Pregnancy is associated with hypotrophy of carotid artery endothelial and smooth muscle cells

Sofija Jovanović1,3,4 and Aleksandar Jovanović2,3

1Department of Anatomy, Veterinary Faculty and 2Department of Clinical Pharmacology, Pharmacology and Toxicology, Medical School, Belgrade, Yugoslavia
3Present address: Division of Cardiovascular Diseases and Clinical Pharmacology, Mayo Clinic and Foundation, Rochester, MN 55905, USA
4To whom correspondence should be addressed at: Division of Cardiovascular Diseases (G-7), Departments of Medicine and Pharmacology, Mayo Clinic and Foundation, Rochester, MN 55905, USA

It is known that blood flow through the carotid artery is decreased during pregnancy, which may be due to a pregnancy-associated increase in the sensitivity of this artery to vasoconstrictors. Recent studies have shown that alteration of blood flow or pressure could remodel some arteries over a short time frame. However, the possibility of remodelling of the carotid artery during pregnancy has not yet been examined. Therefore, the aim of the present study was to study the morphometrical and stereological characteristics of guinea-pig carotid artery during different stages of pregnancy (non-pregnant, early-pregnant, mid-pregnant, late-pregnant, n = 8–10 for each group). The cross-sectional area of the different layers of the carotid artery and the cross-sectional area of endothelial and smooth muscle cells were measured using both light and electron microscopy. The values of internal diameter and cross-sectional area of adventitia were not significantly different, regardless of the pregnancy status. In contrast, external diameter, wall thickness and cross-sectional areas of media and intima progressively and significantly decreased during pregnancy. In addition, volume/surface density ratio of intima and media also significantly and progressively decreased during pregnancy, suggesting hypotrophy of endothelial and smooth muscle cells of carotid artery. Indeed, electron microscopy revealed that the size, defined as cross-sectional area, of endothelial and smooth muscle cells was significantly decreased during different stages of pregnancy. It is concluded that during pregnancy there is thinning of the intimal and medial layers of guinea-pig carotid artery, which reflect pregnancy-associated hypotrophy of carotid artery endothelial and smooth muscle cells.

Key words: carotid artery/endothelium/pregnancy/remodelling/stereology

Materials and methods
Non-pregnant female guinea-pigs and early-pregnant (day 22 of pregnancy), mid-pregnant (day 44 of pregnancy) and late-pregnant (days 64–66 of pregnancy) guinea-pigs were used in this study (eight...
to 10 in each group). On the day of an experiment, guinea-pigs were anaesthetized with sodium pentobarbitone (40 mg/100 g, i.p. injection) and the common carotid artery was removed. For light microscopy, the vascular segments (one segment per animal, 1 cm long) were fixed in 10% buffered neutral formaldehyde (48 h) (Fluka, Buchs, Switzerland), and cut into 2 mm long rings. Subsequently, the specimens were dehydrated through a graded series of ethanol solutions (70–100%) and embedded in paraffin wax. Resultant blocks were cut on a Sorval JB-4 microtome (Newtown, CT, USA) at 3 μm. Sections were stained with haematoxylin and eosin. Each section was examined under a ×20 magnification (Olympus Vanox microscope, Tokyo, Japan). For electron microscopy, the carotid artery from each animal was cut into rings 1 mm long (four rings per artery), fixed in 3% glutaraldehyde (Fluka) for 24 h and after rinsing in 0.1 M cacodylate buffer (pH 7.4) (Fluka), postfixed in a 2% cacodylate solution (Fluka) of osmium tetroxide (1 h). After fixation and postfixation, the specimens were dehydrated in increasing concentrations (70–100%) of ethanol, and then passed through propylene oxide and embedded in Araldite (Lee et al., 1983). Ultrathin sections (50–70 nm) were cut with a diamond knife. The sections were stained with uranyl acetate (Fluka) and Reynold’s lead citrate (Fluka) and then examined and photographed using a Philips EM 400-HMG electron microscope (Amsterdam, The Netherlands). The investigation conformed with the ‘Guide for the Care and Use of Laboratory Animals’ (NIH publication 85-23, revised 1985).

