Vascular dysfunction in preeclampsia
van Wijk, M.J.

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Chapter 11

Endothelial dysfunction in the uterine circulation in preeclampsia: can estrogens improve it?


Abstract

Objective: To evaluate whether a 3-hour incubation with 17β-estradiol will enhance flow- and BK-mediated dilatation and alter pressure-induced basal tone in myometrial resistance arteries from women with preeclampsia, and to evaluate the role of NO in the responses observed.

Methods: Flow- and BK-mediated dilatation and responses to intraluminal pressure of 60 and 80 mmHg were compared before and after 3 hours incubation with 17β-estradiol (10 nmol/L) in isolated myometrial arteries using pressure myography technique. In separate experiments the role of NO on 17β-estradiol-induced responses was evaluated in the presence of the NOS inhibitor (L-NAME, 0.1 mmol/L). Endothelial morphology was evaluated by scanning electron microscopy.

Results: Incubation with 17β-estradiol significantly improved flow-mediated dilatation as compared to initial flow-mediated response in arteries from women with preeclampsia. This effect was NO-mediated, since L-NAME abolished the response. Arteries from women with preeclampsia demonstrated impaired BK-mediated dilatation compared to that obtained in arteries from normal pregnant women. 17β-estradiol had no effect on BK-mediated dilatation in arteries from women with preeclampsia. The enhanced pressure-induced tone at 80 mmHg as compared to tone developed at 60 mmHg in arteries from women with preeclampsia was reduced after incubation with 17β-estradiol. This reduction was also NO-mediated. Morphological signs of endothelial dysfunction were evident in arteries from women with preeclampsia.

Conclusions: 17β-estradiol improved impaired flow-mediated dilatation and reduced basal tone through a NO-mediated pathway in isolated myometrial arteries from women with preeclampsia.
Introduction

Endothelial dysfunction is considered to be a central pathogenic feature of preeclampsia. This is supported by alterations in a variety of biochemical markers, and by functional studies in isolated resistance artery preparations by measuring dilatory response to endothelium-dependent agonists or flow-mediated dilatation [255]. A current hypothesis suggests that compromised endothelial function in preeclampsia may be related to oxidative stress, which is developed under influence of enhanced lipid peroxidation [312] and impaired availability of antioxidant agents [73].

In view of substantial evidence of endothelial dysfunction in preeclampsia treatment options should be designed towards reduction, repair or prevention of endothelial damage. Estrogens are known to have antioxidative effects [313] and to cause both immediate endothelium-independent [314] and -dependent dilatation [315] as well as longer-term endothelium-dependent responses, which may be genomic in origin and primarily involve the regulation of endothelial NOS [316]. Moreover, in cultured endothelial cells estrogens may stimulate a calcium independent pathway of NO synthesis, similar to that obtained by flow-induced shear stress-mediated stimulation of NO synthesis facilitated by heat shock protein 90 [317].

Preeclampsia is associated with reduced placental estrogen production, reduced supply of fetal estrogen precursors and an impaired conversion of these compounds into estrogens by the placental tissue resulting in decreased maternal plasma estrogen levels [318]. The lower risk for breast cancer later in life for women who experienced preeclampsia [319] has been attributed to a reduced estrogen effect.

The present study was undertaken to evaluate whether a 3-hours incubation with 17β-estradiol may improve endothelial function in isolated myometrial arteries from women with preeclampsia, and, if so, whether this improvement is NO-mediated. Endothelial function was estimated by evaluation of flow-mediated dilatation, which is known to be NO-mediated in these arteries during normal pregnancy and is absent in preeclampsia [123]. Impaired BK-mediated dilatation in isolated myometrial arteries from women with preeclampsia has been demonstrated previously by using conventional wire-myography technique [129]. In our study we used a more physiological approach, i.e. a pressure myography system, in order to confirm endothelial dysfunction and to investigate whether estrogens may improve this response. In addition, the role of estrogens on the modulation of pressure-induced tone was determined. Finally, we used scanning electron microscopy technique to compare the surface morphology of the endothelium in myometrial arteries from women with preeclampsia and normal pregnant women.

Methods

Subjects

The ethical committee of Huddinge University Hospital approved this study and all women gave their informed consent to participate. Twenty women with preeclampsia (15 nulliparous)
with a median age of 34 years (range 22-38) and a median gestational age of 35 weeks (range 31-37) undergoing cesarean delivery for deterioration of the preeclamptic condition were enrolled. Women, who had received antihypertensive agents, were excluded. The control group included 27 healthy pregnant women (18 nulliparous) with a median age of 36 years (range 21-42) and a median gestational age of 39 weeks (range 37-41) undergoing elective cesarean delivery due to breech presentation (n=12), previous cesarean delivery (n=8) or psychosocial reasons (n=7). The biopsies were taken immediately following delivery, from the upper edge of the transverse incision in the lower uterine segment.

