Endothelium-derived hyperpolarizing factor in preeclampsia: heterogeneous contribution, mechanisms, and morphological prerequisites

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PREECLAMPSIA (PE) is a multisystem syndrome and remains a leading cause of maternal and perinatal morbidity and mortality. Despite intensive research, the etiology remains elusive. Indeed, opinions regarding the pathogenetic mechanism are divided. Some argue that PE is the result of inadequate trophoblast invasion in the uterine spiral arteries. This may result in placental ischemia and cause the release of placental factors with toxic effects on the maternal vascular endothelium (34). Others propose that PE is caused by an inappropriate or exaggerated maternal inflammatory response toward the presence of trophoblast debris (33).

It is generally believed that endothelial dysfunction is a hallmark of PE, and it causes many of the clinical manifestations of the disorder including hypertension, proteinuria, and edema. Studies of a variety of biochemical markers (35) and functional studies of isolated small arteries (reviewed in Ref. 26) have proposed numerous candidates and/or pathways that could potentially cause compromised endothelial function (2, 25, 39). However, none of them has yet proved to be a universal mediator or mechanism, and a combination is likely to be involved (33). Moreover, not all functional investigations have provided supportive evidence for endothelial dysfunction, and even increased responses to endothelium-dependent agonists have been observed (10). Therefore, endothelial dysfunction in PE could be vascular bed and/or agonist dependent (26), and some pathways of endothelium-dependent relaxation could be compromised when others remain preserved (18).

The role of endothelium-derived hyperpolarizing factor (EDHF) could be of interest. EDHF plays its predominant role in the resistance vasculature (26) and, as such, is ideally suited to the control of local organ blood flow, peripheral resistance, and blood pressure (30), all of which are severely disturbed in PE (46). The contribution of EDHF to enhanced endothelium-dependent relaxation in normal pregnancy has already been suggested (18, 32). Experimental evidence indicates that myoendothelial gap junctions (MEGJs) are commonly involved in EDHF-mediated responses, and this has been confirmed in arteries from the peripheral (23, 27) and the uterine (19) circulations, in which significant increases in blood flow occur in normal pregnancy (17, 36). However, functional studies of the mechanism/s mediating EDHF in PE are still lacking, and the available data pertaining to its role remain controversial and variable between the vascular bed studied and/or agonist used (20, 29, 31, 40).

We have previously reported that PE is associated with pronounced alterations in small artery endothelial morphology (42, 43), which could potentially cause impaired intercellular communication. Consequently, we hypothesized that, in arteries from women with PE, EDHF activity is mediated by one or more alternative pathways that do not require intercellular communication via MEGJs. In this study, we therefore aimed to clarify the contribution of EDHF vs. nitric oxide (NO) in bradykinin (BK)-mediated endothelium-dependent relaxation.
in small subcutaneous arteries from women with PE. We explored the mechanisms of EDHF-mediated responses in these arteries and examined the ultrastructure of vascular wall, paying particular attention to MEGJs.

METHODS

Subjects. The study was approved by the Ethical Committee at Karolinska University Hospital, Huddinge, and all women gave informed consent before participation. PE was defined according to the criteria of the International Society for the Study of Hypertension in Pregnancy. Diagnosis required blood pressure $\geq 140/90$ mmHg after the 20th week of gestation in association with $>300$ mg proteinuria in a 24-h urine collection. Exclusion criteria for both women with PE and normal pregnant (NP) women included diabetes, established atherosclerosis, chronic hypertension, malignancy, hepatic or renal failure, systemic infection, vasculitis, and recent surgery or trauma.

Fifteen women with PE (6 nulliparous) with a median age of 31 yr (range 21–39 yr) and a median gestational age of 34 wk (range 31–38 wk) undergoing cesarean delivery for deterioration of PE were included. Two women with PE fulfilled the criteria for the hemolysis, elevated liver enzymes, and low platelet count (HELLP syndrome) at the time of sampling. During the operation, subcutaneous fat biopsies were collected from the incision margin and immediately placed in ice-cold physiological salt solution (PSS).

