Matrix Metalloproteinase Enhances Big-Endothelin-1 Constriction in Mesenteric Vessels of Pregnant Rats With Reduced Uterine Blood Flow

Ali Abdalvand,* Jude S. Morton,* Stephane L. Bourque,* Anita L. Quon, Sandra T. Davidge

Abstract—Preeclampsia is a leading cause of maternal and fetal morbidity/mortality; however, the pathophysiological mechanisms are unclear. Vascular endothelial dysfunction in preeclampsia has been partially attributed to changes in endothelin-1 (ET-1). Several enzymes, including matrix metalloproteinases (MMPs; particularly MMP-2), cleave the inactive precursor big ET-1 (bET-1) to active ET-1. Notably, expression levels of MMP-2 have been shown to be on the increase in women who subsequently develop preeclampsia. We hypothesized that the increased MMP-2 expression leads to increased bET-1 conversion, thereby increasing vasoconstriction in preeclampsia. A reduced uteroplacental perfusion pressure (RUPP) model of preeclampsia in the rat was used to assess mesenteric artery vascular function. Responses to bET-1 (3–310 nmol/L) and ET-1 (1–200 nmol/L) were studied in the presence or absence of inhibitors of enzymes known to cleave bET-1. Vascular contractility in response to bET-1 was greater in RUPP than Sham (P<0.001), whereas neither responses to ET-1 nor maximal contractility to high potassium salt solution (123.70 mmol/L) were different. MMP inhibition with GM6001 (30 μmol/L) significantly decreased responses to bET-1 in RUPP (P<0.001) but not Sham-operated rats. Interestingly, combined treatment with GM6001 and L-NG-nitroarginine methyl ester (100 μmol/L) revealed a NO modulation of MMPs that was reduced in RUPP. In summary, we found increased vascular contractility to bET-1 in the RUPP model of preeclampsia that was likely attributable to upstream enzymatic pathways. These data are consistent with a greater contribution of MMP to cleavage of bET-1 to ET-1 ex vivo in RUPP, suggesting that this enzyme may be partially responsible for increased bET-1–induced contractility. (Hypertension. 2013;61:00-00.)

Key Words: endothelin-1 □ matrix metalloproteinases □ preeclampsia □ reduced uterine perfusion pressure □ vascular function

Preeclampsia is a common complication of pregnancy that affects 2% to 8% of pregnancies worldwide.1 It is characterized by the de novo appearance of hypertension and proteinuria after mid-pregnancy. Preeclampsia not only poses a significant risk to maternal health, but also accounts for up to 12% of infants born small for gestational age and 20% of preterm births.1

Vascular endothelial dysfunction is one of the well-established pathological features of preeclampsia, and several factors have been proposed to be involved in this process.2 It is thought that multiple etiologic factors involved in preeclampsia, and endothelial dysfunction in particular, may converge on the endothelin-1 (ET-1) pathway.3 Indeed, circulating levels of this highly potent vasoconstrictor have been shown to be significantly increased in women with preeclampsia compared with normal pregnant women.4 Recently, ET-1 signaling has been shown to be involved in the pathophysiology of animal models of preeclampsia, such as the reduced uterine perfusion pressure (RUPP) model.5,6

The production of ET-1 involves enzymatic cleavage of big ET-1 (bET-1), largely via ET converting enzymes (ECEs).7 Several alternative enzymes have also been shown to be capable of cleaving bET-1 to yield active ET-1 peptides of varying length, including neutral endopeptidase (NEP), chymase, and matrix metalloproteinase-2 (MMP-2).7 MMP-2 is particularly noteworthy as circulating levels are elevated in preeclampsia,19 as well as in apparently healthy women who subsequently develop preeclampsia.10 Although MMPs play an important role in healthy pregnancies (eg, in trophoblast invasion of the endometrium),11 their dysregulation has also been implicated in the vascular dysfunction that occurs in preeclampsia;12 however, the precise mechanisms by which this

Received August 20, 2012; first revision December 7, 2012; accepted December 10, 2012.


