Hypertensive Vascular Lesions and Renin or Lysosomal Enzymes in Rats

NOBORU SAITO, MASATO MATSUNAGA, AKIRA HARA, JIN YAMAMOTO, YUKIO YAMORI, SAKAE MUKAINO, KOICHI OGINO, and CHUICHI KAWAI

HYPERTENSION is accompanied by vascular complications of cardiac, renal and cerebral tissues. It is not fully confirmed whether renin is related to hypertensive vascular lesions or not, although renin administration can produce severe vascular damages in rats pretreated with DOCA plus saline. Brunner et al. reported that essential hypertensive patients with normal or high plasma renin activity were associated with higher incidences of heart attacks or cerebral strokes than those with low plasma renin activity. However, there have been many controversies against the vasculotoxic effects of renin. We observed increased activities of cathepsin D and β-glucuronidase in the kidney of hypertensive rats with DOCA plus high salt. Wolinsky et al. reported that lysosomes were more numerous in aortic muscle cells of hypertensive rats. Okamoto et al. recently succeeded in breeding the stroke-prone SHR (SHRSP) with severe hypertension and high spontaneous incidence of stroke. The present study was undertaken to investigate the relations between renin, lysosomal enzymes and hypertensive vascular lesions.

MATERIALS AND METHODS

Key Words:
- Hypertension
- Hypertensive vascular lesions
- SHR, SHRSP
- Renin
- Lysosomal enzymes

1) Experiment 1: Female Wistar-Kyoto(WK) rats aged 2 to 4.5 months were used. Six rats were fed on high salt chow containing 7 to 7.6% NaCl plus 0.9% saline as drinking water for more than 2.5 months. Twelve rats implanted subcutaneously with 30 mg of DOCA were also fed on high salt chow plus 0.9% saline for 0.5 to 3 months. Eight rats were treated with normal chow containing 0.6% NaCl plus tap water as a control. Systolic blood pressure of rat was determined by the plethysmographic method.

2) Experiment 2: Rats aged 3.5 months were used. WK rat and SHR of both sexes were given high salt chow containing 7% NaCl plus 0.9% saline for 28 to 30 days. The control WK rat and SHR were given normal chow plus tap water.

3) Experiment 3: Ten SHRSPs, 10 stroke-resistant SHR (SHRSTRs) and 10 WK rats were used. These male rats were 9 to 10 months of age.

4) Experiment 4: SHRSP, SHRSTR and WK rat were used. These male rats were 7 weeks, 12 weeks and 10 months after birth. In these rats a polyethylene catheter was inserted into the femoral artery and advanced towards the aorta one day before blood sampling. During measurement of the blood pressure the catheter was connected to a transducer and recording equipment. The details of method are described elsewhere.

Tissue fractionations: In Experiments 1 to 3, rats were exsanguinated by decapitation or under ether anesthesia. Rat kidney removed was placed in ice-cold 0.45 M sucrose and homogenized in a
### TABLE I-a DATA IN EXPERIMENT 1. ENZYME ACTIVITY IN THE CYTOPLASMIC FRACTION OF RAT KIDNEY