**Morphometric and stereologic analysis**

**Light microscopy**

Sections (3 μm thick) that included the entire circumference of each ring were cut from five different blocks from each animal and viewed with light microscopy using an ocular micrometer accurate to 0.01 mm. For each block, five sections were examined. Maximal and minimal internal and external diameters were determined at magnification ×20 (using a stage micrometer), and these values were used to determine the mean values of internal and external diameters. Wall thickness was calculated by subtraction of the two diameters and dividing by a factor of 2 (Jovanović and Jovanović, 1997). To determine cross-sectional areas of the different aortic layers, including total cross-sectional area, a standard point-counting system, Weibel M42, was used (Weibel, 1979). In brief, this frame-square test grid is composed of 21-line segments, which are used to count intersects, and 42-line endpoints. During point and intersect counting, each series of horizontal lines was scanned to count all the points and intersects falling on the various components of the vessels. The cross-sectional areas were calculated using the following equation (Weibel, 1979; Lee et al., 1983):

\[
\text{Am} = \left(\frac{\text{Pi}}{\text{Pt}}\right) \times \text{Ag}
\]

where Am = cross-sectional area, Pi = number of points falling on specific layer, Pt = total points of test grid and Ag = area of the whole test grid for the appropriate magnification (×20). Correction for eccentricity due to sectioning angle with reference to the long axis of the vessel was used for all calculations (Lee et al., 1983): correction = d1/d2, where d1 = minimal radius of vessel and d2 = maximal radius of vessel. Thus, the definitive form of the equation used was

\[
\text{Am} = \left(\frac{\text{Pt}}{\text{Pi}}\right) \times \text{Ag} \times (d1/d2).
\]

To determine cellular hypotrophy and/or hypoplasia of intima and media, the volume surface density ratio (V/S) of the examined layers was calculated using the formula: V/S = Pi/Zd4/3, where Pi = total number of points falling on examined layer, Li = total number of intersects with the borders of the layer and Z = length of one test line from test grid at ×20 magnification.

**Electron microscopy**

Morphometric analysis was performed from 20 random electron micrographs (five micrographs per block, four block per aorta) obtained at ×2800–7900 magnification. The size of endothelial and smooth muscle cells, defined as cross-sectional area (Lee et al., 1983), were determined using the B 100 standard point-counting system (Weibel, 1979). This test grid is composed of a coherent square lattice of lines, and a total number of points of 100. To avoid errors in making average estimates of cell objects without having information about individual objects, the point counting was performed by eye, and the cross-sectional area of each individual cell profile was determined. The intersects of the lines were considered as points for point counting. During point counting, each series of points was scanned to count all the points falling on the cross-section of the examined structure.

Cross-sectional area was calculated using the following equation

\[
A = \text{Pi} \times d'
\]

where A = cell cross-sectional area, Pi = number of points falling on cell cross-sectional area, d = distance between the nearest points of the grid and d’ = distance between the nearest points of the grid corrected with magnification, i.e. d’ = d/magnification (Weibel, 1979; Lee et al., 1983).

**Statistical analysis**

For all vessel parameters, separate data measurements of diameters, wall thickness and cross-sectional profiles of vascular components, from each animal were respectively pooled and an average value recorded, so that in analysis each animal contributed only one value for each parameter. All data were tested by the Kolmogorov Goodness of Fit test and were found to be normally distributed. The results are expressed as means ± SEM, with range of values in parentheses; n refers to the number of animals. One-way analysis of variance (ANOVA) was used when more than two groups were analysed and the individual pregnant groups were compared with the non-pregnant one, which served as a control. A value of P < 0.05 was considered to be statistically significant.