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The online-only Data Supplement is available with this article at http://hyper.ahajournals.org/lookup/suppl/doi:10.1161/HYPERTENSIONAHA.111.00055/-/DC1.

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Hypertension is available at http://hyper.ahajournals.org
DOI:10.1161/HYPERTENSIONAHA.111.00055
occurs are not yet clear. We hypothesized that elevated MMPs lead to greater conversion of bET-1 to enhance vasconstriction in preeclampsia.

**Methods**

**Ethics**

This study was approved by the University of Alberta Animal Policy and Welfare Committee and followed the Canadian Council on Animal Care guidelines.

**Reduced Uteroplacental Perfusion Pressure in the Rat**

The RUPP model is a well-established animal model of preeclampsia that has been previously been characterized. Three-month-old female Sprague Dawley rats were used. On day 14 of pregnancy, dams were randomly assigned to receive either a Sham (n=17) or RUPP (n=20) surgical procedure (see Methods in the online-only Data Supplement).

**Vascular Function Studies**

First-order mesenteric resistance arteries (<250 μm internal diameter) were mounted on a wire myograph to measure isometric tension (see Methods in the online-only Data Supplement). The ET pathway was assessed using cumulative concentrations of bET-1 (AnaSpec; 3–310 nmol/L) or ET-1 (Sigma-Aldrich; 1–200 nmol/L) in the presence or absence of inhibitors. Importantly, bET-1 is unable to cause direct vasoconstriction but has to be converted to ET-1 to elicit a vascular response. Enzyme inhibitors used to study various pathways included the following: GM6001 (Calbiochem; 30 μmol/L), a gelatinase inhibitor with high affinity for MMP-2; phosphoramidon (Sigma-Aldrich; 30 μmol/L), an inhibitor of ECE and NEP; CG35066 (Tocris Bioscience; 25 μmol/L), a selective ECE inhibitor; DL-Thiophan (thiophan; Calbiochem; 25 μmol/L), a selective inhibitor of NEP; chymostatin (Sigma-Aldrich; 100 μmol/L), a chymase inhibitor; L-NG-nitroarginine methyl ester (L-NAME; Sigma-Aldrich; 100 μmol/L), an inhibitor of NO synthase (NOS); and the ETB receptor antagonist BQ788 (Calbiochem; 1 μmol/L). Finally, we exposed mesenteric arteries to high potassium salt solution ([K+] =123.70 mmol/L) to compare the innate vascular contractile capacity of the vessels, independent of bET-1 cleavage and ET-1 receptors. To study vascular effects of bET-1 in the absence of endothelium, mesenteric arteries were denuded using intraluminal infusion of 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (1%). Failure of vessels to dilate to methylcholine (3 μmol/L) was used to indicate successful denudation.

**Enzyme Expression Levels**

Western blot experiments were performed on protein homogenates of mesenteric arteries from Sham and RUPP animals using primary antibodies for endothelial NOS (eNOS), MMP-2, and ECE-1. bET-1 and ET-1 expression was also visualized in mesenteric arteries using immunofluorescence microscopy (see Methods in the online-only Data Supplement).

**Statistical Analyses**

Data are presented as mean ±SEM. Responses to bET-1, in the absence or presence of inhibitors, were summarized as maximal responses at 0.3 μmol/L (E_{max}0.3μmol/L) and compared between groups. Responses to ET-1, in the absence or presence of L-NAME, were summarized as maximal responses at 0.1 μmol/L (E_{max}0.1μmol/L) and compared between groups. Comparisons between 2 groups were conducted using Student t test. Two-way ANOVA with Bonferroni post hoc analysis was used to compare vascular responses to bET or ET-1 in Sham and RUPP, with and without inhibitors. Data were analyzed using GraphPad Prism 5.02 Software (GraphPad Software Inc, CA). A P<0.05 was considered significant.

