Pulse Pressure, Endothelium Function, and Arterial Stiffness in Spontaneously Hypertensive Rats

Michel Safar, Philippe Chamiot-Clerc, Georges Dagher, Jean François Renaud

Abstract—In rats, removal of the carotid arterial or abdominal aortic endothelium results in an acute increase of diameter and compliance. In humans, acute local administration of a specific NO synthase inhibitor increases radial artery compliance but not the diameter. The purpose of this review is to determine whether in spontaneously hypertensive rats (SHR), a cause-and-effect relationship may be observed between endothelial function and arterial stiffness with possible consequences on pulse pressure (PP) control. The study is based on a comparative time-dependent analysis of the following in young and old SHR: aortic blood pressure measurements and reactivity, ultrasonographic arterial stiffness assessment, aortic histomorphometry and staining, and molecular biology with evaluation of endothelium function. In young SHR, aortic mean blood pressure and PP increase proportionally, whereas isobaric arterial stiffness is unchanged or poorly modified. The endothelial NO response to norepinephrine is normal or upregulated as a response to predominant vasoconstrictive influences. In contrast, in old SHR, PP and mean blood pressure change disproportionately with age, together with an enhanced isobaric arterial stiffness. The endothelial NO response to norepinephrine is abolished, in association with endothelium-dependent heightened norepinephrine reactivity and enhanced accumulation of vessel extracellular matrix. In this latter case, exogenous NO acutely and selectively lowers the increased PP. Thus, during SHR aging, a negative feedback may be observed between NO bioactivity and PP through changes in arterial structure and function. Whether this alteration contributes to the development of systolic hypertension in old populations remains to be determined. (Hypertension. 2001;38:1416-1421.)

Key Words: rats, inbred SHR • arteries • endothelium • nitric oxide

For many years, studies of spontaneous hypertension in rats focused on the presence of sympathetic hyperactivity and the mechanisms by which this alteration contributes to changing the mean blood pressure (MBP) and the structure and function of arterioles.1,2 Subsequently, the role of the vascular endothelium was principally deduced from the investigation of NO-norepinephrine (NE) interactions.3 Nowadays, hypertension is mainly considered as a cardiovascular risk factor, leading to more attention being focused on the structure and function of hypertensive large arteries, which greatly influence the development of complications in hypertensive vascular disease.4

It has been widely reported that the conduit arteries of spontaneously hypertensive rats (SHR) are stiffer than those of the corresponding normotensive control rats.4,5 In addition to blood pressure level, it seems likely that intrinsic modifications of the arterial wall might contribute to the increased stiffness.4 Because stiffness is influenced by arterial structure and function, changes in vasomotor tone, possibly of endothelial origin, might promote the alterations of the mechanical properties of the SHR conduit arteries.

The purpose of the present review is to evaluate, in SHR, the possible time-dependent relationships between arterial stiffness and endothelial function and to determine whether altered NO-NE interactions in the endothelium might contribute to the extent of arterial stiffness and, consequently, to pulse pressure (PP) control.

Genetic and Environmental Background

Clinical and experimental studies have clearly demonstrated that conduit arteries from hypertensive populations are thicker than those from normotensive control populations,5 according to Laplace’s law. Because arterial hypertrophy occurs very early in SHR, the following question has been raised: do pressure-independent modifications of the arterial wall resulting from environmental and genetic factors predispose to these alterations?6 In the various models of hypertension in rats, genetic and/or predisposing factors either may be
Changes in Hemodynamic Parameters, Histomorphometric Parameters, and Aortic Reactivity Measured From Developed Tension in Organ Chambers