Biopsies were also obtained from 23 NP women (12 nulliparous) with a median age of 26 yr (range 18–39 yr) and a median gestational age of 38 wk (37–40 wk) undergoing planned cesarean section for indications including breach presentation ($n = 8$), previous cesarean section ($n = 4$), and psychological reasons ($n = 11$). In this study, some women of the NP group were also included in the NP group of our previous report about EDHF-mediated responses (27).

Experimental setup. Small subcutaneous arteries were identified and dissected from the fat biopsies by carefully removing surrounding tissue using a stereomicroscope. From each biopsy, two or more arteries of similar size, and preferably from the same vascular segment, were isolated and mounted on two stainless steel wires (25–40 $\mu$m in diameter) in the organ baths of a four-channel wire myograph (multimyograph, model no. 610; Danish Myo Technology; Aarhus, Denmark) as described previously (27). Each organ bath contained warmed (37°C) PSS and was continuously bubbled with 5% carbon dioxide in oxygen. Following a 30-min equilibration period, a passive circumferential-tension curve was created for each segment to set optimum resting tension. This resting tension is calculated to simulate the circumferential-tension curve was created for each segment to set optimum resting tension. This resting tension is calculated to simulate the optimum resting tension to enable optimal contractile conditions with a resting tension. This resting tension is calculated to simulate the optimum resting tension to enable optimal contractile conditions with a resting tension. This resting tension is calculated to simulate the optimum resting tension to enable optimal contractile conditions with a resting tension.

Biopsies and arteries from NP women, we did not find any differences between the inhibitory actions of 18-oGA and those of connexin mimetic peptides (Gap26 and Gap27, a novel group of selective and reversible gap junction inhibitors) (23, 27).

In separate experiments, concentration-response curves for NO donor sodium nitroprusside (SNP) and ATP-sensitive potassium channel opener pinacidil (10 mmol/l to 100 mmol/l) were evaluated before and after incubation with inhibitors used in arteries from NP women.

Transmission electron microscopy. Artery segments were fixed in 2% glutaraldehyde + 0.5% paraformaldehyde in 1 M sodium cacodylate buffer containing 0.1 M sucrose and 3 mM CaCl$_2$, pH 7.4, at room temperature for 30 min followed by 24 h at 4°C. Specimens were rinsed in 0.15 M sodium cacodylate buffer containing 3 mM CaCl$_2$, pH 7.4; postfixed in 2% osmium tetroxide in 0.07 M sodium cacodylate buffer containing 1.5 mM CaCl$_2$, pH 7.4, at 4°C for 2 h; and dehydrated in ethanol followed by acetone and embedded in LX-112 (Ladd, Burlington, VT). Semi-thin sections were cut and stained with toluidin blue and used for light microscopic analysis. Ultra-thin sections (~40–50 nm) were contrasted with uranyl acetate followed by lead citrate (14). Sections were examined in a Tecnai 10 transmission electron microscope at 80 kV, and digital images were captured by a Mega View III digital camera (Soft Imaging System, Muenster, Germany).

Chemicals. The composition of PSS was as follows (in mmol/l): NaCl 119, KCl 4.7, CaCl$_2$ 2.5, MgSO$_4$ 1.17, NaHCO$_3$ 25, KH$_2$PO$_4$ 1.18, EDTA 0.026, and glucose 5.5. The chemicals were obtained from Sigma (St. Louis, MO). To prepare stock solution, the substances were dissolved in distilled water. Indo, pinacidil, and U46619 were dissolved in ethanol. All concentrations represent the final steady-state concentrations in the chamber. Pilot studies showed that the solvent used has no effect on the mechanical responses at their final bath concentrations.

