**Abstract**—Although the role of sodium in hypertension has been documented extensively, its effect on large arteries has not been well documented. We examined the effect of high-sodium (8%) diet and the diuretic indapamide (IND) on systemic hemodynamics and aortic wall structure and composition in collagen, elastin, and hyaluronan. Four groups of spontaneously hypertensive rats (SHR) were studied after 8 weeks: those on a normal diet (SHR), a high-sodium diet (SHR+NaCl), a normal diet with IND (SHR+IND), and a high-sodium diet with IND (SHR+NaCl+IND). Mean BP, which was not normalized with IND, was comparable for all groups. Systemic arterial compliance averaged 3.8, 2.5, 4.9, and 3.3 mL/mm Hg · 10⁻³, respectively, for the SHR, SHR+NaCl, SHR+IND, and SHR+NaCl+IND groups (P<0.003 and <0.05 for NaCl and IND effects). Wall thickness increased only in the SHR+NaCl group (P<0.01). Aortic wall COL decreased from 16 116 in the SHR to 12 382 µm²/mm in the SHR+NaCl+IND (P<0.005) group. IND alone had no effect on elastin, but the elastin/collagen ratio was increased significantly. Aortic hyaluronan averaged 2343, 266, 3243, and 1052 µm²/mm, respectively, for the SHR, SHR+NaCl, SHR+IND, and SHR+NaCl+IND groups (P<0.0001 for NaCl and IND effects). Changes in systemic arterial compliance were significantly and positively correlated with aortic hyaluronan contents. Thus, high-sodium diet affects the structural and functional characteristics of large arteries independently of BP. A high-sodium diet, in addition to a diuretic regimen with IND, affects simultaneously aortic hyaluronan content and large artery mechanical properties through pressure-independent mechanisms that remain to be defined. (Hypertension. 2001;38:1172-1176.)

**Key Words**: arteries ➤ diet ➤ diuresis ➤ proteoglycans ➤ rats, spontaneously hypertensive

Epidemiological studies indicate that in populations of normotensive and hypertensive subjects, a strong association is observed between high sodium intake and increased aortic rigidity, independent of both age and BP level.¹ Conversely, reduced sodium intake is associated with enhanced arterial elasticity.² In hypertensive rats, high sodium intake leads not only to cardiac hypertrophy and fibrosis but also to hypertrophy of large arteries and enhanced extracellular matrix, again independently of BP level.³ However, the potential link between such structural alterations and the mechanical properties of the arteries has not been investigated extensively.

During high sodium intake in experimental animals, concentration of this cation in the vascular smooth muscle cell rises, but most of it remains extracellularly bound to the extracellular matrix, probably to polyatomic mucopolysaccharides.⁴ To our knowledge, whether such macromolecules contribute to mechanical properties of the arteries during high sodium intake has never been reported under in vivo conditions, at least for large conduit arteries. In sodium-dependent hypertensive animal models, diuretics reduce hypertrophy of the arterial wall even if BP remains unchanged.² Interestingly, a concomitant decrease in arterial stiffness has been reported in Dahl,⁵ stroke-prone,⁶ and even spontaneously hypertensive rats (SHR).⁷ However, whether these structural and functional changes on the arterial wall represent direct or indirect effects of diuretics has never been evaluated clearly in vivo.

The purpose of the present in vivo study in SHR is 2-fold: (1) to evaluate carotid artery mechanical properties and arterial wall structural characteristics after administration of a high-sodium diet and (2) to examine the effect of a diuretic drug, indapamide (IND), on mechanical and structural characteristics of the arterial wall under normal- and high-sodium dietary conditions.

**Methods**

**Experimental Design**

All procedures were performed according to the Guide for Care and Use of Laboratory Animals (NIH publication No. 85-23, revised 1985). Male SHR of age 4 weeks were obtained from Iffa Credo, Lyon, France, and divided into 4 groups of 15 rats each. The SHR control group received a standard chow diet (0.2% NaCl), the SHR+NaCl group received additional sodium through drinking water (8% NaCl), the SHR+IND group remained on the standard chow diet...
and received IND through drinking water (1.5 mg · kg⁻¹ · d⁻¹), and the SHR + NaCl + IND group received additional sodium and IND through drinking water (8% NaCl, 1.5 mg · kg⁻¹ · d⁻¹). Duration of treatments was exactly 8 weeks. Animals were individually housed at 25°C and exposed to a daily 12-hour/12-hour light/dark cycle. We and others have reported that the oral dosage of IND required to normalize BP in SHR is >1.5 mg · kg⁻¹ · d⁻¹. Therefore, dosages used in the present study were subtherapeutic. In the rat, a single oral dose of 1 mg/kg is reported to give a peak plasma concentration of 1.3 μg/mL corresponding to 3.5 × 10⁻⁷ mol/L.¹⁴

