Smooth Muscle Tone and Arterial Wall Viscosity
An In Vivo/In Vitro Study

Pierre Boutouyrie, Salïha Boumaza, Pascal Challande, Patrick Lacolley, Stéphane Laurent

Abstract—The relationships between steady and pulsatile pressures, smooth muscle tone, and arterial viscoelastic behavior remain a matter of controversy. We previously showed that arterial wall viscosity (AWV) was 3-fold lower in vivo than in vitro and suggested that in vivo active mechanisms could minimize intrinsic AWV to improve the efficiency of heart-vessel coupling energy balance. The aim of the present study was to determine the role of smooth muscle tone on AWV, under various levels of steady and pulsatile pressures, both in vivo and in vitro. AWV of rat abdominal aorta was studied first in vivo after bolus injections of phenylephrine (PE) or sodium nitroprusside (SNP), then in vitro in response to PE or SNP. In vitro, arterial segments were submitted first to steady pressure (0 to 200 mm Hg) by increments of 20 mm Hg, then to increasing levels of pulse pressure (20 to 50 mm Hg) at various mean arterial pressures (75 to 150 mm Hg). AWV was quantified as the area of the pressure/diameter relationship hysteresis, issued from the simultaneous measurements of pressure (Millar micromanometer) and diameter (NIUS echotracking device). In vivo, AWV increased after PE and decreased after SNP, in parallel with pressure changes. In vitro, AWV was not significantly influenced by PE and SNP. After both PE and SNP, AWV increased with pulse pressure but was not influenced by mean arterial pressure. At any given pulse pressure, AWV was higher in vitro than in vivo. The relation between AWV and pulse pressure was significantly steeper in vitro than in vivo. These results show that AWV is strongly influenced by steady and pulsatile mechanical load but not by smooth muscle tone, both in vivo and in vitro. Factors other than sustained smooth muscle activation should be explored to explain the minimization of AWV in vivo compared with intrinsic in vitro values. (Hypertension. 1998;32:360-364.)

Key Words: viscosity ■ arteries ■ muscle, smooth ■ sodium nitroprusside ■ phenylephrine ■ aorta

Although it is well known that biological tissues, including the arterial wall, respond to stress through both elastic and viscous behaviors, the viscous aspect has often been neglected. Indeed, authors acknowledged the theoretical difficulties raised by taking viscosity into account and the methodological difficulties for measuring it.1,2 In most studies, viscosity was considered a dampening phenomenon and expressed in term of phase delay. An alternative approach, developed by mechanical engineers, was to consider viscosity an energy-dissipating phenomenon during the mechanical transduction.3–5 Indeed, a major function of large arteries is to store mechanical energy generated by the heart during systole and to restore it during diastole to optimize the heart-vessel coupling.6–9 We used this approach in a recent study10 and showed that the viscosity measured in vivo in intact animals was 3-fold lower than viscosity measured in vitro at the same arterial site under similar pressure conditions. We hypothesized that active mechanisms could compensate for intrinsic viscosity under physiological conditions to improve the efficiency of the heart-vessel coupling energy balance. The few studies that determined the effect of smooth muscle tone on AWV yielded complex results, showing either higher AWV in muscular arteries7,11 and increased AWV in response to smooth muscle contraction,12,13 or no effect under sinusoidal pressure waves of small amplitude.14 The objectives of the present study were to determine the role of smooth muscle tone on AWV and to compare in vivo experiments to in vitro ones performed under similar pulsatile pressure conditions.

Methods

Animals

We used 11 male Wistar rats (Iffa-Credo France) aged 12 weeks and weighing 320±20 g (mean±SD). The animals were managed according to the French Ministry of Agriculture guidelines. Experiments were conducted with animals under disodium thiopental anesthesia (50 mg/kg IP). Animals were killed by exsanguination. The influence of PE and SNP on AWV of rat abdominal aorta was determined first in vivo in 5 rats, then in vitro in 6 rats.