**Results**

The RUPP model has been established within our laboratory (Table S1 in the online-only Data Supplement). In addition, we demonstrated an increase of lectin-like oxidized LDL receptor 1 receptor and eNOS expression in the aorta. In summary, compared with Sham-treated rats, RUPP animals had increased systolic blood pressure (+12 mm Hg; P=0.01), evidence of glomerular endotheliosis, and a decreased number of viable but asymmetrically growth restricted offspring.

**Constrictor Capacity**

As an inactive precursor, bET-1 is unable to cause vascular effects without previous conversion to ET-1, a process that is thought to occur in the endothelium. Indeed, vascular responses to bET-1 were completely abolished after endothelial denudation (32.08±2.87% endothelium intact versus −8.06±2.97% endothelial denuded; P<0.001).

bET-1 caused greater constriction in RUPP compared with Sham (Figure 1A; P<0.0001). However, neither responses to ET-1 (Figure 1B) nor high potassium salt solution (E_sham: 4.30±0.16 mN/mm versus RUPP: 4.09±0.30 mN/mm) were different between groups, suggesting that there was no change in ET receptor sensitivity or contractile capacity of vessels from Sham and RUPP animals. To determine if this enhanced constriction was accompanied by increased bET-1 and ET-1 expression, we assessed peptide levels in mesenteric vascular sections by immunofluorescence microscopy. When assessed separately, neither bET-1 nor ET-1 were different between Sham and RUPP (Figure S1). However, because bET-1 and ET-1 levels are not independent (ie, bET-1 is the substrate for ET-1), assessing combined bET-1 and ET-1 expression is indicative of overall ET-1 activity in a given tissue. Using this analysis, RUPP vessels had 32% greater expression compared with Sham vessels (P<0.05; Figure S1).

**Nitric Oxide**

Endothelium-dependent pathways involving NO are reduced in the systemic vessels of RUPP animals; therefore, vessels were preincubated with L-NAME to study the contribution of NO to bET-1 vasoconstriction. Although L-NAME significantly increased vascular responses to bET-1 in both Sham...
(Figure 2A; *P<0.001) and RUPP (Figure 2B; *P<0.05), the delta change (magnitude of potentiation) was reduced in the RUPP group (Figure 2C; *P<0.05). Vascular responses to ET-1 were increased in the presence of L-NAME in Sham (Figure 3A; *P<0.01) and RUPP (Figure 3B; *P<0.001) groups, but there was no difference in the magnitude of potentiation (Figure 3C). Further, eNOS expression was significantly increased in mesenteric arteries from RUPP compared with Sham animals (Figure 4; *P<0.01). Because ET can stimulate endothelial ET_{A} receptors to increase NO, we tested and found that BQ-788 pre-treatment had no differential effects between Sham and RUPP animals, suggesting that differences in ET_{A} receptors were not involved in vascular responses to bET-1 in the presence or absence of L-NAME (data not shown).

**bET-1 Cleavage Enzymes**

To further explore the increased vascular responses to bET-1 observed in RUPP animals compared with Sham, the involvement of various enzymes in the conversion of bET-1 to ET-1 was investigated. GM6001, a gelatinase inhibitor of both MMP-2 and MMP-9, did not alter responses of vessels exposed to bET-1 in Sham but significantly decreased vascular responses to bET-1 in RUPP (Figure 5; *P<0.001). In the presence of L-NAME, however, a small but significant decrease in bET-1 responses was observed in the presence of GM6001 in Sham (E_{max0.3μmol/L} Sham: 4.01±0.37 versus 3.19±0.35 mN/mm; *P<0.05) but not RUPP (E_{max0.3μmol/L} RUPP: 4.80±0.45 versus 4.73±0.25 mN/mm) animals, suggesting NO modulation of MMPs only in the Sham group. In mesenteric arteries, MMP-9 was not detectable by zymography, whereas MMP-2 was highly expressed (data not shown). Thus, we further assessed MMP-2 protein expression and found higher levels in the RUPP compared with the Sham group (Figure 6; *P<0.05).

