Static and Dynamic Mechanical Properties of the Carotid Artery From Normotensive and Hypertensive Rats

Oscar Lichtenstein, Michel E. Safar, Eric Mathieu, Pierre Poitevin, Bernard I. Levy

Abstract—Several recent results obtained in hypertensive animals and subjects under in vivo isobaric conditions do not confirm the classic view of stiffer arteries in hypertensive subjects. We compared the mechanical behavior of in situ isolated common carotid arteries from normotensive Wistar-Kyoto rats (WKY) and age-matched spontaneously hypertensive rats (SHR) under both static and dynamic conditions for transmural pressure ranging from 50 to 200 mm Hg. The static pressure (P)–diameter (D) relationship was shifted to higher values of diameters in the SHR mainly because of a larger unstressed carotid diameter (D₀) in hypertensive rats. The carotid mechanical strain, calculated as (D−D₀)/D₀, was significantly reduced in SHR at pressure levels between 100 and 200 mm Hg. The static carotid compliance and distensibility were markedly smaller in SHR than in WKY carotid arteries, indicating a stiffer wall in hypertensive animals. In contrast, carotid compliance and distensibility were similar under dynamic conditions close to the in vivo pulse pressure (frequency, 300 bpm; peak amplitude of the oscillatory pressure, 20 to 25 mm Hg). However, marked differences in dynamic compliance- and distensibility-strain relationships in SHR and WKY are evidence of clearly different arterial wall material properties in both strains. We therefore conclude that larger lumen carotid arteries in hypertensive rats could compensate for a stiffer arterial wall, resulting in similar dynamic compliance and distensibility in normotensive and hypertensive rats. (Hypertension. 1998;32:346-350.)

Key Words: carotid arteries • stiffness • viscosity • blood pressure

There is general agreement that arterial hypertension, aging, diabetes, and atherosclerosis are associated with marked changes in structure and mechanical properties of large arteries.1

Because of the nonlinear pressure-diameter relationship of large arteries, it is difficult to compare the mechanical properties of the large arterial wall in patients or animals with different arterial blood pressure. Furthermore, most of the experiments to assess the mechanical properties of the arterial wall are performed under static conditions, ie, with the pressure being varied by successive steps.2,3 However, under in vivo conditions with pulsatile pressure generated by the cardiac pump, the large arterial wall must be considered in terms of dynamic behavior, ie, taking into account the viscous, frequency-dependent properties of the vascular wall.

New ultrasonic technologies have been developed that allow the precise measurement of both in vitro and in vivo instantaneous arterial diameter.4 The application of these ultrasonic techniques to the calculation of mechanical properties of large arteries in human and experimental hypertension has led to conflicting results. In contrast to the classically reported stiffer arterial wall in hypertensives, similar or even increased arterial compliance and distensibility were calculated in hypertensive subjects and animals.5,6 However, because of in vivo measurements in the operating arterial pressure range, it was impossible to compare the dynamic mechanical properties of the arterial wall in hypertensives and normotensives under isobaric conditions without an unverified hypothesis and theoretical models of the mechanical behavior of the arterial wall. In fact, to compare the arterial wall properties in vivo under isobaric conditions in normotensives and hypertensives, one must use calculations performed from pressure-diameter values recorded during the systole in the former and during the late diastole in the latter, ie, under markedly different dynamic conditions. It is also possible to modify the operating pressure in 1 group to reduce the arterial pressure in hypertensives and/or to increase it in normotensives, and therefore to compare wall properties in normotensive and hypertensive animals at comparable blood pressures in vivo.7 However, the calculated parameters do not represent their actual operating values; furthermore, a direct effect of the drugs used to modify the arterial pressure on arterial smooth muscle tone and mechanical properties cannot be excluded.