Data analysis. The force developed per millimeter of artery segment during application of each concentration of vasoactive compound was calculated using Myodata (Danish Myo Technology). Data were then transferred to STATISTICA (version 7.0; StatSoft, Uppsala, Sweden), in which all statistical analyses were performed. All absolute measurements were corrected for the baseline force developed. The relaxation response to BK was calculated as a percentage of the contraction. Negative log concentration required to cause 50% of the maximum response (pEC$_{50}$) was calculated by nonlinear regression analysis. ANOVA was used to compare concentration-response curves before and after incubation with different pharmacological inhibitors. Paired and unpaired Student’s t-tests, as appropriate, were used to compare pEC$_{50}$ before and after incubation with different agents in arteries used in different experimental protocols. The number and length ($\mu$m) of protrusions per section, thick-
ness of internal elastic lamina (IEL; µm), and percentage of the area with detached endothelium from the basement membrane were compared among arteries from PE and NP groups. All data are presented as means ± SE unless indicated in the text; n represents the number of patients. Significance was taken at the 5% level for all comparisons.

RESULTS

In total, 44 arteries with an internal diameter of 219 ± 11 µm were dissected from 15 biopsies from women with PE, and 74 arteries with internal diameter of 244 ± 15 µm were dissected from 23 biopsies from NP women. There was no difference in internal diameter, the magnitude of contraction in response to KPSS (2.6 ± 0.2 mN/mm in PE vs. 2.8 ± 0.2 mN/mm in NP), or the precontraction level with NE (2.6 ± 0.2 mN/mm in PE vs. 3.2 ± 0.2 mN/mm in NP) among the arteries used for different experimental protocols.

Arteries from NP women relaxed in response to increasing concentrations of BK up to 85–95%. The concentration-response curves for BK were similar before and after a 30-min incubation in physiological salt solution (PSS) and after incubation with L-NAME (P < 0.05, ANOVA; Fig. 1). In arteries from both groups, incubation with L-NAME+Indo resulted in a significant reduction in relaxation in response to BK compared with that obtained with PSS (P < 0.05, ANOVA; Fig. 1). This suggests that the NO/prostanoid-independent component (i.e., mediated by EDHF) contributes significantly to BK-induced relaxation in isolated arteries from women with PE and NP (up to 60% of the total BK response). This overall reduction was, however, more pronounced in PE arteries compared with NP arteries [%relaxation in response to BK at 3 µmol/l (here and below): 51 ± 2.0 for PE vs. 60 ± 4 for NP, P < 0.05, ANOVA; Fig. 1], although there was no difference in pEC50 between these arteries (6.8 ± 0.1 for PE vs. 7.0 ± 0.1 for NP). Thus the contribution of EDHF- rather than NO-mediated relaxation appeared to be compromised in arteries from women with PE.

Contribution of MEGJs to EDHF-mediated relaxation in PE. While MEGJs are the major component of EDHF-mediated responses in arteries from NP women [19 ± 3% l-NAME+Indo+18-αGA (n = 9) vs. 65 ± 8% l-NAME+Indo (n = 9), P < 0.001; Fig. 2A], in PE we found heterogeneity in the ability of 18-αGA to influence the EDHF-mediated component of the response to BK. On the basis of that, we divided the PE group into two subgroups. In one subgroup (PE-1), composed of seven women with PE, incubation of arteries with 18-αGA produced a substantial reduction in BK-mediated dilatation (i.e., >50% reduction of BK-induced relaxation compared with relaxation after incubation with l-NAME+Indo), and it was comparable with the response seen in arteries from NP women (15 ± 3% vs. 19 ± 3% for NP; Fig. 2A), suggesting an important role of MEGJs. In the second subgroup (PE-2), composed of the remaining women with PE (n = 5), arteries responded differently, and incubation with 18-αGA had a small (i.e., <50% reduction of BK-induced relaxation compared with relaxation after incubation with l-NAME+Indo) but still significant (57 ± 5% l-NAME+Indo+18-αGA vs. 67 ± 4% l-NAME+Indo, P < 0.05; Fig. 2B) influence on EDHF-mediated relaxation. The smaller effect of 18-αGA in the PE-2 subgroup suggests that another pathway, in addition to MEGJs, may be active in mediating EDHF. As reflected in Fig. 3, the difference between the subgroups was not only limited to the mechanism of EDHF-mediated relaxation. The contribution of EDHF to total relaxation was also significantly greater in PE-2 vs. PE-1 (67 ± 4 vs. 45 ± 6%, respectively, P < 0.05), although pEC50 was not different between arteries from the two subgroups (7.0 ± 0.2 for PE-2 vs. 6.9 ± 0.1 for PE-1).