**Experimental set-up**

Intramyometrial small arteries (internal diameter approximately 200 μm and 2.5-3 mm length) were immediately dissected from the biopsies, carefully removing surrounding myometrium, connective tissue and adventitia. The myometrial arteries were then mounted in a pressure arteriograph (Living Systems Instrumentation Inc; Burlington, USA), as previously described [123]. The vessels were orientated in the in vivo direction of flow on a pair of opposing glass microcannulae matched for flow resistance. The organ bath was perfused (7 mL/min) with PSS. A servo-controlled pump maintained the required intraluminal pressure and the internal diameter of the artery was recorded continuously using a video dimension analyzer. “in-line” pressure transducers monitored the proximal and distal pressure on each side of the specimen, enabling calculation of the mean intraluminal pressure. Each artery was equilibrated for 60 minutes while pressurized to 50-60 mmHg.

**Effects of 17β-estradiol on flow-mediated dilatation**

After the equilibration period, the intraluminal pressure was increased to 80 mmHg for 30 minutes and internal diameter was recorded thereafter. NE (1 μmol/L) was then added to the superfusate for 30 minutes and internal diameter was recorded before intraluminal flow was initiated using a flow pump. Flow was increased at 5 minute intervals (from 0 to 204 μL/min) and the internal diameter was recorded at the end of each flow step. During flow establishment at different flow rates, the proximal and distal pressure gradient was controlled by changing the distal pressure in order to keep the intraluminal pressure constant i.e. 80 mmHg. After achievement of the first flow response curve intraluminal flow was stopped and intraluminal pressure was kept at 80 mmHg. The vessel was then incubated with 17β-estradiol in PSS (10 nmol/L, 3 hours, and 37°C). Estradiol was then removed by washing with PSS (30 minutes) and the flow-response assessment was repeated. In order to find out whether the effect of 17β-estradiol on flow-mediated dilatation is NO-mediated, in separate arteries the first flow-mediated response was performed after initial incubation with 17β-estradiol (10 nmol/L) and a washing period for 30 minutes with PSS and repeated after incubation with the NOS-inhibitor L-NAME (0.1 mmol/L). At the end of each experiment, while pressure was maintained at 80 mmHg, the arteries were incubated in calcium-free solution with EGTA (1 mmol/L) and papaverine (0.1 mmol/L) for 30 minutes and the increase in internal diameter was recorded.
Effects of 17β-estradiol on BK-mediated dilatation

In separate arteries after an equilibration period at 60 mmHg NE (1 μmol/L) was added to the superfusate for 30 minutes to achieve a stable constriction. Concentration-response curves were constructed by addition of cumulative concentrations of BK (1 nmol/L to 1 μmol/L) in NE-substituted PSS, application of each concentration lasting 3 minutes. Dilatation to BK was determined before and after pre-incubation with 17β-estradiol (10 nmol/L, 3 hours), as in the flow protocol described above. Finally, while pressure was maintained at 60 mmHg, the arteries were incubated in calcium-free solution for 30 minutes and the increase in internal diameter was recorded.

Scanning electron microscopy

Isolated small myometrial arteries from women with preeclampsia and normal pregnant women were carefully dissected and immersed in 2.5% glutaraldehyde solution in a sodium cacodylate buffer (0.15 mol/L, pH 7.3, 24 hours). The arteries were then post fixed in 1% osmium tetroxide in sodium cacodylate buffer (0.15 mol/L, pH 7.3) containing 75 mmol/L sucrose. They were then dehydrated in acetone and dried in a critical point drier with carbon dioxide. The samples were mounted, coated with gold palladium and examined under a JEOL 820 scanning electron microscope.

Statistical analysis

Values in the text and figures are given as mean ± SEM. Flow- and BK-mediated dilatation were calculated as a percent change in internal diameter from initial preconstriction with NE. The flow- and BK-mediated responses obtained in arteries from preeclamptic and normal pregnant women under different treatment conditions were compared by RM-ANOVA (StatSoft Inc; Tulsa, USA). The difference in internal diameter of the arteries when pressurized at 60 and 80 mmHg before and after equilibration in calcium-free PSS (calcium-free PSS with EGTA) provides an estimate of pressure-induced myogenic tone, which was calculated from the following equation:

Myogenic tone (%) = (ID calcium-free PSS - ID PSS) / ID calcium-free PSS) × 100, in which ID=internal diameter. Pressure-induced tone was compared within and between the groups with paired and unpaired t tests respectively. Significance was assumed if P < .05.