<table>
<thead>
<tr>
<th></th>
<th>Control (a) RVD -</th>
<th>High Salt Loading (b) RVD -</th>
<th>(c) RVD +</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic Blood Pressure</td>
<td>87 ± 16 (8) mmHg</td>
<td>170 ± 43 (10)</td>
<td>216 ± 22 (8)</td>
<td>a: b****</td>
</tr>
<tr>
<td>Body Weight</td>
<td>165 ± 30 (8) g</td>
<td>176 ± 57 (10)</td>
<td>169 ± 48 (8)</td>
<td>a: c****</td>
</tr>
<tr>
<td>Heart Weight per 100g</td>
<td>0.41 ± 0.04 (8) g</td>
<td>0.51 ± 0.11 (10)</td>
<td>0.6 ± 0.1 (8)</td>
<td>a: b**</td>
</tr>
<tr>
<td>of Body Weight</td>
<td></td>
<td></td>
<td></td>
<td>a: c***</td>
</tr>
<tr>
<td>Kidney Weight per 100g</td>
<td>0.78 ± 0.1 (8) g</td>
<td>1.0 ± 0.21 (10)</td>
<td>1.08 ± 0.26 (8)</td>
<td>a: b**</td>
</tr>
<tr>
<td>of Body Weight</td>
<td></td>
<td></td>
<td></td>
<td>a: c**</td>
</tr>
<tr>
<td>Protein</td>
<td>125 ± 15 (8) mg/ml</td>
<td>114 ± 14 (10)</td>
<td>112 ± 19 (8)</td>
<td></td>
</tr>
<tr>
<td>Renin</td>
<td>33.5 ± 14.5 (8) ng/mg</td>
<td>0.4 ± 0.4 (10)</td>
<td>6.0 ± 3.1 (8)</td>
<td>a: c***</td>
</tr>
<tr>
<td>Cathepsin</td>
<td>379 ± 92 (8) cu/mg</td>
<td>547 ± 128 (10)</td>
<td>600 ± 178 (8)</td>
<td>a: b***</td>
</tr>
<tr>
<td>β-Glucuronidase</td>
<td>25.2 ± 2.4 (8) u/mg</td>
<td>30.6 ± 6.7 (10)</td>
<td>42.6 ± 6.9 (8)</td>
<td>a: b*</td>
</tr>
<tr>
<td>β-Glycerophosphatase</td>
<td>21.5 ± 1.0 (8) u/mg</td>
<td>19.9 ± 1.7 (6)</td>
<td>21.6 ± 1.9 (6)</td>
<td>a: c***</td>
</tr>
<tr>
<td>Deoxyribonuclease</td>
<td>0.105 ± 0.018 (8) u/mg</td>
<td>0.108 ± 0.026 (6)</td>
<td>0.17 ± 0.055 (6)</td>
<td>a: c*</td>
</tr>
<tr>
<td>Ribonuclease</td>
<td>0.36 ± 0.07 (8) u/mg</td>
<td>0.33 ± 0.06 (6)</td>
<td>0.42 ± 0.04 (6)</td>
<td>b: c**</td>
</tr>
</tbody>
</table>

**RVD:** Renal Vascular Damages  
Means ± SD are given. The number of cases is shown in parenthesis.  
*p < 0.05, **p < 0.025, ***p < 0.01, ****p < 0.005

Potter-Elvehjem homogenizer with 0.45 M sucrose (1:8, w/v) by two up-and-down strokes within 30 seconds. The cytoplasmic fraction (C.F.) of rat kidney was obtained by centrifuging the homogenate for 2.5 min at 650 g using Kubota KR-6P centrifuge. This C.F. was used for enzyme assays after more than five-time freezing and thawing.

Histologic technique: Neutral 10% formalin was used for fixation of rat kidney. Staining was performed with hematoxyline eosine.

Enzyme assays: The details of enzyme assays are reported previously. For the bioassay of kidney renin activity (KRA), the sample was incubated at 37°C for 45 min at pH 5.5 in the presence of EDTANa2 and DFP, using dialyzed rat plasma as substrate. For β-glycerophosphatase (β-GPase), deoxyribonuclease (DNase) and ribonuclease (RNase), incubations were carried out at 37°C for 30 min, for 60 min and for 30 min, respectively, at pH 5. For β-glucuronidase, β-N-acetylglucosaminidase (β-NAGA) and cathepsin (using denatured hemoglobin as substrate), incubations were performed at 37°C for 120 min, for 20 min and for 30 min, at pH 4.5, at pH 4.4 and pH 3.6, respectively. Protein concentration was determined by the method of Lowry et al. Plasma renin concentration (PRC) was measured by a radioimmunoassay to quantify the angiotensin I formed during one hour incubation at pH 6.5 in the presence of EDTANa2, dimercaprol, DFP, phenylmercuric acetate and enough renin substrate.

