Aortic stiffness in vivo in hypertensive rat via echo-tracking: analysis of the pulsatile distension waveform

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Abstract

Aortic stiffness in vivo in hypertensive rat via echo-tracking: analysis of the pulsatile distension waveform. Am J Physiol Heart Circ Physiol 301: H382–H390, 2011. First published May 20, 2011; doi:10.1152/ajpheart.00094.2011.—Large-artery stiffening is a major risk factor in aging and hypertension. Elevated blood pressure (BP) and vascular wall properties participate in arterial stiffening; we aimed to evaluate their respective role by combining echo-tracking and the spontaneously hypertensive rats (SHR) treated with low doses of a nitric oxide synthase inhibitor, shown to have arterial stiffening. Normotensive [Wistar-Kyoto (WKY)], SHR, and SHR treated for 2 wk with Nω-nitro-arginine methyl ester (SHRLN) were anesthetized; BP and distension (pulsatile displacement) of the aortic walls with the ArtLab echo-tracking device were measured. Stiffness index increased in SHRLN vs. SHR, compliance, distensibility, and the slopes and area of the distension-pressure loop curve decreased. The pulsatile distension and pressure waveforms were strongly altered in SHRLN. The distension (maximal value and AUC/ms) and the area under the curve adjusted to heart rate (AUC/cts) was calculated. Acute BP reductions were induced by diltiazem in SHR and SHRLN, to levels similar to those of WKY. In SHR, compliance, distensibility, stiffness index, and the ascending slope of the distension-pressure loop reached the values of WKY, whereas they were only partially improved in SHRLN. Aortic stiffness (maximal value and AUC/ms) and the area of the distension-pressure loop were improved in SHR, but not in SHRLN. These data confirm the aortic stiffening induced by nitric oxide reduction in SHR. They show that the ArtLab system analyzes aortic stiffness in rats, and that the aortic pulsatile distension waveform is a parameter strongly dependent on the vascular wall properties of blood pressure elevation and of mechanical and functional properties of the arterial wall that lead to alterations of compliance and stiffness.

In humans, the most reliable and most frequently used method to detect regional arterial rigidification is pulse-wave velocity (PWV); other methods used are pulse pressure (PP) wave analysis, as well as echo-tracking, with the evaluation of local compliance and stiffness via the dynamic properties of the arterial wall (17). Concerning pathological animal models, the spontaneously hypertensive rat (SHR) is largely used, and, as the animals get older, some parameters similar to human disease are noted (10, 25). In the old SHR, carotid and aortic remodeling have been shown, with increased collagen-to-elastin ratio (8, 20), increased integrin and fibronectin (4), endothelial dysfunction (4), and stiffening (32); also, cardiac and renal damages were described. More recently, a new model has been proposed using SHR treated with a low dose of the nitric oxide (NO) synthase (NOS) inhibitor Nω-nitro-L-arginine methyl ester (L-NAME) to reduce endogenous NO production. This model (SHRLN) has been shown to rapidly develop renal, cardiac, and arterial alterations (14, 31, 33). We have also recently illustrated that, in these rats, aortic stiffening is associated with endothelial dysfunction, hypertrophy, and fibrosis, comparable to the changes observed in aged SHR (Isabelle et al., unpublished observations); these results point to the interest of this disease model to investigate the vascular changes in hypertension.

The present study was designed 1) to improve our approach of the aortic stiffness analysis using the SHRLN model combined with an echo-tracking technique ArtLab; and 2) to evaluate the role played by blood pressure and arterial remodeling in aortic stiffening.

METHODS

This study conforms with European Community Guidelines for the use of experimental animals, with the National Institutes of Health (NIH) guide for the care and use of laboratory animals, and was approved by the ethical committee on Animal Experiments of the Servier Research Institute.

Male SHR and normotensive Wistar-Kyoto (WKY) rats (from CERJ), 20 wk old, were used. A group of SHR was treated with the NOS inhibitor L-NAME in drinking water at 5 mg·kg⁻¹·day⁻¹ during 2 wk (from 18 to 20 wk of age). Thus three groups of rats were studied: the normotensive WKY group, the SHR group, and the SHRLN group.