Inhibition of ECE by phosphoramidon significantly decreased vascular responses to bET-1 in both Sham and RUPP groups in the absence (E_{max0.3μmol/L} Sham: 0.98±0.20 versus 0.17±0.07 mN/mm, *P<0.001; RUPP: 2.50±0.36 versus 0.57±0.26 mN/mm, *P<0.001) and presence (E_{max0.3μmol/L} Sham: 3.69±0.53 versus 0.26±0.08 mN/mm, *P<0.001; RUPP: 4.11±0.98 versus 0.40±0.18 mN/mm, *P<0.001) of L-NAME. Use of a more specific ECE inhibitor, CGS35066, produced similar results (Table). ECE expression levels were not different between the Sham and RUPP groups (data not shown).

Incubation with the NEP inhibitor, thiorphan, did not have any significant effect on the vascular responses to bET-1 in either Sham or RUPP. Interestingly, in the presence of L-NAME, responses to bET-1 were significantly inhibited by thiorphan in both Sham and RUPP (Table; *P<0.05 and *P<0.001, respectively). Preincubation of vessels with the chymase inhibitor (chymostatin) did not alter responses to bET-1 in either Sham or RUPP (Table). However, when these experiments were performed in the presence of L-NAME, vascular responses to bET-1 were significantly decreased in RUPP (Table; *P<0.01) but not Sham.

**Discussion**

The RUPP animal model used in the current study exhibits several salient features of preeclampsia, including increased blood pressure and fetal growth restriction,14 consistent with other groups.16–18 Using this model, we demonstrated for the first time that contractile responses to bET-1 in mesenteric arteries were greater in RUPP animals compared with Sham, concomitant with increased bET-1/ET-1 staining in the same vessels. Furthermore, contractile responses to high concentrations of potassium and ET-1 were similar, showing not only that the contractile capacity of the vessels was unchanged, but also that differences between bET-1 responses in Sham and RUPP were likely to stem from upstream enzymatic pathways involved in bET-1 to ET-1 cleavage.
Our primary aim was to investigate the role of MMP in the cleavage of bET-1 to ET-1 and its potential upregulation in a preeclamptic-like model. In support of our hypothesis, we demonstrated a significant contribution of MMP to the cleavage of bET-1 to ET-1 in mesenteric arteries in only RUPP animals. Whereas ECE contributed to the majority of enzymatic conversion of bET-1 to ET-1 in the current study, there was no differential contribution of ECE in bET-1 conversion between Sham and RUPP. Similarly, neither inhibition of NEP nor chymase had differential effects on vascular responses to bET-1 between Sham and RUPP. These findings further indicate the importance of MMPs in the pathophysiology of PE and provide mechanistic insights into vascular dysfunction associated with PE.

Because of the well-established link between NO and the ET-1 pathway, we also sought to determine the relative contribution of bET-1 cleavage enzymes in the presence of L-NAME. Consistent with our previous observations, L-NAME potentiated vascular responses to bET-1. Interestingly, this effect was 2-fold greater in Sham rats, a differential effect that could not be attributed to potentiation of ET-1 induced vasoconstriction because these latter responses were similarly affected in both groups. Rather, these data suggest that upstream processing of bET-1 is a likely step of altered NO modulation of bET-1 processing in the vasculature of RUPP animals.

In the presence of L-NAME, MMP contribution to bET-1 conversion was reduced in RUPP rats. We have previously observed enhanced oxidative stress and reduced bioavailability of NO in the vasculature of RUPP animals. Peroxynitrite, the product of superoxide and NO, has been shown to promote conversion of the 72-kDa pro-MMP-2 to the active 64-kDa enzyme.