Therefore, the purpose of the present study was to (1) describe an experimental setup that would allow us to simultaneously evaluate in situ the static and dynamic properties of the CCA in normotensive WKY and SHR and (2) differentiate from the alterations of the mechanical arterial properties in SHR those that are related to the static properties (pressure domain) and those related to the dynamic properties (frequency domain) of the vessels.

Methods

Experimental Setup and Artery Preparation

The experimental setup allowed us to maintain the CCA at constant levels of mean pressure throughout the study and to superimpose to...
the mean pressure a sinusoidal pressure wave of adjustable frequency and amplitude.

Eight 12-week-old normotensive control rats (WKY) and 8 age-matched SHR were anesthetized with sodium pentobarbital (50 mg/kg IP) and kept at constant body temperature (38°C) with a thermostatic operating table (Harvard Apparatus). After anesthesia, the trachea was cannulated and connected to a rodent respirator (model 680, Harvard Apparatus). The left CCA was then exposed, and its upper end was catheterized with a 10-cm-long noncompliant tube (Teflon, 0.9 mm ID) filled with Tyrode’s solution containing albumin (4%). A noncompliant tube, as short as possible, was used to minimally affect the mechanical properties of the carotid segment. The tube used was at least 10,000 times stiffer than the carotid segment. The presence of protein in flushing and incubating solutions preserved the endothelium and maintained a physiological osmotic pressure gradient across the vessel wall. The root of the left carotid was dissected, and a 2F microtip pressure transducer (Millar) was inserted into the CCA. The upper end of the CCA was connected to a pressure chamber with adjustable steady pressure (windkessel system); pressure sinus waves were superimposed in the isolated segment of carotid artery by means of a vibrator (LSD, model 200) with an amplifier (LSD, model PA25E) connected to the upper end of the artery (frequency, 300 bpm; peak amplitude of the oscillatory pressure, 20 to 25 mm Hg).

For the arterial diameter measurement, a high-precision ultrasonic echo-tracking device was used (Asulab). The probe consisted of a 10-MHz strongly focused piezoelectric transducer (6 mm diameter, 10 mm focal length) operated in the pulse-echo mode. The −10 dB beam width is 0.3 mm at the focal point, and the depth of field at −10 dB is 5 mm. A stereotaxic arm permitted motion of the probe perpendicularly to the arterial axis in its largest cross-sectional dimension. The transducer was positioned so that its focal zone was located in the center of the artery; thus, the back-scattered echoes from both the anterior and posterior walls could be easily visualized. A typical RF signal is then displayed on a computer monitor interfaced to the transducer system. Arterial diameter was measured when a clear “double-peak” RF ultrasound signal of the artery was visible as the ultrasound beam crosses the axis of the vessel and are characterized first by a high-amplitude signal followed by a relatively silent acoustic period and then a second high-amplitude signal.

The sample rate of the system is 5000 Hz, and its resolution is close to 1 μm. To enlarge the acquisition capacity, each consecutive 16 points were averaged into a single value, meaning that the actual sample rate is 312.5 Hz. The difference between 2 successive measurements was <2 μm.

Protocol and Data Acquisition

In normotensive WKY and SHR, the in situ isolated CCA was exposed to stepwise pressure increases of 25 mm Hg each step, from 50 mm Hg to 200 mm Hg. Pressure-diameter relationships were determined under static and then under dynamic conditions (5 minutes per step plus 5 minutes for recovery at each step). To obtain oscillatory pressure, a sinusoidal wave of 5-Hz frequency with a peak-to-peak amplitude of 20 to 25 mm Hg was superimposed over each mean pressure. The ultrasound probe was positioned perpendicularly over the artery with an ultrasound gel to avoid direct contact. After each change in pressure, the new conditions were maintained for 4 minutes to let the tissue reach its steady state. At each pressure level, the pressure and diameter signals were continuously measured for 4 seconds.

