Plasma-Mediated Vascular Dysfunction in the Reduced Uterine Perfusion Pressure Model of Preeclampsia: A Microvascular Characterization

Sarah K. Walsh, Fred A. English, Edward J. Johns, Louise C. Kenny

Abstract—Preeclampsia is associated with widespread maternal vascular dysfunction, which is thought to be mediated by circulating factor(s). The aim of the study was to characterize vascular function in the reduced uterine perfusion pressure (RUPP) rat model of preeclampsia and to investigate the role of plasma factors in mediating any observed changes in vascular reactivity. Mean arterial blood pressure and vascular function were measured in RUPP and control rats. Mesenteric vessels from both virgin and pregnant rats were exposed for 1 hour or overnight to plasma from both RUPP and control rats and their vascular function assessed. RUPP rats were characterized by severe hypertension, restricted fetal growth, and reduced placental weight ($P<0.001$). Vasorelaxation was impaired in resistance vessels from RUPP compared with control rats (acetylcholine: $R_{\text{max}}$ 70±3 versus 92±1 [NP] and 93±3% [sham], $P<0.01$; bradykinin: 40±2 versus 62±2 [NP] and 59±4% [sham], $P<0.001$). Incubation of vessels from pregnant (but not virgin) animals with RUPP plasma overnight resulted in an attenuation of vasorelaxant responses (acetylcholine: 63±7 versus 86±2%, $P<0.05$; bradykinin: 35±5 versus 55±6%, $P<0.001$). The residual relaxant response in RUPP plasma-treated vessels was not further attenuated after treatment with $N^\omega$-nitro-L-arginine methyl ester (acetylcholine: 57±7 versus 63±7%, ns; bradykinin: 37±5 versus 35±5%, ns). The RUPP rat model is characterized by an impaired response to vasodilators which may be attributable to one or more circulating factors. This plasma-mediated endothelial dysfunction appears to be a pregnancy-dependent effect. Furthermore, nitric oxide–mediated vasorelaxation appears to be absent in RUPP plasma-treated vessels. *(Hypertension. 2009;54:345-351.)*

Key Words: preeclampsia ■ reduced uterine perfusion pressure ■ endothelium ■ hypertension ■ plasma ■ nitric oxide

Preeclampsia (PE) is a multisystemic disorder of pregnancy which affects more than 8 million pregnancies worldwide annually.1 The underlying etiology is incompletely understood, but current thinking suggests the development of a relatively hyperperfused placenta stimulates the maternal response, including alterations in the maternal vascular endothelium.2 Impaired endothelium-dependent responses have been reported in systemic vessels isolated from women with PE.3,4 Moreover, numerous studies have demonstrated disparities in terms of the relative contributions of nitric oxide (NO), endothelium-derived hyperpolarizing factor (EDHF), and prostacyclin (PGI2) to vasodilatation in both physiological5–8 and pathophysiological pregnancies.5,6,9 In addition, the vascular dysfunction associated with PE has been shown to be induced by one or more circulating mediators as impaired relaxation was detected in resistance vessels exposed to plasma from women with PE.10,11

Several animal models of pregnancy-induced hypertension (PIH) exist including the reduced uterine perfusion pressure (RUPP) rat, which involves the chronic reduction of uteroplacental perfusion resulting in a pregnancy-dependent hypertensive state.12,13 Although a number of gene knockout models of PIH (Comt–/–14 and BPH/515 mice) have emerged, those using chronic reduction of uteroplacental perfusion are still largely considered to be more sympathetic to the human condition as they are characterized by hypertension, reduced glomerular filtration rate, proteinuria, intrauterine growth restriction, and endothelial dysfunction.13 Although PE is a complex condition exclusive to humans, animal models of PIH provide considerable scope for studying numerous aspects of this condition and thus can add substantially to the current understanding of its etiology.

Although the RUPP model has been shown to exhibit impaired vasorelaxation in conduit vessels,12,16 the vasculature of this model has been incompletely characterized. Furthermore, although plasma from the RUPP model has been shown to induce activation of human-derived endothelial cells,17 it is not known whether these plasma-derived
factors are responsible for mediating the vascular dysfunction in this model. We set out to characterize microvascular function in the RUPP rat model of PE and to investigate the possible role of circulating factors in mediating any observed changes in vascular function.

