Differences in the Mechanical Properties of the Rat Carotid Artery In Vivo, In Situ, and In Vitro

Anne Zanchi, Nikos Stergiopulos, Hans R. Brunner, Daniel Hayoz

Abstract—The elastic properties of carotid arteries of spontaneously hypertensive rats (SHR) and normotensive controls (Wistar-Kyoto rats [WKY]) were examined in vivo, in situ, and in vitro. The changes of internal diameter were measured with a high-resolution A-mode echo-tracking device simultaneously with the intra-arterial pressure at the carotid. The internal diameter at mean arterial blood pressure (MBP) was substantially smaller in vitro than in vivo in SHR (−33.8%) and WKY (−48.3%). The arterial distensibility was lower in vitro in all arteries compared with in vivo conditions (SHR, −30.1%; WKY, −60.4%; at MBP) despite a reduced incremental elastic modulus in vitro (SHR, −56.9%; WKY, −45.1%; at MBP). However, the in vitro and in vivo measurements show consistent elastic behavior of the carotid arteries between both strains of rats. Carotid arteries from WKY were also examined in situ. Although no significant reduction in internal diameter could be observed in situ, distensibility was dramatically decreased (−87% at MBP). These results emphasize the importance of considering the original vascular geometry when determining elastic properties of arteries. We conclude that experimental conditions are likely to be a critical determinant for the assessment of the mechanical properties of conduit vessels. (Hypertension. 1998;32:180-185.)

Key Words: arterial distensibility ■ elasticity ■ carotid arteries

Resistance arteries have the capacity to adapt to increased wall stress by reorganization of the components of the media (remodeling) and/or by thickening of the media (hypertrophy).1-3 In contrast to resistance arteries, conductance arteries hardly contribute to peripheral resistance, but their buffering capacity contributes to the pulsatile component of blood pressure, and they are a major determinant of cardiac afterload. The adaptation of large arteries to hypertension is associated with hypertrophy of the media. Recent studies have been carried out to examine whether the hypertrophic process may alter the mechanical properties of the arterial wall.4-9 There has been evidence that arteries demonstrate increased passive stiffness10 and decreased compliance11 during hypertension. However, the development of new methods for in vivo assessment of the mechanical properties of the arterial wall led to controversial findings. In humans, the majority of in vivo assessments of arterial compliance was derived from pulse-wave velocity measurements and demonstrated a reduced compliance in hypertensive subjects.12 In contrast, direct measurements of the diameter-pressure relationship of the radial artery in hypertensive subjects did not show a reduction of distensibility in hypertensive subjects.45,13 Some of these discrepancies may be related to the heterogeneity of the arterial tree. The elastic properties of carotid arteries measured in hypertensive rats, however, were not significantly modified17 when compared with those of control animals. These results differed from those obtained either by in situ measurements in anesthetized animals8,14,15 or by in vitro experiments.11

The objective of this study was to assess the influence of the experimental conditions (in vivo, in situ, in vitro) on the mechanical properties of the common carotid artery in the rat and to examine in which way the differences in mechanical properties observed so far between normotensive and hypertensive animals could be affected by the experimental conditions in which the measurements are performed.

Methods

In Vivo Versus In Vitro Conditions

Twenty-four-week-old male SHR (n=6) and normotensive WKY (n=6) control rats were obtained from Iffa-Credo (Lyon, France). The animals were housed in a conditioned environment, with constant temperature and humidity and regular light/dark cycles (ie, light from 7 AM to 7 PM). Ordinary rat chow (UAR, AO4, Ville-moisson-sur-Orge, France) containing 100 μmol sodium per gram and drinking fluid were provided ad libitum. The procedures used in this study were approved by the governmental ethics committee for animal experiments.

On the day of the experiment, anesthesia was induced and maintained with halothane (Arovet AG) at a concentration of 1.5%. The right common carotid artery was cannulated with a catheter (PE-50, Portex) filled with heparinized 0.9% saline solution. Intra-arterial pressure and heart rate were monitored as described previously with a computerized data acquisition system. The frequency response of the transducer (Micro Switch, Honeywell) is >1 KHz, and the transducer system is automatically zeroed with respect to atmospheric pressure. The ID of the left common carotid artery was measured simultaneously with an A-mode ultrasonic echo-tracking device (NIUS 02, Asulab). This device, which has a dynamic precision close to 1 μm, has been used previously in both animal

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Selected Abbreviations and Acronyms