Systemic Hemodynamics

At the end of the 8-week treatment period, rats were anesthetized with pentobarbital (50 mg/kg IP) and prepared for direct intra-arterial pressure and cardiac output measurements with Doppler probe methodology and a microcomputer system (Vectra model 282, Hewlett-Packard) for data processing, as described in previous articles. Systemic arterial compliance was derived from a simple model of vascular elasticity that assumes that blood is discharged during diastole into a single resistance vessel that represents total peripheral resistance. We used the modification of the traditional Windkessel model described by Yin and Liu and Liu et al, which accounts for a nonlinear exponential pressure-volume relationship by use of the area under the diastolic pressure waveform. Validations have been published previously.¹⁴,¹⁵

Histomorphometric Studies

Histomorphometric analysis of the thoracic aorta was undertaken after the hemodynamic studies when rats were 12 weeks of age. The procedure has been reported in previous papers. The fixation solution was perfused for 30 minutes at a pressure equal to the mean BP recorded before euthanatization of each rat. Thickness of the media was measured by taking the structural components shown by staining each successive section, which different algorithms have been developed to measure each of the aortic media being examined. Morphometric analysis was performed on the structural elements of the thoracic aorta embedded longitudinally in paraffin. In brief, 3 successive sagittal 5-μm tissue sections were stained with Sirius red for collagen, orcein for elastin, and Alcian blue for hyaluronan to obtain monochromatic preparations that corresponded to the particular elements of the aortic media being examined. Morphometric analysis was performed with an automated image processor (NS 15000, Microvision) for which different algorithms have been developed to measure each of the structural components shown by staining each successive section. Mean thickness of the media was measured by taking the distance between internal and external elastic lamina and lumen diameter, measured as the distance between opposite sides of the aortic lumen (70 measurements per section). Reproducibility of this morphometric analysis has been validated and published in detail in previous papers.¹¹,¹⁴

Validation of the Alcian Blue Method

Enzyme-linked immunosorbent assay (ELISA) methodology, as described by 2 independent groups, has been used to measure tissue hyaluronan in 2 groups of SHR to establish whether findings obtained with Alcian blue semiquantitative histomorphometry are reproducible by direct measurement of aortic hyaluronan under similar experimental conditions. In brief, this method is based on the following procedures. Tissues are removed after animal euthanatization and immediately frozen in liquid nitrogen. Samples are lyophilized to dryness after extraction, weighed, and suspended in PBS at 37°C. Protease is inactivated by boiling the samples for 10 minutes. Aliquots are diluted in PBS and assayed by use of ELISA. Eight control animals were given standard and high-sodium diets as described above for the SHR and the SHR + NaCl groups for 8 consecutive weeks. These rats were killed, and 3 to 4 tissue specimens were obtained from the thoracic aorta, skeletal muscle, and duodenum. The latter 2 organs were chosen to validate further results obtained in the main target tissue, aorta of controls and SHR. Animals given the high-sodium diet had a marked decrease in aortic hyaluronan versus control SHR: 301 ± 49 versus 661 ± 61 ng/g tissue (P < 0.001). Interestingly, the high-sodium regimen had no effect in the other tissues examined.

Statistical Analysis

Results are expressed as mean ± SD. Data were examined by use of 2-way ANOVA to distinguish between a sodium-related effect, an IND-related effect, and an interaction between the 2 factors. Single and multiple regression analyses were performed with standard methods. P < 0.05 was considered to be statistically significant.