The rats were anesthetized with pentobarbital sodium (50 mg/kg ip). The anesthesia was maintained with pentobarbital sodium of 5 mg sc/45 min. The trachea was cannulated, and ventilation was maintained with a respirator Hallowell EMC (TEM) at a frequency of 70 cycles/min and a pressure of 10 cmH₂O. Body temperature was maintained at 38°C with a homeothermic blanket (Harvard) connected to a rectal probe.
Two catheters of similar length were introduced in the abdominal aorta via the femoral artery and close to the aortic arch via the carotid artery for recording of distal and proximal aortic pressures, respectively. Both catheters were connected to a Statham P10EZ Gould probe and then visualized and analyzed with an Acknowledge acquisition and analysis system (Biopac).

With the rats laying laterally, the aortic properties could be accurately analyzed with an echo-tracking ultrasonic system ArtLab (Esaote, Maastricht, the Netherland). After skin incision, the ultrasound probe L10–5 (40 mm) was placed above the abdominal aorta, close to the pressure recording site.

Detailed technical description of data acquisition via the ArtLab system has been previously published (7). Briefly, the aorta is visualized, and the diastolic diameter measured in a brightness mode scanning (Fig. 1A). Then a motion acquisition mode scanning is used to track the echoes of the aortic walls and thus to record their pulsatile displacement named distension (Fig. 1B). This recording, together with the ECG and the distal aortic pressure, allows a beat-by-beat analysis (Fig. 1C), including the feet-to-feet alignment of blood pressure and diameter, using a MatLab mathematical software platform (MathWorks). A low-pass filtering with a cut-off frequency of 100 Hz is applied on the wall velocity signal; the blood pressure signal, acquired via the Biopac system and derived to the ArtLab system, is not filtered.

The individual beat-by-beat curves of the aortic pressure, velocity, and distension (Fig. 1C) were acquired at 980 Hz (interval 1.02 ms) on
24–32 cardiac cycles, after synchronization with the ECG. The vascular distension is the diastolic-systolic diameter changes (previously named $\Delta D$ for delta diameter).

The parameters automatically calculated to determine the dynamic properties of the aortic wall are as follows: mean diameter and diastolic diameter ($d_D$); mean distension (in $\mu$m); compliance ($\Delta A/\Delta P$) in mm$^2$/kPa, where $A$ is the transsectional area of the vessel calculated from the diameter, and $P$ is pressure; distensibility ($\Delta A/\Delta P \times A$) in 1/kPa; and stiffness index beta ($([dD \ln(SAP/DAP)]/(sD - dD))$, where $sD$ is systolic diameter, SAP is systolic arterial pressure, and DAP is diastolic arterial pressure.

In addition, in the present study, from the beat-by-beat analysis (Fig. 1B), the pressure/time waveform, the distension/time waveform, and the loop curve of distension-pressure were constructed for each animal by averaging these data in the 24–32 cycles (Fig. 2). Distension was expressed either in micrometers or in percent vs. diastolic diameter ($d_D/100/d_D$).

From the distension-pressure loop, slopes were determined as described in humans (11) using linear regression: the systolic slope in the ascending (upward) part of the loop, according to the minimal and maximal pressure values, and the diastolic slope in the descending (downward) part of the loop, according the maximal and minimal distension values. An isobaric compliance was calculated as the distension-pressure slope at 200–210 mmHg for high blood pressure levels and at 160 mmHg for lower blood pressure levels. The arterial wall viscosity was estimated as previously shown via the area of the distension-pressure loop (3, 5). For individual analysis, blood pressure was expressed in millimeters of mercury and distension in micrometers. To average the data in each group of rats, distension was transformed in percent vs. diastolic diameter. Pressure was also...
calculated in ΔP (pressure-diastolic pressure). To take into account the changes in the kinetics of the distension/time and pressure/time waveforms, we calculated the area under the curve (AUC) individually. These AUC values were adjusted to the heart rate (AUC/ms = AUC × heart rate/60 × 10^3) to avoid heart rate differences between groups. An index of distensibility was calculated as AUC pulse distension/AUC PP.

After stabilization, mean arterial pressure (MAP), SAP, DAP, PP, aortic wall diameter, and dynamic parameters were measured at basal blood pressure. Then the SHR and SHRLN animals received an intravenous injection of diltiazem at 1 mg/kg into the penile vein to increase their blood pressure (2 ml/h, Perfusor Space B Braun). All parameters were then recorded at the decreased (SHRLN, SHR) or increased (WKY) blood pressure levels.