<table>
<thead>
<tr>
<th></th>
<th>Japanese Group</th>
<th>Lyon Group</th>
<th>Group Effect</th>
<th>Pressure Effect</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>133±4</td>
<td>155±3</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.005</td>
</tr>
<tr>
<td>PP, mm Hg</td>
<td>26±1</td>
<td>31±3</td>
<td>NS</td>
<td>0.005</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Distensibility index, 10^{-3} · mm Hg^{-1}</td>
<td>1.20±0.13</td>
<td>2.11±0.56</td>
<td>0.002</td>
<td>0.001</td>
<td>NS</td>
</tr>
<tr>
<td>Medial cross-sectional area, mm²</td>
<td>0.55±0.02</td>
<td>0.84±0.06</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Elastic, %</td>
<td>34.8±0.9</td>
<td>30.9±0.3</td>
<td>0.006</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Collagen, %</td>
<td>10.8±0.7</td>
<td>10.4±0.5</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Collagen I, %</td>
<td>72.4±2.4</td>
<td>65.5±2.9</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Collagen III, %</td>
<td>74.4±1.1</td>
<td>58.1±2.7</td>
<td>&lt;0.005</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>$T_{\text{max}}$</td>
<td>1866±164</td>
<td>1549±145</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>NE (E+)</td>
<td>1567±188</td>
<td>1333±108</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>NE (E−)</td>
<td>2556±246</td>
<td>2496±205</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>$\Delta T ([T_{\text{max}} E−]−[T_{\text{max}} E+])$</td>
<td>1042±179</td>
<td>838±135</td>
<td>0.06</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>pD² (NE (E+))</td>
<td>7.93±0.08</td>
<td>7.57±0.04</td>
<td>&lt;0.005</td>
<td>&lt;0.005</td>
<td>NS</td>
</tr>
<tr>
<td>pD² (NE (E−))</td>
<td>7.46±0.06</td>
<td>7.46±0.11</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Aortic reactivity was measured from developed tension in the presence of NE with (E+) and without (E−) endothelium. $\Delta T$ represents the difference in maximal developed tension ($T_{\text{max}}$) E− and E+. Concentrations inducing 50% of the maximal effects are expressed as pD² values calculated for NE in Japanese (WKY and SHR) and Lyon (LN and LH) rats aged 12 weeks. Values are mean±SEM. See Chambot-Clerc and colleagues.7,8

Specific to the hypertensive process and its causes or may be common to the hypertensive population and its corresponding normotensive control population.

Usually, SHR are compared with their own control, Wistar or Wistar-Kyoto (WKY) normotensive rats. However, the difference in blood pressure between the normotensive and the hypertensive strains is a major confounding factor regarding the mechanical consequences on large-artery structure and function. With this approach, it is difficult to determine whether these alterations are consequent to increased blood pressure or to genetic characteristics of the selected strains. An alternative approach consists of comparing the 2 rat strains of Japanese origin (WKY and SHR) with rat strains of other origins, such as the Lyon strains (LH [hypertensive] and LN [normotensive]). Such comparisons have shown that independently of hypertension, the pooled Japanese strains, SHR plus WKY, differ from the regrouped Lyon strains, LH plus LN, by the following (Table): their stiffer carotid wall material at any given value of wall stress, their lower degree of aortic hypertrophy together with their higher density of collagen III but not collagen I, and the higher affinity of their aortic smooth muscle $\alpha$-receptors.6–8 Most of the other vascular parameters studied were affected by both the origin of the rats (Japanese versus Lyon) and the presence of hypertension. Thus, large-artery structure and function in genetically hypertensive rats are influenced not only by high blood pressure itself but also by specific factors that are also constitutive in their corresponding normotensive control populations. The illustrations show changes in hemodynamic parameters, histomorphometric parameters, and aortic reactivity in the WKY, SHR, LN, and LH groups.

