Vascular dysfunction in preeclampsia
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Citation for published version (APA):
Chapter 9

Resistance artery smooth muscle function in pregnancy and preeclampsia


Abstract

Objective: The purpose of this study was to investigate whether the altered vascular resistance in pregnancy and preeclampsia results from alterations in intrinsic vascular smooth muscle properties or from external influences on vascular smooth muscle function.

Methods: We studied subcutaneous resistance arteries from women with preeclampsia, from normal pregnant women, and from nonpregnant women, that were obtained during cesarean delivery or gynecologic surgical procedures, in a pressure myograph. Arteries were denervated, and smooth muscle cells were loaded with calcium indicator fura-2. Contractile properties were tested in PSS and during potassium- and NE-induced constriction at various pressures. In addition, endothelial function was assessed. Intracellular calcium and tone were measured continuously.

Results: No significant differences in basal tone, constrictor and myogenic responses were found between groups. Contractile element calcium sensitivity was significantly increased in women with preeclampsia. NE caused an increase in calcium sensitivity in all groups.

Conclusion: Vascular smooth muscle calcium sensitivity is increased in preeclampsia.
Introduction

In normal pregnancy extensive changes of the cardiovascular system occur to accommodate the demands of pregnancy. These adaptations are characterized mainly by an increase in blood volume, an increase in cardiac output, a fall in blood pressure, a fall in peripheral resistance, and a generalized vasodilatation. Preeclampsia is a multisystem disorder of pregnancy, in which a malfunctioning vascular system exists. This condition is characterized by an elevation in blood pressure and proteinuria and by a reduction in plasma volume, an increase in peripheral resistance and a generalized vasoconstriction. It usually develops after 20 weeks of gestation and resolves after delivery of the placenta. Preeclampsia is still one of the major causes of maternal and perinatal death and morbidity. Current treatment consists of symptom relief and ultimately delivery [255]. A better understanding of the control of vascular tone in pregnancy and preeclampsia could form a basis for development of new intervention strategies for treatment of preeclampsia.

In vivo vascular tone depends on intrinsic basal tone and on the acute effect of many factors of hormonal, neural, and endothelial origin. Many investigators have reported alterations in endothelial function [4,292] and sympathetic nervous activity [119] in both normal pregnancy and preeclampsia and the presence of factors, which circulate in the maternal blood, that can cause endothelial dysfunction in preeclampsia [4]. It is, however, unclear whether the altered levels of tone that are observed in normal pregnancy and preeclampsia are due only to changes in these external influences on vascular smooth muscle, or whether intrinsic alterations in vascular smooth muscle cell signaling are also involved. The in vitro study of human resistance arteries could resolve this question. However, such studies are rare in pregnancy and preeclampsia and have been limited to the constrictor responses of isolated arteries [89,148,150]. Understanding vascular smooth muscle properties and changes therein could be of pivotal importance for the development of new pharmacotherapeutic intervention strategies in preeclampsia. Accordingly, our goal was to study tone and responsiveness of isolated, cannulated, human subcutaneous arteries in normal pregnancy and in preeclampsia. From animal experiments it has become clear that vascular tone and responsiveness depend on modulations in both the intracellular calcium concentration and the sensitivity of the contractile machinery to calcium [293]. Vascular smooth muscle calcium handling has not been determined in pregnancy and preeclampsia, and measurements of intracellular calcium were therefore included in this study.

Methods

Subjects

This study was performed in the Academic Medical Center and the Onze Lieve Vrouwe Hospital after obtaining approval of the local ethical committees of both hospitals. After obtaining informed consent from the patients, subcutaneous fat biopsy specimens were taken during cesarean delivery from women with preeclampsia and normal pregnant women and at elective abdominal surgical procedures from nonpregnant women. Women with preeclampsia
who had been treated with calcium channel blockers were excluded from the study. Nonpregnant control subjects were women who underwent elective abdominal surgical procedures for nononcologic gynecologic reasons.

