Hyperplasia of rat arteries smooth muscle cells associated with development and reversal of renal hypertension

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Summary

1. Renal hypertension produces a marked and rapidly detectable hypertrophy of the rat aorta, due to smooth muscle cell hyperplasia and connective tissue deposition.

2. As we described previously for collagen synthesis, cell hyperplasia is a very early event which reaches a maximum at a time when the blood pressure is far from its highest level, and thereafter progressively decreases.

3. Reserpine prevents the vascular wall changes on the arterial as well as the venous side of the circulation. On the other hand, captopril although effective in preventing the blood pressure rise does not suppress the hyperplastic response.

4. The arterial hypertensive disease appears to be reversible, when renal ischaemia is corrected. The smooth muscle cell hyperplasia is, however, only partly and slowly reversible.

5. These data suggest that blood pressure is not the only determinant of the vascular wall response, and that the effect of a drug on the blood pressure does not necessarily predict the vascular wall response.

Keys words: arteries, collagen, DNA, smooth muscle cells, renal hypertension.

Introduction

Increased wall thickness of the arteries, particularly of their media, has long been recognized as occurring in hypertension in man as well as in experimental hypertension. Earlier work has stressed the importance of these structural changes in the pathogenesis of chronic hypertension (Folkow, Hallback, Lundgren, Sivertsson & Weiss, 1973) and in the development of atherosclerotic lesions (Hollander, Kramsch, Farmelant & Madoff, 1968). Few studies were devoted to the biochemical determinants of this vascular hypertrophy. Such studies appear, however, of interest since they could provide new therapeutic approaches to hypertensive arterial disease.

We previously reported that, in renal hypertension in the rat, the DNA, collagen and protein content of the aorta are increased (Foidart, Rorive, Nusgens, Carlier & Lapiere, 1979). In order to provide information on the relation between blood pressure and these modifications, we report here their development during the early phase of renal hypertension and also its reversal.

Methods

Eight weeks old female Wistar rats were made hypertensive by applying a silver clip (0.2 mm diameter) on the left renal artery, the right kidney being previously removed. Age-matched, normotensive uninephrectomized rats were used as controls. Experimental groups of normotensive and hypertensive rats were treated from day 1 after surgery with reserpine (0.1 mg day⁻¹ kg⁻¹ body weight) or with captopril (SQ 14 225, 50 mg day⁻¹ kg⁻¹ body weight). In another group, the silver clip was removed 1, 2 or 3 weeks after the onset of hypertension.

After the animals were killed, blood vessels were rapidly and carefully dissected and a intima–media preparation was obtained as previously described (Rorive, 1975). The tissues were then blotted dry, weighed and frozen in liquid nitrogen.

The DNA content was measured by the method developed by Zamenhof, Bursztyn, Rich...
& Zamenhof (1964) and modified by Bevan, Van Marthens & Bevan (1976). DNA rate of biosynthesis was estimated by the determination of the uptake of \(^{3}H\)thymidine into the perchloric acid-soluble fractions after incubation for 2 h in physiological salt solution containing 2 \(\mu\)Ci of \(^{3}H\)thymidine/ml.

The amount of hydroxyproline in the powdered aorta was measured by the method of Bergman & Loxley (1963). The rate of collagen synthesis was estimated by incubation in physiological salt solution containing 50 \(\mu\)Ci of [3, 4-\(^{3}H\)proline and determination of the incorporation of proline into protein-bound hydroxyproline (Foidart et al., 1979).

All experimental values are expressed as the means \(\pm\) SD.

Results

As classically observed, the blood pressure of the rats with renal artery stenosis rapidly increased and reached maximal levels within 3 weeks (190 \(\pm\) 30 mmHg; \(n = 42\)).