Results

As shown in the Table, rat body weight at 12 weeks was comparable in 3 of 4 groups. Only in the SHR + NaCl group was body weight less than in the other groups. Mean arterial BP and heart rate were comparable in the 4 groups both before (unanesthetized state) and after (unanesthetized state) thoracotomy performed for invasive evaluation of hemodynamic parameters. Cardiac output was also similar in SHR, SHR + IND, and SHR + NaCl + IND groups. Only the SHR + NaCl group exhibited a significant reduction in cardiac output: 53 versus 77 mL/min (P < 0.009). When expressed in milliliters per minute per kilogram, no difference was seen between groups. As expected, systemic peripheral resistance was significantly higher in the SHR + NaCl group: 366 versus 215 dyne · s⁻¹ · cm⁻² · 10⁻³. IND per se had no effect on total peripheral resistance but prevented elevation of this parameter in the SHR + NaCl + IND group. Systemic arterial compliance was reduced by 34% in the SHR + NaCl group, from 3.8 to 2.5 mL · mm Hg⁻¹ · 10⁻³ (P < 0.003). Total arterial compliance was increased by IND treatment, from 3.8 to 4.9 mL · mm Hg⁻¹ · 10⁻³, a significant increase (27%; P < 0.05). This drug also attenuated the effect of high sodium intake, given that the total arterial compliance value of 3.3 mL · mm Hg⁻¹ · 10⁻³ observed in the SHR + NaCl + IND group was not statistically different from that measured in the control SHR group.

The Table summarizes morphometric analysis performed in the 4 experimental groups. Thoracic aorta internal radius was not different from 1 group to another, but wall thickness was increased by 25%, from 112 to 140 μm, in the SHR + NaCl group (P < 0.01). IND alone failed to affect the size of the media but prevented its rise in the SHR + NaCl + IND group. Aortic collagen content was not affected by the high salt intake, but IND treatment resulted in a 23% decrease (P < 0.005) in this matrix macromolecule, from 16 116 to 12 382 μm²/mm. High sodium intake failed to affect collagen contents both in SHR and SHR + NaCl + IND groups. Aortic elastin content was slightly elevated (16%) in the SHR + NaCl group, from 25 572 to 30 640 μm²/mm. IND alone had no effect on elastin content but prevented elevation in aortic elastin content in the SHR + NaCl + IND group. The elastin/collagen ratio, an arbitrary structural picture of the aortic wall rigidity, was significantly elevated by IND (P < 0.009), from 1.64 to 2.18. The effect of sodium intake on thoracic aorta hyaluronan in the untreated SHR was highly significant: a 89% decrease, from 2343 to 266 μm²/mm (P < 0.0001). IND alone was associated with a 36% increment in PGL content, from 2343 to 3243 μm²/mm (P < 0.01), and partially prevented, in the SHR + NaCl + IND group, the...
Systemic Hemodynamics and Histomorphometric Changes

<table>
<thead>
<tr>
<th>Parameters</th>
<th>SHR* (n=14)</th>
<th>SHR + NaCl (n=11)</th>
<th>SHR + IND (n=15)</th>
<th>SHR + NaCl + IND (n=15)</th>
<th>NACI Effect</th>
<th>IND Effect</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>326±14</td>
<td>274±4.0</td>
<td>320±22</td>
<td>290±15</td>
<td>0.0001</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before thoracotomy</td>
<td>213±15</td>
<td>228±46</td>
<td>216±9</td>
<td>216±14</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>After thoracotomy</td>
<td>188±17</td>
<td>189±16</td>
<td>172±28</td>
<td>187±21</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Cardiac output</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In mL/min</td>
<td>77±23</td>
<td>53±23</td>
<td>77±30</td>
<td>63±25</td>
<td>0.009</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>In mL·min⁻¹·kg⁻¹</td>
<td>236±75</td>
<td>192±79</td>
<td>239±92</td>
<td>215±87</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>439±42</td>
<td>430±137</td>
<td>450±37</td>
<td>466±24</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Systemic arterial compliance, mL/mm Hg·10⁻³</td>
<td>3.8±1.9</td>
<td>2.5±1.1</td>
<td>4.9±2.0</td>
<td>3.3±1.5</td>
<td>0.003</td>
<td>0.05</td>
<td>...</td>
</tr>
<tr>
<td>Total peripheral resistance, dyne/s·cm⁻²·10⁶</td>
<td>215±75</td>
<td>366±29</td>
<td>231±17</td>
<td>256±99</td>
<td>0.06</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Internal radius, mm</td>
<td>0.82±0.09</td>
<td>0.83±0.07</td>
<td>0.79±0.09</td>
<td>0.80±0.09</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Wall thickness, T, μm</td>
<td>112±11</td>
<td>140±27</td>
<td>118±15</td>
<td>113±11</td>
<td>0.01</td>
<td>0.02</td>
<td>0.0004</td>
</tr>
<tr>
<td>Internal radius/wall thickness ratio</td>
<td>7.5±1.2</td>
<td>6.6±1.3</td>
<td>7.0±1.1</td>
<td>7.1±1.1</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Elastin content, μm²/mm</td>
<td>25572±5529</td>
<td>30640±8520</td>
<td>26667±5581</td>
<td>24442±5383</td>
<td>...</td>
<td>...</td>
<td>0.04</td>
</tr>
<tr>
<td>Collagen content, C μm²/mm</td>
<td>16116±3908</td>
<td>16912±3976</td>
<td>12382±1936</td>
<td>12833±5325</td>
<td>0.005</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Elastin/collagen content ratio unit</td>
<td>1.64±0.09</td>
<td>1.88±0.16</td>
<td>2.18±0.12</td>
<td>2.12±0.18</td>
<td>...</td>
<td>0.009</td>
<td>...</td>
</tr>
<tr>
<td>Nuclei cross-sectional area, μm²</td>
<td>9.0±1.9</td>
<td>12.6±4.1</td>
<td>8.8±2.8</td>
<td>8.7±1.5</td>
<td>0.02</td>
<td>0.006</td>
<td>0.01</td>
</tr>
<tr>
<td>Nuclei content, No./mm</td>
<td>395±112</td>
<td>411±65</td>
<td>435±65</td>
<td>419±55</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Proteoglycans, μm²/mm</td>
<td>2343±701</td>
<td>266±284</td>
<td>3243±1191</td>
<td>1052±398</td>
<td>0.0001</td>
<td>0.0001</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean±SD.
*Control group.

