Increased angiotensin-converting enzyme activity of aorta in two-kidney, one-clip Goldblatt hypertension in rats

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March 1982

There is increasing evidence that conversion of angiotensin I into angiotensin II can occur in the renal vasculature of humans [1], and in the vascular beds of the hind limb of the dog [2]. In previous experiments the specific angiotensin-converting enzyme in rat aorta [3] has been demonstrated. More recently, Mizuno et al. [4] have studied the subcellular localization of the enzyme activity in aortic tissue of rat and found that the enzyme activity was extremely high in the supernatant and microsomal fractions. They have also reported that the enzyme activity in the aortic tissue of spontaneously hypertensive rats was higher than that of normotensive rats [4]. To date, however, the role of the enzyme in arterial tissue in the initiation and/or the maintenance of hypertension has not fully been elucidated. The present study was undertaken to determine the subcellular localization of aortic angiotensin-converting enzyme activity in three different types of experimental hypertension in rats.

Female normotensive rats of the Wistar-Kyoto strain, aged 20–21 weeks, were divided into groups 1–4. Operation for induction of hypertension was performed under ether anaesthesia. Group 1; control rats, given a stock chow and distilled water ad libitum for a period of 4 weeks (n = 10). Group 2; two-kidney, one-clip Goldblatt hypertension. The left renal artery was constricted with a silver clip, leaving the contra-lateral kidney untouched (n = 8). Group 3; one-kidney, one-clip Goldblatt hypertension. The left renal artery was constricted with a silver clip after the contralateral kidney was removed (n = 14). Group 4; two-kidney, two-clip hypertension. The left and right renal arteries were constricted with a silver clip (n = 10). Tail blood pressure of these rats was determined every week for a period of 4 weeks. The rats were killed by decapitation 4 weeks after the operation, and whole aortae without branches were excised immediately at 4°C. Aortic tissue was cleaned of blood with moist gauze, stripped of surrounding tissue, including adventitia, briefly rinsed three to five times in sucrose solution (0-25 mol/l) to remove surface contamination by serum, and minced into 2 mm x 2 mm pieces. Portions of the minced tissue were homogenized with a Virtis homogenizer in sucrose solution (0-25 mol/l) in volumes to give a 10% (w/v) homogenate. The crude homogenate was subfractionated by differential ultracentrifugation as previously described [5], and electron-microscopic appearances of the mitochondrial and the microsomal fractions were similar to that described previously [6]. Angiotensin-converting enzyme activity in each fraction was determined by the spectrophotometric assay with hippuryl-L-histidyl-L-leucine as substrate [4]. One unit of the enzyme activity is defined as 1 nmol of hippuric acid formed min⁻¹ mg⁻¹ of protein at 37°C. Results are presented as means ± SEM. Significance of difference was assessed by analysis of variance.

The systolic blood pressure of Group 1 did not change, whereas in Group 2 it rose from a control of 106 ± 8 to 187 ± 12 mmHg at 4 weeks. In Group 3 the systolic blood pressure rose from 104 ± 5 to 195 ± 14 mmHg at 4 weeks, and in Group 4 from 102 ± 5 to 196 ± 7 mmHg. Table 1 summarizes our principal data on aortic angiotensin-converting enzyme activity. The enzyme activity of the crude homogenate in Group 2 was 6.9 ± 0.7 units, which was significantly higher than in Groups 1, 3 and 4 (P < 0.05), but there was no significant difference in the enzyme activity of the crude homogenate between Group 1 and Groups 3 and 4. The enzyme activity of the mitochondrial, microsomal and supernatant fractions in Group 2 was also significantly higher than in Groups 1, 3 and 4 (Table 1). There was no significant difference in the enzyme activity from the mitochondrial, microsomal and supernatant fractions between Group 1 and Groups 3 and 4.
In the present study, it was clearly demonstrated that aortic angiotensin-converting enzyme activity was consistently high in two-kidney, one-clip Goldblatt hypertensive rats as compared with normotensive, one-kidney, one-clip Goldblatt hypertensive and two-kidney, two-clip hypertensive rats. A new hypothesis has been advanced which suggests that renin accumulates in peripheral arteriolar walls, leading to local generation of angiotensin II [7, 8]. Renin-like activity has been detected in isolated arterial walls [9, 10] and appears to vary quantitatively with sodium balance in exactly the same manner as does plasma renin activity [11]. Since many investigators have presented data which suggest that two-kidney, one-clip Goldblatt hypertension represents a model for renin- and angiotensin-dependent hypertension [12, 13], it is conceivable that in this type of hypertension arterial wall renin is increased. Although we have not elucidated the interaction between arterial renin and converting enzyme activity in the present study, it is likely that production of the enzyme, which might occur in the mitochondrial and/or the microsomal fraction of aorta, is enhanced in this type of hypertension, leading to enhanced conversion of angiotensin I in vascular tissue.

References

Table 1. Angiotensin-converting enzyme activity of the aortic subcellular fractions in normotensive and hypertensive rats

<table>
<thead>
<tr>
<th>Group 1 (n = 10)</th>
<th>Group 2 (n = 8)</th>
<th>Group 3 (n = 14)</th>
<th>Group 4 (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenate</td>
<td>4.1 ± 0.2</td>
<td>6.9 ± 0.7*</td>
<td>3.8 ± 0.5</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>3.2 ± 0.4</td>
<td>30.7 ± 1.0†</td>
<td>3.1 ± 1.1</td>
</tr>
<tr>
<td>Microsomes</td>
<td>14.7 ± 3.3</td>
<td>35.0 ± 1.3**</td>
<td>16.7 ± 1.7</td>
</tr>
<tr>
<td>Supernatant</td>
<td>35.5 ± 5.1</td>
<td>160.0 ± 14.3†</td>
<td>29.9 ± 1.9</td>
</tr>
</tbody>
</table>
| Values are means ± SEM (nmol min⁻¹ mg⁻¹). Significance of differences: * P < 0.05, ** P < 0.01, † P < 0.001 (compared with groups 1, 3 and 4 in the same fractions).