At the end of the experiments, the animals were euthanized with a lethal dose of pentobarbital. The distance between the two catheter tips was measured to calculate the PWV (equal to distance divided by the time between distal and proximal pressures, in m/s), and the aorta was carefully prepared for histological analysis. It was cut in 5-μm-thick sections and stained with hematoxylin-eosin, and intima-media thickness was measured. From a piece of frozen aorta, expression of fibronectin gene was quantified by real-time RT-PCR, as previously described (Isabelle et al., unpublished observations). Drugs used were pentobarbital sodium (SANOFI), L-NAME (Sigma), L-phenylephrine (Sigma), and diltiazem (Sigma); they were dissolved daily in saline.

Data are given as means ± SE. Changes were considered significant when P < 0.05, using a one-way ANOVA. ANOVA was followed by Bonferroni’s complementary test for comparison of blood pressure in all groups or selected groups to assess the effect of diltiazem in SHRLN and SHR and the effect of phenylephrine in WKY, and to compare the parameters in the group with similar blood pressure levels. A two-way ANOVA was used to compare the time curves between groups, followed by a complementary Bonferroni test.

RESULTS

Distension analysis in WKY, SHR, and SHRLN. MAP was 146 ± 3 mmHg in WKY, n = 9; 198 ± 4 mmHg in SHR, n = 11; and 216 ± 4 mmHg in SHRLN, n = 9. SAP and DAP are given in Table 1. The animal weight averaged 461 ± 12 g in WKY, 388 ± 5 g in SHR, and 314 ± 7 g in SHRLN. Heart rate was reduced in SHRLN; the internal aortic diameter did not differ between groups (Table 1). The intima-media thickness was 126 ± 2.0 μm in WKY, 144 ± 2.3 μm in SHR, and increased by 23% in SHRLN vs. SHR (177 ± 4.8 μm).

Fibronectin mRNA expression was significantly higher only in SHRLN (1.14 ± 0.26, 1.16 ± 0.24, and 7.05 ± 1.06 in WKY, SHR, and SHRLN, respectively). Aortic compliance and distensibility were reduced and PWV and stiffness index were increased in SHRLN (Table 2) compared with WKY and SHR values.

The distension-pressure loop (Fig. 2B) was altered in SHR vs. WKY and strongly modified in SHRLN compared with that in SHR. This observation was quantified by the analysis of the slopes of the ascending and descending phases; both were reduced in SHR and in SHRLN, as was the area of the distension-pressure loop, an index of the arterial wall viscosity (Table 2). The individual pressure and distension time waveforms were compared in each rat. When maximal changes were compared, PP and pulse distension were respectively increased and decreased in SHR vs. WKY, and these changes were more pronounced in SHRLN than in SHR (Table 2). We observed that, in addition to changes in maximal values, the waveforms were also modified (Fig. 2A). To illustrate the changes in distension waveforms described above, the percentage of the distension vs. the diastolic diameter was calculated, allowing us to average this parameter and to compare it between groups (Fig. 3A); similarly, pressure waveforms were averaged (Fig. 3B).

Because of these changes in pulsatile pressure and distension waveforms, the AUC for these parameters were analyzed, and AUC values were adjusted to heart rate. Moreover, a distensibility index was calculated via the ratio of distension AUC to PP AUC (Table 2). The PP waveform AUC was increased similarly in SHR and SHRLN. The distension waveform AUC and the distensibility index were greatly reduced in SHR vs. WKY and in SHRLN vs. SHR (Table 2).

Effects of blood pressure changes. MAP was decreased in SHR and SHRLN to a level close to that measured in WKY (134 ± 4 and 140 ± 6 mmHg) by the intravenous administration of the calcium channel blocker diltiazem. In WKY, intravenous administration of the α1-adrenoceptor agonist phenylephrine increased blood pressure to a value comparable to that in SHR (186 ± 6 mmHg). The SAP in SHR and SHRLN after diltiazem was comparable to that in WKY, and, in WKY under phenylephrine, it was comparable to that noted in SHR and SHRLN (Table 1). Heart rate and aortic diameter were not altered by either phenylephrine or diltiazem (Table 1).