In this context, because the SHR strain represents a well-documented model of sympathetic hyperactivity, an important result to consider in Japanese rats is the presence of increased affinity of aortic smooth muscle $\alpha$-receptors.7 In organ chambers, aortic contractions in response to NE are enhanced after endothelium removal or preincubation with a specific NO synthase (NOS) inhibitor, indicating that the vascular response to NE is counterbalanced by NO release of endothelial origin.7 This response is significantly more pronounced in the Japanese (WKY and mainly SHR) than the Lyon (LH plus LN) strains,7,8 implying that for a given environment and diet,9 specific genetic factors play a role in Japanese rats. Indeed, the basal activity and protein expression of endothelial NOS in the aorta are significantly lower in SHR than in WKY.10 Similarly, in human mammary arteries, an heightened response to NE has been demonstrated in the Gly298 mutant allele of the endothelial NOS gene by comparison with the nonmutant allele, suggesting less NO formation and/or release in the presence of NE.11

Taken together, these findings in SHR indicate the following: (1) large-artery walls are subjected to a number of genetic and environmental influences that are not necessarily related to the mechanism and the origin of hypertension itself but that are also constitutive in the corresponding normotensive control strain; (2) because large arteries, but not small arteries, are involved, the hemodynamic stress acting on the arterial wall necessarily implies the role of PP and not only MBP4; and (3) because NE and NO are known to have different and specific effects on large-artery diameter and stiffness, the NE-NO interaction in SHR represents an adequate model for investigation of the links between endothe-
recent years, the use of intra-aortic blood pressure measure-
directly measured and accurately calculated. However, in
arteries. Furthermore, by use of tail SBP, PP cannot be
without significant change of intra-aortic pressure and, there-
erned. In the absence of significant change of intra-aortic
SBP increase markedly from central to peripheral arteries
and PP without any disproportionate increase of SBP and PP
over MBP (Figure 1).13
In young SHR (ie, aged up to 12 weeks), we observed that
developed in SHR, transient increases of cardiac output
and carotid blood flow velocity have been clearly established
and widely reported.13,14 Because arteries are always known
to respond to chronic changes of blood flow velocity with an
acute vasomotor response, vasodilatation for increased flow,
and constriction for decreased flow15 and because, in young
SHR, the transient changes of blood flow velocity are not
associated with a parallel change of carotid diameter, it seems
likely that major alterations of the flow-dilatation mechanism
had occurred in this hypertensive strain. Because this mecha-
anism requires an intact endothelium3,15 and because, in SHR,
the unchanged carotid diameter in the presence of elevated
blood pressure requires a concomitant change of arterial
stiffness, it seems logical to accept that endothelial and
stiffness alterations are temporally associated during the early
phase of hypertension in SHR.
We and others16–18 have previously reported that in 12-
week-old rats studied in vivo, acute removal of the carotid
arterial or abdominal aortic endothelium resulted in increased
diameter and compliance without any blood pressure
change.16 These findings suggested that endothelium function
and arterial stiffness are causally associated and that a
contribution of vasoconstrictive substances is required for
this process, with more pronounced diameter and compliance
enhancement in normotensive control rats than in SHR.16 On
the other hand, when the radial artery of normotensive
humans is studied in vivo, NOS inhibition did not change
arterial caliber but increased arterial compliance, an observa-
tion supporting the presence of compensating vasodilatation
mechanisms.19 Taken together, such findings suggest that the
balance of vasodilating and vasoconstricting effectors of the
endothelium have different effects in SHR and WKY. Be-
cause the NO pathway at the endothelial level is known to
counterbalance the effect of vasoconstrictive substances, such
as NE and angiotensin, a more extensive investigation of
endothelial function is required to determine its links with
arterial stiffness.

