Pulse pressure and arterial stiffness in rats: comparison with humans

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The development of appropriate animal models for investigating human hypertension has been extremely valuable for studies of the natural history and the pathophysiological mechanisms of the disease (21). It has been considered that there is a strong similarity between spontaneously hypertensive rats (SHR) and patients with essential hypertension. Both have their apparent onsets of the condition very early in life, a reflection of their genetic backgrounds. The arterial hypertension involves a progressive increase of vascular resistance that initiates profound cardiac and systemic vascular adaptations and produces parallel increases of systolic (S), diastolic (D), and mean (M) arterial blood pressure (BP). Neural mechanisms seem to predominate in the early stages of both species’ hypertensive diseases (especially in SHR), although in rats, multiple structural and functional disorders seem to be involved.

Partly as a consequence of antihypertensive drug therapy, the clinical aspects of human hypertension have changed considerably in recent years. In younger populations, milder and milder forms of hypertension are observed. In the elderly, more attention is accorded to isolated systolic hypertension and its treatment (7, 20, 42, 50). Systolic hypertension always involves a disproportional increase of SBP over DBP and differs markedly from the proportional increase of SBP and DBP commonly observed in SHR. In this context, relatively few studies on BP measurements and the pathophysiological mechanisms of high BP in old SHRs have been reported. Thus the purpose of this editorial is to provide some new insights into the mechanisms of systolic hypertension in humans and SHR, primarily taking into account the role on SBP, pulse pressure (PP), and PP amplification in the elderly of both populations.

SBP AND PP IN RATS AND HUMANS

Basic Concepts

Studies of pulsatile arterial hemodynamics have shown that the cyclic BP curve may be divided into two components (39, 42): a steady component mean arterial pressure (MAP) and a pulsatile component PP. Because of the propagation of the pressure wave and the summation of the incident and reflected waves at each specific point of the vascular circuit, SBP is physiologically higher, whereas DBP is slightly lower, in peripheral than central arteries. In contrast, MAP is practically constant along the totality of the arterial tree (Fig. 1).

In normotensive and hypertensive humans, studies of pressure wave transmission under basal conditions have shown that the amplification between the aortic arch and brachial PP averaged 18–31%. Nichols and O’Rourke (42, 43) found that brachial amplification was strongly dependent on the duration of ventricular systole, being decreased when it was lengthened, and increased when it was shortened. Thus, in humans, it is clear that brachial artery tracings may give a falsely high SBP value and falsely low DBP value compared with the ascending aorta and the rest of the arterial tree under basal conditions and those with a shortened duration of systole. From these findings, it is important to take into account for hypertensive subjects, not only peripheral (brachial), but also central (thoracic aorta and carotid artery) BP measurements.

In rats, the situation is more complex. Usually, tail SBP is measured. Therefore, it is assumed that tail SBP reflects central SBP and that there are no substantial SBP and PP amplifications in rats. In fact, studies in Wistar-Kyoto (WKY) rats (60, 61), either conscious or under anesthesia, have shown that significant SBP and PP amplifications are observed in these animals and, therefore, that it is not valid to extrapolate central SBP from tail SBP. In contrast, in SHR studied under basal conditions no amplification is observed. This finding in SHR is not surprising. The levels of SBP and PP amplifications are proportional to the length of the arterial tree (42, 43). In hypertensive rats, the increased arterial stiffness and the resulting changes in wave reflections tends to attenuate amplification as a consequence of the reduced length of the arterial tree. Nevertheless, in SHR, SBP and PP amplifications have been noted after administration of vasoactive agents. Angiotensin-converting enzyme inhibitors and calcium-entry blockers, but not dihydralazine, markedly reduce central PP with practically no effect on the terminal aorta PP (60).