Table. Enzymatic Involvement in Mesenteric Artery Responses to bET-1

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Vehicle</th>
<th>CGS35066</th>
<th>Thiorphan</th>
<th>Chymostatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>Inhibitor</td>
<td>2.05±1.02 (3)</td>
<td>0.47±0.38* (3)</td>
<td>2.14±0.79 (3)</td>
<td>1.72±0.46 (3)</td>
</tr>
<tr>
<td>RUPP</td>
<td>Inhibitor</td>
<td>3.25±0.47 (4)</td>
<td>1.03±0.87† (4)</td>
<td>2.46±0.46 (5)</td>
<td>2.93±0.69 (5)</td>
</tr>
<tr>
<td></td>
<td>L-NAME</td>
<td>3.62±0.27 (17)</td>
<td>0.49±0.32† (6)</td>
<td>2.69±0.62* (6)</td>
<td>3.73±0.47 (6)</td>
</tr>
<tr>
<td></td>
<td>Inhibitor</td>
<td>3.63±0.45 (12)</td>
<td>0.35±0.19† (4)</td>
<td>2.10±0.70† (5)</td>
<td>3.14±0.08‡ (5)</td>
</tr>
</tbody>
</table>

Mesenteric artery responses in Sham and RUPP animals to bET-1 (Emax=0.3µmol/L, mean±SEM [n]) in the absence (vehicle) or presence of the enzymatic inhibitors; CGS35066 (a selective ECE inhibitor), thiorphan (a selective NEP inhibitor), or chymostatin (a chymase inhibitor). The modulatory effect of NO on each enzymatic pathway was assessed through coincubation of the enzymatic inhibitors with L-NAME. bET-1 indicates big endothelin-1; L-NAME, NG-nitroarginine methyl ester; and RUPP, reduced uteroplacental perfusion pressure.

*P<0.05, †P<0.001, ‡P<0.01 vs vehicle control.
form,\textsuperscript{21} which is consistent with the observed increased MMP-2 expression in arteries from RUPP animals in the current study. We speculate that in the presence of L-NAME, reduced NO production may limit the production of peroxynitrite, thereby preventing the activation of MMP-2 in the vasculature and reducing the overall contribution of this enzyme to bET-1–induced constriction. Consistent with this hypothesis, Tronc et al\textsuperscript{22} previously demonstrated that L-NAME treatment reduced MMP activity in the vasculature, concomitant with reduced peroxynitrite staining. Although this hypothesis could explain the relative lack of contribution of MMPs to the conversion of bET-1 to ET-1 in L-NAME–treated vessels, it fails to account for the relative increase in overall constriction in the wake of NOS inhibition. NO, however, has also been shown to inhibit ECE-1 expression;\textsuperscript{23} thus, it is possible that in the presence of intact NO, ECE is largely inhibited and the additional bET-1 is processed in RUPP animals by MMPs, which are largely absent in Sham animals. In the presence of L-NAME, the loss of NO-mediated inhibition of ECE may provide a more dominant synthesis pathway for bET-1, with a resultant lowered contribution by MMPs.

Although ECE and MMPs seem to be the dominant pathways of bET-1 conversion in the RUPP model, our findings do not rule out the possibility of NO modulation of other enzymes that convert bET-1 to active ET-1 in the RUPP model. Indeed, when vessels from both groups were coincubated with L-NAME, we found a significant contribution of NEP and chymase. This finding may indicate that NO can also modulate the function of chymase, which may be important with respect to increased bET-1 conversion in the RUPP model. Increased expression and activity of chymase in the maternal vascular endothelium of preeclamptic women has previously been shown.\textsuperscript{24} We, for the first time, have shown that an increased chymase contribution in a preeclampsia-like animal model may directly contribute to increased ET-1 mediated vasoconstriction.

The role of impaired NO signaling in this model is also evidenced by increased expression levels of eNOS in vessels from RUPP animals. The increase in eNOS expression in the systemic resistance arteries of the RUPP model is in line with our previous data showing increased eNOS expression in microvessels obtained from subcutaneous fat biopsies from women with preeclampsia compared with uncomplicated pregnancies or nonpregnant counterparts.\textsuperscript{25} It is tempting to speculate that increased eNOS expression may occur as a maladaptive compensatory response to reduced NO bioavailability, which could be secondary to eNOS uncoupling. Alternatively, soluble endoglin, an antiangiogenic factor that has been shown to be increased in women with preeclampsia (reviewed in Anderson et al\textsuperscript{26}), has been shown to attenuate activation of eNOS\textsuperscript{27} and may, therefore, provide an additional explanation for the disconnect between the observed increase in eNOS expression and the decrease in NO modulation of vascular tone.