Figure 1. Changes in mean arterial pressure (MAP) and PP in
the carotid artery of Japanese rats (WKY and SHR) aged 5, 12,
52, and 78 weeks. See Chamiot-Clerc et al.20

Thoracic Aortas and Carotid Arteries in
Young SHR
During the early phase of genetic hypertension, there are
major limitations for blood pressure determinations in small
animals, such as rats.12 Whereas some authors have described
a prehypertensive period, a number of reports from other
laboratories have indicated a significantly higher blood pres-
sure in SHR than in control rats before weaning.12,13 In
several studies, blood pressure was measured as systolic
(SBP) at the tail artery. Because blood pressure involves a
steady component (MBP) and a pulsatile component (PP) and
because PP and SBP increase markedly from central to
peripheral arteries without a concomitant change in MBP, the
elevation of SBP, when measured only at the tail artery, may
simply represent an alteration of pressure wave transmission
without significant change of intra-aortic pressure and, there-
fore, underestimate the role of mechanical factors in central
arteries. Furthermore, by use of tail SBP, PP cannot be
directly measured and accurately calculated. However, in
recent years, the use of intra-aortic blood pressure measure-
sive animals, NE acts on endothelial cells to increase NO production and/or release, thus attenuating its own contractile effect on vascular smooth muscle. Nevertheless, in young SHR, numerous molecular biology studies have shown that NO formation and/or release is upregulated and should be considered a compensatory mechanism for the presence of neurogenic vasoconstriction (Figure 2). In parallel, we and others have shown that carotid diameter and isobaric distensibility and their age-related changes do not differ significantly between WKY and SHR until the age of 12 weeks, whereas blood pressure is higher and arterial walls are thicker in SHR than in control rats. Thus, in young SHR, it seems likely that the NE-NO interactions in endothelium contribute to the preservation of arterial function and proportional increases of MBP and PP with age, despite severe constrictive (NE) influences. Pertinently, experimental studies on endogenous NO formation and/or release have clearly shown that NO release is frequency dependent and inversely related to PP. Finally, in young SHR with sympathetic hyperactivity, NO upregulation contributes to maintaining arterial function as well as an adequate PP.

**Thoracic Aortas and Carotid Arteries in Old SHR**

The elevated values of SBP, DBP, MBP, and PP in SHR have a spontaneous tendency to decline after 36 weeks of age. This finding is generally associated with a smaller stroke volume with aging and reflects an incipient congestive heart failure. In fact, SBP and PP are reduced with age to a lesser extent than are MBP and DBP, indicating that a significant statistical interaction may be observed for SBP and PP (and not MBP and DBP) between age and strains (SHR and normotensive control rats). Study of old conscious survivor SHR (aged >60 weeks) has shown that although aortic MBP remains relatively stable with aging, PP increases significantly (see Figure 1 at weeks 52 and 78), thereby indicating that despite the smaller stroke volume, a parallel increase of aortic stiffness is able to produce an absolute increase of PP in these surviving animals. It should be noted that a significant increase of PP (but not MBP) with age has been previously observed in normotensive rats. In contrast to the results obtained in young SHR, isobaric aortic pulse wave velocity and incremental elastic modulus are significantly increased in old SHR compared withagematched WKY. These findings indicate that the elastic properties in SHR aortas are intrinsically modified by aging and are independent of blood pressure level. This aortic stiffening cannot be related to wall thickening itself because the medial thickness/ internal diameter ratio remains constant with age in both SHR and control rats. Therefore, other determinants of aortic wall elastic properties (eg, relative proportions and/or interactions between smooth muscle cells and extracellular matrix, or altered functional factors) may change during the aging of hypertensive rats.

Regarding the extracellular matrix components, modifications of collagen content are not dominantly involved in age-linked aortic stiffening in SHR, because fibrosis does not develop markedly with aging in this strain (or develops to the same extent in SHR and Wistar rats when the latter are taken as normotensive controls) (Figure 3). On the other hand,
even though the elastin content decreases slightly with age, it
does not decline faster in SHR than in WKY, and the
elastin/collagen ratio decreases similarly with aging in both
strains. Therefore, changes of blood pressure, aortic wall
thickening, or scleroprotein contents do not play a major role
in the age-linked aortic stiffening observed in SHR. In
addition, studies using aminoguanidine in old SHR have
shown that a pressure-independent decrease of carotid stiff-
ness may be obtained without changing wall thickness and
the amount of extracellular matrix as a consequence of rapid
changes in end-glycosyl products. Furthermore, the pres-
ence of the age-related aortic structural changes results in
substantial modifications of vascular smooth muscle tone,
involving increased endothelium-independent aortic reactiv-
ity to potassium chloride, a response that is known to be
proportional to the level of wall thickness and mean arterial
pressure.