Table 1. Hemodynamic parameters and aortic diameter

<table>
<thead>
<tr>
<th></th>
<th>WKY</th>
<th>WKY + Phenylephrine</th>
<th>SHR</th>
<th>SHR + Diltiazem</th>
<th>SHRLN</th>
<th>SHRLN + Diltiazem</th>
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</thead>
<tbody>
<tr>
<td>Systolic AP, mmHg</td>
<td>181 ± 4*</td>
<td>242 ± 7*</td>
<td>239 ± 5*†</td>
<td>171 ± 6*</td>
<td>259 ± 7</td>
<td>167 ± 8*</td>
</tr>
<tr>
<td>Diastolic AP, mmHg</td>
<td>127 ± 3*</td>
<td>151 ± 2*</td>
<td>163 ± 7*†</td>
<td>113 ± 3*</td>
<td>188 ± 4</td>
<td>126 ± 8*</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>422 ± 9*</td>
<td>410 ± 6*</td>
<td>362 ± 7*†</td>
<td>353 ± 19*</td>
<td>327 ± 12</td>
<td>292 ± 19*</td>
</tr>
<tr>
<td>Pulse pressure, mmHg</td>
<td>51 ± 2*</td>
<td>90 ± 5*</td>
<td>70 ± 4*</td>
<td>56 ± 4*</td>
<td>68 ± 6</td>
<td>40 ± 2*§</td>
</tr>
<tr>
<td>PWV, m/s</td>
<td>5.7 ± 0.2*</td>
<td>8.3 ± 0.5*</td>
<td>7.5 ± 0.5</td>
<td>5.9 ± 0.3*</td>
<td>8.3 ± 0.3</td>
<td>6.4 ± 0.4*</td>
</tr>
<tr>
<td>Aortic diameter, μm</td>
<td>1,882 ± 138</td>
<td>1,971 ± 116</td>
<td>1,983 ± 133</td>
<td>1,821 ± 8</td>
<td>1,831 ± 56</td>
<td>1,737 ± 63</td>
</tr>
<tr>
<td>Aortic distension, μm</td>
<td>110 ± 16*</td>
<td>66 ± 8*</td>
<td>67 ± 10*†</td>
<td>95 ± 6*</td>
<td>34 ± 9</td>
<td>44 ± 6*§</td>
</tr>
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</table>

Values are means ± SE; n, no. of rats. Hemodynamic parameters and aortic diameter and pulse distension were measured in normotensive [Wistar-Kyoto (WKY)] rats, spontaneously hypertensive rats (SHR), SHR treated for 2 wk with N^6-nitro-L-arginine methyl ester (SHRLN), SHR and SHRLN after blood pressure reduction with diltiazem, and WKY after increase in blood pressure with phenylephrine. AP, arterial pressure; PWV, pulse-wave velocity. Group differences: *WKY, SHR vs. SHRLN; †SHR vs. low pressure groups: $SHR + diltiazem, SHRLN + diltiazem vs. WKY; §SHRLN + diltiazem vs. SHR + diltiazem (one-way ANOVA followed by Bonferroni’s tests, P < 0.05).
Table 2. Aortic dynamic parameters

<table>
<thead>
<tr>
<th></th>
<th>WKY</th>
<th>WKY + Phenylephrine</th>
<th>SHR + Diltiazem</th>
<th>SHR</th>
<th>SHRLN + Diltiazem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compliance, 10^-3 mm²/kPa</td>
<td>45.3 ± 9.4*</td>
<td>17.4 ± 2.6*</td>
<td>24.4 ± 5.9*</td>
<td>36.8 ± 4.0*</td>
<td>9.0 ± 1.5</td>
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<tr>
<td>Distensibility, 10^-3/kPa</td>
<td>16.0 ± 1.7*</td>
<td>5.9 ± 0.7*</td>
<td>8.1 ± 1.0*†</td>
<td>14.7 ± 1.7*‡</td>
<td>3.7 ± 0.6</td>
</tr>
<tr>
<td>Stiffness index</td>
<td>6.9 ± 0.5*</td>
<td>16.0 ± 1.2*</td>
<td>11.4 ± 1.4*</td>
<td>8.1 ± 0.7</td>
<td>25.9 ± 3.4</td>
</tr>
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</table>