**RESULTS**

1) Experiment 1 (Table I-a): All rats treated with DOCA plus high salt developed hypertension higher than 150 mmHg, one half of which were associated with renal vascular lesions. Four of rats given high salt developed hypertension and one half of hypertensive rats were complicated with renal vascular lesions. Macroscopically renal vascular lesions consisted of cellular hyperplasia and fibrinoid necrosis in arterioles and small arteries. Kidneys of hypertensive rats with renal vascular lesions showed some renin activity under high salt loading while those of rats without vascular lesions demonstrat-
TABLE I-b  DATA IN EXPERIMENT 2. ENZYME ACTIVITY IN THE CYTOPLASMIC FRACTION OF RAT KIDNEY

<table>
<thead>
<tr>
<th></th>
<th>WK rat</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(a) Control</td>
<td>(b) High salt loading</td>
</tr>
<tr>
<td></td>
<td>(c) Control</td>
<td>(d) High salt loading</td>
</tr>
<tr>
<td></td>
<td>n = 6</td>
<td>n = 7</td>
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<tr>
<td></td>
<td>c: d****</td>
<td>b: c****</td>
</tr>
<tr>
<td></td>
<td>a: c***</td>
<td>b: c****</td>
</tr>
<tr>
<td></td>
<td>c: d****</td>
<td>a: d****</td>
</tr>
</tbody>
</table>

- **Systolic Blood Pressure**
  - WK rat: 120 ± 17 mmHg
  - SHR: 176 ± 13 mmHg
- **Body Weight**
  - WK rat: 227 ± 17 g
  - SHR: 186 ± 15 g
- **Heart Weight per 100g of Body Weight**
  - WK rat: 0.37 ± 0.03 g
  - SHR: 0.45 ± 0.02 g
- **Kidney Weight per 100g of Body Weight**
  - WK rat: 0.68 ± 0.05 g
  - SHR: 0.72 ± 0.06 g
- **Protein**
  - WK rat: 114 ± 16 mg/ml
  - SHR: 114 ± 18 mg/ml
- **Renin**
  - WK rat: 25.1 ± 9.7 ng/mg
  - SHR: 24.9 ± 7.8 ng/mg
- **Cathepsin**
  - WK rat: 335 ± 239 cu/mg
  - SHR: 421 ± 160 cu/mg
- **β-Glucuronidase**
  - WK rat: 19.9 ± 3.2 u/mg
  - SHR: 19.7 ± 2.1 u/mg
- **β-Glycerophosphatase**
  - WK rat: 21.4 ± 2.9 u/mg
  - SHR: 25 ± 3.1 u/mg
- **Deoxyribonuclease**
  - WK rat: 0.09 ± 0.02 u/mg
  - SHR: 0.11 ± 0.01 u/mg
- **Ribonuclease**
  - WK rat: 0.36 ± 0.04 u/mg
  - SHR: 0.37 ± 0.09 u/mg

Means ± SD are given.
* p < 0.05,  **p < 0.025,  ***p < 0.01,  ****p < 0.005

ed almost no renin activity (Table I-a). Both cathepsin and β-glucuronidase activities of kidney were higher in rats given high salt than in the control rats. Moreover, β-glucuronidase activity was highest in rats with renal vascular lesions. RNase activity was greater in rats with renal vascular lesions than in rats without renal vascular lesions under high salt loading. DNase activity was greater in rats with renal vascular lesions than in rats without renal vascular lesions.

2) Experiment 2 (Table I-b): SHR given high salt for 28 to 30 days showed the highest blood pressure above 200 mmHg. Body weight of SHR was less than that of WK rat and least in SHR given high salt. During the period of salt loading body weight of SHR tended to be decreased, but that of WK rat tended to be increased. KRA of female WK rat was more suppressed than KRA of female SHR under high salt loading, but not significantly different between them (TABLE I-b). In rats of both sexes, KRA of WK rat was 1.5 ± 1.5 ng/mg (Mean ± SD, 13 cases) and KRA of SHR was 5.1 ± 2.4 ng/mg (9 cases) under high salt loading, and the difference between them was significant. Microscopically, angionecrosis of renal arterioles and small arteries was observed only in SHR fed on high salt. Both cathepsin and β-glucuronidase activities in the female SHR fed on high salt were highest in average, but not significantly different from those in other groups. In rats of both sexes, cathepsin activity of SHR was 700 ± 399 cu/mg (9 cases) under high salt or 358 ± 147 cu/mg (7 cases) in the control, and that of WK rat was 434 ± 130 cu/mg (13 cases) under high salt or 341 ± 188 cu/mg (10 cases) in the control. Significant difference was found between SHR treated with high salt and the control SHR or WK rat in cathepsin activities. Beta-GPase, DNase and RNase activities were greater in SHR than in WK rat under high salt loading.