Perspectives

In the context of the overall pathophysiology of preeclampsia, the current study offers new insights into the late-stage events that occur downstream of MMP activation in endothelial cells which culminate into the vascular dysfunction and increased vasoconstriction observed in the RUPP model. Although beyond the scope of the current study, the mechanisms leading to the activation of MMPs and subsequent bET-1 conversion to ET-1 are of interest. Roberts et al\textsuperscript{28} and Walsh et al\textsuperscript{18} have independently shown that healthy vascular tissue, whether endothelial cells or whole blood vessels, cultured in the presence of serum obtained from RUPP rats results in increased ET-1 release. These findings provide convincing evidence for a role of circulating factor(s) leading to endothelial dysfunction in the maternal system. Although the factor(s) have not been fully elucidated, there is compelling evidence for the involvement of inflammatory mediators, such as angiotensin receptor type 1 auto-antibodies and the cytokines tumor necrosis factor α and interleukin 6\textsuperscript{70}; these factors are particularly noteworthy as they have been shown to have a direct link to ET-1 production,\textsuperscript{15} although the link to MMPs as an upstream mediator has yet to be shown in this model of disease. The use of the RUPP model in the current study has identified key components contributing to maternal vascular dysfunction, and future studies will endeavor to elucidate their specific interactions, with the intention of devising therapeutic interventions for preeclampsia.

Acknowledgments

We thank Yanyan Jiang for her technical assistance.

Sources of Funding

This work was supported by a research grant from the Canadian Institutes of Health Research (CIHR). A. Abdalvand, J. S. Morton, and S. T. Davidge are supported by the Women and Children’s Health Research Institute of the University of Alberta. S. L. Bourque is supported by CIHR and Alberta Innovates-Health Solutions (AIHS). S. T. Davidge is a Canada Research Chair in Women’s Cardiovascular Health and is funded by AIHS.

Disclosures

None

References

Preeclampsia is a pregnancy-related disorder with no known cure; elucidation of the mechanisms of increased blood pressure will be instrumental in resolving these complications.

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Increased contribution of matrix metalloproteinases and chymase to bET-1 cleavage.

No modulation of bET-1 cleavage enzymes matrix metalloproteinases, neutral endopeptidase, and chymase.

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Hypertension. published online January 7, 2013;
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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http://hyper.ahajournals.org/content/early/2013/01/07/HYPERTENSIONAHA.111.00055

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**MATRIX METALLOPROTEINASE ENHANCES BIG-ENDOTHELIN-1 CONSTRICTION IN MESENTERIC VESSELS OF PREGNANT RATS WITH REDUCED UTERINE BLOOD FLOW**

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*AA, JSM and SLB contributed equally.

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Methods

Reduced Uteroplacental Perfusion Pressure Surgical Procedure in the Rat

Three month old, female Sprague Dawley rats were bred and pregnancies confirmed by the presence of sperm in a vaginal smear (designated day (d) 0 of pregnancy). On d14 of pregnancy, dams were randomly assigned to receive either a Sham (n=17) or RUPP (n=20) surgical procedure. Under inhaled isoflurane anesthesia, a 2 cm midline incision was made in the abdominal wall and a silver clip (0.203 mm ID) was placed over the abdominal aorta to restrict blood flow. Similarly, silver clips (0.100 mm ID) were used to constrict both proximal uterine arteries in order to prevent collateral blood flow to the uteroplacental unit. In the Sham group, equivalent manipulation of the arteries was made and the silver clips were attached to perivascular fat tissue.

