Effects of Valsartan on Mechanical Properties of the Carotid Artery in Spontaneously Hypertensive Rats Under High-Salt Diet

Carlos Labat, Patrick Lacolley, Malika Lajemi, Marc de Gasparo, Michel E. Safar, Athanase Benetos

Abstract—The aim of this investigation was to evaluate the influence of a high-salt diet (HSD) on the effects of valsartan, an angiotensin II type 1 (AT₁) receptor antagonist, on carotid arterial stiffness and structure in spontaneous hypertensive rats (SHR). Carotid arterial stiffness was studied in SHR receiving a HSD or a normal-salt diet (NSD) from the 10th to 20th week of age. Within each of the 2 groups, the animals received treatment with either placebo or valsartan (30 mg · kg⁻¹ · d⁻¹) administered on the 4th to 20th week of age. Arterial pressure, wall stress, incremental elastic modulus (Einc), medial cross-sectional area, and EIIIA fibronectin isoform were significantly increased in placebo-HSD rats compared with placebo-NSD rats with no change in the ratio of collagen to elastin. Valsartan reduced mean arterial pressure in both NSD and HSD rats but reduced pulse pressure only in NSD rats. In NSD rats, valsartan reduced Einc and medial cross-sectional area. In HSD, valsartan increased Einc and did not modify medial cross-sectional area and fibronectin. In valsartan-treated rats, the ratio of collagen to elastin was greater in HSD than in NSD rats. In conclusion, the effects of AT₁ blockade are greatly influenced by salt intake in SHR. Despite a reduction in mean arterial pressure in HSD rats, AT₁ blockade was not able to prevent the effects of a HSD on pulse pressure, carotid artery stiffness, and hypertrophy. (Hypertension. 2001;38:439-443.)

Key Words: salt ● angiotensin II ● AT₁ blockade ● large artery stiffness ● carotid artery

Previous reports have shown that plasma renin-angiotensin activity is reduced in rats receiving a high-salt diet (HSD). However, a long-term HSD in salt-sensitive animals—Dahl salt-sensitive rats, stroke-prone hypertensive rats, or ANP knockout mice—is known to stimulate plasma renin activity. These observations suggest that the effects observed during perturbation of the renin-angiotensin aldosterone system by a high sodium intake may depend on the animal model used. Recently, Wang and Du observed an increased expression of angiotensin II type 1 (AT₁) receptor mRNA in arterial preparations derived from Wistar and Sprague-Dawley rats maintained on a HSD. In contrast, when Dahl rats were treated with a HSD, decreases in aortic mRNA and AT₁ receptor density were observed. Together, these results suggested that 1 modification associated with high sodium intake may be at the level of the AT₁ receptor. Interestingly, in spontaneously hypertensive rats (SHR) treated with a HSD, an enhanced functional response to angiotensin II and AT₁ receptor antagonists was demonstrated, suggesting that sodium modified AT₁ activity in SHR. Although AT₁ receptor antagonists have been shown to reduce mortality in salt-sensitive animal models (Dahl rats or stroke-prone rats), there is little evidence available that AT₁ receptor antagonists continually block the elevated blood pressure and arterial abnormalities observed in SHR during high sodium intake.

The aim of this investigation was to examine the preventive effects of valsartan, an AT₁ receptor antagonist, on blood pressure, carotid artery (CA) structure, and functional elastic properties in SHR maintained on different sodium diets.

