by hormonal and mechanical factors (5, 6). Hypoxia-induced maternal endothelial dysfunction has the effect of both reducing important endothelial-derived vasodilators such as placental NO and prostacyclin and enhancing production of the potent vasoconstrictors endothelin-1 and thromboxane A_2 (7).

The relative lack of availability of a reliable animal model of preeclampsia has delayed progress on the elucidation of the underlying mechanisms contributing to the vascular hyperresponsiveness characteristic of human preeclampsia. Recently, Granger et al. (8) championed the use of a rat model of pregnancy-induced hypertension that involves mechanical constriction of the major arteries feeding the maternal–fetal unit. The reduced uteroplacental perfusion pressure (RUPP) model in the Sprague-Dawley rat is one of the most authentic models of human preeclampsia (7, 9). The surgical induction of placental hypoxia promotes a phenotype mimicking many of the same clinical characteristics seen in the human condition, including hypertension and fetal growth restriction (10, 11). These findings are consistent with the altered vascular function in other animal models and in women with preeclampsia.

Investigation into the functional alteration of resistance vessels in the RUPP model has been limited. Previous

reports of vascular function in aortic conductance vessels demonstrated a reduction in endothelial-dependent vascular relaxation and release of nitric oxide (NO) from endothelial cells (12). Further, L-arginine supplementation and blockade of endothelin receptors and angiotensin II synthesis were found to attenuate hypertension in RUPP dams (10, 13, 14). A recent study by our lab of uterine artery function in the RUPP model demonstrated enhanced responsiveness to vasoconstrictors and impaired endothelial-dependent relaxation (15). The RUPP dams developed significantly elevated systolic blood pressure by gestational Day 20 in response to reduction in placental perfusion (11). The functional consequences of RUPP on mesenteric resistance arteries in pregnant Sprague-Dawley rats are currently unknown. The purpose of this study was to examine the influence of placental hypoxia on small mesenteric resistance artery responses in pregnant Sprague-Dawley rats.

Materials and Methods

Approval from the University of North Dakota Animal Care Committee in accordance with the *Guide for Care and Use of Laboratory Animals* (16) was obtained prior to conducting any procedures involving animals. Male and female virgin Sprague-Dawley rats were purchased from Charles River Laboratories (Wilmington, MA) for use in this study. The use of male rats was limited to breeding purposes only. Animals were group housed in an environmentally controlled vivarium prior to surgery and were individually housed after surgery. A 12:12-hr light:dark cycle was provided, and animals were allowed free access to water and a standard pellet diet. Individual female rats weighing between 250 and 300 g were paired with a single male and mated overnight for a maximum of 4 days or until the vaginal plug was identified. This was defined as gestational Day 1.

RUPP Model. On gestational Day 14 of the anticipated gestation period of 22 to 23 days, animals underwent either a surgical procedure to reduce uteroplacental perfusion pressure (RUPP) or a sham operation, as previously reported by our lab (11, 15). In brief, dams were given an injection of buprenorphine (0.25 mg/kg body weight) as a preemptive analgesia 15 mins prior to surgery and were anesthetized with 2% isoflurane by controlled continuous inhalation. An abdominal midline surgical incision was made, and the lower abdominal aorta was isolated below the renal arteries. Perfusion pressure was decreased by approximately 40% *via* placement of a silver clip around the aorta above the iliac bifurcation (0.203-mm i.d.) and around the main uterine branches of both the right and left ovarian arteries (0.100-mm i.d.). Sham operations in the control group included isolation of vessels without clip placement. Incisions were closed in layers using appropriate suture material.

