INCREASED LYSYL OXIDASE ACTIVITY IN BLOOD VESSELS OF HYPERTENSIVE RATS AND EFFECT OF β-AMINOPROPIONITRILE ON ARTERIOSCLEROSIS

AKIRA OOSHIMA, M.D. AND OSAMU MIDORIKAWA, M.D.

The activity of lysyl oxidase which catalyzes the initial step of cross-linking of collagen and elastin polypeptides was measured in blood vessels of the hypertensive rat. The enzyme activity was increased in the aorta and mesenteric artery when hypertension was induced in 8-week-old rats with administration of deoxycorticosterone acetate (DOCA) and 1% saline. Reserpine diminished this increase in vascular lysyl oxidase activity concomitant with reduction in blood pressure. When β-aminopropionitrile, a specific inhibitor of lysyl oxidase, was administered before the onset of DOCA-salt hypertension, the aortic collagen content was reduced markedly. Concomitant with reduction in the aortic collagen content, the development of hypertension and arteriosclerotic changes in the kidney was partially prevented. These results would indicate that hypertension increases the amount and the degree of cross-linking of vascular collagen and that the deposition of excess collagen in the vascular wall contributes to the development of hypertension and arteriosclerosis.

It has been shown that hypertension elevates collagen biosynthesis in peripheral blood vessels and that antihypertensive agents, reserpine and chlorothiazide, prevent or reverse the hypertension-induced collagen biosynthesis. The biochemical markers of collagen biosynthesis used in our previous report were prolyl hydroxylase activity, prolyl hydroxylase related antigen, the rate of $^3$H- or $^{14}$C-proline incorporation into collagen and the net collagen content in the blood vessels. All the markers were shown to be elevated in the vascular system of hypertensive rats.

In the present report, we have measured the activity of lysyl oxidase which catalyzes the formation of α-aminoadipic-δ-semialdehyde (allysine) and is necessary for the initial step in the cross-linking of collagen and elastin polypeptides. We also examined the results of preventing the increase in vascular collagen synthesis of hypertensive rats by administering β-aminopropionitrile, a specific inhibitor of lysyl oxidase.

METHODS

Hypertension was induced in uninephrecto-

Key Words:
- Hypertensive rats
- Arteriosclerosis
- Lysyl oxidase
- Reserpine
- β-Aminopropionitrile
- Collagen
- Cross-Linking

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mized, 8-week-old, male Wistar rats by subcutaneous injection of deoxycorticosterone acetate (DOCA) (5 mg per rat) twice weekly and maintained on 1% saline as a source of drinking water. Concomitant with deoxycorticosterone acetate (DOCA)-salt treatment, reserpine (0.75 mg per kg of body weight) or β-aminopropionitrile fumarate (150 mg per kg of body weight) was administered to some of the DOCA-salt treated rats by daily intraperitoneal injection. Blood pressure was monitored indirectly without anesthesia by the tail cuff method. Controls were uninephrectomized and maintained on 1% saline. After 4 weeks of DOCA-salt treatment, the rats were killed by decapitation. The aorta and mesenteric artery were immediately excised and perivascular adipose tissues carefully removed. Each tissue was kept frozen overnight, then homogenized either with Polytron ST-10 (aorta) or a ground glass homogenizer (mesenteric artery) in 30 volumes of 0.05 M potassium phosphate, pH 7.4, containing 4 M urea and 0.16 M NaCl. The homogenate was centrifuged at 105,000 x g for 30 min and supernatant was dialyzed against 3 changes of 0.05 M potassium phosphate, pH 7.4, containing 0.16 M NaCl for 24 h. The dialyzed extract was used for the assay of lysyl oxidase activity. The substrate for the assay of lysyl oxidase activity was prepared from 20 calvaria parietal bones of 17-day-old chick embryos according to the procedure of Giegel and Martin with some modifications noted below. DL-[6-3H] lysine (New England Nuclear, specific activity > 5 Ci/m mole) was added to each flask twice the amount (400 µCi) and Eagle's minimum essential medium lacking lysine and glutamine was reduced to 4 ml per flask. Portions of the dialyzed enzyme extract containing 10 mg of tissue (0.3 ml) were mixed with 0.3 ml of substrate protein (300,000–400,000 CPM). The final volume was adjusted to 1 ml with 0.05 M potassium phosphate, pH 7.4, containing 1 M NaCl and incubated for 4 h at 37°C. The reaction was stopped by adding 0.1 ml of 50% trichloroacetic acid. Trinitated water was separated by vacuum distillation of the whole reaction mixture and 0.8 ml of the distillate was added to 10 ml of Bray's solution. Radioactivity was then measured in a Packard Tri-Carb scintillation spectrometer model 2002. Hydroxyproline was measured in the aortic tissue homogenized in 20 volumes of 0.01 M Tris-HCl buffer, pH 7.4, by the method of Kivirikko et al after hydrolysis in 6 N HCl. These values yield collagen content when multiplied by 6.98. For histological examinations, the aortic arch and the kidney were fixed in 10% formalin solution, embedded in paraffin and sectioned in 5 µ thick. Hematoxylin-eosin and elastica-van Gieson's staines were employed. The numerical data obtained in this experiment were analysed by Student's small sample t-test.