Vascular Function Studies

First order mesenteric resistance arteries (~2mm long, <250μm internal diameter) were mounted on two 40 μm wires attached to a wire myograph (Danish Myo Technology A/S Inc., Aarhus, Denmark) for measurement of isometric tension. After normalization to 0.8xIC₁₀₀ (the internal circumference equivalent to a transmural pressure of 100 mmHg) and equilibration, vascular integrity of the vessels was confirmed using a single dose of phenylephrine (PE) (10 μmol/L) followed by methylcholine (MCh) (3 μmol/L). Vascular responses to agonists were expressed as units of force normalized to vessel length (mN/mm). All antagonists were administered to the bath at least 30 minutes prior to performing bET-1 or ET-1 cumulative concentration response curves.

Enzyme Expression Levels
Western blot experiments were performed on protein homogenates of mesenteric arteries from Sham and RUPP animals using primary antibodies for eNOS (BD Bioscience; mouse monoclonal antibody, 1:250), MMP-2 (Santa Cruz Biotechnology; rabbit polyclonal antibody, 1:200) and ECE-1 (Santa Cruz Biotechnology; mouse monoclonal antibody, 1:200). β-actin was loaded as a control (Abcam; β-actin rabbit polyclonal primary antibody, 1:1500). Goat anti-rabbit and donkey anti-mouse fluorochrome conjugated antibodies (LI-COR Bioscience; 1:10000) were used as the secondary antibodies. The protein bands were imaged and analyzed using the Li-Cor Odyssey version 3.0 imager and software system (Mandel Scientific Company). Expression data were presented as ratios of β-actin.

For immunofluorescence microscopy, mesenteric arteries were fixed in ice-cold acetone for 20 minutes, washed in PBS, and blocked for 1 hour in 1% BSA. Slides were then incubated overnight at 4°C with a primary antibody for bET-1 (anti-rat bET-1 (22-38) rabbit IgG; IBL America; 1:50) or ET-1 (anti ET-1 mouse monoclonal; Calbiochem; 1:200) and subsequently incubated for 1 hour with a goat anti-rabbit or anti-mouse secondary respectively (Alexa Fluor 546: 1:250, Invitrogen). Slides were then mounted using a 4,6-diamidino-2-phenylindole containing mounting medium to stain for cell nuclei and cover slipped. Slides were imaged using an IX81 Olympus fluorescence microscope.
Reference

**Table S1.** Previously reported biometric characteristics of the RUPP and Sham animals and their offspring [1]. * = p<0.05, † = p<0.01, ‡ = p<0.001.

<table>
<thead>
<tr>
<th>Biometric Measurements</th>
<th>Sham</th>
<th>RUPP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maternal factors:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>104 ± 4</td>
<td>116 ± 3 †</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>84 ± 5</td>
<td>93 ± 4</td>
</tr>
<tr>
<td>Heart Rate (bpm)</td>
<td>333 ± 25</td>
<td>314 ± 27</td>
</tr>
<tr>
<td>Proteinuria, albumin/creatinine ratio</td>
<td>0.39 ± 0.07</td>
<td>0.37 ± 0.14</td>
</tr>
<tr>
<td><strong>Offspring:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Live births (n)</td>
<td>13.69 ± 1.07</td>
<td>4.20 ± 0.71 ‡</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>3.59 ± 0.07</td>
<td>3.38 ± 0.08 (p=0.058)</td>
</tr>
<tr>
<td>Crown-rump length/abdominal girth ratio</td>
<td>1.12 ± 0.02</td>
<td>1.05 ± 0.02 *</td>
</tr>
<tr>
<td>Placental weight (g)</td>
<td>0.46 ± 0.01</td>
<td>0.44 ± 0.01</td>
</tr>
</tbody>
</table>
Figure S1. (A) Big endothelin-1 (bET-1) and (B) endothelin-1 (ET-1) immunofluorescence staining in mesenteric vessels of Sham and RUPP animals. Data in C reflect average intensities obtained from respective data sets in (A) and (B). MFI: mean fluorescence intensity.