The relationship between blood pressure and aortic collagen metabolism in renal hypertensive rats

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Summary

1. Biosynthesis and deposition of collagen, as well as DNA and total proteins, are increased in aortae of rats after 1, 3 and 6 weeks of hypertension.
2. The maximal increase in the rate of synthesis of collagen is observed within one week of hypertension when the stress to the arterial wall is maximal.
3. Reserpine administration prevents hypertension and inhibits the increase of collagen metabolism.
4. At any time of evolution of the hypertension, a linear positive correlation is found between the collagen content in the aorta and the level of blood pressure.
5. These data suggest that synthesis of matrix components by the arterial smooth-muscle cells is controlled by variation in the blood pressure level and is not a direct consequence of circulating humoral factors liberated by the ischaemic kidney.

Key words: blood pressure, collagen, hypertension.

Introduction

It has been reported that the synthesis and the deposition of collagen are increased in the arteries, but not in veins of rats made hypertensive by deoxycorticosterone and those changes are suppressed by antihypertensive drugs such as thiazides and reserpine (Wolinsky, 1970; Ooshima, Fuller, Cardinale, Spector & Udenfriend, 1977). To provide additional support for a possible relationship between blood pressure and collagen metabolism in the vascular wall, we studied the collagen content and the rate of biosynthesis in aortae of renal hypertensive rats, untreated or treated with reserpine. This experimental model allows avoidance of drugs which could directly modify collagen metabolism.

Methods

Renal hypertension was produced by clipping the left renal artery with a silver clip 0.2 mm wide and removing the right kidney in eight-week-old female Wistar rats. Normotensive, uninephrectomized rats were used as controls. After death, portions of the aortae from the origin of the left subclavian to the renal arteries were carefully excised and dissected free from the adventitia (Rorive & Van Cauwenberge, 1973). Groups of normotensive and hypertensive rats were treated with reserpine (0.1 mg/kg body wt. per day) from the first post-operative day.

For collagen content determination, the aortae were blotted dry, weighed and frozen in liquid N₂. They were then smashed to powder under liquid N₂, freeze-dried and stored at −20°C. The amount of hydroxyproline was measured by the method of Bergman & Loxley (1963). One μmol of hydroxyproline was considered to represent one mg of collagen. The collagen content was expressed as total content in the segment of aorta dissected or in terms of concentration, i.e. per mg of powdered dry aorta.

The rate of collagen synthesis was measured in vitro by incubation for 5 h in the presence of [3,4-3H]proline (50 μCi/ml of incubation medium). The incorporation of [3H]proline into protein bound [3H]hydroxyproline, which was linear at least up to
8 h, was used as an index for the synthesis of collagen (Peterkofsky & Prockop, 1962; Foidart, Rorive & Nusgens, 1978).

Total protein nitrogen was determined by the Kjeldahl method and the DNA content in the powdered aortae was measured by the method of Burton (1956), modified by Bevan, Van Marthens & Bevan (1976).

Results

The blood pressure of rats with renal artery stenosis rapidly increased and reached maximal levels within three weeks. Aortae dissected 1, 3 and 6 weeks after clipping the renal artery were markedly hypertrophied as illustrated by weight (Table 1), and there was a significant increase in total protein DNA and collagen content. However, the concentration of DNA and protein were not significantly modified. For instance, six weeks after surgery, DNA concentration was 2.54 ± 0.14 mg of DNA/g of aorta in normotensive, and 2.76 ± 0.13 mg of DNA/g of aorta in hypertensive rats. In contrast, after three and six weeks of hypertension, both the concentration and the total content of collagen were significantly increased in hypertensive arteries. The ratio of collagen to DNA content increased with time in hypertensive vessels.

The rate of collagen biosynthesis was markedly stimulated in the hypertensive aortae, the largest increase being observed after the first week. At that time, the rate of incorporation of [3H]proline into collagen was 12 times higher in hypertensive arteries. Successively, the stimulation of collagen biosynthesis decreased progressively but remained significantly higher than in control vessels (Table 1).

Reserpine administration during 6 weeks slightly but significantly decreased collagen content in normotensive rats. This effect might be related to the slight hypotensive effect observed. Reserpine treatment almost completely prevented the rise in blood pressure in the animals with renal artery constriction. In these rats, the collagen content as well as the rate of collagen biosynthesis was markedly decreased compared with hypertensive animals, and was similar to the values found in normotensive animals.