Determination of Dynamic Diameter-Pressure Relationship

Because viscosity is measured as the area of hysteresis of the diameter-pressure relationship, we first simultaneously measured pulsatile changes in diameter and pressure to determine their relationship. In vivo and in vitro measurements were made in the same way. Pulsatile changes in distending pressure were measured...
using a 2F high-fidelity microtransducer (Millar Instruments, Nycom). Pulsatile changes in arterial diameter were measured with the NIUS 1 echotracking device (Asulab Research Laboratory) as previously described.15–17 Briefly, the device measures internal diameter and its systolic-diastolic variations with a 10-MHz ultrasound transducer, with precision close to 50 μm and 1 μm, respectively. The software was adapted for viscosity measurements.10 Diameter and pressure were simultaneously digitized and stored at a frequency of 2.5 kHz, giving a 4 × 10⁻⁴-second time domain definition (0.0125 radian for a frequency of 5 Hz). The computerized acquisition system derived the LCSA-pressure curve from the 2 continuous signals of arterial diameter and pressure and fitted it using an arctangent model with 3 independent parameters as described by Langewouters et al.16 This model allows the calculation of geometric and functional parameters at any pressure value within the systolic-diastolic range. Because AWV determines hysteresis in the pressure-diameter relationship, the distension limb is distinct from the recoil limb. The calculation of distensibility and compliance at MBP was performed on the diastolic part of the curve, as previously described.3,15

### Evaluation of AWV

AWV was estimated by the area of hysteresis of the pressure-volume relationship, both in vivo and in vitro.3 As previously described,10 the energy per unit of arterial length exchanged from the blood to the arterial wall during distension can be written as:

$$\frac{dW}{dL} = P \cdot d\text{LCSA}$$

where P is pressure, LCSA is lumen cross-sectional area, dLCSA is the elementary variation in lumen area, and dL is an arbitrary length.

AWV determines the emergence of a hysteresis loop, where it is possible to distinguish the “distension” limb during systole from the “recoil” limb during diastole. Arterial diameter is smaller during distension than recoil at a given distending force. The hysteresis loop corresponds to a classic adiabatic cycle in thermodynamics. Indeed, the energy stored during the distension phase (elastic energy or WE) is not fully restored during recoil. The energy dissipated by the arterial wall (dW) per unit length during 1 cycle (ΔL) represents the viscous energy (Wv) and can be written as:

$$W_v = \text{distension} \int_{A}^{B} \frac{dW}{dL} - \text{retraction} \int_{A}^{B} \frac{dW}{dL}$$

$$W_v = \left[ \text{distension} \int_{A}^{B} P \cdot d\text{LCSA} - \text{retraction} \int_{A}^{B} P \cdot d\text{LCSA} \right] \cdot \Delta L$$

The quantity given by Equations 2 and 3 corresponds graphically to the area of the hysteresis loop of the pressure-LCSA relationship. We developed dedicated software to measure this area directly from the original pressure and diameter recordings, using an integrative algorithm. Energies are expressed as joules per meter during 1 cycle. Viscous energy (Wv) can be expressed either in absolute values or as a percentage of total energy (Wt) with Wv = 100 × (Wv − Wd)/Wt. We chose to use the latter expression of AWV thereafter as Wv. This software permitted us to detect a phase lag as small as 10⁻³ radian on simulated sinusoidal time series. The practical precision of this method is given by the precision of pressure and diameter measurements (0.5 mm Hg and 1 μm, respectively) and can be estimated as better than 1 × 10⁻¹⁰ joule · m⁻¹. Viscosity was measured on all cardiac cycles recorded during 3 periods of 4 seconds (ie, ~64 cycles) and was averaged. Reproducibility coefficient was 10% within a given measurement (ie, 20 successive cycles without replacement of the probe) and 8% between measurements (ie, coefficient of variation on 2 successive measurements). The major source of variability in hysteresis loop area was the variability in PP from pulse to pulse. The viscosity measurement has been validated in vitro through an experimental setup previously described.10 which included a pressure wave synthesizer designed to generate in vitro pressure waves similar to in vivo ones. We found that the combination of a rectangular negative ramp electric signal and an adjustable proximal windkessel provided realistic pressure, not significantly different from in vivo, in time and frequency domains.10 We checked the absence of time delay between pressure and diameter signals.