**Experimental set-up**

Subcutaneous fat biopsy specimens that were taken during operation were placed immediately in ice-cold PSS and stored in the refrigerator until dissection was started. The biopsy specimens were placed in a cooled dissection chamber that was kept continuously at 0 to 4°C; resistance arteries in the range of 100 to 200 µm in diameter were identified and carefully dissected from the surrounding tissue. The arteries were cannulated at both ends on glass microcannulas and sutured with 17 µm nylon filaments. After cannulation the arteries were pressurized to 100 mmHg for approximately 1 minute and stretched until they appeared straight. Arteries were pressurized through both cannulas, with the use of a Fairchild voltage-pressure converter. A pressure gradient between both pipettes of approximately 1 mmHg was applied to provide a small refreshment flow in the artery. This subphysiological flow did not cause flow-induced dilatation or constriction. Arteries were immersed in PSS. The intraluminal perfusion fluid consisted of PSS that was supplemented with 0.5% BSA. The inner diameter of the artery was determined continuously from processing of a video signal by self-built analogue hardware. For the measurement of fura-2, excitation was achieved with a 75-W xenon light source and a filter wheel that rotated at approximately 40 Hz and contained 340- and 380-nm interference filters. The 510-nm fluorescence intensities at each excitation wavelength were measured by a photomultiplier tube, recorded at a time interval of 250 msec, and stored with the diameter signal on a computer.

**Experimental protocol**

First, viability of the arteries was tested. Arteries that failed to constrict to NE 1 µmol/L were excluded from the study. After an equilibration period of 30 minutes at 60 mmHg, the arteries were denervated by superfusion with 6-hydroxy-dopamine (0.3 mg/mL) in PSS, without NaHCO₃ and with 10 mmol/L HEPES buffer (pH 4.0), at 30°C for 10 minutes, followed by a washout period of 20 minutes at 37°C. This denervation step was performed to prevent the possible release of neurotransmitters from nerve endings during potassium-induced depolarization later in the experiment. Subsequently, the arteries were loaded with the calcium indicator Fura-2 AM. Fura-2 AM (50 µg) was dissolved in 50 µL dimethyl sulphoxide that contained 2% pluronic, and suspended in 5 mL of PSS. The arteries were then superfused with this solution in the dark at approximately 30°C for 1 hour, followed by a washout period of 30 minutes at 37°C. After denervation and fura-2 loading, the arteries were preconstricted with NE 1 µmol/L and endothelium-dependent dilatation to ACh 1 µmol/L and to BK 0.1 µmol/L was determined at 60 mmHg. Both dilators were applied for 4 minutes with a washout period of 4 minutes between and afterwards. Tone and calcium responses of the arteries were determined under basal conditions in PSS and during NE 1 µmol/L- and potassium 36 mmol/L-induced
constriction at pressures of 20, 60, and 100 mmHg, each pressure being applied for 3 minutes. At the end of the experiments, maximal and minimal fluorescence values for the 340/380 fura-2 ratio were determined in the presence of 2 μmol/L ionomycin and, respectively, 10 mmol/L CaCl₂ and 10 mmol/L EGTA in calcium-free PSS. During the application of the calcium-free solution the passive diameter-pressure relationship was determined at pressures 5, 10, 20, 30, 60, and 100 mmHg. Finally, the background fluorescence level was determined after quenching with 20 mmol/L manganese chloride.

**Chemicals**

All vasoactive agents were applied by changing the superfusion solution. NE, ACh, BK, and ionomycin were obtained from Sigma Chemical Co (St Louis, USA). Fura-2 AM was obtained from Molecular Probes (Leiden, The Netherlands). All substances were dissolved in PSS, unless described otherwise.