Values are means ± SE; n, no. of rats. Aortic dynamic parameters measured were via the ArtLab system in normotensive (WKY), SHR, SHRLN, SHR and SHRLN after blood pressure reduction with diltiazem, and WKY after increase blood pressure with phenylephrine. AUC, area under the curve; AUCp/ms, AUC from pulse pressure waveform, adjusted to heart rate; AUCd/ms, AUC from distension waveform adjusted to heart rate. Group differences: *WKY, SHR vs. SHRLN; †SHR vs. WKY. Effect of acute blood pressure changes: #WKY + phenylephrine vs. WKY, SHR + diltiazem vs. SHR, SHRLN + diltiazem vs. SHRLN. Comparison of low-pressure groups: $SHR + diltiazem, SHRLN + diltiazem vs. WKY; §§SHRLN + diltiazem vs. SHR + diltiazem (one-way ANOVA followed by Bonferroni’s tests, P < 0.05).

In normotensive rats, phenylephrine decreased compliance, distensibility, and pulse distension. It increased PP, PWV, and stiffness index and did not alter arterial wall viscosity index (Tables 1 and 2). The values obtained reached those noted in SHR, but not those noted in SHRLN, except for PWV and PP. In SHR, diltiazem restored the values of compliance, distensibility, and pulse distension close to those of WKY. In contrast, in SHRLN, diltiazem restored the values of compliance, distensibility, and stiffness index only to those observed in SHR and thus remained different from WKY values, despite comparable blood pressure levels (Table 2). The arterial wall viscosity index and the pulse distension were not improved in SHRLN after diltiazem administration, remaining lower than in SHR.

The analysis of the pulsatile distension waveform confirmed this observation: the curve in WKY under phenylephrine was similar to that in SHR (Fig. 4B), and the curve in SHR after diltiazem was similar to that of WKY. However, in SHRLN, it was only moderately improved after diltiazem (Fig. 4A), despite a blood pressure level similar to that of WKY. The distension waveform AUC, adjusted to the heart rate, remained unchanged after diltiazem (Fig. 5B).

The averaged percent arterial distension and mean pressure were plotted together to compare distension-pressure loop curves between groups (Fig. 6), thus visualizing the modest changes observed in SHRLN after acute blood pressure reduction. The distension-pressure slopes, systolic and diastolic, were only partially improved after diltiazem in SHRLN (Table 2).

The isobaric compliance taken as the slope of the ascending part of the distension-pressure curve at a given pressure was reduced in SHRLN by ~55% after diltiazem (0.82 ± 0.08 μm/mmHg) compared with WKY (1.87 ± 0.26 μm/mmHg) and by ~50% compared with SHR (1.66 ± 0.26). This parameter was not different at 200–210 mmHg in WKY with increased blood pressure (0.54 ± 0.12 μm/mmHg), in SHRLN (0.50 ± 0.09 μm/mmHg), or in SHR (0.74 ± 0.10 μm/mmHg). The values of these isobaric slopes were very close to that of the whole ascending slope shown in Table 2.

Thus PP appears as expected, dependent on basal pressure, but not aortic pulse distension. To confirm this observation, we calculated the correlation coefficients between either PP or pulse distension values and the systolic pressure levels. PP was significantly correlated with systolic pressure in all rats (Pear-
son $r$ was 0.95 for WKY, 0.68 for SHR, and 0.79 for SHRLN), whereas there was a significant correlation between pulse distension and systolic operating pressure for normotensive rats and SHR (Pearson $r$ = 0.53 and 0.62, respectively), but no correlation for hypertensive rats (Pearson $r$ = -0.20).

**DISCUSSION**

The present data show that rat aortic stiffness can be accurately analyzed with the ArtLab device, and that distensibility and stiffness indexes depend both on the blood pressure level and on the vascular wall properties. The main conclusion of our study is that the analysis of the dynamic aortic distension waveform presented here for the first time appears to be specifically dependent on the vascular wall properties and thus relevant for a pressure-independent stiffening evaluation, together with the distension-pressure loop.