All these observations taken together show that pressure wave transmission should be considered for BP measurements in rats and more generally in small rodents. Rats and mice have the same MAP but the mouse PP is half that in rats together with a significantly higher heart rate (37).
Age-Associated Changes in SBP and PP in Rats

During the early phase of genetic hypertension in rats, there are major obstacles to accurately determine BP in such small animals. Whereas some authors have described a prehypertensive period, a number of other reports have indicated a significantly higher BP in SHR than in control rats before weaning (15, 17). In recent years, the use of intra-aortic BP measurements in conscious animals has clearly indicated that central BP increases with age more rapidly in SHR than in normotensive controls and that this increase involves enhancement of SBP, DBP, MAP, and PP but without any disproportional increase of SBP over DBP or PP over MAP (Fig. 2) (15).

The proportionally elevated SBP, DBP, MAP, and PP in SHR are known to decline spontaneously after 36 wk of age (36, 47). This finding is generally associated with a smaller stroke volume with aging and often considered to be due to incipient congestive heart failure (12, 47). In fact, SBP and PP are reduced with age to a lesser extent than MAP and DBP, which results in a statistically significant age-strain (SHR and WKY rats) interaction for SBP and PP but not MAP and DBP (36). Study of subpopulations of old (>60 wk) conscious SHR, which may be considered “survivors,” has shown that, although aortic MAP remains relatively stable with aging, PP increases significantly from 52 to 78 wk of age (Fig. 2B). This observation indicates that, in rats, despite a decreased stroke volume with age, a parallel increase of aortic stiffness is able to produce an absolute PP increase in survivors (12). It should be noted that a significant increase of PP (but not MAP) with age has previously been reported in normotensive rats (38, 57).

In conclusion, few data are available on SBP and PP in older subjects (42). This finding is generally associated with a smaller stroke volume with aging and often considered to be due to incipient congestive heart failure (12). It should be noted that a significant increase of PP (but not MAP) with age has previously been reported in normotensive rats (38, 57).

In contrast to the results obtained in young SHR, the isobaric aortic pulse wave velocity (PWV) and incremental elastic modulus are significantly increased in old (52–78 wk) SHR compared with age-matched controls (36). These findings indicate that in SHR the elastic properties of the aorta are intrinsically modified with aging independently of MAP level. This aortic stiffening cannot be attributed to wall thickening itself because the medial thickness-to-internal diameter ratio remains constant with age in both SHR and control rats (36). Therefore, other determinants of aortic wall elastic properties, i.e., the relative proportions of and/or interactions between smooth muscle cells and extracellular matrix, are affected during the aging of hypertensive rats. This process involves disproportional increases of collagen fibers and various adhesion molecules, such as fibronectin and proteoglycan, which contribute not only to increasing arterial stiffness but...
also to altering the circulation of fluids in the interstitial milieu and favors cell death (51). It is noteworthy that, in such cases, cardiovascular (CV) death does not result from any lipid infiltration, as observed in atherosclerosis, but rather to other factors implied in the aging process. Thus rats have numerous biological factors capable of modulating arterial stiffening, SBP, PP, and PP amplification with age, and it is important to study these factors to develop novel approaches to treat hypertension.

**BIOLOGICAL FACTORS MODULATING SBP AND PP IN RATS**

Biological factors modulating SBP, PP, and PP amplification have been studied mainly in genetic models of hypertension and involve mainly nitric oxide (NO) and vasoconstrictive agents, extracellular matrix, and sodium.

**NO and Vasoconstrictive Substances**

In anesthetized Sprague-Dawley rats, a bolus injection of NO synthase (NOS) inhibitor is able to significantly increase PWV, in parallel with an increase of BP (19). Because the BP changes are known by themselves to affect PWV, phenylephrine (PE) was administered in a control group to mimic the MAP changes induced by NO inhibition, thus compensating for the pressure-dependent component of the PWV changes. Under those conditions, at each given level of MAP, PWV was significantly higher with NO blockade than with PE treatment. Similar findings have been reported using intra-arterial administration of suppressive doses of NO blocker in sheep (63). In Sprague-Dawley rats, the PWV changes were associated with an increase of central SBP and decrease of central DBP (i.e., PP increase) under NO blockade but not under PE (19). In elderly WKY rats with systolic hypertension, the increased SBP was reversed with L-arginine and angiotensin-converting enzyme inhibition (57). Taken together, these findings suggest that acute withdrawal of endogenous NO increases arterial stiffness independent of MAP changes and that an intact endogenous NO system is required to maintain arterial elasticity.