Concentration-Effect Curves for Determination of Isometric Tension. On gestational Day 20, rats were anesthetized. Fetal pups and placentas were removed, placed on ice, and weighed. Mesenteric arteries were excised, dissected free of surrounding tissues and mounted on a small vessel wire myograph in physiologic saline solution (PSS) containing (in m*M*): 119 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 25 $NaHCO_3$, 1.2 NaH_2PO_4, 0.03 EDTA, and 5.5 glucose, adjusted to pH 7.4 as previously described (15). Normalized mesenteric arteries were set at 0.9× internal circumference when internal pressure reached 100 mm Hg. Exposure to increasing concentrations of phenylephrine (0.001–1000 μ*M*), angiotensin II (0.001-1000 n*M*), and potassium chloride (4.7–120 m*M*) to determine maximal isometric tension was completed in maternal vessels after equilibration and priming. Concentration-effect curves in arteries preconstricted with 3 μ*M* phenylephrine followed by exposure to acetylcholine (0.001–1000 μ*M*), calcium ionophore (A23187; 3–100 n*M*) and sodium nitroprusside (0.1–1000 n*M*) were generated in maternal vessels. Animals were euthanized by cardiac transection.

Statistical Analyses. Isometric tension in mesenteric arteries was graphically displayed and measured using concentration-effect curves generated from exposure to vasoactive agents. Drug concentrations producing a response of 50% (EC_{50}) and maximal tension (T_{max}) were used to compare responses between control and experimental groups. Data are expressed as means ± SEM. Statistical analysis was performed using a one-tailed, unpaired Student's *t* test with the Prism software analysis (GraphPad Software Inc., San Diego, CA). $P \leq 0.05$ was considered significant.

Results

Effects of Placental Hypoxia on Vasoconstrictor Responses. Small mesenteric resistance arteries were challenged with incremental concentrations of phenylephrine, potassium chloride, and angiotensin II to determine vasoconstrictor responsiveness. There were no significant differences in normalized internal vessel diameters between the RUPP and sham-operated animal groups. Concentration-effect curves were analyzed for each drug in mesenteric arteries from RUPP and sham-operated animals on gestational Day 20. Dose-dependent contraction was evident with all vasoconstrictor agonists examined. Figure 1 represents receptor-mediated vasoconstriction induced by phenylephrine. While responses to phenylephrine did not produce significant differences in EC_{50} (RUPP: 1.86 ± 0.34 μ*M*; sham: 1.91 ± 0.6 μ*M*), maximal tensions were significantly increased ($P < 0.001$) in the RUPP group compared with the sham-operated controls. Increased maximal tension in the mesenteric arteries of the RUPP group ($P < 0.05$) was also seen in response to angiotensin II (Fig. 2). Angiotensin II–induced maximal tension was significantly increased in the RUPP group (2.59 ± 0.42 m*N*/mm) compared with the sham-operated controls (1.51 ± 0.22 m*N*/mm). The responsiveness of the mesenteric artery to angiotensin II in the RUPP group (EC_{50} 2.14 ± 0.36 n*M*) was not

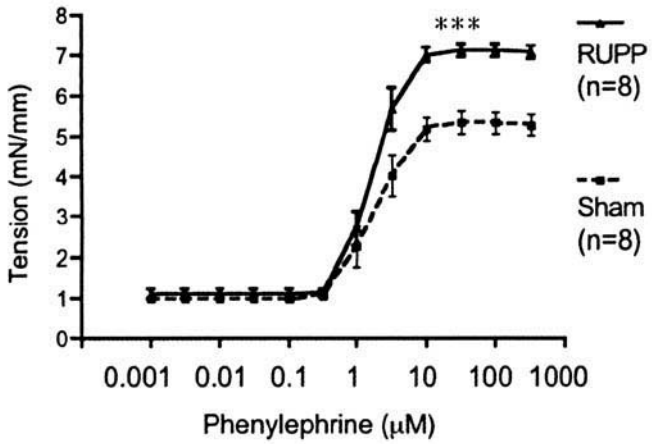

Figure 1. Vascular response of mesenteric arteries to increasing doses of phenylephrine. Maximal tension was increased in the RUPP group (*** $P < 0.001$) compared with sham-operated controls.