Thus the similar BK-induced relaxation in PSS and the variability in responses after incubation with l-NAME+Indo strongly suggest that women with PE in this study differed in terms of the relative contributions made by EDHF and NO toward overall endothelium-dependent relaxation in isolated arteries. Moreover, among the women with PE and with reduced EDHF contribution (PE-1), MEGJs seemed to be the main mechanism responsible for EDHF activity. On the other hand, in women with PE in whom EDHF made a larger contribution (similar magnitude to that seen in NP women, PE-2), MEGJs were involved to a lesser extent, and one or more additional or alternative mechanisms appeared to predominate.

Contribution of endogenous H2O2 and arachidonic acid metabolites to EDHF-mediated relaxation in PE. The contribution of endogenous H2O2 to EDHF-mediated relaxation was evaluated in 12 women with PE. Catalase significantly attenuated the response to BK after NOS and COX inhibition in arteries from three women, all from the PE-2 subgroup (Fig. 4A). After incubation with catalase, responses to BK were significantly different between arteries from the PE-2 subgroup (n = 3) and those from the NP group (25 ± 6 vs. 47 ± 5, P < 0.05; Fig. 4A). In arteries from the rest of the women with PE (n = 9), catalase failed to attenuate the EDHF-mediated response [pEC50: 6.6 ± 0.1 after vs. 6.7 ± 0.1 before incubation with catalase (n = 9), P > 0.5; Fig. 4B], and relaxation in response to BK after incubation with catalase was similar in
arteries from PE and NP women \([pEC_{50}: 6.6 \pm 0.1 \ (n = 9) \) vs. \(6.7 \pm 0.2 \ (n = 12), P > 0.5\).

The mechanism of EDHF-mediated relaxation in subcutaneous arteries from the last two women of the PE-2 subgroup involved the metabolite of CYP450 epoxygenase, presumably epoxyeicosatrienoic acids (EETs). Simultaneous incubation with \(l\)-NAME+Indo and sulfaphenazole markedly attenuated BK-induced relaxation by up to 23\% (Fig. 5A). In contrast, in arteries from other women with PE (3 from PE-2 and 5 from PE-1), sulfaphenazole did not affect the EDHF-mediated relaxation, and concentration-response curves for BK were similar in arteries after incubation with \(l\)-NAME+Indo vs. \(l\)-NAME+Indo+sulfaphenazole \([pEC_{50}: 6.9 \pm 0.1 \) vs. \(6.8 \pm 0.1 \ (n = 8), P > 0.5; \) Fig. 5B] and were also similar in arteries from NP women \([pEC_{50}: 6.8 \pm 0.2 \) vs. \(6.7 \pm 0.2 \ (n = 6); \) Fig. 5C].

Thus, in addition to MEGJs, endothelial H\(_2\)O\(_2\) and CYP450 epoxygenase metabolites of AA were involved in EDHF-mediated relaxation of small subcutaneous arteries from women in the PE-2 subgroup. This is in contrast to NP, where MEGJs appeared to be the main mechanism responsible for EDHF-mediated relaxation of subcutaneous arteries. Subgroup analysis was performed with respect to the demographic characteristics of the women with PE and included pregnancy history, age, parity, gestational age, birth weight, and blood pressure. However, this did not yield any helpful information for interpreting the basis of the observed functional heterogeneity in the pathways involved in EDHF-mediated relaxation.