**Results**

**Light microscopy**

The values of external and internal diameters and wall thickness of aorta from guinea-pigs in different stages of pregnancy are depicted in Table I. The values of internal diameter were not significantly different (P > 0.05, n = 8–10), while the values of external diameter and wall thickness decreased during pregnancy and reached minimal values at the time of delivery (Table I). Analysis of cross-sectional values of different layers revealed that the cross-sectional area of the adventitia was not affected by pregnancy status (data not shown). In contrast, the cross-sectional areas of the carotid artery media and intima significantly and progressively decreased during pregnancy (Figure 1). Since the cross-sectional area of the intima and media reflect the sum of the cross-sectional areas of endothelial

<table>
<thead>
<tr>
<th>Stage of pregnancy</th>
<th>External (10² μm)</th>
<th>Internal (10² μm)</th>
<th>Wall thickness (10² μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-pregnant</td>
<td>22.4 ± 3.0</td>
<td>10.9 ± 1.4</td>
<td>5.8 ± 0.8</td>
</tr>
<tr>
<td>Early-pregnant</td>
<td>20.9 ± 2.7</td>
<td>10.5 ± 1.1</td>
<td>5.7 ± 0.7</td>
</tr>
<tr>
<td>Mid-pregnant</td>
<td>19.2 ± 1.8</td>
<td>10.5 ± 1.7</td>
<td>5.3 ± 0.6</td>
</tr>
<tr>
<td>Late-pregnant</td>
<td>17.3 ± 2.1</td>
<td>10.3 ± 1.5</td>
<td>4.8 ± 0.5</td>
</tr>
</tbody>
</table>
cells and smooth muscle cells, we postulated that the observed changes may be due either to changes in endothelial/smooth muscle cell numbers or size. To investigate these possibilities, we calculated the volume/surface density ratio (V/S) for intima and media. V/S values for both intima and media were significantly decreased during pregnancy (Figure 2). Since the decrease in V/S ratio was concomitant with the decrease in intimal/medial cross-sectional area, it is possible that thinning of intimal and medial layers may be due to decreased size of endothelial and smooth muscle cells (Lee et al., 1983).

Electron microscopy

In order to examine the hypothesis that decrease of intimal and medial cross-sectional areas of guinea-pig carotid artery during pregnancy is due to endothelial and smooth muscle cell hypotrophy we used electron microscopy. Indeed, under ×2800–7900 magnification, electron microscopy revealed that the cross-sectional areas of individual endothelial and smooth muscle cells were significantly and progressively decreased during pregnancy (endothelial cells: 75.2 ± 8.0 µm² in non-pregnant versus 31.9 ± 4.3 µm² in late-pregnant animals, n = 8–10, P < 0.01; smooth muscle cells: 119.2 ± 11.0 µm² in non-pregnant versus 81.0 ± 8.0 µm² in late-pregnant animals, n = 8–10, P < 0.01). The original photomicrographs of endothelial and smooth muscle cells of carotid artery from guinea-pigs in different stages of pregnancy are depicted in Figures 3 and 4, while mean ± SEM values of endothelial and smooth muscle cells cross-sectional area are shown in Figure 5.

