Pulse Pressure, Aortic Reactivity, and Endothelium Dysfunction in Old Hypertensive Rats

Philippe Chamiot-Clerc, Jean François Renaud, Michel E. Safar

Abstract—The reactivity of old hypertensive rat aortas has not been investigated in relation to each phenotype of the blood pressure curve, mean arterial pressure (MAP), and pulse pressure (PP). Aortic reactivities from 3- to 78-week-old Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) were studied with the use of organ chambers and invasive blood pressure, carotid diameter, and histomorphometry. MAP and PP were elevated in SHR, but at 78 weeks, a selective increase of PP without further MAP increase was observed for the same carotid diameter as WKY. Aortic relaxation in response to carbamylcholine decreased similarly with age in both strains. With (+) or without (−) endothelium (E), maximal developed tension (MDT) under KCl increased linearly with age in SHR, proportionally to wall thickness and MAP increase. Under norepinephrine (NE), MDT of E− aortas from SHR and controls increased with age and reached plateaus at 12 weeks, whereas MDT of E+ aortas from SHR increased linearly with age. Because the NE-induced MDT was higher for E+ than E−, the difference estimated endothelial function. This difference reached plateaus from 12 to 78 weeks in WKY but was abolished beyond 12 weeks in SHR, a finding also observed under NO-synthase inhibition. In old hypertensive rats, (1) increased KCl reactivity is endothelium independent but influenced by the MAP-dependent aortic hypertrophy with resulting increased vascular smooth muscle reactivity, whereas (2) increased NE reactivity is endothelium dependent in association with increased PP, altered endothelial function, and extracellular matrix, with resulting enhanced intrinsic arterial stiffness. (Hypertension. 2001;37:313-321.)

Key Words: rats, spontaneously hypertensive arteries endothelium pulse pressure

In hypertension, the blood pressure (BP) curve results from the summation of a steady component, mean arterial pressure (MAP), and a pulsatile component, pulse pressure (PP).1 The relative contributions of the two components of the BP curve are influenced significantly by age, hence by the level of arterial stiffness, one of the major determinants of PP. In younger populations of rats and humans, isobaric carotid and aortic rigidities are similar to those of normotensive controls2–4 in association with proportional increases of MAP and PP. In the older populations, particularly in humans and some strains of hypertensive rats,6–8 isobaric carotid and aortic rigidities are reduced, indicating pressure-independent alterations of the stiffness of wall material,6–8 with a disproportional increase of PP over MAP but no increase of stroke volume.1,6–9 This hemodynamic pattern is usually considered a direct consequence of structural changes within the aortic wall.1,6–8 However, acute administration of the exogenous NO-donor sodium nitroprusside is able to reverse the increase of PP (see reviews in References 1 and 10).10,11 thus, independent of structural vascular changes. In other words, in old hypertensive animals and humans, the contributions of vasomotor tone and of NO-dependent alterations should be taken into account in the mechanism of increased aortic stiffness and PP. Because endothelial function is highly influenced by age,11–13 its change should be considered in parallel.

We have previously shown that in Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR), an intact endothelium (E+) is required to achieve a normal relation between PP and pulsatile diameter.14,15 In the absence of endothelium (E−), carotid and abdominal arterial diameters are increased, suggesting a major role of vasoconstrictive agents in this alteration. Because the function of endothelium is not only to relax vascular smooth muscle (VSM) through NO formation and/or release but to modulate the response of the vessels to vasoconstrictive stimuli,11,13,16 this latter adjustment may interfere critically in the control of the relation between PP and pulsatile diameter and hence arterial stiffness and PP.

In SHR, sympathetic overactivity is present.9,17 Central conduit arteries are hyperresponsive to α-adrenergic receptor stimulation and blockade,5 associated with an increased affinity of VSM α-adrenergic receptors.18 This characteristic feature is of major importance because at the level of the carotid arterial bed, we and others have shown that the vasoconstrictive properties of norepinephrine (NE) are coun-
terbalanced by NO formation and/or release. This mechanism is operating in young SHR, but its relevance in old hypertensive rats has not been fully investigated. In this study, our working hypothesis is that an age-related change of the endothelial NE–NO interaction is an important target mechanism that explains, in old hypertensive rats, the presence of a pressure-independent increase of arterial stiffness with resulting PP increase.