Results

The arteries obtained from women with preeclampsia and women with normal pregnancy had similar diameters when equilibrated in PSS alone at 60 mmHg (212 ± 24 μm (n=23) vs 228 ± 37 μm (n=24), respectively).
17β-Estradiol and flow-mediated dilatation

In accordance with a previous study [123], we found that myometrial arteries from women with preeclampsia failed to dilate in response to increasing intraluminal flow (% change from initial preconstriction -8 ± 6% at the maximum flow rate of 204 µl/min, figure 1). In contrast, following 3 hours incubation with 17β-estradiol these arteries demonstrated a substantial dilatation in response to flow (60 ± 19% at the maximum flow rate of 204 µl/min, RM-ANOVA, F = 4.6, P = .006, n=7, figure 1). This estrogen-induced upregulation of flow-mediated dilatation was NO-mediated, since in separate arteries the increased vasodilatation in response to increase in intraluminal flow obtained after 3 hours incubation with 17β-estradiol was reversed after NOS-inhibition with L-NAME (50 ± 15% after 17β-estradiol vs 5 ± 15% after L-NAME at the maximum flow rate of 204 µl/min, RM-ANOVA; F = 8.7, P = .02, n=4, figure 2).

Figure 1. Flow-mediated dilatation in myometrial arteries from women with preeclampsia in PSS (open circles) vs after incubation with 17β-estradiol (black circles) (n=7).
17β-Estradiol and BK-mediated dilatation

Arteries obtained from women with preeclampsia demonstrated a reduced dilatation to increasing concentrations of BK in comparison with dilatation obtained in arteries from normal pregnant women (e.g. at concentration of 1 µmol/L, % change from initial preconstriction was 90 ± 25% (n=12) vs 175 ± 14% (n=24), RM-ANOVA; F = 6.4, P = .02, figure 3). Pre-incubation with 17β-estradiol did not affect BK-mediated dilatation in arteries obtained from women with preeclampsia (figure 4).

Figure 2. Flow-mediated dilatation in myometrial arteries from women with preeclampsia after incubation with 17β-estradiol (black circles) vs L-NAME (open circles) (n=4).

Figure 3. BK-mediated dilatation in PSS in isolated myometrial arteries from normal pregnant women (black circles, n=24) and women with preeclampsia (open circles, n=12).
**Figure 4.** BK-mediated dilatation in isolated myometrial arteries from women with preeclampsia in PSS (black circles) and after incubation with 17β-estradiol (open circles) (n=7).

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**17β-Estradiol and pressure-induced myogenic tone**

Myometrial arteries from women with preeclampsia developed pressure-induced tone at 60 mmHg that was similar before and after incubation with 17β-estradiol (11 ± 2% vs 11 ± 2%, n=7). When pressurized to 80 mmHg, developed tone was enhanced in comparison with that obtained at 60 mmHg (33 ± 4%, n = 7 vs 13 ± 3%, n=12, P < .001, fig 5). In contrast, 3 hours incubation with 17β-estradiol significantly reduced pressure-induced tone at 80 mmHg (33 ± 4% in PSS vs 11 ± 2% after 17β-estradiol, n=7, P < .001, figure 5). The effect of 17β-estradiol on the reduction of pressure-induced myogenic tone was due to a dilatory influence of NO, since in separate arteries, L-NAME significantly reversed the myogenic tone developed at 80 mmHg after initial incubation with estradiol to a value similar to that obtained in PSS alone (13 ± 1% after 17β-estradiol vs 42 ± 5%, P < .01, n=4, figure 5).
Figure 5. Pressure-induced tone in isolated myometrial arteries from women with preeclampsia in PSS and after incubation with 17β-estradiol or L-NAME.

* P < .001 for tone in PSS at 60 mmHg (n=12, 5 additional arteries included, that were not exposed to 17β-estradiol) vs 80 mmHg (n=7).
§ P < .001 for tone at 80 mmHg in PSS (n=7) vs after incubation with 17β-estradiol (n=7).
# P < .01 for tone at 80 mmHg after incubation with 17β-estradiol (n=4) vs L-NAME (n=4).