### In Vivo Experiment

A 2F microtransducer was introduced through the left femoral artery of anesthetized animals for aortic blood pressure measurements. A venous PE-10 catheter was inserted in the femoral vein for drug injections. A median laparotomy was then performed, and the abdominal aorta was exposed but not dissected. The echotransducer was stereotactically positioned 1 cm above the aortic trifurcation, using 37°C warm isotonic saline as coupling medium. The position of the pressure probe was adjusted visually and assumed to be correct when the tip of the catheter was detected on the radiofrequency signal of the aorta.

After establishing the dose-response curve for blood pressure with each drug, we chose 2 different doses of PE and SNP to determine subpressor responses (0.75 μg/kg bolus) or large changes in blood pressure (7.5 μg/kg bolus). PE and SNP were given in random order. We began with saline, followed by the subpressor dose and then by the large dose. Each injection was followed by a washout time of >5 minutes after the blood pressure had returned to the baseline value. Pulsatile changes in diameter and pressure were then measured during 3 periods of 4 seconds framing the maximal blood pressure change. The average of the 3 measurements was retained as a single value.

### In Vitro Experiment

The experimental setup developed for in vitro determination of AWV has been described in detail previously.10 Arterial diameter and pressure were measured and computed in the same way as in vivo. Briefly, segments from the abdominal aorta were quickly removed and placed in ice-cold Krebs buffer. The proximal end of the abdominal aorta was cannulated with a 2F cannula and connected to a perfusion line. The distal end was cannulated with a 2F high-fidelity microtransducer to measure intraluminal pressure. The arterial segment was then mounted in a specially designed organ chamber containing oxygenated Krebs buffer at 37°C, extended to its in vivo length, and pressurized with Krebs at a constant pressure of 100 mm Hg for 30 minutes. The perfusion line was composed of low-compliance polyethylene tubing, a pressure reservoir containing oxygenated Krebs buffer at 37°C, a proximal adjustable windkessel segment, and the PP synthesizer.

We used the following protocol for studying the interrelationship between smooth muscle tone and steady and pulse pressures. After the equilibration period was completed, the bath was changed and replaced by the same buffer in addition to the drug of interest. We chose to study the drug concentration producing a maximal response, which corresponded to final PE and SNP concentrations of 10⁻⁴ mol/L. The order of drug administration was random. Under each drug, the protocol began by determining the quasi-steady-state pressure-diameter relationship between 0 and 200 mm Hg, up and down by 2-minute steps of 20 mm Hg. The artery was set for 10 minutes at 75 mm Hg without PP; then 4 levels of PP (20, 30, 40, and 50 mm Hg) were successively applied in increasing order for 5 minutes each at a fixed frequency of 5 Hz. This procedure was repeated for successive mean pressure levels of 100, 125, and
150 mm Hg. The whole procedure was repeated for the second drug after a 30-minute washout period at 100 mm Hg.

Statistical Analysis
Data are expressed as mean±SEM. The effects of in vivo administration of PE and SNP on AWV were analyzed with repeated-measures ANOVA, with dose as a within-subject factor and drug (PE, SNP) as a between-subject factor. Post hoc Bonferroni’s test was performed. In vitro experiments were analyzed with repeated-measures ANOVA with dose as a within-subject factor and drug (PE, SNP) as a between-subject factor. We tested the linear relationship between AWV and PP level as a within-subject factor and drug (PE, SNP) as a between-subject factor. We considered to denote significant differences. All tests were bilateral, and values of \( P < 0.05 \) were considered to denote significant differences. All statistical tests were performed with Systat 5.0 software.\(^{19}\)

Results

In Vivo Experiment
The highest dose of SNP induced a significant decrease in MBP, PP, SBP, DBP, and AWV, and a 2-fold increase in distensibility and compliance (Table 1). The highest dose of PE induced opposite changes: a significant increase in MBP, PP, SBP, DBP, and AWV, and a 2-fold increase in distensibility and compliance. The abdominal aorta LCSA did not change under SNP despite the decrease in blood pressure, whereas it increased significantly with the highest dose of PE (Figure 1).