In this study, we investigated at different ages the aortic reactivity of SHR by comparison with normotensive WKY controls. The first objective was to show that, with the use of organ-chamber experiments, the contractile responses to NE of E’ and E” thoracic aortas reflected specific age-related changes of the NE–NO interactions in SHR and differed from the responses to KCl. The second objective was to attempt to correlate such alterations to the increased arterial stiffness and PP already observed and described in old hypertensive animals.

Table 1. Histomorphometric Parameters of Thoracic Aorta From SHR and WKY Between 3 and 78 Weeks

<table>
<thead>
<tr>
<th>Histomorphometry</th>
<th>WKY</th>
<th>SHR</th>
<th>Age Effect</th>
<th>Strain Effect</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCSA, mm²</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Wk 3</td>
<td>0.221±0.003** (5)</td>
<td>0.286±0.022† (4)</td>
<td>0.454±0.019 (4)</td>
<td>0.589±0.099§ (5)</td>
<td>P&lt;0.005</td>
</tr>
<tr>
<td>Wk 12</td>
<td>0.284±0.013 (5)</td>
<td>0.326±0.036‡ (5)</td>
<td>0.637±0.039 † (5)</td>
<td>1.022±0.026 (4)</td>
<td></td>
</tr>
<tr>
<td>Total collagen content, µm²/mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY Wk 3</td>
<td>314±18* (5)</td>
<td>690±64† (4)</td>
<td>1350±72 (4)</td>
<td>1887±14° (5)</td>
<td>P&lt;0.005</td>
</tr>
<tr>
<td>SHR Wk 3</td>
<td>332±30 (5)</td>
<td>482±21† (5)</td>
<td>1585±63 (5)</td>
<td>2502±137 (4)</td>
<td></td>
</tr>
<tr>
<td>Total elastin content, µm²/mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY Wk 3</td>
<td>2786±191 (5)</td>
<td>2850±427 (4)</td>
<td>3613±437 (4)</td>
<td>4233±701 (5)</td>
<td>P&lt;0.005</td>
</tr>
<tr>
<td>SHR Wk 3</td>
<td>2907±200 (5)</td>
<td>2833±132 (5)</td>
<td>4247±225 (5)</td>
<td>2506±301 (4)</td>
<td></td>
</tr>
<tr>
<td>Collagen/elastin ratio</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>WKY Wk 3</td>
<td>0.115±0.012* (5)</td>
<td>0.250±0.026† (4)</td>
<td>0.383±0.044 (4)</td>
<td>0.460±0.050° (5)</td>
<td>P&lt;0.005</td>
</tr>
<tr>
<td>SHR Wk 3</td>
<td>0.115±0.006 (5)</td>
<td>0.170±0.007‡ (5)</td>
<td>0.376±0.014 (5)</td>
<td>1.037±0.159§ (4)</td>
<td></td>
</tr>
</tbody>
</table>

Values are ± 1 SEM. Numbers of animals are shown in parentheses.

*P<0.005, 3 weeks vs 5 weeks.
†P<0.05, †P<0.005, 5 weeks vs 12 weeks.
‡P<0.005, 12 weeks vs 78 weeks.
§P<0.05; ¶P<0.01; **P<0.005 SHR vs WKY.
bated for 45 to 60 minutes. They were stretched progressively and exposed repeatedly to 40 mmol/L KCl to induce contraction for each new level of stretching until a maximal contractile response to KCl was obtained (40 mmol/L, a submaximal concentration independent of age and species). This basal tension was considered the optimal point on a length-tension curve. E1 and E2 experiments were performed in parallel. Endothelium was considered to be intact when carbamylcholine (10^{-9}/10^{-5} mol/L) caused complete relaxation of rings precontracted with 3×10^{-7} mol/L NE and effectively removed when carbamylcholine did not induce relaxation.11–13,18 The α-adrenergic contraction was evaluated by addition of cumulative NE concentrations (10^{-9}/10^{-5} mol/L). Thereafter, the endothelium-independent relaxation elicited by 10^{-5} mol/L papaverine was studied on the same aortic preparations precontracted by 3×10^{-7} mol/L NE, and, finally, vasorelaxation in response to carbamylcholine was investigated. Concentration of the drugs are expressed as final molar (mol/L) or millimolar (mmol/L).