Arterial stiffening has been correlated with cardiovascular events and is considered to be a major risk factor in aging, hypertension, end-stage renal disease, and metabolic pathologies (17). It is related to, and likely a consequence of, the increased blood pressure, the vascular wall inflammation, remodeling, and endothelial dysfunction, which all influence each other. Thus it is difficult to evaluate in humans or in animal models the changes in arterial stiffness due to pressure recovery and/or to vascular wall protection (6, 21). To clarify this point, a method has been developed to measure compliance and stiffness indexes at isobaric pressure, using an echo-tracking technique, with the limitation that only radial, brachial, or carotid artery parameters may be recorded in humans (11, 18, 24). In animal models, aortic parameters are directly analyzed with PWV and have also been measured with the echo-tracking devices NIUS2 (13) and Walltrack (29). In an effort to evaluate the pressure-independent stiffening, isobaric data have been taken, with gradual changes in blood pressure for PWV measurements (19), or with a determination of isobaric distensibility using the NIUS2 (4, 15), and at last with data measured at low pressure using the WallTrack device (29, 30). With this approach, PWV was slightly increased only in old SHR (20), and this increase seemed dependent on the pressure (19). Aortic distensibility and/or compliance were reduced in old SHR (4, 15, 29, 30, 32), but discrepancies appeared with isobaric parameters. In some studies, distensibility, isobaric or adjusted to blood pressure, was not decreased in old SHR (4, 15). Van Gorp and collaborators (30), in contrast, showed that, at a low level of blood pressure, a decreased compliance appeared already in young pre-hyper-

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**Fig. 4.** Mean of the aortic systolic-diastolic distension waveforms showing the effect of phenylephrine in WKY (open squares; $n = 6$; $A$), diltiazem in SHR (shaded triangles; $n = 8$; $B$), and diltiazem in SHRLN (open circles; $n = 8$; $C$). Values are means ± SE. The curves for WKY, SHR, and SHRLN are shown for comparison. Aortic distension in SHRLN after diltiazem remains lower than in SHR, whereas it reached in SHR after diltiazem the values of WKY and reached in WKY under phenylephrine the values of SHR. For clarity, only one-third of the values were plotted in this graph (initial interval, 1.02 ms), and SE are plotted only for the curves after treatment.

**Fig. 5.** The figure illustrates that aortic distension ($A$) is altered by phenylephrine in WKY and diltiazem in SHR, but not by diltiazem in SHRLN, despite a level of mean arterial pressure (MAP) comparable to that of WKY ($B$). $C$: distensibility reached WKY values in SHR, but was only partially restored in SHRLN. Open bars = before; solid bars = after acute increase (WKY) or decrease (SHR and SHRLN) in blood pressure by phenylephrine and diltiazem, respectively. Values are means ± SE. Areas under the curve (AUC) were calculated from distension pulsatile waveforms in percent. Group differences: *WKY, SHR vs. SHRLN; †SHR vs. WKY. Effect of acute blood pressure changes: #WKY + phenylephrine vs. WKY, SHR + diltiazem vs. SHR, SHRLN + diltiazem vs. SHRLN. Comparison of low pressure groups: $§$ SHR + diltiazem, SHRLN + diltiazem vs. WKY; §§SHRLN + diltiazem vs. SHR + diltiazem (one-way ANOVA followed by Bonferroni’s tests, $P < 0.05$).
tensive SHR. Our laboratory previously showed that, in old SHR, isobaric and operational compliance and distensibility were decreased when measured at a pressure maintained elevated, as in conscious animals (32).

Thus, from these studies, a number of controversial data were obtained showing that, despite differences in age, parameters, and experimental conditions, it remained difficult to separate the role of blood pressure and the role of the mechanical properties of the artery in the compliance and stiffness indexes measured.

SHR treated with a low dose of the NOS inhibitor l-NAME rapidly develop increases in arterial blood pressure and aortic stiffening associated with fibrosis, hypertrophy, and endothelial dysfunction comparable to that of old SHR (Ref. 15; Isabelle et al., unpublished observations). The purpose of the present study was to take this model as a tool to improve the analysis of arterial stiffening using a recently developed echotracking device, the ArtLab, which is presently only investigated in human studies (2, 28).

The different parameters measured in the present study at basal pressure, compliance, distensibility, and stiffness index were, as expected, altered in hypertensive vs. normotensive rats and more so in SHRLN than in SHR. The changes measured were comparable to those measured previously with NIUS2, with decreases of compliance ~50% in SHRLN vs. SHR; stiffness index was greatly increased in SHRLN vs. SHR (+155%), as described with the NIUS2 (+121%) (31). These parameters were measured after acute changes in blood pressure, and we observed that compliance, distensibility, and stiffness index were more profoundly altered in WKY after phenylephrine and in SHR after diltiazem than in SHRLN after diltiazem compared with the matched blood pressure groups SHR/SHRLN and WKY, respectively. The same observation was made for PWV. These data are in line with the hypothesis that both pressure and vascular wall properties influence these parameters.