In Vitro Experiment
LCSA of the abdominal aorta (Figure 2) was significantly lower after PE than after SNP, at any blood pressure level, both in static and dynamic conditions (Table 2). At any combination of mean and pulse pressures, AWV was not significantly different between PE and SNP. AWV increased significantly with PP but was not influenced by MBP. Figure 3 shows that the linear relationships between AWV and PP after PE and after SNP (\( r = 0.99, P < 0.001 \), and \( r = 0.98, P < 0.001 \), respectively) are superimposable.

In Vivo/In Vitro Comparison
In vitro relationships between AWV and PP were significantly \( (P < 0.001) \) shifted upward compared with in vivo ones, with a significantly steeper slope \( (0.22±0.01 \) versus \( 0.06±0.01\%/\text{mm Hg}, \) respectively; \( P < 0.01 \)). The in vivo AWV/PP relationship tended toward a plateau for the higher and lower PPs. This pattern was not observed in vitro.

Discussion
The main result of the present study is that at any combination of mean and pulse pressures in vitro, AWV was not significantly different between PE and SNP. The second result of this study is that in vitro relationships between AWV and PP were significantly shifted upward, and with a steeper slope compared with in vivo.

Consideration of Methods
The 2 prerequisites of the present study were (1) accurate measurement of AWV in vivo, under physiological pulsatile pressure and flow conditions, and (2) comparability of these measurements with those obtained on the same arterial
segments in vitro under similar pressure conditions. We had to make theoretical choices as to the characterization of AWV. Indeed, although many models of viscoelasticity have been proposed, none has been proved fully able to describe the behavior of the vascular wall.20 Thus, instead of using a specific model, we took advantage of the ability of the NIUS system to accurately determine the pressure-diameter relationship and chose to measure AWV directly through its thermodynamic expression, ie, the hysteresis loop area of the pressure-diameter curve. This approach has the advantage of stressing the physical consequence of viscosity, which is to dissipate energy. Brodley4 and Bertram5 applied this method to arterial wall mechanics. As explained in the Methods section, a prerequisite for measuring the hysteresis loop of the 2 signals. In a dedicated experimental setup including a highly elastic membrane, we have already shown that this was effectively the case, with a negligible hysteresis loop area.10

The second challenge was to generate in vitro physiological pressure waveforms. Previous works on AWV have involved low-amplitude sinusoidal pressure waveforms. Under these conditions of low-amplitude deformation, AWV was fairly insensitive to frequency,20 a result that does not reflect the true response of the arterial wall to the physiological mechanical load. Indeed, viscous phenomena depend not only on frequency but above all on the amplitude and velocity of PP change.6,21 The natural pressure waveform exhibits both high-amplitude and high-frequency components that predominate during the distension phase. We thus developed a pressure wave synthesizer to generate a pressure waveform similar to the natural counterpart. We have shown that the synthesized pressure waveforms did not differ significantly from in vivo ones.10

Consideration of Findings
The present study was undertaken to test the hypothesis that smooth muscle tone could explain the in vivo/in vitro difference in AWV.10 Indeed, we showed that the viscosity measured in vivo in intact animals was 3-fold lower than viscosity measured in vitro at the same arterial site under similar pressure conditions. Although dissipation of pulsatile energy through viscosity is only 1 aspect of the energy exchanges in the cardiovascular system, it may become important under abnormal conditions. Bertram5 showed that in vivo viscoelasticity “contributes relatively little to energy dissipation per cardiac cycle and pulse wave attenuation.” O’Rourke22 has shown that in vivo, the ratio of pulsatile to pressure-diameter relationship was the synchronization of the 2 signals. In a dedicated experimental setup including a highly elastic membrane, we have already shown that this was effectively the case, with a negligible hysteresis loop area.10

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**TABLE 2. AWV of Abdominal Aorta During PR or SNP Perfusion Under In Vitro Conditions**

