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

Leanid Luksha,1 Henry Nisell,1 Natallia Luksha,1 Marius Kublickas,1 Kjell Hultenby,2 and Karolina Kublickiene1

1Institution for Clinical Science, Technology and Intervention, Department of Obstetrics and Gynecology, Karolinska Institutet, Karolinska University Hospital, Huddinge Campus, Stockholm; and 2Clinical Research Center, Department of Laboratory Medicine, Karolinska Institutet, Novum, Stockholm, Sweden

Submitted 27 June 2007; accepted in final form 19 November 2007

Luksha L, Nisell H, Luksha N, Kublickas M, Hultenby K, Kublickiene K. Endothelium-derived hyperpolarizing factor in preeclampsia: heterogeneous contribution, mechanisms, and morphological prerequisites. Am J Physiol Regul Integr Comp Physiol 294: R510–R519, 2008. First published November 21, 2007; doi:10.1152/ajpregu.00458.2007.—We hypothesized that in preeclampsia (PE), contribution of endothelium-derived hyperpolarizing factor (EDHF) and the mechanism/s of its action differ from that in normal pregnancy (NP). We aimed to assess endothelial function and morphology in arteries from NP and PE with particular focus on EDHF. Arteries (≈200 μm) were dissected from subcutaneous fat biopsies obtained from women undergoing cesarean section. With the use of wire myography, responses to the endothelium-dependent agonist bradykinin (BK) were determined before and after inhibition of pathways relevant to EDHF activity. The overall responses to BK in arteries from PE (n = 13) and NP (n = 17) were similar. However, in PE, EDHF-mediated relaxation was reduced (P < 0.05). All women within the PE group were divided into two subgroups: with more (group 1) or less (group 2) than 50% reduction of EDHF-typed responses after 18-α-glycyrrhetinic acid (an inhibitor of myoendothelial gap junctions, MEGJs). The division showed that I MEGJs are principally involved when the EDHF contribution is reduced; and 2) when the EDHF contribution is similar to that in NP, the H2O2 and/or cytochrome P-450 epoxygenase products of arachidonic acid (AA), along with MEGJs, confer EDHF-mediated relaxation. In contrast, MEGJs were the main pathway for EDHF in NP. The abundant presence of MEGJs in arteries from NP but deficiency of them in PE was observed using transmission electron microscopy. We conclude that PE is associated with heterogeneous contribution of EDHF, and the mechanism behind EDHF-typed responses is mediated either by MEGJs alone or in combination with H2O2 or cytochrome P-450 epoxygenase metabolites of AA.

gap junctions; small arteries; pregnancy

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 (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

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
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 MEJs.

**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 ≥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 μm 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 circumference-tension curve was created for each segment to set optimum resting tension. This resting tension is calculated to simulate an in vivo transmural pressure of 100 mmHg. Arteries were then set at 90% of this tension to enable optimal contractile conditions with a low resting tension, as described previously. Calibration and data processing were performed using Myodac software (version 2.1, Danish Myo Technology) on a personal computer. All solutions were refreshed every 30 min.

Five reference constrictions were elicited. The first, second, and fifth contractions were produced using a high (124 mmol/l) potassium solution (KPSS, made by equimolar substitution of KCl for NaCl in PSS) containing 1 μmol/l norepinephrine (NE). The third was obtained with NE (1 μmol/l) alone and the fourth with KPSS alone.

**Experimental protocols.** All arteries were preconstricted with NE (3 μmol/l), and a concentration-response curve was obtained using incremental concentrations of BK (1 μmol/l to 3 μmol/l). Arteries were then incubated for 20 min in either PSS alone or with the nitric oxide synthase (NOS) inhibitor Nω-nitro-l-arginine methyl ester (l-NAME; 300 μmol/l) and the cyclooxygenase (COX) inhibitor indomethacin (Indo; 10 μmol/l) to block the production of nitric oxide (NO) and prostacyclin (PGI2), respectively. Subsequently, arteries were preconstricted again, and a second concentration-response curve for BK was obtained. The term “EDHF” used in this study refers to the l-NAME- and Indo-insensitive component of endothelium-dependent vasodilatation in response to BK. To establish whether adequate NOS inhibition was achieved with l-NAME, a concentration-response curve for BK was assessed in a separate set of arteries after incubation with l-NAME + Indo alone or in the presence of the selective guanylate cyclase inhibitor 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ).

To assess whether arachidonic acid (AA) metabolites play a role in EDHF-mediated relaxation, concentration-response curves for BK were constructed in the presence of l-NAME and Indo after co-incubation with sulfaphenazole (10 μmol/l, 30 min), a specific inhibitor of cytochrome P-450 (CYP450) epoxygenase. To address the role of H2O2 in the mediation of EDHF-type responses to BK, similar experiments were performed in the presence of l-NAME and Indo with catalase (1,250 U/ml). Catalase causes the enzymatic dismutation of H2O2 to form water and oxygen. Finally, to evaluate the contribution of gap junctional communication in BK-induced EDHF-type responses, the protocols above were performed after 15-min incubation with 18-α-glycyrrhetinic acid (18-αGA; 100 μmol/l), a reversible inhibitor of gap junctional communication. We elected to use 18-αGA, despite recent debate about its specificity (7, 16), because when we previously assessed BK-induced relaxation of subcutaneous arteries from NP women, we did not find any differences between the inhibitory actions of 18-αGA 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 μmol/l to 100 μmol/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 CaCl2, 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 CaCl2, pH 7.4; postfixed in 2% osmium tetroxide in 0.07 M sodium cacodylate buffer containing 1.5 mM CaCl2, pH 7.4, at 4°C for 2 h; and dehydrated in ethanol followed by acetic 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, CaCl2 2.5, MgSO4 1.71, NaHCO3 25, KH2PO4 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 by the arteries. The relaxation response to BK was calculated as a percentage of the contraction. Negative log concentration required to cause 50% of the maximum response (pEC50) 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 pEC50 before and after incubation with different agents in arteries used in different experimental protocols. The number and length (μm) 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 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 PSS alone, indicating that BK-mediated dilatation was reproducible (pEC50: 7.4 ± 0.2 first vs. 7.7 ± 0.2 second concentration-response curve, n = 5).