Methods

All procedures were carried out in accordance with institutional guidelines for animal experimentation. SHR (n=58) male rats that were 4 weeks of age were obtained from Iffa Credo (France). In SHR, a HSD (7% NaCl in the food) was administered from the 10th to 20th week of age. Control SHR received a normal-salt diet (NSD; 0.4% NaCl) during the same period. Within each of the 2 groups, the animals received treatment with either placebo or valsartan (30 mg · kg⁻¹ · d⁻¹ in the food) administered on the 4th to the 20th week of age. In pilot experiments, we have observed that when high salt was given starting at the 4th week of age, 80% of the placebo-treated rats died, most of them 4 to 6 weeks after the beginning of the experimental protocol. Under the same conditions, valsartan reduced mortality by 50%. Therefore, we decided to start a HSD later in life (from the 10th week of age) to avoid this excessive mortality. Because our aim was to assess the preventive effects of AT₁ blockade in SHR maintained on different sodium diets, administration of valsartan was started early in life, from the 4th week of age, following the same design we have previously used with blockers of the renin-angiotensin aldosterone system.
Previous studies have shown that valsartan administered at 30 mg · kg$^{-1}$ · d$^{-1}$ PO significantly decreases blood pressure over 24 hours in SHR with a NSD. Oral absorption of (14C) valsartan in rats was rapid, and peak plasma concentrations were attained within 0.5 hour. Absolute bioavailability of unchanged valsartan was high in rats (73%).

The determination of the circumferential wall stress ($\sigma$) and Einc required the value of media cross sectional area (MCSA).

\[
\text{MCSA} = \frac{\pi}{2} \tan^{-1} \left( \frac{P - \rho \cdot \gamma}{\rho \cdot \gamma} \right)
\]

\[
\text{Dist}(P) = \frac{1}{\text{LCSA}} \times \frac{\delta \text{LCSA}}{\delta P}
\]

\[
\sigma = \frac{2 \text{LCSA} \times P}{\text{MCSA}}
\]

\[
\text{Einc} = \frac{3}{\text{Dist}(1 + \frac{\text{LCSA}}{\text{MCSA}})}
\]

The determination of the circumferential wall stress ($\sigma$) and Einc required the value of media cross sectional area (MCSA).

We determined the structure of the CA in 4% formaldehyde–fixed arteries. Sirius red was used for collagen staining, orcein for elastin, and hematoxylin for nucleus. CA thickness, MCSA, and composition of the arterial wall were quantified by computer-directed color analysis.

Immunohistological staining for cellular EIIIA fibronectin (Fn) was performed on 5-µm-thick, freeze-dried, paraffin-embedded sections as previously described. Briefly, samples were treated with mouse monoclonal antibodies (mAb) reactive with cellular EIIIA Fn isoform (Sera Laboratory) and total Fn isoforms (Valbiotech). After 3 washes in Tris buffer solution, the biotinylated anti-mouse Ab was added. After washes in Tris buffer solution, the slides were incubated with streptavidin-peroxidase. The presence of peroxidase was revealed after incubation with diaminobenzidine. Controls were performed by omission of the first or second Ab.

Data were analyzed by use of 2-way ANOVA, followed by a Tukey-Kramer test for multiple comparisons. A value of $P=0.05$ was considered significant.

### Results

**Effects of Valsartan on Blood Pressure and CA**

All SHR maintained on a NSD survived the treatment period. Of 15 SHR receiving a HSD in the placebo group, 2 (13%) died compared with none of the valsartan-treated rats. SHR maintained on a HSD had lower body weight and higher mean arterial pressure (MAP) and pulse pressure (PP) compared with those of rats receiving a NSD (Table 1). Arterial distensibility was lower, with no change in arterial diameter.

The Einc–wall stress curve in HSD rats receiving placebo was shifted to the right in the prolongation of the Einc–wall stress curve observed in the placebo-NSD rats (Figure 1). No significant differences in collagen, elastin densities, and ratio of collagen to elastin were observed between the 2 groups.

The higher collagen and elastin contents under a HSD were related to the significant increase in CA MCSA (Table 2) because MCSA was correlated with MAP ($r=0.63$) and PP ($r=0.80$).

Valsartan reduced MAP similarly in both NSD and HSD rats (21% versus 16%) (Table 1). A reduction in carotid PP was observed only in NSD rats treated with the AT1 antagonist. Valsartan administered to NSD rats produced a significant decrease in carotid diameter and a 2-fold increase in arterial distensibility. Valsartan did not modify arterial diameters.