In hypertensive animals, the hypertrophy of the aorta was already detectable at the end of the first week. At this time, the wet weight of the aorta was 11% higher in the hypertensive animals. This difference in weight increased progressively with the further duration of hypertension. After 6 weeks, the weight of the aorta of hypertensive rats was 40% higher than in normotensive rats (63.6 \(\pm\) 2.6 mg, \(n = 12\), in hypertensive rats vs 44.4 \(\pm\) 2.0 mg, \(n = 10\), in normotensive rats).

Simultaneously, an increase in the DNA content was observed but, in contrast to the rise in weight, the DNA increase reached a maximum at the end of the first week. At that time, the DNA content was 180 \(\pm\) 15 \(\mu\)g (two aortas pooled, \(n = 8\)) in hypertensive rats and 132 \(\pm\) 7 \(\mu\)g (\(n = 7\)) in normotensive rats. Afterwards, the high level remained unchanged in spite of the fact that blood pressure still increased. In contrast, the protein and the collagen content as well as the collagen concentration (mg of collagen/mg of tissue) progressively increased with the duration of hypertension. This implies that the ratio total collagen/total DNA also rises with time (from 14 after 1 week to 32 after 6 weeks). This reflects a progressive increase of the proportion of connective tissue.

The rate of incorporation of thymidine into DNA was markedly increased in the early phase of hypertension, whether the data are expressed in c.p.m./total DNA or in c.p.m./\(\mu\)g of DNA. This modification, which was observed in vitro as well as in vivo, occurred very early. Indeed, in hypertensive animals the highest values were observed 4 days after surgery. At this time, the rate of incorporation of thymidine was 15 times higher in the aortas of hypertensive rats than in the aortas of normotensive rats (34.8 \(\pm\) 10.2 c.p.m./\(\mu\)g of DNA in hypertensive rats, \(n = 10\), and 2.19 \(\pm\) 7.6 c.p.m./\(\mu\)g of DNA in normotensive, \(n = 8\)). After this peak, the rate of biosynthesis of DNA in hypertensive vessels progressively decreased but remained significantly higher than in normotensive vessels. Thus, after 6 weeks, the rate of incorporation was 2.69 \(\pm\) 0.56 \(\mu\)g (\(n = 8\)) in hypertensive and 1.36 \(\pm\) 0.46 \(\mu\)g (\(n = 6\)) in normotensive vessels (\(P < 0.05\)).

Autohistoradiographic studies confirmed these data. If it was unlikely to encounter a labelled nucleus in vessels of normotensive rats (less than 1/10 000) this labelling was more frequent in arteries from hypertensive rats. The maximum labelling was also observed at the end of the first week (11/1000) to decrease to 5.7 \(\pm\) 2.0/1000 after 2 weeks. It is noteworthy that in nephrectomized controls the labelling of nuclei was increased to 1.2 \(\pm\) 0.5/1000. The nuclei labelled were those of the smooth muscle cells of the media; they were generally associated in small groups of several nuclei, suggesting recent mitosis.

As described in previous reports, the rate of incorporation of proline into protein-bound hydroxyproline, e.g. collagen, followed a similar time curve, reaching a peak after 1 week, to decrease rapidly thereafter. Autohistoradiography demonstrated the accumulation of \(^{3}H\)proline in all cell layers although predominantly in the media around the smooth muscle cell (Foidart, Rorive, Nusgens & Lapiere, 1978; Foidart et al., 1979).

Reserpine administration slightly but significantly decreased blood pressure in normotensive rats, but prevented almost completely the rise in blood pressure in the clipped animals. We reported previously that, in such reserpine-treated rats, the collagen content as well as the rate of biosynthesis was strongly correlated with the blood pressure. The same observation was done with DNA concentration and thymidine incorporation into DNA. Reserpine almost prevented the DNA increase in hypertensive rats, and a direct correlation between blood pressure and DNA content or thymidine uptake could be found; for instance:

Thymidine uptake (c.p.m./\(\mu\)g of DNA) =

\[2.18 \times \text{blood pressure (mmHg)} - 153\]