Studies on aortic reactivity in organ chambers have provided an explanation for the links between NO and arterial stiffness in rats. Young SHR aortic smooth muscle reaches maximal tension under norepinephrine (NE), and it is markedly higher in the absence than in the presence of endothelium (12) (Fig. 3). This heightened response, which is also produced by preincubation with a specific NOS inhibitor (12), indicates that NO modulates the SHR vascular smooth muscle cell response to the contractile agent NE. As previously observed in normotensive animals (14), NE acts on endothelial cells to increase NO production and/or release, thereby attenuating its own contractile effect on vascular smooth muscle. Numerous molecular biology studies on young SHR with sympathetic overactivity have shown that NO formation and/or release is upregulated (27, 35, 48) and should be considered a compensatory mechanism for the presence of neurogenic vasoconstriction. In vivo or in vitro NO blockade un masks sympathetic overactivity, leading to increased arterial stiffness and PP (19). Finally, in young SHR, NO upregulation contributes to maintaining adequate arterial function and PP (12, 48).

The situation is completely different in aortic rings of 78-wk-old SHR. Compared with aortic rings from age-matched WKY or Wistar rats, the increase of maximal tension developed under NE obtained after deendothelialization (or under NO blockade) is significantly lower or even abolished (12) (Fig. 3). This observation suggests a modification with age of the interactions among endothelial function, arterial stiffness, and PP, an interpretation supported by several other results. First,
under physiological conditions, the NO release is modulated both by frequency and amplitude of pulsatile flow, resulting in a PP decrease (8, 23). Second, this mechanism is disrupted in the elderly, in which conduit arteries are stiffened (46). Indeed, NO bioactivity and endothelial NOS mRNA and protein, which are markedly influenced by age and substantially lowered in the elderly (38), result in oxidative stress and are more substantially associated with pulsatile than with steady mechanical stress (3, 10, 13, 27, 35, 49, 51). Third, in old hypertensive rats and predominantly in men, exogenous NO donors acutely and selectively normalize PP with minor MAP changes and without any supplementary structural alteration of the hypertrophied arteries (see reviews in Refs. 42, 51, and 52). Finally, nitrates are known to dilate larger rather than smaller arteries, to decrease markedly arterial stiffness independently of MAP changes, and to produce a greater diminution of central than peripheral SBP, thereby increasing PP amplification (42, 52).

The endothelial NO-NE interaction can be easily studied in SHR, where its main characteristic is sympathetic overactivity. However, the interactions of NO with other vasoconstrictors, such as ANG II or endothelin, should also be considered. A specific receptor-mediated effect of ANG II on endothelial cells may be postulated. ANG II specifically constricts the carotid artery and increases cGMP levels via the ANG II type 1 (AT1)-receptor subtype in the in vitro intact rat carotid artery (9, 31). The mechanism underlying this change is thought to be mediated through endothelial NOS stimulation by ANG II. ANG II-dependent NO production by the endothelial cells could modulate the peptide-induced smooth muscle cell contraction. Thus ANG II contractile and trophic vascular effects could be exaggerated under pathological heterogeneous conditions such as accelerated aging and contribute by themselves to the mechanisms of systolic hypertension.