### Table 1. Effect of Valsartan on Body Weight, Blood Pressure, and CA Parameters in SHR Receiving a NSD or a HSD

<table>
<thead>
<tr>
<th></th>
<th>NSD Placebo (n=14)</th>
<th>Valsartan (n=15)</th>
<th>HSD Placebo (n=13)</th>
<th>Valsartan (n=14)</th>
<th>$P$ Interaction (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>412±8</td>
<td>389±10</td>
<td>382±8†</td>
<td>378±11</td>
<td>NS</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>182±6</td>
<td>144±3*</td>
<td>214±6†</td>
<td>180±5††</td>
<td>NS</td>
</tr>
<tr>
<td>PP, mm Hg</td>
<td>60±3</td>
<td>42±3*</td>
<td>69±2†</td>
<td>69±3††</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>349±11</td>
<td>336±10</td>
<td>347±12</td>
<td>363±11</td>
<td>NS</td>
</tr>
<tr>
<td>Distensibility, mm Hg·10$^{-3}$</td>
<td>2.01±0.16</td>
<td>4.43±0.40*</td>
<td>1.37±0.11†</td>
<td>1.61±0.21†</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Einc, kPa</td>
<td>1176±121</td>
<td>539±53*</td>
<td>1659±137†</td>
<td>1679±279†</td>
<td>0.07</td>
</tr>
<tr>
<td>Wall stress, kPa</td>
<td>210±9</td>
<td>168±9*</td>
<td>242±11†</td>
<td>198±12†*</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean±SEM. *$P<0.05$ vs placebo for the same salt diet; †$P<0.05$, HSD vs NSD for the same treatment.
TABLE 2. Effect of Valsartan on CA Composition in SHR Receiving a NSD or a HSD

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=14)</th>
<th>Valsartan (n=15)</th>
<th>Placebo (n=13)</th>
<th>Valsartan (n=14)</th>
<th>P Interaction (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCSA, mm² · 10⁻³</td>
<td>287±9</td>
<td>223±11</td>
<td>325±12</td>
<td>319±18</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Nucleus surface, μm²</td>
<td>6.2±0.2</td>
<td>6.1±0.2</td>
<td>6.1±0.2</td>
<td>7.2±0.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Nucleus number/1-mm section</td>
<td>294±11</td>
<td>284±16</td>
<td>275±12</td>
<td>280±11</td>
<td>NS</td>
</tr>
<tr>
<td>Collagen density, %</td>
<td>16.9±0.6</td>
<td>16.9±0.6</td>
<td>17.7±0.8</td>
<td>17.5±0.6</td>
<td>NS</td>
</tr>
<tr>
<td>Collagen content, mm² · 10⁻³</td>
<td>49±2</td>
<td>37±2</td>
<td>58±4</td>
<td>51±5</td>
<td>NS</td>
</tr>
<tr>
<td>Elastin density, %</td>
<td>36.2±1.1</td>
<td>38.8±1.8</td>
<td>36.9±1.6</td>
<td>31.0±1.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Elastin content, mm² · 10⁻³</td>
<td>103±3</td>
<td>86±5</td>
<td>121±8</td>
<td>94±10</td>
<td>NS</td>
</tr>
<tr>
<td>Collagen/elastin ratio</td>
<td>0.57±0.03</td>
<td>0.56±0.02</td>
<td>0.57±0.04</td>
<td>0.69±0.03</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

*P<0.05 vs placebo for the same salt diet; †P<0.05, HSD vs NSD for the same treatment.

Discussion

In this study, long-term treatment of SHR with the AT₁ receptor antagonist valsartan was investigated in animals maintained on a HSD. The HSD induced an increase in MAP and PP associated with carotid artery hypertrophy and wall stiffness. Valsartan significantly reduced MAP but had no effect on PP, arterial wall hypertrophy, and stiffness.