CSA = cross-sectional area
Em = incremental modulus of elasticity
ID = internal diameter
IMT = intimal-medial thickness
RF = radio frequency
SHR = spontaneously hypertensive rats
WKY = Wistar-Kyoto rats

Carotid arteries were then fixed at the mean blood pressure found in vivo during 30 minutes with a 4% paraformaldehyde solution. Paraffin-embedded tissue blocks were sectioned at a thickness of 5 μm and stained with hematoxylin and eosin. Histomorphometric measurements were performed under a microscope (Diaphot, Nikon). The IMT and ID measurements were performed with 200-fold magnification in a blinded fashion. The measurements carried out on 2 carotid sections and on 6 fields per section were averaged. The intima-media CSA of the fixed arteries was determined according to the following formula: CSA = π[(internal radius + IMT)^2 - (internal radius)^2].

To have an estimation of the incremental modulus of elasticity in vivo and in vitro, the arterial wall thickness was derived for each level of blood pressure from the CSA measured in vitro and ID (d) measured in vivo and in vitro. This was done assuming that the CSA remains constant in vivo and in vitro and is not influenced by the changes in diameter.17,20 The in vitro experiments on the excised arteries were carried out at in situ length. Thus, the length between 2 arbitrarily chosen anatomic markers was set to the same level as before excision and kept for the entire inflation experiment protocols. Therefore, despite large changes in pressure, the length of the segment as well as the longitudinal stretch ratio were kept constant. This means that the local wall CSA, by virtue of the incompressibility condition, remained constant. The wall thickness (h) was calculated according to the following formula: h = 214 [(CSA + π(d/2)^2)/π] - d/2. The circumferential stress (σ) for each level of blood pressure (p) and ID (d) was derived from the following formula: σ = p/d^2h. Finally, the incremental modulus of elasticity was defined as Em = Δσ/Δstrain = [σ(n+1) - σ(n)]/[d(n+1) - d(n)] and was calculated for each increase in intraarterial blood pressure of 2.5 mm Hg within the operational blood pressure range.

In Vivo Versus In Situ Conditions

Twenty-four-week-old male normotensive WKY controls were housed in a conditioned environment as described above. On the day of the experiment, anesthesia was induced and maintained with halothane (Arovet AG) at a concentration of 1.5%. The right internal carotid artery was cannulated with a catheter (PE-50, Portex) filled with heparinized 0.9% saline solution. A loose knot was carefully placed around the proximal common carotid artery. Intra-arterial pressure and heart rate were monitored as described previously. The ID of the right common carotid artery was measured simultaneously with a 10-MHz A-mode ultrasonic echo-tracking device (NIUS 02, Asulab). For the recordings, the probe was placed perpendicularly over the artery at 8 mm away from the knot. Doppler mode was used to guide the probe, and warmed ultrasonic gel was used for signal transmission. The simultaneous arterial diameter and blood pressure measurements allowed calculation of a diameter-pressure relation, which was subsequently converted into cross-sectional compliance and distensibility-pressure curves. After the measurements performed in vivo, the knot was tightened and the catheter was filled with an oxygenated (95% O2-5% CO2), warmed (37°C) physiological salt solution identical in composition to that used in vitro and described above. The intraluminal pressure was again manually altered similarly to the experiment performed in vitro over the systolo-diastolic pressure range measured in vivo after 10 to 15 minutes of preconditioning. After completion of the measurements, the rat was killed with a lethal dose of pentobarbital (90 mg/kg IA); immediately afterward, the intraluminal pressure was again modified

Table 1. Characteristics of Study Groups

<table>
<thead>
<tr>
<th></th>
<th>Weight, g</th>
<th>SBP, mm Hg</th>
<th>DBP, mm Hg</th>
<th>MBP, mm Hg</th>
<th>PP, mm Hg</th>
<th>HWI, mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR (n=6)</td>
<td>419±6*</td>
<td>176±4*</td>
<td>141±3*</td>
<td>153±3*</td>
<td>34±3</td>
<td>3.18±0.07*</td>
</tr>
<tr>
<td>WKY (n=6)</td>
<td>390±6</td>
<td>132±4</td>
<td>102±3</td>
<td>112±3</td>
<td>29±2</td>
<td>2.78±0.04</td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; PP, pulse pressure; and HWI, heart weight index.

*P<0.05 vs WKY.
as described above. The positions of the electronic trackers on the RF lines yielding both proximal and far-wall signals were not altered during all these procedures.