Discussion

Recently it has been recognized that changes in blood flow may be associated with the consequent alterations in the morphology of affected blood vessels (Baumbach et al., 1991; reviewed by Angus, 1994). In different animal species,
including humans, it has been reported that during pregnancy blood flow through different organs is changed (Peeters et al., 1980; Easterling et al., 1991; Magness et al., 1991). It has been postulated that this cardiovascular adaptation may be due to altered vascular reactivity toward neurotransmitters and humoral factors, leading to general maternal vasodilatation (Conrad et al., 1991; Grbović and Jovanović, 1996). As opposed to this concept, it has been established that blood flow through the carotid artery is decreased during pregnancy, without changes in blood pressure, which could be associated with pregnancy-induced increased responsiveness of the carotid artery to vasoconstrictors and decreased responsiveness to vasodilators (Hull et al., 1992), although this concept is not yet definitely accepted (Weiner et al., 1991, 1992). Previous studies addressing the effect of pregnancy on the structure of the carotid artery failed to demonstrate differences between arteries taken from non-pregnant and pregnant experimental animals (Griendling et al., 1985; Hull et al., 1992). In contrast, in the present study, we observed that pregnancy is accompanied by a significant decrease in carotid artery intimal and medial cross-sectional area, reaching a minimum at the time of delivery. This effect of pregnancy has not been previously recognized. The reason for this may be the fact that the stereological methods used in this study are more precise than ordinary histological examination. In order to determine the nature of the alterations in intimal and medial cross-sectional area during pregnancy, we calculated volume/surface density ratio. It is known that changes in cross-sectional area of some structures are not accompanied by changes in volume/surface density ratio, suggesting changes in the number of units (cells) that form this structure. On the other hand, if volume/surface density ratio is changed in parallel with changes in cross-sectional area of some structures, changes in cell volume can be predicted (Lee et al., 1983). In the present study, the intimal and medial volume/surface ratio decreased in parallel with the decrease in cross-sectional area. Therefore, it was possible that the pregnancy-induced decrease in cross-sectional areas of intima and media reflected the decreased size of cells that form these layers, i.e. endothelial and smooth muscle cells. In order to examine this possibility, we determined the cross-sectional areas of carotid artery endothelial and smooth muscle cells. Electron microscopy revealed that the cross-sectional area of endothelial and smooth muscle cells significantly and gradually decreased during pregnancy, confirming our findings obtained with light microscopy. These results represent a previously unrecognized effect of pregnancy on carotid artery smooth muscle and endothelial cells. It should be mentioned that the features studied were profiles of cells or walls, rather than the cells and walls themselves. Although this fact may call into question the conclusions drawn, their reliability has been verified from the analysis of a large number of random sections. It is generally accepted that under such circumstances cross-sectional areas genuinely reflect the three-dimensional size of the examined layer or cells (Weibel, 1979; Lee et al., 1983; Jovanović and Jovanović, 1997). Additionally, since the results were not obtained with native (unprepared) tissue, it is possible that the presented findings may represent artefacts of the applied procedures, such as sectioning, fixing or counting (Weibel, 1979). However, since the experiments were done according to the established principles of morphometry and stereology (Weibel, 1979) and since the results obtained with light and electron microscopy were in accord with each other, this possibility is rather unlikely.

At present, it is not possible to define the factors that are responsible for the remodelling of the carotid artery during pregnancy. Previously, it has been shown that, besides uterine artery (Cipolla and Osol, 1994), aorta is also subject to vascular remodelling (Jovanović and Jovanović, 1997). It has been postulated that this effect of pregnancy may be due to oestrogen action (Leiberman et al., 1993; Keyes et al., 1997). However, it is not possible to comment on this aspect of our findings obtained with carotid artery, since it is not yet known whether carotid artery is an oestrogen-targeted organ. Nevertheless, the present study shows for the first time that carotid artery is subject to remodelling during pregnancy. Despite the yet unknown mechanism(s) of endothelial and smooth muscle cell hypertrophy in pregnancy, it is possible that this may have important consequences for the processes of maternal vascular adaptation. At present, it is not possible to draw any conclusions on the significance of these observed structural changes in the carotid artery during pregnancy. Nevertheless, on the basis of the obtained results, we suggest that during pregnancy, there is thinning of intimal and medial layers of guinea-pig carotid artery, which reflects hypertrophy of the endothelial and smooth muscle cells.

Acknowledgements

We thank Dr Andre Terzic, Mayo Clinic and Foundation, for providing osmium tetroxide and Araldite. A.I. is the recipient of a Merck Sharp & Dohme International Fellowship in Clinical Pharmacology.

References


Figure 5. The effect of pregnancy on endothelial and smooth muscle cell cross-sectional areas of guinea-pig carotid artery. Bars represent mean ± SEM (n = 8–10). *P < 0.05 with respect to the non-pregnant animals.


Received on July 14, 1997; accepted on December 8, 1997