Methods

Animals

Pregnant Sprague Dawley rats (12 weeks; supplied and maintained by the Biological Services Unit, University College Cork) were housed in the Biological Services Unit at University College Cork. Animals were maintained at a temperature of 21 ± 2°C, with a 12-hour light/dark cycle and with free access to food and tap water. All procedures were performed in accordance with national guidelines and the European Community Directive 86/609/EC and approved by the University College Cork Local Animal Experimentation Ethics Committee.

Protocol for RUPP Procedure

On day 14 of pregnancy, animals destined for the RUPP experimental group were anesthetized with isoflurane (2% to 5% inhalation) and the abdominal cavity opened via a midline incision to expose the lower abdominal aorta. A silver clip (0.203 mm ID) was placed around the aorta (above the iliac bifurcation) to reduce uterine perfusion pressure by 7 to 9 mm Hg. Animals were anesthetized with 2.5% isoflurane in a mixture of 95% O2 and 5% CO2. KPSS was produced as per PSS with the addition of 300 mmol/L KCl. Vessels were thereafter washed with KPSS, allowed to return to basal tone, and the procedure repeated with PSS, allowed to return to basal tone, and the procedure repeated.

In Vitro Experimental Protocols

Mesenteric vessels from virgin (n = 7 to 11) and NP (n = 7 to 9) rats were incubated for 1 hour at 37°C in 3% normal pregnant plasma (3% NPP) or 3% RUPP plasma (3% RP). This plasma concentration was chosen as increasing concentrations have not been shown to impair vasorelaxation further.

Mesenteric vessels from virgin and NP rats were incubated overnight at 4°C in 3% NPP or 3% RP solutions containing either vehicle (n = 9 to 10), N\textsuperscript{0}-nitro-L-arginine methyl ester (L-NAME; 10\textsuperscript{-4} mol/L; n = 10) to assess NO contribution, indomethacin (INDO; 10\textsuperscript{-5} mol/L; n = 9 to 10) to assess prostanoïd contribution, or a combination of both (n = 9 to 10) to assess EDHF contribution to receptor-mediated vasodilatation.

After subsequent washes with PSS, concentration response curves to U46619, ACh, and BK were carried out as previously described. In an additional series of experiments, endothelial-independent vasorelaxation was assessed in both 3% NPP and 3% RP-treated (overnight) vessels from NP rats using sodium nitroprusside (SNP; 10\textsuperscript{-9} to 10\textsuperscript{-6} mol/L; n = 9).

Statistical Analysis

For both hemodynamic and weight data a 1-way ANOVA and Dunnett post hoc test were used. Concentration response curves for all vascular data were generated using GraphPad Prism. Concentration responses between groups were compared via a repeated measures ANOVA and Bonferroni post hoc test. Both EC\textsubscript{50} values (expressed as $-\log [\text{mol/L}]\pm\text{SEM}$) and R\textsubscript{max} values (maximal relaxation as a percentage of induced tone) were compared using either a 1-way ANOVA and Dunnett post hoc test or a t test where appropriate. For all experimental groups, data were expressed as the mean±SEM, and significance was determined as P < 0.05.