Specificity of the inhibitors. Sulfaphenazole (10 \(\mu\)mol/l) or catalase (1,250 U/ml) in PSS did not affect the vasodilator responses to both SNP and pinacidil (Table 1). Because of our previously reported observation that incubation with 18\(\alpha\)-GA alone but not in combination with L-NAME+Indo significantly reduced NE-induced tone (27), all specificity experiments for 18\(\alpha\)-GA were performed after preincubation of arteries with L-NAME+Indo. Similar responses to SNP and pinacidil after incubation with 18\(\alpha\)-GA alone vs. L-NAME+Indo (Fig. 5A) was not seen in arteries incubated with L-NAME+Indo vs. L-NAME+Indo+sulfaphenazole (Fig. 5B).

**Fig. 2.** Concentration-response curves for BK in isolated subcutaneous arteries from NP and PE women after incubation with \(l\)-NAME+Indo alone or in combination with 18\(\alpha\)-glycyrrhetinic acid (\(l\)-NAME+Indo+18\(\alpha\)-GA). A: comparison between arteries from NP and PE [i.e., arteries that demonstrate a substantial reduction of endothelium-derived hyperpolarizing factor (EDHF)-mediated relaxation by 18\(\alpha\)-GA]. B: comparison between arteries from NP and PE (i.e., arteries that demonstrate a minor reduction of EDHF-mediated relaxation by 18\(\alpha\)-GA). *\(P < 0.05, \) PE \((l\)-NAME+Indo\) vs. NP \((l\)-NAME+Indo\); #\(P < 0.05, \) PE \((l\)-NAME+Indo\) vs. PE \((18\alpha\)-GA+\(l\)-NAME+Indo\); &\(P < 0.05, \) NP \((l\)-NAME+Indo\) vs. NP \((18\alpha\)-GA+\(l\)-NAME+Indo\); §\(P < 0.05, \) PE \((18\alpha\)-GA+\(l\)-NAME+Indo\) vs. NP \((18\alpha\)-GA+\(l\)-NAME+Indo\).

**Fig. 3.** A heterogeneous contribution of EDHF (after incubation with \(l\)-NAME+Indo) to BK-induced relaxation in arteries from women with PE. PE-1 group, arteries that demonstrated a substantial reduction of EDHF-mediated relaxation by 18\(\alpha\)-GA; PE-2 group, arteries that demonstrated a minor reduction of EDHF-mediated relaxation by 18\(\alpha\)-GA. *\(P < 0.05, \) PE-1 vs. PE-2.
EDHF IN PREECLAMPSIA

l-NAME + Indo vs. l-NAME + Indo + 18α-GA led us to suggest that the inhibitory effect of 18α-GA at the concentration used in the present study on BK-induced relaxation is mostly due to its specific action on gap junctions.

Incubation with l-NAME, the NOS inhibitor, was sufficient to block NO generation, as dilatation to BK was not further reduced by ODQ (pEC\textsubscript{50}: 7.0 ± 0.1 vs. 6.8 ± 0.2, n = 4).

Transmission electron microscopy. Arteries used for morphological evaluation had an internal diameter that was the same as arteries used for functional studies in NP women and women with PE. The morphological prerequisites taken to be indicative of the presence of gap junctions between endothelial cells (ECs) and smooth muscle cells (SMCs) in arteries from PE (n = 4) and NP women (n = 4) were obtained using transmission electron microscopy, and the images are presented in Figs. 6 and 7.

Ultrastructural micrographs of arteries from NP women indicated a relatively large number (40 ± 14 per artery section) of long protrusions (3.8 ± 0.9 μm), mainly from ECs (Fig. 6, A–D). These protrusions were observed penetrating the relatively large IEL (3.5 ± 0.7 μm) to form close contacts between ECs and SMCs within the vascular wall (Fig. 6, F–J). This type of contact previously referred to pentalaminar structures (13) considered to be prerequisite for MEGJs (Fig. 6, F–H).