SBP, PP, and Extracellular Matrix

The balance between rigidity and elasticity of all blood vessels is determined by the mechanical properties of extracellular matrix proteins. This balance involves not only elastin but also the formation of heterologous collagen I, III, and V fibrils synthesized by the smooth muscle cells of the arterial media. The texture and, therefore, the tensile strength of any tissue are characterized by the diameter of the fibrils and their ability to form bundles (26, 40, 44, 53). Collagen I is the most abundant and forms thick fibrils leading to rigidity, but the cross-linkage of this molecule with collagen III (and perhaps collagen V) decreases fibril diameter and increases the extensibility of the tissue. Hence, regulating the synthesis and thus the balance between collagens I and III is crucial for the mechanical properties of the blood vessel. This balance depends on the strict regulation of the biosynthesis and degradation of collagens I and III. For instance, the proportions of collagens I and III differ markedly within the arterial wall of Lyon and Japanese rats, resulting in a higher isobaric elasticity in the former (11).

In cell cultures, ANG II stimulates the production of various types of collagen fibers (25) and also several growth factors (22). In vivo, angiotensin-converting enzyme inhibition and ANG II AT1 receptor blockade have been used as tools to demonstrate that the chronic blockade of the AT1 receptor prevents the accumula-
tion of aortic collagen in SHR (1, 6). This effect is independent of BP changes and bradykinin release but involves the blockade of either AT1 or mineralocorticoid receptors or a combination of both (5, 6). Finally, such findings are observed exclusively on a normal, but not a high-sodium diet (28), a situation during which collagen accumulation is associated with an increase of isobaric arterial stiffness. Through their binding to cations, proteoglycans in the arterial wall may be one of the predominant contributors to this process (18).

Independently of the renin-angiotensin system, glycosylated end products may also participate in the biosynthesis and degradation of arterial collagen (24, 40). They may accentuate the cross-linking of collagen fibers and thereby increase isobaric arterial stiffness, particularly in experimental and human models of diabetes mellitus and aging. The drug aminoguanidine or derivatives can reverse the stiffness alterations (24).

Sodium and Arterial Stiffness

Most of the data on sodium-induced changes of arterial structure and function were obtained from genetic models of hypertension in rats. Tobian (59) was the first to show that, in stroke-prone SHR, high sodium intake is associated with more pronounced structural alterations of cerebral and renal arteries than when a low-sodium diet is consumed. Under high-salt conditions, the increased wall thickness involves a substantial increase of collagen content together with abnormal cross-linking and enhanced arterial stiffness (32, 40). These alterations are reversed with lowering of sodium intake without any change of intra-arterial MAP but in parallel with a reduced incidence of cerebrovascular accidents.

In stroke-resistant SHR, the temporal relationship between high BP hypertension and the appearance of vascular lesions during salt loading has been investigated starting at 5 wk of age (34). Neither intrarterial BP nor vascular morphology of WKY rats is affected by 1% NaCl in the drinking water (34, 45). In SHR, BP is not affected by the addition of salt for at least 11 wk, but vascular morphology is significantly altered within 5 wk, resulting in significant thickening of the aortic media associated with increased arterial stiffness, marked modification of extracellular matrix, collagen, and proteoglycans (18, 34, 45). Similar results, associated with an isobaric increase of arterial stiffness have been obtained in Dahl salt-sensitive rats (4).

Taken together, these findings show pressure-independent interactions between sodium ions and arterial structure and function in various models of hypertension in rats. They also indicate that genetic factors, particularly those involving sodium sensitivity, are highly contributive to stiffness changes.

APPLICATIONS TO SYSTOLIC HYPERTENSION IN HUMANS

Hypertension in the elderly has two distinct features: isolated systolic hypertension with normal or low DBP and systolic-diastolic hypertension with a disproportional increase of SBP over DBP. Both of them result in a higher SBP and PP due to increased arterial stiffness and disturbed wave reflections (42, 52). Compelling evidence is now available that both varieties of hypertension involve a high degree of CV risk, as evaluated from PP and PWV measurements (50), and that drug treatment is highly effective against CV morbidity and mortality (55). In addition, Framingham studies (20) have shown that the age-related increase of SBP and PP vary widely from one individual to another after 50 years of age. The factors modulating this variability should be evaluated extensively if the goal of drug treatment is to adequately reduce CV risk. Thus, in older populations, the purpose of antihypertensive therapy should be not only to reduce BP in all cases of isolated systolic hypertension, but also to reduce the physiological decrease of DBP with age, hence to minimize the increase of PP with age, in populations at high CV risk.