**Data analysis**

ACh- and BK-mediated dilatation were calculated by the formula:

\[
\% \text{ dilatation} = \left( \frac{D_{\text{DH}} - D_{\text{NE}}}{D_P - D_{\text{NE}}} \right) \times 100\%,
\]

where \(D_{\text{DH}}\) is the diameter with ACh or BK, \(D_{\text{NE}}\) is the diameter after preconstriction with NE, and \(D_P\) is the passive diameter at 60 mmHg. Basal tone in PSS and NE- and potassium-induced tone were calculated as the percentage constriction from the passive diameter at equal pressure. The intracellular calcium concentration was expressed as the 340/380 fura-2 ratio, normalized to the minimal ratio and corrected for the background fluorescence. We tested for differences in calcium sensitivity between the groups in the following manner: in a general linear ANOVA model over all data in PSS, tone was assumed to depend on calcium and on patient group. In this model, calcium was treated as a continuous covariate; hence the effect of calcium was determined by linear regression, while the patient group is treated as a fixed factor, as usual. Bonferroni post-hoc tests were then performed for testing which of the patient groups were different in respect to calcium sensitivity. To visualize these differences, we have plotted both the calcium-tone relations for the 3 groups (figure 3) and the determined deviation from the overall calcium-tone relation for individual data points (figure 4). ANOVA and RM-ANOVA were also used to test for other differences between groups and changes within groups. All statistical analyses were performed with SPSS for Windows, version 10.0.7 (SPSS Inc., Chicago, USA). All data are presented as mean ± SEM, unless indicated otherwise. Differences were considered to be significant when \(P \leq .05\).

**Results**

Subcutaneous resistance arteries from 24 women were used for this study, 1 artery per woman. The demographic data of these women are presented in table I. Systolic and diastolic blood pressures were significantly higher in the women with preeclampsia, compared with the normal pregnant and nonpregnant women. Gestational age at delivery tended to be lower in the women.
Table I. Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Preeclampsia</th>
<th>Normal pregnant</th>
<th>Nonpregnant</th>
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<tbody>
<tr>
<td>n</td>
<td>7</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Age (y)</td>
<td>29.8 ± 1.7</td>
<td>32.0 ± 1.2</td>
<td>34.0 ± 1.8</td>
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<tr>
<td>Parity</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>177.5 ± 14.4*</td>
<td>112.2 ± 3.0</td>
<td>121.6 ± 6.6</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>115.0 ± 2.7*</td>
<td>69.8 ± 2.8</td>
<td>74.6 ± 2.2</td>
</tr>
<tr>
<td>Gestational age (wk)</td>
<td>34.1 ± 1.4</td>
<td>38.5 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>Birthweight (g)</td>
<td>1959 ± 316†</td>
<td>3317 ± 244</td>
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Demographic data of women with preeclampsia, normal pregnant and nonpregnant women, who were included in this study. Values represent mean ± SEM, except for parity, where values represent the median.

* P < .001, vs both normal pregnant and nonpregnant women
† P < .05, vs normal pregnant women

with preeclampsia (ANOVA P = .07) and birthweight was significantly lower in the women with preeclampsia compared with the normal pregnant women. Two women with preeclampsia received methyldopa therapy, for 1 and 3 days before delivery, respectively; 1 patient was treated with ketensin for 5 days before delivery. No other medication was taken by the women with preeclampsia or the normal pregnant women. Of the nonpregnant women, 2 women were taking oral contraceptives, and 1 woman was taking a β-blocker (propranolol) for palpitations. No obvious effect of the use of any medication on in vitro vascular function was observed. None of the women with preeclampsia, but 4 normal pregnant women, had contractions before their cesarean delivery.

No significant differences in endothelial function, tone and calcium concentrations in PSS and in response to NE and potassium were found between normal pregnant women who did and did not have contractions before delivery. The median interval between obtaining the biopsy specimen and cannulation of the artery was 2 hours (range, 1-23 hours) and was similar in all groups. When we subdivided all women in groups with a cannulation interval of 0 to 8, 8 to 16 and 16 to 24 hours, we found no differences in endothelial function, tone, and calcium concentrations in PSS and in response to NE and potassium.

The mean inner diameter of the pressurized arteries was 164.4 ± 11.9 μm and was similar in all groups. Passive mechanical properties of the arteries, as assessed from the pressure-diameter relation, were similar in all groups (figure 1). There were no differences in dilatation of preconstricted arteries to ACh 1 μmol/L and BK 0.1 μmol/L between groups (table II). All arteries developed some basal tone in PSS at all pressures (figure 2A).
Figure 1. Passive properties of subcutaneous resistance arteries of women with preeclampsia (grey diamonds), normal pregnancy (open circles) and nonpregnant women (black triangles).