Results

Effects of Chronic Reduction in Uterine Perfusion Pressure

RUPP rats were characterized by a significantly higher MABP (131 ± 4 mm Hg) when compared with NP (99 ± 4 mm Hg; P = 0.001) and sham-operated (108 ± 2 mm Hg; P = 0.001) rats (Figure 1A). RUPP rats were also associated with restricted fetal growth when compared with NP (2.2 ± 0.1 versus 3.2 ± 0.1 g; P < 0.001) and sham-operated (2.2 ± 0.1 versus 3.2 ± 0.1 g; P < 0.001) rats (data not shown). Furthermore, placental weights from RUPP rats were also significantly reduced compared with both NP (0.33 ± 0.01 versus 0.43 ± 0.01 g; P < 0.001) and sham-operated (0.33 ± 0.01 versus 0.42 ± 0.02 g; P < 0.001) rats (data not shown). Third order mesenteric arteries from RUPP rats displayed significantly impaired relaxation to both BK (R\textsubscript{max} 40.2 ± 2 versus 62.2 ± 2 [NP] and 59.2 ± 4%[sham]; P < 0.001; Figure 1B) and ACh (R\textsubscript{max} 70.2 ± 3 versus 92 ± 1[NP] and 93.2 ± 3%[sham]; P < 0.01; Figure 1C) when compared with control vessels. In contrast, significant differences in EC\textsubscript{50} values were not demonstrated between groups in response to either BK (EC\textsubscript{50} [-log] 8.59 ± 0.1 [RUPP] versus 8.82 ± 0.1[NP] and 8.49 ± 0.1[sham] mol/L; ns) or ACh (EC\textsubscript{50} 7.85 ± 0.2[RUPP] versus 7.71 ± 0.1[NP] and 7.57 ± 0.1[sham] mol/L; ns). U46619 induced a concentration-dependent increase in active wall tension in all groups, but the maximum contraction to the vasocon-
strictor did not differ significantly between groups (Figure S2; please see http://hyper.ahajournals.org).

**Effect of Plasma Mediators on Vascular Function After 1-Hour Incubation**

Incubation of vessels from virgin rats with 3% RP for 1 hour did not significantly affect vasorelaxation to either BK ($R_{\text{max}}$ 39±3 versus 45±2%; ns; Figure 2A) or ACh ($R_{\text{max}}$ 77±4 versus 79±4%; ns; Figure 2B) when compared with vessels incubated with 3% NPP. Similarly, exposure of pregnant vessels to 3% RP for 1 hour did not significantly affect the vasorelaxant response to either BK ($R_{\text{max}}$ 46±9 versus 50±3%; ns; Figure 2A) or ACh ($R_{\text{max}}$ 76±4 versus 81±2%; ns; Figure 2B) when compared with control vessels. Furthermore, EC$_{50}$ values for RUPP-plasma–treated vessels did not significantly differ from their respective controls in response to either BK or ACh (Table S1; please see http://hyper.ahajournals.org). Treatment with 3% RP for 1 hour did not significantly affect the maximum contraction of either virgin or pregnant vessels compared with their respective controls (Figure S3; please see http://hyper.ahajournals.org).

**Effect of Plasma Mediators on Vascular Function After Overnight Incubation**

Overnight incubation of virgin vessels with 3% RP did not significantly affect maximum relaxation to either BK ($R_{\text{max}}$ 34±3 versus 37±4%; ns; Figure 3A) or ACh ($R_{\text{max}}$ 74±5 versus 78±5%; ns; Figure 3B). In contrast, impaired relaxation to both BK ($R_{\text{max}}$ 35±5 versus 55±6%; $P<0.001$; Figure 3A) and ACh ($R_{\text{max}}$ 63±7 versus 86±2%; $P<0.05$; Figure 3B) was detected following overnight incubation of vessels from NP rats in 3% RP. However, endothelial-independent relaxation, mediated by SNP, did not significantly differ between plasma-treated vessels from pregnant rats ($R_{\text{max}}$ 83±4 (3% RP) versus 79±4% (3% NPP); ns; Figure 3C). Furthermore, significant differences in EC$_{50}$ values were not demonstrated between any of the groups investigated (Table S1; please see http://hyper.ahajournals.org). Treatment with 3% RP did not significantly affect the maximum relaxation to either BK (Table S1; please see http://hyper.ahajournals.org).
contractions to U46619 following overnight incubation in any groups (Figure S3; please see http://hyper.ahajournals.org).