As observed in animals, the accelerated increases of PP and PWV with age are modulated by a number of genetic and environmental factors. Some gene polymorphisms as those associating the C allele of the ANG II AT₁ receptor gene and the T allele of the constitutive

**Table 1. Pharmacological agents capable of selectively reducing SBP and PP, and increasing arterial stiffness independent of MAP**

<table>
<thead>
<tr>
<th>Mechanism(s) of Action</th>
<th>Compound</th>
<th>References</th>
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<tbody>
<tr>
<td>NO and endothelial function</td>
<td>Isosorbide dinitrate</td>
<td>16, 51, 62</td>
</tr>
<tr>
<td>Nitrates*</td>
<td>Sinitrodiol</td>
<td>12, 62</td>
</tr>
<tr>
<td>Others</td>
<td>Nebivolol</td>
<td>12, 62</td>
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<tr>
<td></td>
<td>Cyctelantine</td>
<td>12, 62</td>
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<tr>
<td>Collagen and extracellular matrix</td>
<td>Blockers of renin-angiotensin system</td>
<td>1, 6, 28, 31</td>
</tr>
<tr>
<td>Collagen content*</td>
<td>Acteon</td>
<td>24</td>
</tr>
<tr>
<td>Collagen cross-linking*</td>
<td>Thiazide diuretics</td>
<td>56, 58</td>
</tr>
<tr>
<td>Sodium and related compounds*</td>
<td>Indapamide</td>
<td>2, 4, 18, 32</td>
</tr>
<tr>
<td></td>
<td>Spironolactone</td>
<td>5, 29</td>
</tr>
<tr>
<td></td>
<td>Sodium diet</td>
<td>28, 29, 34, 59</td>
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MAP, mean arterial pressure; systolic blood pressure; PP, pulse pressure; NO, nitric oxide. *Both experimental and clinical studies.
NOS (G894T) gene are accompanied by accelerated increases PP and arterial stiffness with age (30, 41). We have previously shown that the presence and/or the combination of the C and/or T alleles is associated with PP steeper increases with age than in the non-C, non-T allele subgroups (41). Thus, in individuals with a genetic predisposition, both an increase of ANG II-induced aortic collagen accumulation and impaired NO bioactivity might contribute to enhancing arterial stiffness, thereby increasing SBP and PP with age. In hypertensive subjects, the frequencies of these alterations have been underestimated, because in the majority of genetic studies, DBP, and not SBP, was used as the sole criterion of selection for the diagnosis of hypertension. In addition, other candidate genes have been described, particularly those combining the alleles of the angiotensin-converting enzyme and the α-adducin genes (56).

In humans, environmental factors also contribute to the increases of aortic stiffness and PP with age. In particular, it is well established that sodium sensitivity rises with age in parallel with the increase of PP. These effects have been noted in association with some gene polymorphisms, like those combining the angiotensin-converting enzyme and α-adducin genes (56). Finally, the role of genetic factors on the age-PP relationship is more pronounced in women than in men, possibly as a consequence of their constitutive short stature with resulting gender-related changes of arterial stiffness and wave reflections (41, 54).

In conclusion, it has been shown in this editorial that new aspects of the similarities and dissimilarities of hypertension in humans and rats may be noted. They mainly reflect changes in SBP, PP, and arterial stiffness associated with age. Furthermore, they suggest that the standard drug treatment of hypertension, which in the past focused on decreasing vascular resistance, is no longer adequate to obtain a parallel reduction in arterial stiffness. New aspects of CV pharmacology should be defined to respond to this important challenge as summarized in Table 1.

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DISCLOSURES

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