Figure 2. Percentage tone (A), and fura-2 ratios (B) in isolated subcutaneous resistance arteries from women with preeclampsia (PE), normal pregnant (PR) and nonpregnant women (NP), superfused with PSS at pressures of 20 (white boxes), 60 (grey boxes), and 100 (black boxes) mmHg.

Table II. Endothelium-dependent dilatation.

<table>
<thead>
<tr>
<th></th>
<th>Preeclampsia</th>
<th>Normal pregnant</th>
<th>Nonpregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilatation to ACh</td>
<td>67.8 ± 6.8</td>
<td>74.3 ± 9.3</td>
<td>77.4 ± 13.3</td>
</tr>
<tr>
<td>Dilatation to BK</td>
<td>64.6 ± 10.9</td>
<td>80.7 ± 4.5</td>
<td>68.0 ± 12.9</td>
</tr>
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</table>

Values represent mean percentage dilatation ± SEM to ACh $10^{-6}$ M and BK $10^{-7}$ M in women with preeclampsia, normal pregnancy and nonpregnant women. There were no statistically significant differences between groups.
The amount of tone that was developed was not statistically different when comparing the groups, although there was a clear trend towards a decreased tone in arteries from normal pregnant women, compared with the women with preeclampsia and nonpregnant women (ANOVA, $P = .13$, $P = .07$ and $P = .19$ at 20, 60 and 100 mmHg).

An increase in pressure did cause an increase in tone in all groups (RM-ANOVA, $P = .006$; figure 2A). Intracellular calcium concentrations rose in response to increases in pressure in all groups (RM-ANOVA, $P = .05$; figure 2B). These calcium elevations substantiate the observation from figure 2A that tone increases with pressure, which indicates the presence of myogenic responsiveness in these subcutaneous arteries, even though the responses were weak. Although calcium concentrations seem to be lower in normal pregnancy, there were no significant differences between groups.

A plot of tone as a function of intracellular calcium concentration reveals an upward shift of this relation in preeclampsia, compared with normal pregnancy and the nonpregnant state (figure 3). Thus, these data indicate that for a given calcium concentration, arteries from women with preeclampsia have more tone, i.e. a higher calcium sensitivity. An analysis of calcium and tone indeed reveals a highly significant increase in calcium sensitivity in arteries from women with preeclampsia: figure 4 plots the deviation of tone in individual arteries from the values that were predicted on basis of the intracellular calcium concentrations (linear regression over all data in PSS). These deviations were similar in arteries from normal pregnant and nonpregnant women ($-3.26\% \pm 0.74\%$ and $-1.26\% \pm 1.68\%$), but were significantly higher in arteries from women with preeclampsia ($+4.85\% \pm 2.11\%$ deviation), compared with both the arteries from normal pregnant and nonpregnant women (ANOVA with Bonferroni post hoc test, $P = .001$ and $P = .03$, respectively).

**Figure 3.** Percentage basal tone plotted against fura-2 ratios in subcutaneous resistance arteries from women with preeclampsia (grey diamonds), normal pregnant (open circles) and nonpregnant women (black triangles) in PSS at pressures of 20, 60 and 100 mmHg. SEM for fura-2 ratios and percentages basal tone are presented in figure 4.
Figure 4. Percentage deviation of tone from the regression line depicts the calcium-tone relationship in women with preeclampsia (PE), normal pregnant (PR) and nonpregnant women (NP) in PSS at pressures of 20, 60 and 100 mmHg.