Relative Contributions of NO, Prostanoids, and EDHF to Vasorelaxation in 3% NPP–Treated Pregnant Vessels

Resistance vessels from NP rats incubated overnight in 3% NPP relaxed by approximately 50% ($R_{\text{max}}$) in response to BK (Figure 4A). However, treatment with L-NAME ($10^{-5}$ mol/L) significantly impaired relaxation of vessels to the maximum concentration of BK ($R_{\text{max}}$ 27±2 versus 55±6%; $P<0.001$; Figure 4A). Furthermore, overnight incubation of vessels with INDO ($10^{-8}$ mol/L) significantly impaired vasorelaxation ($R_{\text{max}}$ 32±6 versus 55±6%; $P<0.001$; Figure 4A). Overnight incubation of vessels in 3% NPP containing both AP and CHX (K$^+$ channel blockers) displayed significantly impaired relaxation compared with control vessels ($R_{\text{max}}$ 23±4 versus 55±6%; $P<0.001$; Figure 4A). Plasma-treated mesenteric vessels from pregnant rats relaxed by approximately 80% ($R_{\text{max}}$) in response to the maximum concentration of ACh an effect which was significantly attenuated following overnight L-NAME treatment ($R_{\text{max}}$ 43±8 versus 86±2%; $P<0.001$; Figure 4B). Similarly, overnight incubation with INDO significantly impaired ACh-mediated vasorelaxation ($R_{\text{max}}$ 51±7 versus 86±2%; $P<0.001$; Figure 4B). Concomitant treatment of vessels with AP and CHX significantly impaired relaxation compared with control vessels ($R_{\text{max}}$ 19±5 versus 86±2%; $P<0.001$; Figure 4B). Furthermore, significant differences in EC$_{50}$ values were not demonstrated between any of the groups investigated (Table S1; please see http://hyper.ahajournals.org). None of the treatments investigated significantly affected the maximum contraction of pregnant vessels compared with the respective controls (Figure S4A; please see http://hyper.ahajournals.org).
versus 63%; Figure 5B). However, treatment with INDO 0.05 vs 3% NPP, SEM (n expressed as mean calculated as a percentage of the maximum contraction and the presence of various inhibitors assessed. Relaxation is calculated as a percentage of the maximum contraction and expressed as mean±SEM (n=6 to 10). *P<0.05 vs 3% NPP, †P<0.05 (+INDO), and ‡P<0.05 (+AP and CHX) vs 3% RP.

Figure 5. Role of NO, PGL2, and EDHF in mediating the vasorelaxant responses of RUPP plasma-treated resistance vessels from pregnant rats. Third order mesenteric vessels from gestational day 19 pregnant rats were incubated in 3% RP overnight from pregnant rats. Third order mesenteric vessels from gestational day 19 pregnant rats were incubated in 3% RP overnight from pregnant rats. Thus, treatment with INDO 0.05 rather than 3% NPP had a significantly more profound effect on relaxation. However, the concomitant treatment of AP and CHX significantly attenuated the residual relaxant response to BK when compared with vessels from both healthy pregnant and sham-operated dams. The findings from our study are in support of those of Crews et al22 and Barron et al16 who found an impaired relaxant response of conduit vessels (thoracic aorta) from RUPP rats when compared with normal pregnant females. Furthermore, a study by Anderson et al21 demonstrated an impaired relaxant response (on comparison of EC50 values) of RUPP uterine arteries when compared with arteries from sham rats. In a subsequent study, Anderson et al22 investigated vasorelaxant responses in RUPP compared with sham mesenteric vessels and found no significant differences in EC50 values (in response to ACh) between groups, which is in agreement with the present study. However, in the present study a significant difference in maximal relaxation in response to 1 μmol/L ACh was detected. In contrast, although there appeared to be a difference in the vasorelaxant responses between both groups to 1 μmol/L ACh in the study by Anderson et al22 this was not statistically significant. One possible explanation for the discrepancy between this and the present study may be the large standard errors associated with the ACh-induced vasorelaxant responses in the work by Anderson et al,22 which is most likely a consequence of the limited number of vessels used in that particular series of experiments (n=5 for each group).