Analysis of Einc–wall stress curves showed that the increased stiffness of the CA in the HSD group was due to a higher level of wall stress with no changes in the intrinsic elastic properties of the vascular wall. Preservation of elastic properties is in accordance with the absence of modification in collagen and elastin densities and the ratio of collagen to elastin with a HSD. In SHR, salt loading has been shown to result in a significant thickening of the aortic media between 10 and 20 weeks of age. This increase in wall thickness is associated with an enhanced accumulation of Fn. Previous findings in genetic hypertensive rats indicate marked interactions between a HSD, increased wall thickness, and increase in Fn. The increase in wall thickness and Fn may contribute to the increase in vascular wall elastic modulus in proportional to the level of circumferential wall stress through an increased number of cell matrix attachment sites.

Interestingly, the decrease in MAP in HSD rats treated with valsartan was not associated with a significant reduction in CA wall thickness. One possible explanation is that despite similar changes in MAP with valsartan in NSD- and HSD-fed rats, blood pressure values at the end of the treatment period were still too high in HSD rats (180 mm Hg). We can suggest that MAP should achieve a lower blood pressure threshold to significantly reduce arterial wall hypertrophy and to improve arterial compliance. The persistence of arterial wall hypertrophy may also be explained by the absence of reduction in PP, which is a main determinant of arterial hypertrophy. Augmentation of Einc for a given value of wall stress in valsartan-treated HSD rats compared with all other groups demonstrates a marked increase in intrinsic stiffness of the wall material. The antihypertensive efficacy of AT₁ antagonism, despite a NaCl-induced decrease in the renin-angiotensin system, may be explained by an increase in AT₁ receptor messenger RNA levels and by an increase in AT₁ receptor density. The present study shows that AT₁ blockade in the presence of a HSD was also associated with an increase in the ratio of collagen to elastin compared with the NSD rats receiving the same treatment. These data support the suggestion that this increase is at least partially responsible for the CA wall stiffness observed in HSD rats receiving the AT₁ blockade. However, there was no significant decrease in Fn with valsartan in SHR receiving a NSD, a result that is in agreement with previous reports showing the effects of AT₁ antagonists and ACE inhibitors on in vivo and in vitro Fn.
expression. In contrast, in the presence of a HSD, valsartan was not able to reduce Fn content. Therefore, persistence of arterial wall rigidity in HSD valsartan-treated rats may also be explained by the maintenance of relatively high levels of Fn despite the decrease in wall stress.

The absence of a reduction in PP by the AT1 antagonist may be responsible for incomplete results on mortality reduction in SHR receiving a HSD. Clinical studies have pointed out the predominant role of PP in the cardiovascular morbidity and mortality in several populations, making PP a major cardiovascular risk factor independent of MAP. Previous studies have shown that salt is a determinant of aortic stiffness and arterial wall hypertrophy. PP may aggravate the effects of a HSD on vascular structure and cardiovascular morbidity and mortality observed in experimental models of hypertension.

In conclusion, the present study showed that the effects of AT1 blockade are greatly influenced by high salt intake in SHR. Valsartan reduced MAP but was not able to diminish large artery stiffness and hypertrophy and PP.

Acknowledgments
This work was supported by grants from INSERM (5A090B and 5Z090B). We thank Research Department Novartis (Switzerland) for their help and for providing valsartan. We also thank C. Perret and K. Le Dudal for technical assistance.

References


Effects of Valsartan on Mechanical Properties of the Carotid Artery in Spontaneously Hypertensive Rats Under High-Salt Diet
Carlos Labat, Patrick Lacolley, Malika Lajemi, Marc de Gasparo, Michel E. Safar and Athanase Benetos

Hypertension. 2001;38:439-443
doi: 10.1161/01.HYP.38.3.439

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/38/3/439

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/