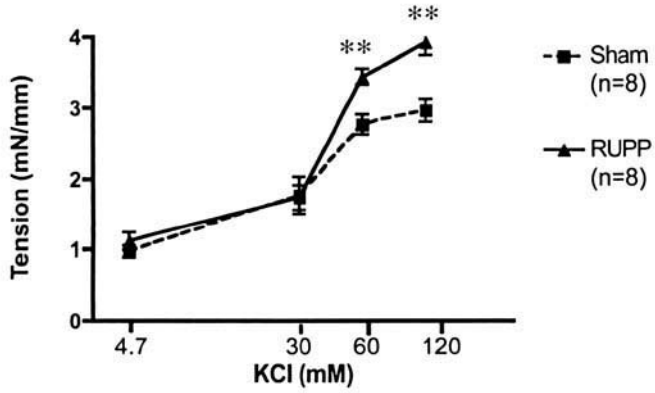

Figure 3. Effect of cumulative concentrations of potassium chloride in mesenteric arteries. Maximal tension was increased at 60-m*M* and 120-m*M* concentrations of KCl in mesenteric arteries of RUPP dams compared with control dams (** $P < 0.01$).

significantly different when compared to the sham-operated group (EC_{50} 4.90 ± 2.13 n*M*).

Nonreceptor-mediated vasoconstriction was increased in the RUPP group ($P < 0.01$) in response to potassium chloride at higher concentrations (Fig. 3). At 60 m*M* maximal tension reached 2.77 ± 0.14 m*N*/mm in the sham-operated controls, compared with 3.43 ± 0.11 m*N*/mm in the RUPP group. A similar pattern was apparent at 120 m*M* (sham: 2.97 ± 0.16 m*N*/mm; RUPP: 3.92 ± 0.18 m*N*/mm). No differences in maximal tension were evident at lower concentrations.

Effects of Placental Hypoxia on Endothelial-Dependent Relaxation. Mesenteric arteries were preconstricted with phenylephrine and subjected to increasing concentrations of acetylcholine (ACh; Fig. 4). There were no significant differences in maximal relaxation between the RUPP and sham-operated groups. Calcium ionophore (A23187) at low concentrations promotes the release of NO by promoting Ca^{2+} entry into endothelial cells (17). At higher concentrations (>30 μ*M*) the calcium begins to exert its effects on the contractile apparatus, serving as a vasoconstrictor. In order to assess endothelial function, mesenteric and uterine arteries were exposed to concentrations of 1, 3, 10, 30, and 100 n*M* of A23187, and concentration-effect relationships were analyzed. There were no significant differences in relaxation responses (RUPP EC_{50}: 0.75 ± 0.36 n*M*, $n = 5$; sham EC_{50}: 2.63 ± 1.19 n*M*, $n = 5$) to A23187 in mesenteric arteries (Fig. 5).

Effects of Placental Hypoxia on Endothelium-Independent Relaxation. Sodium nitroprusside (SNP) provides vasorelaxant effects through the donation of NO, and this action is independent of the endothelium. SNP-induced vasorelaxation may provide insights into the functional responses of vascular smooth muscle. Mesenteric arteries were preconstricted with phenylephrine as previously described in this study. Vessels were challenged with increasing concentrations of SNP, and tension changes were recorded. There were no significant differences in maximal relaxation responses to SNP in the RUPP (EC_{50}: 1.02 ± 9.17 n*M*; $n = 5$) and sham-operated (EC_{50}: 1.86 ± 3.28 n*M*; $n = 5$) groups (Fig. 6).

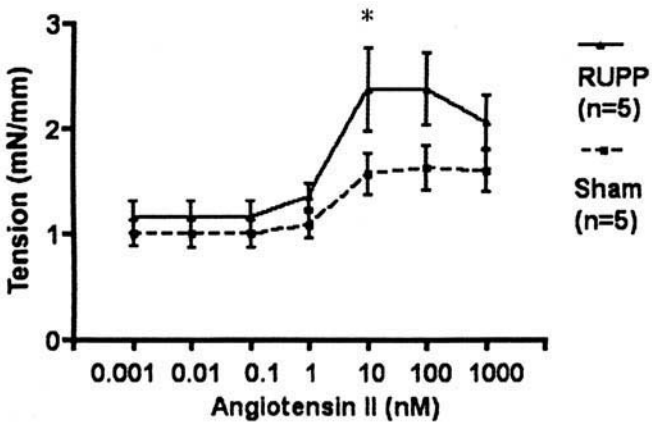

Figure 2. Concentration-effect curve in mesenteric arteries exposed to increasing concentrations of angiotensin II. Maximal tension was increased in arteries from the RUPP group (* $P < 0.05$) compared with controls.