The decrease in hyaluronan associated with high salt intake in the SHR + NaCl group: 1052 versus 266 μm²/mm³ (P<0.0001).

In the overall population, body weight and systemic arterial compliance did not correlate significantly with wall thickness, collagen and elastin content, or nuclei cross-sectional area, although each of these parameters was affected by ≥1 of the chosen treatments. However, a strong correlation developed between thoracic aorta hyaluronan and total arterial compliance (r=0.65, P<0.001; Figure). Taking together all 4 experimental groups, the data showed a positive linear correlation between amount of hyaluronan in the wall of the thoracic aorta and total systemic arterial compliance. Reduction in hyaluronan observed after sodium was associated with a proportional marked decrease in total arterial compliance. IND alone was associated with a rise in hyaluronan and a proportional elevation in compliance. Interestingly, this diuretic agent partially restored the hyaluronan level and the systemic arterial compliance.

**Discussion**

The present results show (1) that a high-sodium diet affects structural and functional characteristics of large arteries independently of mean BP and (2) that a high-sodium regimen as well as a diuretic regimen with IND affects in parallel aortic hyaluronan contents and systemic compliance through mechanisms independent of mean BP, collagen, and elastin contents.

In the present study, total systemic arterial compliance could be regarded not only as a marker of the exchange capacity of the thoracic aorta but also as an index of stiffness of the vessel wall. According to the Moens-Korteweg equation, the elastic modulus, the classic index for evaluating vascular wall stiffness, is calculated from the lumen/wall ratio, and from the arterial distensibility (compliance×vascular volume). In the present study, no difference existed between groups in aortic radius and the radius/thickness ratio. Because BP values were comparable among the 4 groups, changes in systemic arterial compliance undoubtedly represented changes in the intrinsic stiffness of...
aortic wall material: lower systemic compliance, higher elastic modulus, and stiffer wall material, as observed during high salt diet. Conversely, stiffness of wall material decreased during IND treatment. In the latter case, similar findings have been reported with IND in deoxycorticosterone acetate–salt hypertensive as well as Dahl\(^{6}\) rats.

The histomorphometric method with Alcian blue that was used in the present study clearly has been validated. First, by use of the ELISA method, which specifically measures hyaluronan, the high-sodium diet–induced reduction in aortic hyaluronan evaluated by the Alcian blue method was confirmed and amplifies the interesting relationship between changes in total arterial compliance and aortic hyaluronan contents, a unique observation. Second, the high-sodium diet specifically reduced aortic hyaluronan contents without affecting 2 important fluid reservoirs, duodenum and skeletal muscle.