RESULTS

As shown in Table I, the blood pressures of DOCA-salt treated animals elevated to over 200 mm Hg while control and reserpine treated rats remained at the normal level. It is of interest that the administration of β-aminopropionitrile reduced the blood pressure by 38 mm Hg as compared to the DOCA-salt hypertensive rats. The lysyl oxidase activities in Table II are expressed in terms of enzyme activity per mg of

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Blood Pressure (mmHg)</th>
<th>Body Weight (g)</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>119 ± 3</td>
<td>316 ± 7</td>
</tr>
<tr>
<td>DOCA-salt</td>
<td>218 ± 7**</td>
<td>276 ± 4**</td>
</tr>
<tr>
<td>DOCA-salt + BAPN</td>
<td>180 ± 10**</td>
<td>280 ± 13**</td>
</tr>
<tr>
<td>DOCA-salt + Reserpine</td>
<td>124 ± 9</td>
<td>252 ± 4**</td>
</tr>
</tbody>
</table>

Uninephrectomized, 8-week-old, male Wistar rats were given DOCA (5mg per rat) twice weekly for 4 weeks and maintained on 1% saline. Control animals were uninephrectomized and maintained on 1% saline. Concomitant with DOCA-salt treatment, BAPN (150mg per kg of body weight) or reserpine (0.75mg per kg of body weight) was administered daily by intraperitoneal injection. Each value represents the mean±standard error of 5 rats. **: statistically significant compared to controls, P < 0.01.
TABLE II  LYSYL OXIDASE ACTIVITY OF AORTA AND MESENTERIC ARTERY IN DOCA-SALT TREATED RATS WITH AND WITHOUT β-AMINOPROPIONITRILE (BAPN) OR RESERPINE, AND OF CONTROLS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Lysyl oxidase activity</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aorta</td>
<td>Mesenteric artery</td>
</tr>
<tr>
<td>Control</td>
<td>12.5 ± 1.1</td>
<td>20.0 ± 1.7</td>
</tr>
<tr>
<td>DOCA-salt</td>
<td>22.0 ± 1.2**</td>
<td>37.2 ± 4.1**</td>
</tr>
<tr>
<td>DOCA-salt + BAPN</td>
<td>9.2 ± 1.2</td>
<td>11.9 ± 1.1</td>
</tr>
<tr>
<td>DOCA-salt + Reserpine</td>
<td>11.3 ± 1.4</td>
<td>13.7 ± 1.3</td>
</tr>
</tbody>
</table>

Enzyme activity is expressed as counts per minute per mg of tissue. Each value represents the mean ± standard error of 5 rats. *, **: statistically significant compared to controls; * 0.05 > P > 0.01, ** P < 0.01.