For the analysis of dose-response curves, the response was expressed as the percentage of the preceding contraction for relaxation-eliciting agents. For contraction-inducing agents, the response was expressed as the absolute change of maximal developed tension (MDT, in mg). On the basis of the analysis of dose-response curves to KCl and NE obtained from preliminary experiments, MDT was achieved with 30 mmol/L KCl and 3×10^{-7} mol/L NE concentrations, independent of age and strain. For both relaxing and contracting agents, the concentrations inducing 50% of the maximal effect were expressed as pD2 values and calculated with the use of specific software (Microcalô Software Inc).18 Finally, from the dose-response curves studied plotted for each aorta’s E1 and E2 responses, we defined 2 indexes of endothelial function for this, and calculated the differences between the NE-induced MDT of E1 and the MDT of E2. The (E2–E1) difference was called ΔNE and used as an index of the NE-dependent participation of the endothelium during aging. In some experiments, the NO-synthase inhibitor N\textsubscript{o}-nitro-L-arginine (LNNA) (10^{-4} mol/L) was added to the preparation in which the α-adrenergic contractions were measured. The increase of MDT under this inhibitor, called ΔLNNA, was used as an index of NO-dependent endothelial function and, consequently, NE-NO interactions. Potassium chloride, carbamylcholine chloride, and norepinephrine bitartrate salt (Arterenol) were purchased from Sigma Chemical Co. All the drugs were dissolved in distilled water and prepared daily. At the end of the experiments, the histomorphometric parameters of the thoracic aorta were determined, as previously described for rats 3, 5, 12, and 78 weeks old.23

For statistical analyses, mean±1 SEM values are given. For each animal, each dose-response curve was derived from the means of 3 to 6 aortic rings. For all the animals in each subgroup, MDT and pD2 were calculated. The aortic responses were analyzed by a 2-way ANOVA. To evaluate the age-related changes of MDT, 3 different models (linear, semilogarithmic, and hyperbolic) were used, enabling linear relation to be distinguished from those reaching a plateau. A 2-way ANOVA was used to show that for each strain, MDT E2 was significantly higher than MDT E1 at different ages. A value of P<0.05 was considered to be significant after Bonferroni and post hoc tests had been performed.

Results

Intra-Arterial BP, Carotid Diameter, and Histomorphometric Changes

Figure 1 summarizes the BP changes between 5 and 78 weeks of age. For both strains, MAP increased with age (P<0.01), reaching plateaus at 12 weeks of age (Figure 1, upper panel). MAP was significantly higher in SHR than in WKY (P<0.01). At 12 and 52 weeks, carotid diameters measured in SHR at operational MAP were, respectively, 1048±41 and 1315±24 μm (NS). At 78 weeks of age, MAP was 183±7 mm Hg in SHR and 136±4 mm Hg in WKY.
(P<0.001), whereas operational carotid diameters did not differ significantly (1149±26 versus 1124±29 μm). Thus, at 78 weeks of age, the same diameter was achieved in the presence of higher MAP values in SHR, indicating a pressure-independent increase of carotid stiffness in old SHR.

PP was significantly higher in SHR than in WKY (P<0.01) (Figure 1, lower panel). Although PP was practically unchanged with age in WKY, it increased significantly with age in SHR (P<0.05), particularly from 52 to 78 weeks (P<0.01), resulting in a significant interaction (P<0.01) with normotensive WKY controls.

Table 1 indicates that medial cross-sectional area (MCSA), collagen content, and the collagen/elastin ratio increased significantly with age (P<0.005), with significantly higher values in SHR, particularly at 78 weeks of age (interaction: P<0.005). For the elastin content, there was a highly significant age effect without strain effect and a slight significant interaction (P<0.05).

VSM Relaxation Induced by Carbamylcholine and Papaverine

For both strains, endothelium-dependent carbamylcholine-induced relaxation was reduced significantly (P<0.005) with age in association with previously described prostaglandin-mediated contractions at the higher concentrations (Figure 2). Between 3 and 12 weeks, age-related changes were more marked in SHR than WKY, causing slightly less relaxation in SHR than in WKY at 12 weeks (P<0.05).
Finally, obvious differences in the age-related kinetics of relaxation were seen between the two strains (interaction: $P<0.05$). In contrast, non–endothelium-dependent relaxation by papaverine was the same for WKY and SHR and decreased slightly with age ($P<0.02$) (data not shown).