Scanning electron microscopy

The entire surface of arterial lumen obtained from arteries from women with preeclampsia (n=6) and arteries from women with normal pregnancy (n=5) was examined by using scanning electron microscopy with particular attention to the quality of endothelial cell plasma membranes, the presence of degenerated/detaching cells, and the presence of attached erythrocytes, platelets or other particles. Striking differences in respect to morphology between arteries from normal pregnant women and women with preeclampsia were noted (figure 6). In normal myometrial arteries, a continuous sheath of elongated endothelial cells with apparently thick intact plasma membranes covered the luminal surface of the intima. These cells were tightly connected (figure 6A-C). In arteries from women with preeclampsia, many endothelial cells were shrunken, with blebbing and thin plasma membranes detaching from basal lamina. This morphology, which was observed in all arteries from women with preeclampsia, could be compatible with apoptotic cell death (figure 6D). On the other areas of the vascular intima platelets and nonspecific protein aggregates were adhering (figure 6E). In addition, endothelial cell layers with disruption of intercellular junctions were observed in myometrial arteries from women with preeclampsia (figure 6F).
Figure 6. Scanning electron microscopy pictures (× 1125 fold magnification) of the endothelial cell layer: myometrial artery from a normal pregnant woman (A, B, C), arteries from women with preeclampsia (D, E, and F). For details see “Results” section.
In this study we have confirmed that endothelium-dependent dilatation in response to flow-mediated shear stress and BK are impaired in small arteries from the uterine circulation in preeclampsia. Both shear stress, generated by flow, and BK, produced by endothelial cells, has been suggested to be important physiological agonists for the release of endothelium-derived factors and contribute significantly to the vasodilatation seen in normal pregnancy [255]. The impairment of these responses would lead to the development of increased vascular resistance in the uterine circulation in preeclampsia. Morphological alterations (i.e. shrunken, detached, degenerated thin cell membranes or signs of endothelial cell death, cells adhesion, enlarged intercellular junctions) in small myometrial arteries give further support for an important role for endothelial malfunction in the uterine circulation in this disease. These morphological findings would at least partly explain the enhanced vascular permeability through enlarged intercellular junctions and is in line with an activated coagulation system in preeclampsia. Whether endothelial cell death in vascular endothelium found in our study is compatible with apoptosis needs to be confirmed with other techniques for assessment of apoptosis, although increased apoptosis and altered expression of different mediators for apoptosis in the placenta from women with preeclampsia has been demonstrated by others [320]. Whether persistence of endothelial cell death in the endothelium of myometrial arteries from women with preeclampsia is a consequence of estrogen deficiency, oxidative stress or impaired NO production remains unknown. However, a similar morphological pattern has been observed in our laboratory when using isolated arteries from women after menopause (unpublished observation).

Estrogens are considered to confer cardiovascular protection in women after menopause or in the aging population with hypertensive disorders [321]. To our knowledge, this is the first in vitro study demonstrating beneficial effects of estrogen on dysfunctional endothelium in women at reproductive age. Incubation with estrogens significantly improved the impaired flow-mediated dilatation in isolated myometrial arteries from women with preeclampsia. In addition, it also reduced pressure-induced myogenic tone known to be enhanced in this disease [123]. NO mediated both effects, since inhibition of NOS abolished the responses. In the in vivo situation this would reflect an increase in blood flow with the reduction of the vascular resistance in the uteroplacental circulation. Our results are thus in line with other studies demonstrating estradiol-induced reduction in basal tone and up-regulation of flow-mediated dilatation in small arteries from hypertensive male and female rats [322,323] or in small subcutaneous arteries from postmenopausal women [324]. Thus, reduced pressure-induced tone and improved flow-mediated dilatation seem to be important mechanisms of estrogen action in the vasculature. In contrast, impaired BK-mediated dilatation was not affected by three-hour incubation with 17β-estradiol in our study. This might indicate the presence of different regulatory mechanisms by which 17β-estradiol upregulates release of vasodilators in response to pressure, flow-mediated shear stress and BK.
Initial studies suggested that the beneficial effects of estrogen on vascular tone are related to pathways associated with calcium dynamics in smooth muscle cells [325]. However, the physiological relevance of these findings is far from clear, since pharmacological concentrations used were far above those achieved under *in vivo* conditions. At physiological levels, the main effect of estrogens on pressure-induced tone is to open vascular smooth muscle calcium-activated potassium channels, which occurs via a NO/cyclic GMP-dependent pathway as demonstrated in pressurized small arteries obtained from ovariectomized animals [326]. In our study, estrogen-induced reduction in pressure-induced tone was NO-mediated. However, it has to be proven whether this effect occurred through the same mechanism as described above.