Such findings suggest that an increased isobaric stiffness in
the SHR could not simply be consequent to a change in wall
structure but also to a change in both the organization and
content of the media with resulting changes in vascular tone.
The relationship between the smooth muscle cell and the
matrix elements is thought to be critical for the regulation
of several cell functions, including differentiation and elastic
properties. We have recently demonstrated that the integrity
of microtubules and extracellular matrix elements such as
collagen, fibronectin, or laminin can modulate the angioten-
sin II–induced signaling events leading to calcium increase in
aortic smooth muscle cells. Furthermore, this regulation is
different in SHR compared with WKY and could be related to
the organization profile of focal adhesion sites and mechano-
transduction mechanisms. Such findings even suggest that a
synergy between integrin and inositol signaling pathways
would probably affect the tone of smooth muscle cells and,
therefore, arterial stiffness. Finally, all these alterations
taken together may contribute to change aortic reactivity and
in turn modify the mechanical properties of SHR conduit
arteries.

Using 78-week-old SHR aortic rings studied in organ
chambers, we observed that compared with aortic rings from
WKY or Wistar rats of the same age, the increase of maximal
developed tension under NE obtained after deendotheli-
alization (or under LNNA incubation) was significantly
reduced or even abolished (Figure 2). Because this effect
was observed in old and not young SHR, this age-dependent
observation points to a modification with age of the interac-
tions between endothelial function, arterial stiffness, and PP
regulation. Several arguments support this interpretation.
First, in vitro experiments showed that NO bioactivity and
endothelial NOS mRNA and protein, which are markedly
influenced by age and substantially lowered in the elderly,
are more significantly associated with pulsatile than steady
mechanical factors. Second, in old hypertensive animals and mostly men, exogenous NO donors are able to
normalize acutely and selectively a disproportionate increase
of PP, with minor changes of MBP and without any structural
alteration of the hypertrophied arteries (see reviews). Such
acute changes occur in old but not young subjects, ie,
in populations characterized by an age-induced alteration of
endothelium function. Finally, in a recent study on the
response of rat aortic rings to NE in organ chambers after the
local administration of the diuretic agent cicletanine, we
observed an NO-dependent and an endothelium-dependent
relaxation, which was mainly due to NOS stimulation and
was significantly more pronounced in older than in younger
animals. Pertinently, cicletanine given in vivo to SHR
produced an MBP-independent decrease of arterial stiffness
and PP.

In conclusion, animal studies have indicated significant
interactions between NO-dependent endothelium function,
arterial stiffness, and PP in SHR, with substantially different
patterns in young and old animals. The findings are consistent
with the possibility that in the long term, a negative feedback
may be established between NO bioactivity and PP through
concomitant changes in arterial structure and function. Fur-
ther experiments are needed to investigate this important
aspect as well as its possible contribution to the mechanisms
of systolic hypertension in the elderly.

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References
1. Folkow B. Structural factor in primary and secondary hypertension.
2. Pfeffer MA, Frohlich ED, Pfeffer JM, Weiss AK. Pathophysiological
implications of the increased cardiac output of young spontaneously
3. Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: physiology, patho-
4. Safar ME, London GM. The arterial system in human hypertension. In:
5. Nichols WW, O’Rourke M. McDonald’s blood flow in arteries. In:
Theoretical, Experimental and Clinical Principles. 4th ed. London, UK:
Edward Arnold Publishers Ltd; 1998:54–113, 201–222, 284–292,
347–401.
6. Chamiot-Clerc P, Renaud JF, Blacher J, Legrand M, Samuel JL, Levy BI,
Sassard J, Safar ME. Collagen I and III and mechanical properties of


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