In arteries from women with PE, the morphology contrasted with that seen in arteries from NP women and suggested an obstruction to the formation of intercellular contacts (Fig. 7). The percentage of the area with detached endothelium from the basement membrane was 25 ± 8% in PE (Fig. 7, B–E) vs. 4 ± 1.8% in NP (P < 0.05). In arteries from women with PE, the thickness of IEL had a tendency to be enhanced compared with controls (5.1 ± 0.6 vs. 3.5 ± 0.7 μm, P = 0.08), probably because of an increased presence of collagen-like fibers (Fig. 7, B–D), and there were alterations in the size and shape of the ECs themselves (the thinned part of ECs: 0.3 ± 0.1 μm in PE vs. 1.4 ± 0.6 μm in NP, P < 0.05). In one of four preparations from the PE group, only sporadic and short protrusions of ECs (0.6 ± 0.2 μm) were observed in the PE (Fig. 7C) compared with the NP group (3.8 ± 0.9 μm, P < 0.05). In the PE group, rather than the ECs extending protrusions, it was the SMCs that sent out relatively long protrusions (up to 1.5 μm; Fig. 7C) to establish contacts with ECs.

DISCUSSION

This study shows that, in terms of sensitivity and magnitude, the overall endothelium-dependent response to BK in small subcutaneous arteries from women with PE is similar to that seen in NP women. However, in PE the component of endothelium-dependent relaxation insensitive to NOS and COX inhibition (i.e., EDHF) is reduced. Our results suggest heterogeneity in the relative contribution of endothelium-derived factors involved in BK-induced relaxation and in the mechanisms responsible for the EDHF-mediated component in PE. MEGJs and/or H\textsubscript{2}O\textsubscript{2} and/or CYP450 epoxygenase metabolites of AA appear to be involved in the EDHF-mediated response in PE, while in NP women, communication via MEGJs seems to be the common pathway responsible for EDHF action.

In PE, impaired relaxations in response to both ACh (29) and BK (20) have been reported in the same vascular beds. These are not universal findings, as abolished ACh-mediated dilatation but preserved relaxation to substance P (47) or similar responses to ACh and BK in NP and women with PE (46) have been reported by others. Our finding is in line with those reporting similar overall responses in NP and women with PE. However, it contrasts with one study (20), in which comparable protocols were used to examine arteries from one-half as many women with PE as examined here (20). Inconsistent responses to endothelium-dependent agonists in PE have also been reported in other vascular beds including omental and myometrial arteries (3, 18, 31, 40), and these
divergent results are likely to result from heterogeneity of endothelial dysfunction in PE or may reflect the different agonists and methods employed.

The pathways involved in compromised endothelial function in PE have been suggested previously. McCarthy et al. (29) addressed reduced ACh-induced relaxation in response to the attenuated PGI2-mediated component. However, changes in NO- and EDHF-mediated responses have been suggested to confer blunted relaxation in response to BK (20). Our study argues that it is the EDHF- rather than the NO-mediated component of BK-mediated relaxation that is compromised in PE. Indeed, a difference in BK-mediated dilatation was observed between PE and NP only after the inhibition of NOS and COX products. Our results concur with one study in small myometrial arteries (18), in which BK evoked similar overall vasorelaxant responses in NP women and women with PE, but the EDHF-mediated component of relaxation was impaired in the arteries from women with PE.

Although the role of MEGJs in EDHF-mediated responses in NP women has been repeatedly confirmed, not only in subcutaneous (23, 27) but also in other vascular beds (19), to our knowledge, we are the first to report the mechanism/s behind EDHF-mediated relaxation in PE. Indeed, a difference in BK-mediated dilatation was observed between PE and NP only after the inhibition of NOS and COX products. Our results concur with one study in small myometrial arteries (18), in which BK evoked similar overall vasorelaxant responses in NP women and women with PE, but the EDHF-mediated component of relaxation was impaired in the arteries from women with PE.