Statistics
Between-group comparisons of body weight; heart weight index; CSA; IMT; ID at histology; and mean, diastolic, and systolic blood pressures were made by Scheffé’s test. The diameter, distensibility, and \( E_{\text{inc}} \) curves were established within operating pressures, the upper and lower limits representing the mean systolic and mean diastolic values for each group, respectively. For the statistical evaluation of the diameter-, distensibility-, and \( E_{\text{inc}} \)-pressure curves, the areas under the curves for operating blood pressures were compared using Scheffé’s test. Results are given as mean±SEM.

Results
In Vivo Versus In Vitro
Table 1 summarizes the characteristics of the study groups at the age of 24 weeks. The SHR were slightly heavier than the control WKY. Systolic, diastolic, and mean blood pressures were significantly higher in the SHR than in the control WKY. Pulse pressure was not different among the 2 groups. The presence of cardiac hypertrophy in the SHR was demonstrated by a higher heart weight index [heart weight (mg)/body weight (g)] when compared with controls.

Figure 1 illustrates the diameter-pressure curves for the 2 groups obtained in vivo and in vitro over the systolic-diastolic range of pressure. As demonstrated, the ID was significantly smaller in vitro than in vivo in all groups, and this was more pronounced in the WKY (\( P<0.001 \)). The distensibility-pressure curves differed substantially and in a similar fashion in vivo and in vitro in the 2 groups (Figure 2). The distensibility observed in vitro was dramatically reduced when compared with in vivo conditions.

The calculated \( E_{\text{inc}} \) was markedly lower in vitro than in vivo in the SHR (\( P<0.005 \)) and WKY (\( P<0.05 \)) (Figure 3).

The morphometric characteristics were measured first with the ultrasonic device in vitro to assess thickness and diameter of the artery and subsequently by histology after perfusion fixation with paraformaldehyde (Table 2). The CSA of the carotid artery was significantly increased in the SHR compared with the WKY controls when estimated in vitro and by histology, demonstrating a strong positive correlation between the values of CSA obtained in vitro and by histology, indicating that histological processing induced proportionally the same changes in all arteries (\( r=0.92, P<0.001 \)).

The rate-dependent changes in vascular mechanics in vitro is shown in Figure 4, where an inverse relationship between frequency of the pulsatile pressure and distensibility of the common carotid artery was observed.

In Vivo Versus In Situ
At 24 weeks, the WKY weighed 372±4 g, and their mean systolic and diastolic blood pressures were 106±4 and 82±3 mm Hg, respectively. The changes in ID in the systolic-diastolic pressure range of the WKY are shown in Figure 5. The pulse-induced variations in diameter were most evident in vivo. In situ, the diameter was similar at 80 mm Hg, but it barely increased with rising pressures, whereas in situ postmortem, the artery showed a substantial vasoconstriction (\( P<0.001 \) versus in vivo) in addition to very low pulsatile-induced changes. As a consequence of the preceding findings, the distensibility-pressure curves did not differ between the 2 types of in situ measurements. However, both were dramatically lower than the in vivo measurements (\( P<0.001 \)) (Figure 6).

Table 2. Morphometric Studies

<table>
<thead>
<tr>
<th></th>
<th>CSA, ( \mu m^2 )</th>
<th>Diameter, ( \mu m )</th>
<th>IMT, ( \mu m )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NIUS Histology</td>
<td>NIUS Histology</td>
<td>NIUS Histology</td>
</tr>
<tr>
<td>SHR</td>
<td>271±24*</td>
<td>104±3*</td>
<td>90.6±5.3</td>
</tr>
<tr>
<td></td>
<td>855±34*</td>
<td>725±19*</td>
<td>42.5±1.5*</td>
</tr>
<tr>
<td>WKY</td>
<td>173±4</td>
<td>50±3</td>
<td>89.5±5.1</td>
</tr>
<tr>
<td></td>
<td>546±61</td>
<td>545±34</td>
<td>28.4±1.9</td>
</tr>
</tbody>
</table>

\( *P<0.05 \) vs WKY.
Discussion

These results clearly demonstrate that the elastic properties of the rat carotid artery differ substantially between in vivo and in vitro conditions. Furthermore, when compared with intact in vivo conditions, the differences are still significant when the artery is examined in situ in the alive animal. Although death of the animal is associated with a constriction of the artery, the distensibility in situ did not depend on whether the animal was alive.