Further investigation for possible additional and new ways to analyze the changes observed lead us to compare in each animal individually the time-pressure and the time-distension waveforms and the distension-pressure relationship by collecting all of the recorded points in the beat-by-beat analysis. In preliminary experiments, it was verified that the pressure waveform was exactly similar when recorded via a catheter or a Millar probe. We observed a deformation of these curves, modest in SHR, but stronger in SHRLN. The double peak in systolic blood pressure noted in SHRLN is likely due to an earlier return of the reflection wave, as shown in humans (see Refs. 16, 23), and the distension-pressure curve was strongly smoothed. Altogether, these changes explain the striking distension-pressure loop observed in these animals. Therefore, we believe that it is extremely important to carefully analyze the individual curves, as shown in Fig. 2. This approach was already performed for the distension-pressure loop in humans (11, 18) and rats (1).

Then to average the data, the arterial distension was transformed in percent of the diastolic diameter. Blood pressure may be averaged directly, to show the basal level or when transformed in delta (see Fig. 3). In the normotensive rats, after an acute increase in blood pressure, the pressure wave and the distension wave were both completely similar to those of SHR, reflecting the role of blood pressure on the aortic distension. In SHR, after an acute decrease in blood pressure, the distension waveform, like the pressure waveform, was similar to that in WKY. But in SHRLN, different observations were made. First, the decrease in the distension waveform in these rats was more pronounced than could be expected regarding the pressure values, which were close to those in SHR. Then in SHRLN after diltiazem, mean blood pressure and pressure waveform were similar to those of WKY, whereas the pulse distension waveform was not improved. To quantify the whole waveform, we calculated the AUC, from delta and percent values respectively, adjusted to heart rate. These values clearly confirm the above observations as in SHRLN, in contrast to SHR: the distension AUC was not modified after the acute decrease in blood pressure.
Thus in 20-wk-old SHR in which the aorta does not present remodeling, the aortic distension recovers after an acute decrease in blood pressure, whereas, in SHRLN, which presents aortic remodeling, aortic distension does not recover after an acute decrease in blood pressure. The aortic remodeling in SHRLN is assessed by an increased intima-media thickness and fibrosis. These observations suggest that pulse distension depends on arterial wall properties, and this was confirmed by the absence of correlation between distension and pressure in SHRLN, whereas PP was, as expected, highly correlated with SAP.

The distension-pressure loop previously taken as an index of the vascular wall viscosity appears here, after averaging, to be a good tool to show the aortic properties in the different groups. In humans, it is correlated with distensibility (18), and both loop area and slopes are strongly decreased with age (11). In our model, a strong reduction of distension-pressure loop area and slopes is also observed, in agreement with the hypothesis that this model is an early vascular aging model (33) (Isabelle et al., unpublished observations). Acute changes in blood pressure modify the distension-pressure relationship in WKY and SHR, but not in SHRLN. In SHRLN, the slopes are only partially restored, as observed for the distensibility, and the area is not changed. By averaging this parameter for group comparison, we show that it may be used to evaluate the effectiveness of a treatment in experimental models (see Fig. 6).

Our data indicate that compliance, distensibility, stiffness index, and arterial wall viscosity index may be measured in animals via the ArtLab system, and that these parameters are dependent on both pressure and vascular wall properties.

Moreover, we have performed, to our knowledge for the first time, the analysis of the pulsatile aortic distension waveform and show that this parameter is strongly dependent on vascular wall properties. Interestingly, it can be analyzed together with blood pressure through the distension-pressure relationship, but appears interesting even without blood pressure recording through the pulse distension waveform as such. Thus arterial distension appears relevant in animal models to investigate the protective effect of a drug on the vascular wall and for investigation in humans using the carotid artery.

In conclusion, altogether, the results show that compliance, distensibility, and stiffness index evaluate the overall arterial stiffening, and that an accurate evaluation of the role of mechanical properties of the artery may be added via the pulsatile distension waveform analysis and distension-pressure loop analysis.

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DISCLOSURES

All authors are employees of the Server Research Institute.

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