Values are means ± SE; n = no. of patients. Sensitivity (negative log concentration required to cause 50% of the maximum response, pEC50) and relaxation to highest concentration (100 μmol/l) of sodium nitroprusside (SNP) and pinacidil (PIN) before and after incubation with inhibitors of pathways relevant for endothelium-derived hyperpolarizing factor (EDHF)-typed responses. Indo, indomethacin; 18-α-GA, 18-α-glycyrrhetinic acid.

### Table 1. Sensitivity and relaxation to highest concentration of SNP and PIN before and after incubation with inhibitors of pathways relevant for EDHF-typed responses

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<th>pEC50</th>
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<td>6</td>
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<td>94±1</td>
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<td>3</td>
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<td>97±1</td>
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<td>SNP after catalase</td>
<td>3</td>
<td>6.7±0.2</td>
<td>93±3</td>
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<tr>
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<td>6.1±0.2</td>
<td>74±5</td>
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<tr>
<td>PIN</td>
<td>6</td>
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Fig. 5. Concentration-response curves for BK in isolated subcutaneous arteries from NP, PE, and PE-2 after incubation with 1-NAME+Indo in combination with sulfaphenazole (Sulf+1-NAME+Indo; 30 min). A: EDHF-mediated responses in arteries from PE-2. B: EDHF-mediated responses in arteries from PE (arteries from the rest of the women with PE, where sulfaphenazole failed to reduce EDHF-mediated response). C: EDHF-mediated responses in arteries from NP.
teers (8), their involvement has not been confirmed in the same vascular bed in patients with cancer or cardiovascular disease (5).

In this study, we have shown that heterogeneous EDHF pathways exist in PE. We also identified mechanisms for EDHF activity that may either be alternative to or complementary to a requirement for MEGJs in EDHF-mediated relaxation in this disorder. The PE group was divided into two subgroups stratified by the magnitude of the inhibition of EDHF achieved by an antagonist of gap junction-mediated communication. This suggested that ~60% of patients with PE in this investigation had a reduced contribution of EDHF to endothelium-dependent relaxation, and, in this subgroup (PE-1), communication via MEGJs was preserved as a central mechanism for the EDHF-mediated response. However, it should be noted that we did not achieve a 100% abolishment of EDHF-mediated responses after incubation with 18-αGA in arteries from NP women as well as in the PE-1 group. One explanation could be that the concentration of 18α-GA used was not high enough to block completely gap junctions, and we intentionally did not use a higher concentration to minimize unspecific effects of the inhibitor suggested by others (9). There is also a theoretical possibility that another pathway could be involved along with MEGJ, although the latter plays a primary role.

In contrast, in arteries from the other PE subgroup (PE-2), EDHF made a contribution to BK-induced relaxation similar to that seen in NP. However, in this PE subgroup, MEGJs appeared to play a minor role, and H2O2 and CYP450 metabolites of AA appeared as alternative and/or additional candidates for EDHF-mediated relaxation in subcutaneous arteries. Although the reasons behind these disparities remain unknown and warrant further investigation, it is possible that the similar contribution of EDHF to BK-induced relaxation in NP and in some PE women is maintained because of additional pathways (e.g., H2O2 or CYP450 metabolites of AA) invoked to compensate for the compromised MEGJ-mediated EDHF component of relaxation. Indeed, there is some evidence that H2O2 and CYP450 metabolites of AA might serve to control vascular tone in PE. The oxidative stress associated with PE (37) may influence the production of H2O2, which can act as an inhibitor of gap junctions itself (45) or, if produced in ECs, can influence vascular tone (28). The increased urinary excretion of EETs has also been reported in pregnant women with hypertension (6).
Similar changes in EC morphology have also been reported in microscopy in subcutaneous (42) and myometrial (43) arteries. Epithelial abnormalities in PE, confirmed by use of scanning findings concur with our previous report demonstrating endothelial projections toward SMCs (see Fig. 7, A–E). The low-magnification pictures show an overview of vascular wall with ECs and SMCs separated by IEL. The thickness of IEL is irregular and nonhomogenous (B) and contains relatively more collagen-like fibers (A, B, and D; asterisks). Many vacuoles are observed in the intima, and there are also many areas with detached endothelium from the basement membrane (B, D, and E; arrowheads). The endothelium becomes very thin in some places (C). Long projections of SMCs rather than ECs are observed (C; arrow up to 1.5 μm). Occasionally, contacts between ECs and SMCs are observed (A, C, F, and G) that concur with functional findings, supporting a contribution of gap junctions in EDHF-mediated responses in some women with PE. The areas denoted by the boxes are magnified and show the close contact between the ECs and SMCs (F and G). The width of the gap is ~10–12 nm (G; arrows) and ~10 nm (C; arrows), Bar: A, 3 μm; B and F, 2 μm; C, 1 μm; D, 5 μm; E, 4 μm; G, 0.1 μm.