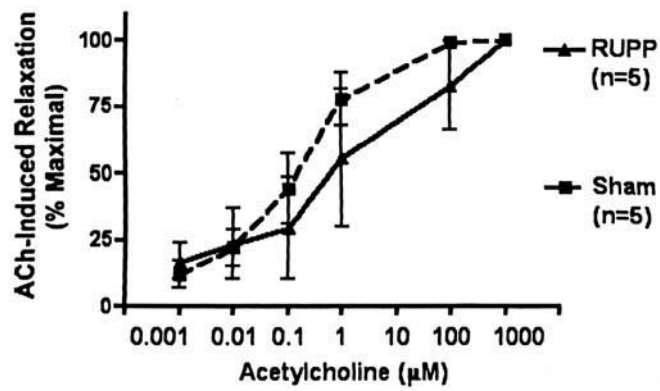

Figure 4. Endothelial-dependant relaxation response to acetylcholine. There were no significant differences in relaxation response to ACh in mesenteric arteries from RUPP and control dams.

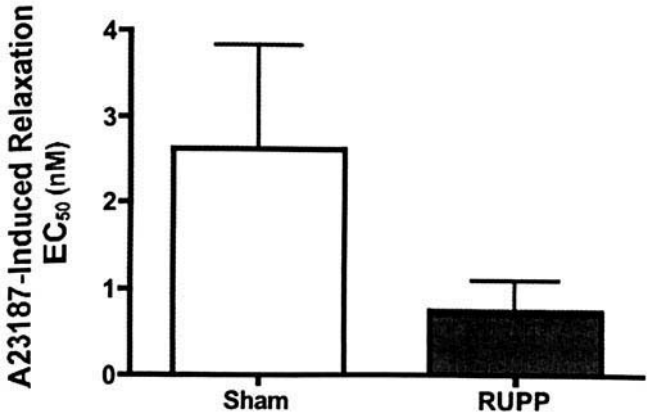

Figure 5. Relaxation response of mesenteric arteries to A23187. No significant difference in endothelium-dependent relaxation response was evident in mesenteric arteries from RUPP and sham-operated control dams.

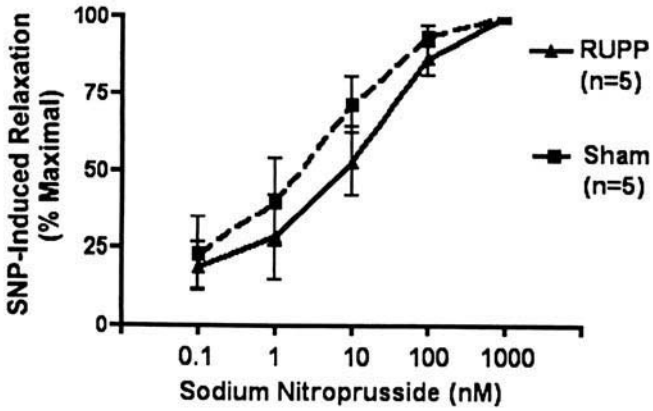

Figure 6. SNP-induced relaxation response in mesenteric arteries. There was no difference in endothelium-independent responses of mesenteric arteries between experimental and control groups.

Discussion

The development of vascular smooth muscle dysfunction as a direct response to decreased placental perfusion provides a mechanism for progressive vasoconstriction, hyperresponsiveness, and impaired relaxation of resistance vessels (3). The ensuing pathology in the resistance vessels is responsible for the progressive alteration in perfusion to all major organs. Perfusion is decreased throughout the maternal system in response to the overabundance of vasoconstrictors generated from the damaged endothelium (18). This is of particular concern in relation to the placenta, a highly vascular organ.