\((r = 0.692, P < 0.001)\)
Hyperplasia of arterial cells in hypertension

TABLE 1. Effect of the correction of hypertension on the biochemical composition of the rat aorta

<table>
<thead>
<tr>
<th></th>
<th>Blood pressure (mmHg)</th>
<th>Heart weight (mg/100 g)</th>
<th>Aorta weight (mg/100 g)</th>
<th>Aorta DNA content (µg)</th>
<th>Aorta collagen content (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensive rats</td>
<td>122 ± 4</td>
<td>343 ± 13</td>
<td>54 ± 2</td>
<td>62 ± 2</td>
<td>5·322 ± 371</td>
</tr>
<tr>
<td>(n = 8)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Hypertensive rats</td>
<td>158 ± 6</td>
<td>456 ± 20</td>
<td>68 ± 3</td>
<td>90 ± 4</td>
<td>8·330 ± 790</td>
</tr>
<tr>
<td>(n = 8)</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Corrected hypertensive rats (n = 7)</td>
<td>105 ± 5</td>
<td>397 ± 8</td>
<td>58 ± 3</td>
<td>74 ± 7</td>
<td>5·655 ± 469</td>
</tr>
</tbody>
</table>

However, such a relation is found only when age-matched rats were used, and for a given time of development of the hypertension. But, even in normotensive rats, age-related biochemical modifications of the arterial wall were characterized by a DNA decrease and a collagen increase. Reserpine also decreased the thymidine uptake in vena cavae from normotensive or hypertensive rats, which could suggest that the effect of the drug is not necessarily related to its hypotensive effect. Captopril also completely inhibited the blood pressure increase, and had a slight hypotensive effect in normotensive rats; but contrary to what was observed with reserpine, the biochemical alterations of the hypertensive arteries were not inhibited by this drug and in this group no correlation could be found between DNA content and blood pressure.

If after 2 or 3 weeks of hypertension, the silver clip was removed, the blood pressure returned rapidly to and even, sometimes, below, normal levels. Simultaneously, and in a few days, the rates of thymidine uptake into DNA, and of proline into collagen, markedly decreased, far below levels found in age-matched normotensive rats. Within 3 weeks, the hypertrophy of the aorta (as well as of the heart) markedly regressed, at least on a weight basis. Collagen content followed the course of the blood pressure and markedly decreased within 1 week to reach normal levels 3 weeks after the reversal of hypertension (Table 1). DNA content of the aorta also markedly decreased but, even 3 weeks after normalization of blood pressure, remained significantly higher than in the control animals. Similar changes were observed in smaller vessels, such as caudal and mesenteric arteries.

Discussion

The biochemical modifications typical of the hypertensive disease (the smooth muscle cell hyperplasia and the connective tissue deposition) are very early events. They already reach a maximum during the phase of blood pressure increase, to diminish later. As reported by Bevan, Eggena, Hume, Van Marthens & Bevan (1980), the time course of cell proliferation closely parallels the rise in arterial pressure, and is not correlated to the blood pressure level itself, except when animals matched for age and duration of hypertension are used.

Reserpine completely inhibits the vascular alterations, but captopril does not. These discrepancies suggest that blood pressure by itself or blood pressure changes are not the only determinants of the response of the vascular wall and the humoral factors, such as the adrenergic or the renin–angiotensin systems, could modulate the vascular response. This observation is also of interest from the therapeutic point of view and suggests that the effect of a drug on blood pressure does not necessarily predict the vascular wall response.

After removal of the clip, arterial hypertrophy appears to be reversible, but as reported for the left ventricular hypertrophy, some of the biochemical changes do not completely disappear. In the heart, it has been reported that the collagen content remains high; in the vessel, the increase in the number of cells appears to be a long-lasting phenomenon.

These results, observed in a female rat model of severe hypertension of renal origin and characterized by a very rapid rise, have to be confirmed in other models.

References


BEVAN, R.D., EGGENA, P., HUME, W.R., VAN MARTHIENS, E. &