Figure 5. Percentage of tone and fura-2 ratios in isolated subcutaneous resistance arteries from women with preeclampsia (PE), normal pregnancy (PR) and nonpregnant women (NP), superfused with NE 1 μmol/L (A and B) or potassium 36 mmol/L (C and D) at pressures of 20 (white boxes), 60 (grey boxes) and 100 (black boxes) mmHg.
NE- and potassium-induced tone were similar in all groups at all pressures, as well as the calcium concentrations (figure 5). While tone was significantly higher in arteries constricted with NE, compared with arteries constricted with potassium at all pressures (RM-ANOVA, P < .001), calcium concentrations were comparable in these arteries at 20 and 60 mmHg and even significantly higher in arteries constricted with potassium at 100 mmHg (RM-ANOVA, P = .001). Thus, arteries that are constricted with NE produce more tone at comparable or lower calcium levels than arteries constricted with potassium, which suggests the presence of an NE-induced calcium sensitization in these human subcutaneous resistance arteries. The differences between groups in the calcium-tone relation that were found in PSS were not detectable during potassium- or NE-induced constriction (ANOVA after linear regression, P = .52 and P = .42, respectively).

Comments

This study reports on contraction and calcium handling of cannulated human subcutaneous resistance arteries in relation to pregnancy and preeclampsia. We found no significant differences in passive pressure-diameter relations, basal tone, and NE- and potassium-induced tone among arteries from women with preeclampsia, women with normal pregnancy, and nonpregnant women, although tone tended to be reduced in normal pregnancy. Calcium sensitivity was significantly increased in arteries from women with preeclampsia compared with both normal pregnant and nonpregnant women. NE induced an increase in calcium sensitivity. There are, however, some comments to be made with respect to the chosen tissue and methods.

Human tissue available from cesarean deliveries is limited to subcutaneous fat, omentum and myometrium. We only studied the subcutaneous vascular bed. Because preeclampsia is a systemic disease that is believed to affect the vasculature of all organs, we expect vascular smooth muscle function of the resistance vasculature in the other vascular beds to be similar.

Several studies have addressed endothelial reactivity of arteries that were isolated from the resistance vasculature [255]. Only few also addressed vascular smooth muscle reactivity, and in these studies arteries were mainly studied under isometric conditions with wire myography. We chose to perform isobaric studies on cannulated arteries (pressure myography) rather than the more common isometric wire studies, to more closely approximate in vivo conditions and to include the effects of changing pressure and to study basal tone. With the use of isobaric conditions, we found NE- and potassium-induced constriction in subcutaneous arteries to be unaffected by pregnancy and preeclampsia. NE-induced constriction has been reported also to be similar in pregnancy and preeclampsia in the omental vascular bed in isobaric conditions [150] and in isometric conditions in subcutaneous arteries [86,87], but was found to be increased in preeclampsia at high concentrations of NE under isometric conditions in omental [131] and myometrial arteries [148]. Potassium-induced constriction has also been reported to be increased in preeclampsia in omental arteries in response to a high concentration of potassium under isometric conditions [89]. It is well known that the vasoconstrictor sensitivity of arteries in isobaric and isometric conditions is different, for reasons related to both the mounting technique.
and the mechanical loading [294,295]. In particular, medial hypertrophy might result in a higher maximal tension development to vasoconstrictors in isometric arteries, while isobaric arteries would show similar maximal constrictions as long as the pressure load is not too high. Because of the hypertension some medial hypertrophy might exist in preeclampsia, as described by Aalkjaer et al. [296] in omental arteries; this could explain the increased vasoconstrictor sensitivity found in the isometric studies [131,148].

We only applied a single concentration of NE and potassium to test contractile responses rather than to quantify the vasoconstrictor sensitivity from concentration-response curves. This choice was based on the limited time for experimentation available in fura-2 loaded arteries, because of the loss of the calcium dye over time. To determine as well as possible the responsiveness to the vasoconstrictors, concentrations of NE and potassium were used that should give submaximal contractions. The choice of the concentrations was based on the extensive literature on animal resistance arteries, the limited studies on isometric human resistance arteries in preeclampsia, and data from additional experiments, in which we obtained complete concentration-response curves to NE and potassium.

Another point that needs to be addressed is the loading of the arteries with fura-2. Fura-2 is a calcium buffer, and overloading will result in blunted and slow calcium responses. On the basis of previous work on animal vessels [293], 1 hour of loading at 30° C, using 10 μg/mL fura-2 AM ester, provides sufficient fluorescence signal without signs of buffering effects (as judged from vasoconstrictor responses and vasomotion). Furthermore, the concentration of pluronic, which was used to facilitate dissolving the fura-2 ester, was kept low (0.02%). Vessels remained viable and did not shown signs of deterioration in the hours of experimentation after loading.