17β-estradiol at physiological concentrations may upregulate calcium-dependent endothelial NOS, as demonstrated in cultured endothelial cells [327] and in isolated arteries obtained from female animals in different hormonal environments [316]. In addition, increasing evidence suggests that estrogens may upregulate flow-mediated NO release through mechanisms peculiar to this process. Thus estradiol has been shown to activate endothelial NOS via tyrosin kinases or mitogen-activated protein kinase-dependent mechanisms, through heat shock protein 90-binding [317] or through activation of endothelial NOS, involving phosphatidylinositols binding 3-kinase-Akt [328]. These pathways are unique to a limited number of stimuli, including primarily flow-induced shear stress, but not agonist, such as BK, dependent pathways. This could explain the effects of 17β-estradiol on flow- but not on BK-mediated dilatation in myometrial arteries from women with preeclampsia. We have previously suggested, that preeclampsia might be associated with impairment of several shear-mediated signal transduction pathways involved in the activation of endothelial NOS, rather than changes in NO activity *per se*, since the basal release of NO was similar in arteries from both normal and preeclamptic women [123]. Taken together it seems that estradiol might upregulate one or several signal transduction pathways involved in the activation of endothelial NOS and leading to NO release in response to flow-induced shear stress in myometrial arteries from women with preeclampsia. Recently, it has been demonstrated that reactive oxygen species are involved as physiological signaling mediators in activation of endothelial NOS and NO release in response to shear stress [329,330], although the biological effects are critically dependent on reactive oxygen species concentration. Whether estrogens in the presence of oxidative stress in preeclampsia may act as a stabilizer between pro- and antioxidant reactions to regulate production of reactive oxygen species remains to be determined.

It cannot be assumed that the lack of effect of estradiol on the blunted BK response would pertain to other endothelium-dependent vasodilators e.g. ACh. It also remains to be determined whether the abnormal BK responses in these vessels are primarily due to a lack of NO or other vasodilators and whether the effects are tissue specific since incubation with estradiol significantly improved BK-mediated dilatation in small subcutaneous arteries from postmenopausal women (unpublished observation).

In our study we have not elucidated whether the effects of 17β-estradiol on NO release were rapid or longer-term genomic in origin. Estrogens may evoke responses within several minutes in cell cultures, and during *in vivo* experiments. The rapid responses associated with increase in NO
Estrogens and endothelial dysfunction

The synthesis in endothelial cell cultures has been shown to be short lived and reversible [331], but the physiological role of these rapid effects in vivo remains poorly understood and needs to be clarified. Our intention was to investigate the longer-term genomic effect. We can, however, not exclude the possibility that the achieved effects in our study are rapid in origin or a combination of both. In this context it should also be emphasized the mechanism of estrogen induced NO release when extraluminal incubation with 17β-estradiol is applied, as in the present study, the ligand may need a relatively longer time to reach the endothelial cells as compared with a cell culture model.

Several studies in isolated small arteries from hypertensive animals [322] and cultured endothelial cells [327] have suggested a predominant role for estrogen receptor-alpha in estrogen-induced upregulation of NO release. There is evidence for the presence of a rapid acting membrane receptor for the estrogen [332]. This corroborates with a recent finding of a subpopulation of estrogen receptor-alpha to be localized in the endothelial cell caveolae, where they are coupled to endothelial NOS in a functional signaling module [331]. It has been recently suggested that the rapid effect caused by physiological levels of estrogen is mediated, at least in part, by the action of the same receptor that could act as a transcription factor to mediate the genomic effects of estrogen on vascular gene expression [332]. Ongoing studies in our laboratory will aim to elucidate the role of this receptor in the initiation of responses in arteries from women with preeclampsia.

Both estrogen and NO play critical roles in blood vessel development, function or remodeling [321]. Understanding of the interaction between these two powerful, interdependent vascular modulators may be the first step in designing strategies for treatment in conditions such as ischemic, diabetic, postmenopausal and preeclamptic vascular dysfunction. We have presented data demonstrating that estrogen improves endothelial function through upregulation of flow-mediated dilatation and reduction of pressure-induced myogenic tone in resistance myometrial arteries from women with preeclampsia. These effects were NO-mediated and might suggest a possible role for estrogen to improve uteroplacental blood flow in preeclampsia.

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