**VSM Contraction Under KCl**

The dose-response curves of MDT in response to increasing KCl concentrations are shown in Figure 3, A and B, respectively. In both strains, significantly ($P<0.005$) higher MDT values were obtained at 12, 52, and 78 weeks than at 3 and 5 weeks, with markedly enhanced SHR responses especially at 78 weeks. In Figure 3C, MDT was evaluated as function of age in WKY and SHR. Whereas MDT increased with age until 12 weeks in WKY and then did not change significantly until 78 weeks, a progressively increasing linear relation was observed for SHR, resulting in a significantly higher MDT in SHR than in WKY at 78 weeks of age ($P<0.005$). In SHR,
MDT and MCSA were significantly, positively and linearly correlated ($r=0.70$). In WKY, a plateau was reached at 12 weeks ($r=0.41$). All these results were observed with $E^{-}$ aortas but did not differ for $E^{+}$ preparations (data not shown).

### VSM Contraction Under NE

The dose-response curves, plotting MDT against increasing NE concentrations obtained with $E^{-}$ (upper panels) and $E^{+}$ (lower panels) thoracic aortas from WKY (left side; A, C) and SHR (right side; B, D), are shown in Figure 4. First, regardless of the strain and the presence or absence of endothelium, MDT increased with age, with significantly higher values at 12, 52, and 78 weeks than at 3 and 5 weeks. Second, MDT was significantly higher with $E^{-}$ than with $E^{+}$ regardless of age and strain ($P<0.001$), except for older SHR (interaction: $P<0.01$) (see below). Third, the MDT responses of SHR were higher than those of WKY, but this enhancement was observed only for $E^{-}$ aorta, particularly in older animals (see below).

Table 2 indicates the $pD_2$ values for WKY and SHR. With $E^{-}$ preparations, $pD_2$ decreased with age ($P<0.005$), and this reduction was more pronounced in SHR ($P<0.05$), particularly at 52 and 78 weeks. No significant changes were seen with $E^{+}$ aortas.

NE-induced changes of MDT as a function of age were significant for both $E^{-}$ and $E^{+}$ preparations ($P<0.005$) (Figure 5). Although the MDT of $E^{-}$ aortas from WKY remained relatively stable after 12 weeks of age, SHR value increased linearly with age, resulting in significantly higher MDT in SHR than in WKY at 52 and 78 weeks ($P<0.005$). With $E^{-}$ aortas, MDT did not differ and increased similarly for both strains, stabilizing between 12 and 78 weeks.

### Indexes of NE-Dependent Endothelial Function

$\Delta NE$, indicated as $T_{max}(E^-)-T_{max}(E^+)$ in Figure 6, increased with age up to 12 weeks for WKY and then achieved a plateau. A similar pattern was observed for $\Delta L_{NN}$ (data not shown). For SHR, $\Delta NE$ also increased with age, with even higher values than in WKY between 3 and 12 weeks of age (Figure 6). Thereafter, $\Delta NE$ fell sharply and was significantly lower than in WKY ($P<0.005$). A similar pattern was observed for SHR $\Delta L_{NN}$, which decreased markedly from 52 to 78 weeks of age (Figure 7). Finally, for 78-week old SHR, $\Delta NE$ (Figure 6) and $\Delta L_{NN}$ (Figure 7) were strongly reduced, whereas PP was selectively increased (Figure 1).

### Comments

In agreement with results previously reported in the literature, we observed that MAP and PP were increased in SHR as compared with normotensive controls. However, whereas in both strains MAP almost reached a plateau at 12 weeks of age, PP increased sharply at 78 weeks only in SHR. This enhancement was associated with increased intrinsic carotid arterial stiffness, aortic MCSA, and collagen/elastin ratio but mostly with substantial changes of aortic reactivity. First, endothelium-dependent and non–endothelium-dependent relaxations were significantly reduced with age but were not influenced by the presence of hypertension. Second, VSM contractions were stronger in old hypertensive rats, but their extent differed markedly depending, on whether membrane depolarization was induced by KCl or NE. Contractions under KCl were endothelium independent and associated MAP and the degree of VSM hypertrophy. Contractions under NE were endothelium dependent and involved complex interactions between NE and NO, particularly at 78 weeks of age, in association with the selective PP increase.

It has previously been reported that arterial endothelium-dependent relaxation is reduced in mature SHR. However, this alteration is known to be not uniform, depending on the model and the vascular bed studied. In this report, the carbamylcholine-induced relaxation was slightly reduced in 12-week-old SHR compared with WKY controls, but the overall reduction of relaxation was mainly influenced by age. For both strains, similar decreases with age were observed but with obvious kinetic differences, leading to steeper reduction of relaxation with age in younger SHR than in controls (Figure 2). These results strongly suggest that age, more than the increase of BP, was the main factor acting on the reduction of aortic smooth muscle relaxation in rats.