<table>
<thead>
<tr>
<th>PP, mm Hg</th>
<th>MBP, mm Hg</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE</td>
<td>75</td>
<td>3.52±0.10</td>
<td>4.98±1.27</td>
<td>7.99±1.87</td>
<td>9.06±2.48*</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2.65±0.63</td>
<td>4.64±0.94</td>
<td>6.53±1.29</td>
<td>7.93±1.74*</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>1.96±1.03</td>
<td>3.50±1.45</td>
<td>6.65±5.49</td>
<td>9.04±3.03*</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>2.34±1.08</td>
<td>4.32±1.62</td>
<td>7.20±2.79</td>
<td>9.24±3.62*</td>
</tr>
<tr>
<td>SNP</td>
<td>75</td>
<td>2.41±0.41</td>
<td>4.28±0.70</td>
<td>6.48±1.24</td>
<td>9.02±1.32*</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2.65±0.57</td>
<td>4.01±0.67</td>
<td>6.56±1.40</td>
<td>8.29±1.45*</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>3.27±1.11</td>
<td>4.85±1.03</td>
<td>6.32±1.47</td>
<td>8.00±1.61*</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>1.63±0.66</td>
<td>3.52±1.00</td>
<td>5.42±1.22</td>
<td>7.74±1.64*</td>
</tr>
</tbody>
</table>

Arterial segments (n=6) were submitted to increasing levels of PP at various levels of MBP (see text). Data are mean±SEM.

*Significant (P<0.001) linear relationship between AMV and PP (ANOVA). No significant difference was observed between PE and SNP.
total external work, although averaging only 10% under control conditions, increased markedly when heart rate or arterial distensibility decreased.

The fact that AWV was very low in vivo suggests that active mechanisms could minimize the intrinsic viscosity of the arterial wall. The main result of the present study is that in vitro AWV was not significantly different between PE and SNP at any combination of mean and pulse pressures, indicating that sustained changes in smooth muscle tone have no influence on AWV. In vivo, changes in AWV in response to PE and SNP were parallel to those of MBP and PP. Thus, it was difficult to determine whether in vivo the changes in AWV were due to changes in smooth muscle tone, MBP, or PP. The fact that in vitro, PP, but not MBP or smooth muscle tone, influenced AWV suggests that in vivo the changes in AWV in response to PE and SNP were primarily caused by changes in PP. We may thus propose that AWV is not influenced by sustained changes in smooth muscle tone either in vivo or in vitro under similar pressure conditions.

This finding is in agreement with previous studies that found no influence of smooth muscle tone on AWV under dynamic conditions. These studies used small sinusoidal waves to generate small-amplitude stress oscillations. The authors put forward that the amplitude of stress oscillations was insufficient to allow breakage of actin-myosin bridges, a phenomenon classically associated with viscous behavior. Our results extend their finding to large physiological deformations and establish evidence that the amplitude of stress oscillations is the major determinant of AWV.

The fact that sustained changes in smooth muscle tone do not influence AWV does not preclude that rapidly occurring changes in smooth muscle tone may modulate AWV. Indeed, in the present study, AWV changed within seconds after the bolus injection of PE or SNP. In addition, the slope of the AWV-PP relationship was lower in vivo than in vitro, suggesting that acute changes in smooth muscle tone could compensate for the intrinsic in vitro AWV, even during rapid changes of hemodynamic conditions. An additional explanation for the in vivo/in vitro difference in AWV may involve the physical properties of the arterial wall components. Indeed, in contrast to very slow-reacting components (such as collagen I) that behave like stiff material under rapidly changing stress, filamentous biopolymers such as vimentin may undergo a change of phase (crystalline versus soluble) in response to changes in the ionic environment. This has been shown to affect the behavior of isolated cells and could therefore influence the viscosity of the arterial wall.

In conclusion, AWV of the rat abdominal aorta is strongly influenced by steady and pulsatile mechanical load but not by smooth muscle tone. Factors other than sustained smooth muscle tone should be explored to explain the optimization of AWV in vivo.
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Hypertension. 1998;32:360-364
doi: 10.1161/01.HYP.32.2.360

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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