Concentration-response curves for BK in PSS were similar between arteries from PE and NP (pEC50: 7.3 ± 0.1 vs. 7.4 ± 0.1, 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% for PE 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
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 [pEC₅₀: 6.9 ± 0.1 vs. 6.8 ± 0.1 (n = 8), P > 0.5; Fig. 5B] and were also similar in arteries from NP women [pEC₅₀: 6.8 ± 0.2 vs. 6.7 ± 0.2 (n = 6); Fig. 5C].

Thus, in addition to MEGJs, endothelial H₂O₂ 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 μmol/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 18α-GA alone but not in combination with L-NAME+Indo significantly reduced NE-induced tone (27), all specificity experiments for 18α-GA were performed after preincubation of arteries with L-NAME+Indo. Similar responses to SNP and pinacidil after incubation with L-NAME+Indo alone or in combination with 18α-GA were observed.

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 18α-glycyretinic acid (L-NAME+Indo+18α-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 18α-GA]. B: comparison between arteries from NP and PE (i.e., arteries that demonstrate a minor reduction of EDHF-mediated relaxation by 18α-GA). *P < 0.05, PE (L-NAME+Indo) vs. NP (L-NAME+Indo); #P < 0.05, PE (L-NAME+Indo) vs. PE (18α-GA+L-NAME+Indo); &P < 0.05, NP (L-NAME+Indo) vs. NP (18α-GA+L-NAME+Indo); §P < 0.05, PE (18α-GA+L-NAME+Indo) vs. NP (18α-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 18α-GA; PE-2 group, arteries that demonstrated a minor reduction of EDHF-mediated relaxation by 18α-GA. *P < 0.05, PE-1 vs. PE-2.

arteries from PE and NP women [pEC₅₀: 6.6 ± 0.1 (n = 9) vs. 6.7 ± 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...
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 H2O2 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. Our study further strengthens the increasingly accepted hypothesis that a single factor or mechanism acting as a “universal” EDHF does not exist (26). Accordingly, its identity or mechanism may vary depending on the species or type of artery examined. Furthermore, as exemplified in our study, its identity may be altered in the diseased state, and there is further evidence to support this. For example, dyslipidemia may duplicate the mechanisms responsible for EDHF-induced dilatation in small arteries of mice (22); although it has been demonstrated that CYP450 AA metabolites can account for EDHF activity in healthy nonpregnant volun-

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>pEC50</th>
<th>Relaxation, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNP</td>
<td>6</td>
<td>6.5±0.1</td>
<td>94±1</td>
</tr>
<tr>
<td>SNP after sulfaphenazole</td>
<td>3</td>
<td>6.5±0.1</td>
<td>97±1</td>
</tr>
<tr>
<td>SNP after catalase</td>
<td>3</td>
<td>6.7±0.2</td>
<td>93±3</td>
</tr>
<tr>
<td>SNP after l-NAME+Indo</td>
<td>5</td>
<td>6.6±0.2</td>
<td>85±7</td>
</tr>
<tr>
<td>SNP after l-NAME+Indo+18-αGA</td>
<td>5</td>
<td>6.1±0.2</td>
<td>74±5</td>
</tr>
<tr>
<td>PIN</td>
<td>6</td>
<td>5.7±0.1</td>
<td>85±3</td>
</tr>
<tr>
<td>PIN after sulfaphenazole</td>
<td>3</td>
<td>6.1±0.3</td>
<td>87±4</td>
</tr>
<tr>
<td>PIN after catalase</td>
<td>3</td>
<td>6.7±0.5</td>
<td>85±1</td>
</tr>
<tr>
<td>PIN after l-NAME+Indo</td>
<td>5</td>
<td>5.5±0.2</td>
<td>60±8</td>
</tr>
<tr>
<td>PIN after l-NAME+Indo+18-αGA</td>
<td>5</td>
<td>5.2±0.1</td>
<td>52±8</td>
</tr>
</tbody>
</table>

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-αGA, 18-α-glycyrrhetinic acid.

Fig. 5. Concentration-response curves for BK in isolated subcutaneous arteries from NP, PE, and PE-2 after incubation with l-NAME+Indo in combination with sulfaphenazole (Sulf+l-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. The findings concur with our previous report demonstrating endothelial projections toward SMCs (see Fig. 7, 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).

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-mediated 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.

ACKNOWLEDGMENTS

We are grateful to Dr. Ninian N. Lang for critical reading of the manuscript.

GRANTS

This work was supported by grants from the Swedish Heart and Lung Foundation, Center of Gender-Related Medicine at Karolinska Institutet, the Swedish Society of Medicine, and Anders Otto Swards and Lars Hiertas Minne Foundations.

REFERENCES