Ultrastructural analysis of the vascular wall indicated various differences between arteries from NP women and women with PE. In arteries from NP women, the regular projections sent from ECs through the IEL toward SMCs represent areas in which MEGJs are typically found. Classically, in transmission electron micrographs, gap junctions appear as pentalaminar-like structures at points of close membrane apposition (13). We believe that we have shown close associations between ECs and SMCs (see Fig. 6, E–H) and that these represent typical MEGJs. However, at this level, the junctional membranes could be less closely apposed as a result of either oblique visualization or a lack of organized gap junctional plaques. Indeed, it has been suggested that only individual gap junctional channels rather than gap junctional plaques provide the pathway for EC-SMC communication at the MEGJ (13). In contrast, deficiency of pentalaminar-like structures was confirmed in vessels from women with PE, and communications between ECs and SMCs were impaired, probably because of a number of changes observed in the vascular wall that could cause an obstacle to the establishment of tight interactions between ECs and SMCs. Those changes primarily included alterations in the morphology and size of ECs themselves (see Fig. 7, C and D) accompanied by an apparent deficiency of projections toward SMCs (see Fig. 7, B and E). Overall, these findings concur with our previous report demonstrating endothelial abnormalities in PE, confirmed by use of scanning microscopy in subcutaneous (42) and myometrial (43) arteries. Similar changes in EC morphology have also been reported in umbilical (48), fetal villous (11), and omental (41) arteries obtained from women with PE.

It could be suggested that the ultrastructural and functional abnormalities that develop in small arteries during PE may confer a vascular phenotype that predisposes one to the development of cardiovascular disorders later in life. This is reflected in several studies examining women with a history of NP vs. women who have had a pregnancy complicated by PE (1, 4, 15). Endothelial repair by the recruitment of endothelial progenitor cells or by the replication of neighboring ECs is associated with normalization of some, but not all, aspects of endothelial function (12). In those areas that are repaired, the disturbances in endothelium-dependent relaxation may be critically dependent on the upregulation of EDHF-mediated vasodilatation to preserve overall endothelium-dependent dilatation (21, 24, 38, 44).

In summary, we have demonstrated that PE is associated with apparent functional and morphological differences in small peripheral arteries. Although the overall endothelium-dependent response to BK was preserved in PE, the contribution of EDHF to relaxation is significantly reduced and heterogeneous. In PE the mechanism of EDHF-typed responses is mediated either by MEGJs alone or in combination with H2O2 or CYP450 epoxygenase metabolites of AA. We have suggested that the morphological alterations within the vascular wall may underlie the diminished contribution that MEGJs make in the mediation of EDHF vasorelaxation in PE. The clear heterogeneity in the contribution of EDHF to endothelium-depen-
dent relaxation and its dynamic nature provides an intriguing challenge for further investigations, which need to be performed in other vascular beds from women with PE and women of reproductive age with a prior history of PE.

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