Previous studies involving the RUPP rat model have implicated altered vascular function as a consequence of reduced uteroplacental perfusion in cell models and in conductance vessels (12, 19, 20). In addition, endothelial dysfunction and enhanced vasoconstriction as a local response of uterine arteries have been demonstrated (15). The consistent verification of hypertension as a response to reduced placental perfusion suggests a systemic role for vascular dysfunction in resistance arteries. In studies from our lab and others using the RUPP rat model, increased blood pressure in pregnant dams exposed to RUPP is consistently identified (10, 11, 21).

The functional responses in mesenteric arteries subjected to RUPP are unknown. Characterization of mesenteric resistance arteries' vascular responsiveness to vasoconstrictors and vasorelaxants was completed in order to further elucidate mechanistic details contributing to the development of hypertension. Uterine artery vasoconstrictor responses were consistent with the findings in mesenteric arteries under similar conditions (15). Mesenteric artery maximal tension responses to vasoconstrictors in the RUPP model were significantly greater when compared to their sham-operated counterparts. Smooth muscle responses to KCl were not significantly different at baseline physiologic levels (4.7 m*M*). Vascular hyperresponsiveness was evident at KCl concentrations of 60 and 120 m*M*. Maximal responses to phenylephrine, an α_1-receptor agonist, were greater in the RUPP group. Increased intracellular calcium may play a role in this phenomenon, as this response has been previously identified in the renal arterial smooth muscle as the source of enhanced contraction in the RUPP model (20). Enhanced mechanisms of calcium entry, rather than release from intracellular calcium stores, was suggested as contributing to the increased $[Ca^{2+}]_i$ and smooth muscle cell contractions in response to angiotensin II and KCl in the pregnant RUPP animals.

The significant contribution made by angiotensin II to homeostasis is well known (22). While absolute levels of angiotensin II are believed to be unchanged in normal and preeclamptic human pregnancies, the sensitivity of the contractile apparatus to angiotensin II is increased in preeclampsia. Responsiveness to angiotensin II in the mesenteric arteries in both the sham and RUPP groups demonstrated patterns similar to other reported vasoconstrictive agents. In the sham and RUPP groups there were no significant differences in EC_{50}, whereas maximal tension was significantly increased in the RUPP group in the mesenteric arteries.

Endothelial cell dysfunction as a response to reduced uteroplacental perfusion is believed to be responsible for the hyperresponsiveness to vasoconstrictors seen in small resistance vessels. It would then be logical also to expect impairment of relaxation in these same vessels. As expected, impaired endothelial-dependent relaxation was demonstrated in aortic strips in the RUPP model (12). In uterine arcuate resistance arteries from dams subjected to RUPP, impaired endothelial relaxation also was characterized (15). While the significance of endothelial dysfunction in conductance vessels is important, the resistance arteries represent a vital link to the determination of blood pressure. Arterial responsiveness to the endothelium-dependent vasorelaxant ACh was not significantly decreased in the RUPP mesenteric artery. Variability in endothelium-dependent relaxation response has been identified in other animal

models of preeclampsia, including those inducing preeclampsia with NO synthase inhibition, suggesting model- and tissue-specific responses (23–25). These data suggest the potential for the contribution of damaged endothelium in impaired relaxation responses and have particularly important consequences when coupled with increased responsiveness to vasoconstrictive agents.

The development of hypertension as a result of decreased placental perfusion is a hallmark of preeclampsia and serves as the clinical manifestation of vascular tone. Hyperresponsiveness of small resistance arteries appears to play a significant role in the development of pregnancy-induced hypertension induced by reduced placental perfusion. The findings of this study were consistent with the differences in systolic blood pressure (11) that define preeclampsia and which are supported by the identification of vascular dysfunction resulting from reduced uteroplacental perfusion, consistent with those findings reported by other labs. The results of this study support the prevalent theory of reduced uteroplacental perfusion as the most likely etiology of pregnancy-induced hypertension and highlight the influence of reduced placental perfusion on the development of vascular dysfunction. This model of placental insufficiency-induced vascular hyperresponsiveness provides an opportunity to identify the mechanism mediating vascular dysfunction, with implications for improving health outcomes of both mothers and their offspring.

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