From the instantaneous pressure and diameter signals, the mean diameter and mean pressure were calculated. The power spectrum analysis of the signals was done using the standard signal processing toolbox of MATLAB and the Welch method of power spectrum estimation. The sequence of N points was divided into K sections of M points each (M must be a power of 2). Using an M-point fast Fourier transform, successive sections were Hanning windowed, analyzed, and accumulated. After processing of the spectral analysis of the signal, the amplitudes of the first harmonic of pressure (dP) and diameter (dD) were obtained.

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<th>Lumen Diameter and Wall Strain of Common Carotid Artery Submitted to Increasing Levels of Static Transmural Pressure</th>
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*p < 0.001 for differences between WKY and SHR.
Calculation of Mechanical Properties
Using the measured static values of the “in situ” carotid lumen diameter (D, \(\text{mm}\)) from 50 to 200 mm Hg and the measured dynamic values \(dD\) (\(\text{mm}\)) and \(dP\) superimposed at each pressure (P) step, several variables were calculated.

For static variables, unstressed diameter \(D_o\) and volume \(V_o\) were considered to be diameter and volume values at 50 mm Hg transmural pressure. Strain was calculated as \(\frac{(D^2 - D_o^2)}{D_o}\) (dimensionless parameter). The static compliance per unit length was defined for each pressure step as \(C = \frac{DV}{DP}\) (\(\text{mm}^2 \text{L/mm Hg}\)), where \(DV\) is the change in volume \(V\) (with \(V = \pi D_o^2/4\)) induced by a transmural pressure variation of \(DP\) (25 mm Hg) in an artery segment of 1 mm in length. The static distensibility was defined for each step of transmural pressure as \(\text{Dist} = \frac{C}{V_o}\) and expressed per unit length (\(10^{-3} \text{ mm Hg}^{-2}\)).

For dynamic variables, measurements were performed at each step of transmural pressure \(P\), corresponding to a static diameter \(D\). At each value of \(P\), a sinus pressure wave signal \(dP\) produced changes in diameter \(dD\) and corresponding changes in cross-sectional area \(dS\), assuming a cylindrical model of the artery. In this system, dynamic compliance and distensibility, expressed per unit length of the artery, were respectively calculated as \(\frac{dS}{dP}\) and \(\frac{(dS/S_o)}{dP}\), with \(S_o\) (unstressed lumen area) = \(\pi D_o^2/4\).

Statistical Analysis
Results are expressed as mean \(\pm\) SEM. Two-way ANOVA was used to test for significant differences between groups. Bonferroni’s test was used to detect differences for determined levels of pressure with correction for multiple comparisons. A level of \(P<0.05\) was considered significant.

Results

Under Static Conditions
The Table reports the diameter-pressure and strain-pressure static values in WKY and SHR; static diameters were significantly higher in SHR than in WKY at any given value of transmural pressure \((P<0.001)\). For transmural pressure values >75 mm Hg, strain was significantly higher in WKY than in SHR \((P<0.001)\).

Figure 1 shows the curve relating transmural pressure to static compliance and distensibility of the isolated segment of the CCA. In both WKY and SHR, static compliance (Figure 1A) decreased when transmural pressure increased. Except for the extremities of the curves (50 to 75 and 175 to 200 mm Hg), static compliance was significantly lower \((P<0.02)\) in SHR than in WKY. Figure 1B illustrates similar findings for the distensibility-pressure curves; static carotid distensibility was significantly lower \((P<0.001)\) in SHR than in WKY for pressures ranging from 75 to 175 mm Hg.

Under Dynamic Conditions
Figure 2 shows the experimental relationships between transmural pressure and carotid lumen diameter in both normotensive and hypertensive rats. Curvilinear curves (thin lines) were obtained by joining the mean values of oscillatory pressures and diameters at each pressure step. The phasic changes in diameter \((dD)\) induced by sinus pressure waves \((dP)\) were superimposed (bold lines) at each pressure steps. Two major points resulted from these data: (1) there was no difference between the static pressure-diameter carotid relationship and that obtained under dynamic conditions by using the mean pressure and diameter calculated from their phasic values; and (2) at each transmural pressure step, the slope of the pressure-diameter static relationship was steeper under static than under dynamic conditions, indicating stiffer arteries under static pressure conditions.