The mechanical properties of the arteries were examined in vivo, in situ, and in vitro by deriving the cross-sectional distensibility from the diameter-pressure curve without trying to match vascular strain by either pharmacological or physical means. The measurements were performed on the assumption that the common carotid artery is a cylindrical vessel, and since changes in arterial volume are mainly due to changes in cross section, distensibility is thus defined as the cross-sectional changes induced by pulse pressure. Assuming that the ligature of the artery does not affect the changes in length per se. Indeed, the artery was mounted on a 5-mm needle, so there was no significant collapse. From a solid mechanical point of view, the “end effects” are created by the local bending moments, which in the present case of a relatively thin artery, dissipate very fast, in the order of 1 to 2 diameters. Since the segment under study was typically 10 mm long, this implies that at the middle of the segment where measurements were carried out, interference of “end effects” was negligible. The main advantage of cross-sectional analysis of the mechanical properties of the carotid artery is that it allows for the first time, in contrast to volume-pressure measurements, the comparison of in vivo, in situ, and in vitro measurements using the same method.

The lack of hemodynamically generated shear stress at the intima, the difference in the quality of the perfusate, the absence of blood-borne vasoactive substances and of circulating elements, and the low frequency of blood pressure oscillations (0.2 Hz) were conditions prevailing both in the in vitro and in situ measurements that could be responsible for some of the differences found with the in vivo measurements. This may indeed affect to different extents the arterial diameter of the carotid arteries of WKY and SHR, in part due to a possible alteration of the endothelial function (Table 2) as discussed below. However, the manually generated low-frequency oscillations should increase the distensibility of the vascular wall by virtue of a reduced viscoelasticity, although the velocity of stretching was not taken into consideration. Figure 4 shows an inverse relationship between the frequency of pulsations and the distensibility of excised rat carotid arteries. Furthermore, the arterial wall viscosity differs significantly between in vivo and in vitro conditions, and an active modulation of the viscous component of the arterial wall seems to depend on the integrity of the endothelial function. The high oxygen concentration (95%) used in vitro instead of air may be responsible for the generation of an increased reactive oxygen species that can interfere with smooth muscle tone.

Strong evidence exists that mechanical strain modulates the secretion of various vasoactive factors by the endothelial cells. Pressurization produces simultaneous strains and stresses in all directions that are associated with insignificant flow rate in situ and in vitro because of the presence of a ligature around the artery. Shear stress increases as a function of the product of flow rate and fluid viscosity. Both param-

Figure 4. Line plots show the effect of pulse pressure frequency on the distensibility-pressure relationship of isolated carotid arteries in Krebs buffer at 37°C (n=5; mean±SEM).

Figure 5. Line plots show the relation between intra-arterial pressure and diameter of the common carotid artery in vivo, in situ, and in situ postmortem in WKY. In vivo vs in situ: P<0.001; in vitro vs in situ postmortem: P<0.001; in situ vs in situ postmortem: P>0.05. Lines represent means; shadows, SEM.

Figure 6. Line plots show the relation between intra-arterial pressure and distensibility of the common carotid artery in vivo, in situ, and in situ postmortem in WKY. In vivo vs in situ: P<0.001; in vitro vs in situ postmortem: P<0.001; in situ vs in situ postmortem: P>0.05. Lines represent means; shadows, SEM.
eters are very low in the in situ and in vitro measurements, and consequently shear stress is abnormally low. A reduced release of nitric oxide and endothelin-derived hyperpolarizing factor related to low shear stress, and conversely an increased release of endothelin, may favor a constriction of the vessel and presumably be partially responsible for the decreased diameter found in vitro in the SHR and WKY.\textsuperscript{28,29,31–33} However, the fact that the ID in situ in the alive animal was close to the ID values in vivo illustrates that factors other than flow-induced and blood-borne substances may participate in the dilation of the vessel, in particular the central nervous system via the $\beta$-adrenergic system. Nevertheless, whatever the differences in diameter in situ, in vitro, and in vivo, arterial distensibility was dramatically decreased in the arteries of WKY when examined in situ in the alive animal or immediately after death and in vitro in all arteries obtained from normotensive or hypertensive animals. This experiment did not allow us to discriminate the effects linked to different mechanical environments, ie, low shear stress or pattern of variation in blood pressure, from those related to the absence of blood-borne vasoactive factors. Indeed, further studies are needed to investigate the importance of shear stress in the mechanical properties of the carotid artery and also the influence of cyclic stretch, its amplitude, and its frequency. Modulation of the arterial tone in vitro should also give an indication of the influence of this parameter on the frequency. Modulation of the arterial tone in vitro should also the influence of cyclic stretch, its amplitude, and its frequency. Modulation of the arterial tone in vitro should also give an indication of the influence of this parameter on the frequency. Modulation of the arterial tone in vitro should also give an indication of the influence of this parameter on the frequency. Modulation of the arterial tone in vitro should also give an indication of the influence of this parameter on the frequency. Modulation of the arterial tone in vitro should also give an indication of the influence of this parameter on the frequency.