TABLE III  COLLAGEN CONTENT OF AORTA IN DOCA-SALT TREATED RATS WITH AND WITHOUT β-AMINOPROPIONITRILE (BAPN) OR RESERPINE, AND IN CONTROLS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Collagen content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>97.0 ± 7.7</td>
</tr>
<tr>
<td>DOCA-salt</td>
<td>127.7 ± 10.5*</td>
</tr>
<tr>
<td>DOCA-salt + BAPN</td>
<td>87.3 ± 8.4</td>
</tr>
<tr>
<td>DOCA-salt + Reserpine</td>
<td>93.5 ± 10.5</td>
</tr>
</tbody>
</table>

Collagen content is expressed as mg per g of tissue. Each value represents the mean ± standard error of 5 rats. *: statistically significant compared to controls, 0.05 > P > 0.01.

tissue. The values calculated per mg of protein were proportionately the same. The lysyl oxidase activity in blood vessels of the DOCA-salt treated rats were markedly elevated over control. As expected, the DOCA-salt animals treated with β-aminopropionitrile yielded a value lower than controls. It should be noted that the administration of reserpine also causes a decrease in lysyl oxidase activity. Aortic collagen content is presented in Table III. The DOCA-salt hypertensive rats showed the highest value among experimental groups and the administration of β-aminopropionitrile reduced the aortic collagen content. The decrease in vascular collagen produced by reserpine is consistent with our previous report.1,2

Histological examination of the rat showed that after 4 weeks of DOCA-salt treatment there were necrotic and hyperplastic changes in the small blood vessels and glomeruli of the kidney in all cases examined, which correspond to malignant nephrosclerosis. However, the DOCA-salt rat treated with β-aminopropionitrile only showed slight vascular changes in 2 cases out of 5. No vascular damage was observed in controls and reserpine treated rats. The thickness of the aorta, which is primarily due to the increased connective tissue matrix, was greatest in DOCA-salt hypertensive rats. The aortic wall of hypertensive rats also showed hyperplastic and hypertrophic changes of smooth muscle cells. The thickness of the aorta in β-aminopropionitrile treated DOCA-salt rats was intermediate between that of the DOCA-salt hypertensive rats and controls. In this experiment, the blood vessels of β-aminopropionitrile treated rats did not show any histological changes such as edema and elastolysis of the medial elastin observed in experimental lathyrisim induced in young growing animals.9

DISCUSSION

The finding reported here indicates that there is increased lysyl oxidase activity in the blood vessels of the DOCA-salt hypertensive rat and that an antihypertensive agent, reserpine, when administered before the onset of hypertension, prevented or diminished the increase in enzyme
activity. The present finding suggests that there might be increased cross-linkings in collagen and elastin in the vascular wall of the hypertensive rat, providing more vascular rigidity. It has previously been shown that reserpine decreases collagen biosynthesis in blood vessels and other tissues.\textsuperscript{1,2,11} The inhibitory effect of reserpine on lysyl oxidase activity may be either due to decreased synthesis of collagen or due to reduction in blood pressure. Since β-aminopropionitrile reduced the amount of collagen in the blood vessels of DOCA-salt treated rats, there is no doubt that β-aminopropionitrile decreases vascular fiber formation by inhibiting the cross-linking of collagen. In this experiment, it was also found that administration of β-aminopropionitrile to the DOCA-salt rats brought about a decrease in blood pressure. The cause of this hypotensive effect is not known, but the effect may be due to reduced vascular rigidity. Since the formation of collagen is an important step in the development of arteriosclerosis, it is reasonable to assume that β-aminopropionitrile may prevent hypertensive vascular damage (arteriosclerosis) by inhibiting abnormal accumulation of fibrous tissue in the blood vessels. This concept could therefore provide a new aspect of therapy for arteriosclerosis.

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REFERENCES