Relative Contributions of NO, Prostanoids, and EDHF to Vasorelaxation in 3% RP–Treated Pregnant Vessels

Overnight exposure of resistance vessels from pregnant rats to 3% RP resulted in significantly impaired relaxation to BK compared to control vessels (Rmax 35±5 versus 55±6%; P<0.001; Figure 5A). However, the concomitant treatment of healthy vessels with both 3% RP and L-NAME did not significantly affect the residual relaxant response to BK when compared with 3% RP–treated vessels (Rmax 37±5 versus 35±5%; Figure 5A). In contrast, inclusion of either INDO or a combination of AP and CHX significantly attenuated the resultant vasorelaxant response to BK after 3% RP treatment (Rmax 19±4 versus 35±5%; P<0.01 [INDO]; Rmax 18±4 versus 35±5%; P<0.01 [AP and CHX]; Figure 5A). After incubation in 3% NPP, mesenteric vessels relaxed by approximately 80% (Rmax) to the maximum concentration of ACh, whereas vessels incubated in 3% RP only relaxed by approximately 60% ([Rmax] P<0.01; Figure 5B). The residual relaxant response to ACh (after 3% RP treatment) was not further attenuated after treatment with L-NAME (Rmax 57±7 versus 63±7%; Figure 5B). However, treatment with INDO (Rmax 44±6%; P<0.05) or a combination of AP and CHX (Rmax 20±4%; P<0.001) significantly impaired vasorelaxation when compared to vessels treated with 3% RP alone (63±7%; Figure 5B). Furthermore, significant differences in EC50 values were not demonstrated between any of the groups investigated (Table S1; please see http://hyper.ahajournals.org). None of the treatments investigated significantly affected the maximum contraction of pregnant vessels compared with the respective controls (Figure S4B; please see http://hyper.ahajournals.org).

Discussion
As previously published,20 chronic reduction of uteroplacental perfusion in day 14 pregnant dams resulted in a hypertensive condition associated with fetal growth restriction and impaired placental growth when compared with healthy pregnant rats. In addition, our study demonstrated for the first time that mesenteric arteries from RUPP rats display impaired vasorelaxation to the classic vasodilators BK and ACh when compared with vessels from both healthy pregnant and sham-operated dams. The findings from our study are in support of those of Crews et al22 and Barron et al16 who found an impaired relaxant response of conduit vessels (thoracic aorta) from RUPP rats when compared with normal pregnant females. Furthermore, a study by Anderson et al21 demonstrated an impaired relaxant response (on comparison of EC50 values) of RUPP uterine arteries when compared with arteries from sham rats. In a subsequent study, Anderson et al22 investigated vasorelaxant responses in RUPP compared with sham mesenteric vessels and found no significant differences in EC50 values (in response to ACh) between groups, which is in agreement with the present study. However, in the present study a significant difference in maximal relaxation in response to 1 μmol/L ACh was detected. In contrast, although there appeared to be a difference in the vasorelaxant responses between both groups to 1 μmol/L ACh in the study by Anderson et al,22 this was not statistically significant. One possible explanation for the discrepancy between this and the present study may be the large standard errors associated with the ACh-induced vasorelaxant responses in the work by Anderson et al,22 which is most likely a consequence of the limited number of vessels used in that particular series of experiments (n=5 for each group).

The exact cause of PE has yet to be elucidated, but it appears that placental ischemia is emerging as one of the key mechanisms involved in the etiology of this condition. Circulating factors released from a compromised placenta are thought to be responsible for many of the adverse events associated with PE, including endothelial dysfunction.23 Results from the present study demonstrated for the first time that incubation of resistance vessels from healthy pregnant rats with plasma from RUPP rats resulted in a significantly blunted vasorelaxant response to several vasodilators, which was endothelial-dependent. Furthermore, this plasma-mediated endothelial dysfunction was found to be both a time- and pregnancy-dependent effect. Our findings using the rat model of PE are comparable to those from numerous in vitro studies in which exposure of isolated human vessels (both myometrial and omental) to plasma from women with PE resulted in significantly impaired receptor mediated vasorelaxation.10,11,24
Human studies have suggested that vasorelaxation is predominantly mediated by either NO, EDHF, or a combination of both, and the vascular dysfunction documented in PE is attributable to aberrant activity of either or both of these pathways. Similarly in pregnant rats, vasorelaxation is suggested as being mediated primarily through a combination of NO- and EDHF-mediated pathways. Results from the present study demonstrated a significant contribution of NO, EDHF, and PGF2α to vasorelaxation (induced by either BK or ACh) in normal plasma-treated vessels. In contrast, the residual relaxation after treatment with RUPP plasma appeared to be mediated via pathways independent of NO. A diminished or absent NO-mediated vasodilatory response has previously been demonstrated in vessels from women with PE. Moreover, studies have also detected a lack of NO-mediated vasodilatation in conduit vessels from RUPP rats, however the present study is the first to demonstrate this in resistance vessels treated with RUPP plasma. The remaining relaxant response in RUPP plasma-treated vessels was significantly attenuated after both cyclooxygenase inhibition and K+ channel blockade indicating vasorelaxant roles for both PGF2α and EDHF, respectively.