The excision of vessels with resulting retraction followed by elongation and distension has been reported to change the intrinsic properties of the arterial wall.\textsuperscript{34} This procedure alone cannot explain the differences found in vitro and in vivo because during the in situ measurements the artery length remained unchanged. Nevertheless, striking differences between in situ and in vivo measurements were also observed. Surgical exposure of the artery might also alter its properties. Thus, to obtain similar conditions in vivo and in situ, exposure of the artery was performed before the in vivo measurements.

With a more constricted artery as found in vitro, the connective tissue retracts and the load is borne by more elastic components. In WKY and SHR, the reduced diameter in vitro was associated with a decreased elastic modulus, ie, a low stiffness. Although the intrinsic characteristics of the arteries were less stiff in vitro than in vivo in the WKY and SHR, the distensibility of the arteries was dramatically decreased. This clearly illustrates that the mechanical properties of the artery are not solely dependent on the intrinsic properties of the artery. The $E_{\text{inc}}$ gives an indication of the stiffness of the arterial wall material independent of vessel geometry. However, distensibility depends both on the geometry of the artery and the intrinsic properties of the arterial wall. Between the 2 conditions, in vitro and in vivo, the $E_{\text{inc}}$ changes because of a modification of the smooth muscle tone leading to changes in geometry. The decrease in passive stiffness found in vitro was evidently exceeded by the change of arterial geometry, ie, wall thickening and reduction in diameter. It is possible to mathematically represent the relationship between the distensibility of an artery, its material properties, and its geometry by the following formula (d, diameter; h, thickness):

$$E_{\text{inc}} = \frac{6\pi (d+2h)^2}{8} \frac{1}{CSA/D}$$

where the following formula for the incremental modulus was used:\textsuperscript{35}

$$E_{\text{inc}} = \frac{3(d(d+2h))}{8(h(d+h))} \frac{dP}{d(d)}$$

For a given artery, the CSA remains constant (incompressibility of the wall) and the incremental modulus becomes proportional (assume $h << d$) to:

$$E_{\text{inc}} \approx \frac{d^2}{D} \text{ or } D \approx \frac{d^2}{E_{\text{inc}}}$$

The above relation explains the seemingly controversial finding where the arteries in vitro have lower $E_{\text{inc}}$ (softer material) but are still less distensible (lower $D$). This is due to the severe reduction in diameter, which, being raised to the second power, overrides the change in $E_{\text{inc}}$ and yields a lower distensibility. The above can be demonstrated using the values for WKY at 120 mm Hg as an example (Figures 1 through 3).

$$\left( \frac{D_{\text{in vivo}}}{D_{\text{in vitro}}} \right)^2 \approx \left( \frac{d_{\text{in vivo}}}{d_{\text{in vitro}}} \right)^2 \times \frac{E_{\text{inc in vitro}}}{E_{\text{inc in vivo}}}$$

$$\approx \left( \frac{1.1}{0.58} \right)^2 \times \left( \frac{3.6}{7.0} \right) = 1.85$$

This simple calculation predicts a 2-fold decrease in the distensibility in vitro, which is in good agreement with the values shown in Figure 2.

There was a strong positive correlation between the CSA obtained in vitro by ultrasonography with the 17-MHz probe and the CSA obtained at histology. This demonstrates that histological processing induces a similar and consistent degree of volume reduction in all arteries. It also indicates that with the 17-MHz ultrasonic probe, it is possible to determine accurately an arterial wall thickness as small as 70 $\mu$m. However, this was only possible in vitro in the dissected artery and not in vivo in the presence of the surrounding structures.

In conclusion, the mechanical properties of the carotid artery substantially differ in vivo, in situ, and in vitro. However, the data from WKY and SHR show consistent elastic behavior in vitro and in vivo in both strains of rats. Cross-sectional distensibility is dramatically decreased in vitro in hypertrophic and normal arteries, and this phenomenon is not related to an increased stiffness of the arterial wall but rather to a change in geometry of the artery in situ and in vitro. These results demonstrate that in vitro and/or in situ measurements cannot automatically be extrapolated to in vivo conditions.

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