There is a difference in gestational age between the women with preeclampsia and normal pregnant women at the moment of cesarean delivery, obviously related to the need to terminate pregnancy earlier in preeclampsia because of fetal or maternal distress. In all published studies concerning isolated arteries, this gestational age difference has been present. No longitudinal data on vascular function in isolated arteries in pregnancy are available, but from longitudinal data on hemodynamics in normal pregnancy, it is known that the peripheral resistance reaches a plateau beyond the second trimester [297]. Because peripheral resistance is, for a large part, generated in the resistance vasculature, no major changes are expected to occur in systemic vascular function in normal pregnancy during the third trimester. Therefore, the difference in gestational age between women with preeclampsia and normal pregnant women is not likely to influence the results.

We found that the sensitivity of the contractile apparatus to calcium is significantly increased in arteries from women with preeclampsia, compared with arteries from normal pregnant and nonpregnant women. This indicates that the increased peripheral resistance in preeclampsia is not only under the influence of external factors, but may result also from the intrinsically higher calcium sensitivity in the resistance vasculature. The cause of this altered calcium sensitivity remains to be established. The reason that the observed differences in calcium sensitivity in these arteries are only present under basal conditions and the reason that differences disappear during potassium- or NE-induced constriction remain to be established. In our ex vivo system the
increased calcium sensitivity could result from intrinsic alterations either in the vascular smooth muscle cells or in the endothelium. Endothelial cells could reduce calcium sensitivity by production of NO [298]. Thus, endothelial dysfunction, if associated with a decreased NO production, indeed would result in an increased calcium sensitivity. It was not the main purpose of our study to assess endothelial function. Therefore, we tested only single concentrations of BK and ACh, which produced a submaximal dilatation, as based on previously reported concentration-response curves [86,87]. With this test we found no differences in BK- and ACh-mediated dilatation in pregnancy and preeclampsia, which indicates an absence of gross endothelial damage. The possibility, however, remains that the altered calcium sensitivity in preeclampsia results from an altered endothelial function, due to changes in the release or action of endothelial products that could not be detected with our tests.

In vivo vascular smooth muscle function is under the influence of the sympathetic nervous system. Sympathetic nervous activity has been reported to be decreased in normal pregnancy and increased in preeclampsia in vivo [119]. NE is the most important neurotransmitter involved in sympathetic nervous activity. In resistance arteries from animals, NE has been reported to cause constriction not only by increasing the calcium concentration, but also by upregulating calcium sensitivity [293]. We also observed this acute upregulation of calcium sensitivity in response to NE in isolated human subcutaneous arteries. Elevated NE levels in preeclampsia that result from increased sympathetic nervous activity could acutely upregulate vascular smooth muscle calcium sensitivity. Because the sympathetic nervous system is obviously absent in our ex vivo system, this cannot explain the rise in intrinsic calcium sensitivity in the isolated arteries from women with preeclampsia. The observed upregulation of calcium sensitivity by NE could provide an explanation for the disappearance of the differences in calcium sensitivity between groups during NE-induced constriction. Intrinsinc differences in calcium sensitivity that are present during the basal state are then likely to be overruled by upregulation in arteries from all groups, thereby equalizing calcium sensitivities. However, the pathways that result in the observed changes in intrinsic properties and the upregulation of calcium sensitivity by NE are still unknown; further investigations are needed to clarify them.

In conclusion, we found an increased vascular smooth muscle calcium sensitivity in isolated subcutaneous arteries in preeclampsia. Intrinsic alterations in resistance artery calcium sensitivity could therefore be involved in the increased peripheral resistance in preeclampsia. Pharmacological modulation of calcium sensitivity may provide a future means for the treatment of preeclampsia-associated hypertension.

We thank the Onze Lieve Vrouwe Gasthuis, in particular Jan M. van Lith, for their active participation in inclusion of patients in this study.