The identity of the circulating factor(s) responsible for mediating this endothelial dysfunction have yet to be identified, but studies in both humans and experimental models of PIH may provide a number of possible candidates including inflammatory cytokines and antiangiogenic mediators, among others. Within these groups likely culprits may be TNF-α or soluble fms-like tyrosine kinase-1 as levels of both mediators have been shown to be elevated in both patients with preeclampsia and in the RUPP model of PIH. Furthermore, both factors have been shown to mediate vascular dysfunction in pregnant rats, however whether or not these mediators are responsible for the RUPP plasma-induced vascular dysfunction demonstrated in the present study has yet to be determined.

In conclusion, the present study has demonstrated for the first time that chronic reduction of uteroplacental perfusion in the pregnant rat results in impaired vasorelaxation in the microvasculature. Furthermore, our results demonstrated that this endothelial dysfunction was induced by one or more circulating mediators and was a pregnancy-dependent effect. Finally, additional characterization of the vasorelaxant responses in plasma-treated resistance vessels has demonstrated a loss or absence of NO-mediated vasorelaxation in RUPP plasma-treated vessels.

Perspectives

Observations from the present study and those of others have demonstrated a considerable homology between vascular function in the gravid human and animal under both physiological and pathophysiological conditions. However, our study is the first to demonstrate that endothelial dysfunction in the RUPP rat is mediated by 1 or more circulating factor(s), which is in agreement with studies using human samples. It may therefore be tempting to suggest that common mediators induce vascular dysfunction in both the clinical condition and experimental representation of PE (animal models). Previous work has demonstrated the presence of plasma-derived vasoactive mediators in the plasma of women destined to develop PE many weeks before the development of the clinical syndrome. The characterization of these circulating factor(s) would prove extremely beneficial in terms of providing novel insight into the etiology of PE and also the possible identification of therapeutic targets for the treatment of this condition. Although it is evident that a considerable amount of information regarding the pathogenesis of PE has been garnered from clinical studies using plasma, urine, placental, and vascular samples from preeclamptic women, the difficulty of performing mechanistic studies in pregnant women necessitates the use of animal models of this condition. Thus although the identification of these plasma borne mediators was not within the scope of the current study, it is envisioned that future work will attempt to characterize these circulating factors with the use of the RUPP model of PE.

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Disclosures

None.

References


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PLASMA-MEDIATED VASCULAR DYSFUNCTION IN THE REDUCED UTERINE PERFUSION PRESSURE MODEL OF PREECLAMPSIA: A MICROVASCULAR CHARACTERIZATION

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### TABLE S1. EC<sub>50</sub> values for BK, ACh and SNP concentration responses in plasma treated resistance vessels.

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All data expressed as mean±s.e.m. No significant differences between any groups.
Figure S1. Comparison of the contractile responses of U46619 treated resistance vessels from control pregnant rats, sham-operated rats and rats with reductions in uterine perfusion pressure (RUPP). Data is calculated as active wall tension (change in tension (mN)/wall length (mm); mN/mm) and is expressed as mean±s.e.m. (n=9 for all groups)
Figure S2. Comparison of the contractile responses of U46619 treated resistance vessels from both virgin (A) and pregnant (B) rats which have been pre-exposed to either 3% NP or 3% RUPP plasma. Data is calculated as active wall tension (mN/mm) and is expressed as mean±s.e.m. (n=7-15)
Figure S3. Comparison of the contractile responses of U46619 treated resistance vessels from pregnant rats which have been pre-exposed to either 3% NP (A) or 3% RUPP (B) plasma with or without L-NAME, indomethacin or charybdotoxin (CHX) and apamin (AP). Data is calculated as active wall tension (mN/mm) and is expressed as mean±s.e.m. (n=6-10)