In this study, aortic smooth muscle contractions under KCl were increased in old hypertensive rats and were proportional to the development of VSM hypertrophy. Folkow et al postulated that according to LaPlace’s law, resistant vessels in hypertensive rats undergo structural changes, causing the medial layer to thicken and resulting in a geometrically related increased response to vasoactive stimuli. The data reported here extend this alteration to hypertensive conduit arteries, with 3 particularities. First, according to LaPlace’s law, this change concerns exclusively the steady component of BP, MAP. Second, the increased reactivity occurs in proportion with the degree of increased MCSA that develops.
very early (Table 1), at a period during which there is no pressure-independent increase of arterial stiffness.\textsuperscript{2,3,8,10} Third, the KCl-induced contractions are not endothelium dependent and thus differ markedly from those observed under NE.

In SHR, although a number of vasoactive stimuli such as endothelin or prostanoids may act on the vessel wall through changes in endothelial function,\textsuperscript{11,24} NE is certainly of major importance because activation of the sympathetic nervous system is a characteristic hallmark of these animals. In agreement with a previous study,\textsuperscript{18} our VSM pD\textsubscript{2} values obtained with E\textsuperscript{2} aortic rings were significantly lower for SHR, indicating an altered muscle structure-activity coupling. Within this framework, a major function of endothelium is to modulate the response of VSM cells to contractile agents. NE acts on the endothelial cells to increase NO formation and/or release, thus attenuating its own contractile effect on VSM.\textsuperscript{19–21} In our experiments, this mechanism was operating, as shown from the experiments involving LNNA. However, whereas these responses, represented by the two calculated indexes of endothelial function, \(\Delta NE\) and \(\Delta LNNA\), were observed at all ages in WKY, they differed markedly in younger and older SHR.

In younger SHR, we found that \(\Delta NE\) was maintained and was even higher than in controls (Figure 6). Numerous molecular biology studies have shown that NO formation and/or release is upregulated in young SHR and should be considered a compensatory mechanism for the presence of neurogenic vasoconstriction.\textsuperscript{10,11,13,18–21,24} In parallel, we and others\textsuperscript{2–5} have observed that in younger rats, carotid arterial

Figure 5. E\textsuperscript{+} or E\textsuperscript{−} thoracic aorta under NE: Relation between MDT (at 3\(\times\)10\textsuperscript{−2} mol/L NE) and age in WKY and SHR with or without endothelium. Probability values: \(f, P<0.05; \#f, P<0.005\) (WKY vs SHR).
diameter and isobaric distensibility did not differ in WKY and SHR strains, whereas BP and arterial wall thickness were higher in hypertensive than in normotensive animals. Thus in SHR, the altered NE-NO interaction contributes to the maintenance of normal arterial function and proportional increases of MAP and PP in the face of severe constrictive influences.

In 78-week-old hypertensive rats, we observed that compared with WKY of the same age, ∆NE and ∆LNNA were significantly reduced or even abolished, in association with increased intrinsic arterial stiffness and PP. Several reported findings may point to a specific link between altered endothelial function and increased PP. First, in vitro experiments showed that NO release and endothelial NO-synthase mRNA and protein, which are markedly influenced by age, are more significantly associated with pulsatile than steady mechanical factors. Second, in old hypertensive animals and humans, exogenous NO donors are able to normalize PP acutely and selectively, with minor changes of MAP and no alteration of the structure of the hypertrophied and stiffened arterial vessels. This acute change occurs in older but not in younger populations, for example, in the presence of an age-induced alteration of the endothelium. Finally, in a recent study on the response of aortic rings to the diuretic agent cicletanine, we observed that this compound induced NO- and endothelium-dependent relaxation, which was mainly due to stimulation of NO-synthase and more pronounced in older animals. In vivo, cicletanine was shown to produce a MAP-independent decrease of arterial stiffness and PP.

In conclusion, KCl- and NE-dependent aortic reactivities are increased in old SHR. The former is endothelium independent and associated with MAP, whereas the latter is endothelium dependent and more directly related to PP. Increased PP may be due not only to modified composition of the arterial wall but also to changes in vasomotor tone of endothelial origin mainly involving altered NE-NO interactions.

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References


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