The present results revealed (1) that high sodium intake was associated with hypertrophy of the media, (2) that collagen content of the vascular wall was not influenced by increased salt intake, and (3) that arterial-wall hyaluronan diminished markedly. These structural and functional changes developed under stable systemic BP values. The reduction in total systemic arterial compliance under high-sodium diet could be related to increased media thickness, and, more importantly, to reduced aortic hyaluronan content. The latter observation has not yet been reported, whereas the relationship between compliance reduction and salt-induced changes in total arterial compliance and aortic hyaluronan contents, a unique observation. Second, the high-sodium diet specifically reduced aortic hyaluronan contents without affecting 2 important fluid reservoirs, duodenum and skeletal muscle.

In the present study, no change occurred in aortic extracellular collagen and elastin levels. However, hyaluronan content decreased markedly. From data obtained in the present study, the reduction in total systemic arterial compliance under high-sodium diet could be related to increased media thickness, and, more importantly, to reduced aortic hyaluronan content.

In SHR, cellular hyperplasia has been mainly reported in the small resistance arteries, whereas vascular smooth muscle cell hypertrophy has been observed mainly in the large conduit arteries. In the present experiments, cellular hyperplasia was documented in the thoracic aorta but nonetheless was recognized to develop mainly in peripheral resistance arteries. Stimulation of the sodium-calcium exchanger secondary to high sodium intake could be involved in the changing phenotype pattern of vascular smooth muscle cells.

In the present study, no change occurred in aortic extracellular collagen and elastin levels. However, hyaluronan content decreased markedly. From data obtained in the present study, the reduction in total systemic arterial compliance can be related logically to the decrease in interstitial hyaluronan. Subpressor doses of angiotensin II have been reported to enhance hyaluronan synthesis. The renin-angiotensin-aldosterone system probably is suppressed during high sodium intake, and the eventual angiotensin-mediated production of hyaluronan probably can be turned off.

IND, a nonthiazide diuretic, long has been considered to be a drug that causes a high level of vascular and renal pharmacological actions in the relatively low doses recommended for treatment of arterial hypertension in humans. Previous studies in hypertensive patients suggested that this drug ameliorates aortic compliance and decreases pulse pressure in elderly hypertensive subjects, an expected consequence of the former vascular effect. To our knowledge, the present study reveals, for the first time, a potential effect of IND on aortic wall hyaluronan in SHR. This effect on wall structure is accompanied by a significant improvement in aortic compliance, as if these macromolecules were directly or indirectly involved in arterial wall mechanics. The amount of sodium ions bound to PGLs in the SHR and SHR+IND groups is unlikely to be identical under baseline dietary conditions. Therefore, the enhanced hyaluronan staining pattern observed in the SHR+IND group could be due to 1 of the following possibilities: (1) IND could enhance synthesis or decrease degradation of these macromolecules in the interstitial matrix; (2) even if IND fails to affect synthesis or degradation of hyaluronan, IND might interfere with some intermediary steps of their synthesis, such as the sulfation process, responsible in part for the anionic properties of these macromolecules; (3) Untreated SHR on a normal salt diet could contain an increased amount of sodium bound to hyaluronan that could be displaced either directly, through chemical interactions with hyaluronan-bound sodium, or indirectly, through an IND-induced enhanced natriuresis. It is reasonable to retain the first or second hypothesis to explain the unique effect of IND on aortic interstitial hyaluronan. Preliminary results from our laboratory indicate that normal rats treated for 10 days with IND at the dosage used in the present study exhibited a more thorough incorporation of radiolabeled sulfur into aortic proteoglycans than did untreated control rats (2808 versus 1610 cpm mg\(^{-1}\) h\(^{-1}\)), whereas incorporation measured in other tissues was also negligible.

In the present study, we have shown that, independent of BP levels in the SHR, high sodium intake is associated with low systemic arterial compliance and low aortic interstitial hyaluronan but no change in collagen and elastin content. More interesting, IND, a diuretic compound with a high vascular/renal action ratio, exerts a newly described and unexpected pharmacological effect on interstitial hyaluronan contents, in addition to its small effect on collagen. Together, these structural changes contribute to improvement in total arterial compliance, a major functional characteristic of large conduit arteries.

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Sodium Intake, Large Artery Stiffness, and Proteoglycans in the Spontaneously Hypertensive Rat
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