Including all the data observed in the four series of animals (normotensive, normotensive-treated, hypertensive and hypertensive-treated), a positive linear correlation was found between the collagen concentration in the aorta and the blood pressure ($r = 0.87, P < 0.01$), as well as between the total collagen content and the blood pressure ($r = 0.90 P < 0.01$).

In some animals, the reserpine treatment was stopped after 2 weeks to observe 1 week later, a marked elevation of the blood pressure, and, simultaneously, an increase of the rate of collagen synthesis and collagen deposition.

Discussion

Renal hypertension produces a marked and rapidly detectable hypertrophy of the rat aorta. This hypertrophy appears to be partly due to smooth-muscle cell hyperplasia, as shown by DNA increase (Bevan et al., 1976). The observation that at least from the third week of hypertension, not only the content, but also the concentration of collagen are increased in hypertensive vessels, implies that the collagen accumulation is not only secondary to the smooth-muscle cell hyperplasia, but also to an increase in collagen synthesis by each smooth-muscle cell; this is also suggested by the increase of the collagen/DNA ratio. The stimulation of collagen biosynthesis reaches a maximal rate one week after the production of renal ischaemia when the blood pressure is far from its maximum level.

Table 1. Modifications of the composition of the rat aorta 1, 3 and 6 weeks after clipping the left renal artery

<table>
<thead>
<tr>
<th>Time after clipping (weeks)</th>
<th>Aorta wet wt. (mg)</th>
<th>DNA content (μg)</th>
<th>Protein content (mg)</th>
<th>Collagen content (mg)</th>
<th>Relative rate of collagen synthesis (HT/NT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 NT</td>
<td>41.4 ± 2.9</td>
<td>132 ± 7</td>
<td>6.72 ± 0.42</td>
<td>2.24 ± 0.05</td>
<td>12.2 ± 3.50</td>
</tr>
<tr>
<td>HT</td>
<td>46.1 ± 0.8</td>
<td>180 ± 15</td>
<td>8.20 ± 0.92</td>
<td>2.58 ± 0.20</td>
<td></td>
</tr>
<tr>
<td>3 NT</td>
<td>37.6 ± 1.1</td>
<td>128 ± 7</td>
<td>6.14 ± 0.42</td>
<td>2.54 ± 0.11</td>
<td>3.37 ± 0.40</td>
</tr>
<tr>
<td>HT</td>
<td>47.3 ± 3.2</td>
<td>180 ± 11</td>
<td>8.40 ± 0.83</td>
<td>3.96 ± 0.28</td>
<td></td>
</tr>
<tr>
<td>6 NT</td>
<td>44.4 ± 2.0</td>
<td>112 ± 8</td>
<td>6.72 ± 0.34</td>
<td>3.35 ± 0.05</td>
<td>1.25 ± 0.10</td>
</tr>
<tr>
<td>HT</td>
<td>63.6 ± 2.6</td>
<td>171 ± 5</td>
<td>9.63 ± 0.62</td>
<td>5.46 ± 0.34</td>
<td></td>
</tr>
</tbody>
</table>
However, it is in the early stages of the disease that the smooth-muscle cells are submitted to the maximal distension stress. Subsequently, cell hyperplasia, thickening of the vascular wall, and decrease of compliance due to connective tissue accumulation reduce the wall stress (Wolinsky, 1970).

Our results also show that the arterial hypertrophy and collagen metabolism are related to the level of blood pressure and to the mechanical distension of the medial smooth-muscle cells. Reserpine treatment indeed decreases the blood pressure in hypertensive as well as in normotensive control animals, and reduces the vascular collagen synthesis. Collagen accumulation in aorta during hypertension appears to be independent of renal factors and more closely related to changes in blood pressure. Similar conclusions are also reached by Iwatsuki, Cardinale, Spector & Udenfriend (1977), who, in deoxycorticosterone hypertensive rats and in spontaneously hypertensive rats, observed that collagen synthesis and deposition are increased in arteries but not in veins of hypertensive animals. However, it has been suggested that the effect of reserpine could be mediated through changes in the adrenergic tone and a decrease of growth hormone secretion. Although this mechanism is possible, the close relationship between blood pressure and collagen synthesis shown in our observations is better supported by a direct mechanism or at least, if a circulating factor plays a role in the vascular response, it appears to be a plasma factor which is only operative in association with increased blood pressure (Bevan et al., 1976).

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