Figure 3 reports the dynamic pressure-compliance (Figure 3A) and pressure-distensibility (Figure 3B) relationships. For both carotid compliance and distensibility, there was no
significant difference between WKY and SHR, suggesting similar dynamic behavior of the CCA in normotensive and hypertensive strains.

Figures 4 and 5 show the static and dynamic carotid compliance (Figure 4A and 5A) and distensibility (Figure 4B and 5B) with their respective static and dynamic strain values. Both compliance- and distensibility-strain relationships are markedly different between SHR and WKY either under static or dynamic conditions, indicating stiffer material in hypertensive animals.

Discussion

The structural changes of the arterial wall observed in hypertensive patients and animals may reflect either a primary defect or a consequence of elevated blood pressure. Increased vascular wall thickness, a constant feature reported in sustained hypertension, is thought to be a key determinant of the mechanical behavior of large arteries. Several physical parameters have been used to assess the influence of hypertension on the arterial mechanical properties. Pulse wave velocity, analysis of the exponential diastolic pressure wave decay, and analysis of the pressure-diameter relationship are among the approaches used to characterize the mechanical properties of the arterial wall associated with hypertension. Compliance, which can be defined as the blood volume stored or released for a given arterial pulse pressure, decreases when mean blood pressure increases. Because the pressure-volume relation is clearly nonlinear, compliance changes dramatically with variations in blood pressure. Therefore, pressure has to be accounted for as a variable. This is not possible in vivo when mechanical properties of arteries have to be compared under normal and hypertensive conditions.

In the present series of experiments, the in situ isolated segment of rat CCA was studied under controlled values of transmural pressure (50 to 200 mm Hg), allowing us to correctly compare the calculated mechanical parameters in normotensive and hypertensive rats. In terms of functional changes in pathological conditions, information from the carotid bulb would be more relevant; however, the ultrasonic system used in this and in most experimental and clinical studies does not allow reliable recording of diameters in noncylindrical vessels.

Furthermore, in most previous studies, the mechanical properties of the arterial wall have been considered under static conditions. Basically, when blood pressure increases, the lumen arterial diameter increases and energy is stored in the vascular wall. Purely elastic materials allow the whole stored energy to be restored during diastole. However, the arterial wall exhibits viscoelastic behavior, and a part of the stored energy is dissipated within the arterial wall because of viscous properties of the wall. In a recent extensive in vivo and in vitro study, Boutouyrie et al showed that the viscous loss of energy appeared to be small at the cross-sectional level but cannot be
neglected if it is integrated along the arterial tree. We used a pulse pressure value of 25 mm Hg to mimic the in vivo pressure. The pulse pressure values are different in normotensive and hypertensive animals, and our experimental conditions were as close as possible to the in vivo conditions but did not perfectly fit to the in vivo models.

One of the main findings of the present work is that the mechanical properties of the carotid arterial wall are markedly different under steady (static) and pulsatile conditions, indicating stiffer arterial wall for pulsatile pressure. Under static conditions, carotid compliance and distensibility were markedly reduced in the SHR, in agreement with previously reported results under similar conditions.17,18 In contrast, under dynamic conditions, carotid compliance and distensibility appeared to be very similar in normotensive and hypertensive rats. This latter result can be compared with those obtained by several investigators5,6 under in vivo hypertensive rats. This latter result can be compared with similar values of compliance and distensibility under dynamic conditions, carotid compliance and distensibility were reportedly results under similar conditions.17,18 In contrast, dynamic conditions: compliance (A) and distensibility (B) of the in situ isolated CCA vs carotid strain. ○ indicates WKY; ●, SHR. Values are mean±SEM.

References
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