3) Experiment 3 (Table II-a): SHRSP aged 10 months showed the highest blood pressure. Body weight of SHRSP was least among three groups. Although KRA of SHR was less than KRA of WK rat, KRA of SHRSP was more than KRA of SHRSR (Table II-a). When expressed in nanogram per gram of wet weight, KRA of SHRSP,
### TABLE II-a DATA IN EXPERIMENT 3. ENZYME ACTIVITY IN THE CYTOPLASMIC FRACTION OF RAT KIDNEY

<table>
<thead>
<tr>
<th></th>
<th>WK rat</th>
<th>SHR</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(a)</td>
<td>(b) SHR</td>
<td>(c) SHRSP</td>
</tr>
<tr>
<td>n = 10</td>
<td></td>
<td>n = 10</td>
<td>n = 10</td>
</tr>
<tr>
<td>Systolic Blood Pressure</td>
<td>116 ± 10 mmHg</td>
<td>181 ± 17</td>
<td>206 ± 17</td>
</tr>
<tr>
<td>Body Weight</td>
<td>407 ± 28 g</td>
<td>410 ± 18</td>
<td>352 ± 31</td>
</tr>
<tr>
<td>Kidney Weight per 100g of Body Weight</td>
<td>0.67 ± 0.05 g</td>
<td>0.75 ± 0.06</td>
<td>0.81 ± 0.06</td>
</tr>
<tr>
<td>Protein</td>
<td>116 ± 8 mg/ml</td>
<td>113 ± 7</td>
<td>128 ± 12</td>
</tr>
<tr>
<td>Renin</td>
<td>100 ± 24 ng/mg</td>
<td>35.6 ± 14.4</td>
<td>70.2 ± 29.8</td>
</tr>
<tr>
<td>Cathepsin</td>
<td>100 ± 31 cu/mg</td>
<td>79.6 ± 33.3</td>
<td>121.2 ± 49.5</td>
</tr>
<tr>
<td>β-Glucuronidase</td>
<td>100 ± 13.8 u/mg</td>
<td>105.2 ± 19</td>
<td>116 ± 20.3</td>
</tr>
<tr>
<td>β-Glycerophosphatase</td>
<td>100 ± 17.9 u/mg</td>
<td>101.2 ± 15</td>
<td>102.8 ± 12.4</td>
</tr>
<tr>
<td>Deoxyribonuclease</td>
<td>100 ± 17.5 u/mg</td>
<td>129.8 ± 22.6</td>
<td>158.9 ± 64.3</td>
</tr>
<tr>
<td>Ribonuclease</td>
<td>100 ± 21.3 u/mg</td>
<td>116.7 ± 17.2</td>
<td>134.2 ± 45.5</td>
</tr>
<tr>
<td>β-N-Acetylglucosaminidase</td>
<td>100 ± 14.7 u/mg</td>
<td>138.9 ± 33.7</td>
<td>129.5 ± 15.8</td>
</tr>
</tbody>
</table>

Means ± SD are given. Control value of enzyme activity is expressed as 100.
*p < 0.05,  **p < 0.025,  ***p < 0.01,  ****p < 0.005

SHRSR or WK rat was 935 ± 407 ng (10 cases), 420 ± 173 ng (10 cases) or 1204 ± 275 ng (10 cases), respectively, and the difference between SHRSR and WK rat was not significant. Cathepsin activity was greater in SHRSR than in SHRSR. DNase and β-NAG activities were greater in SHR than in WK rat. Beta-glucuronidase and RNase activities in SHSP were greatest in average, but not significantly different from those in other groups.

4) Experiment 4 (Table II-b): The mean blood pressure of SHR was higher than that of WK rat. In 7 weeks of age the body weight of SHR was less than that of WK rat. In 12 weeks of age the body weight of SHRSR was least of the three groups. PRC of SHRSR was more than PRC of SHRSR in 7 weeks of age (Table II-b). PRC of rats aged 12 weeks showed no significant difference among the three groups. PRC of SHRSR was significantly increased as compared to PRC of WK rat in 10 months of age.

**DISCUSSION**

The such tendencies as kidneys of rats with renal vascular lesions similar to malignant hypertension showed some renin activity under high salt loading were also shown in SHR treated with high salt. These findings indicate an incomplete suppression of KRA in rats with renal vascular lesions under high salt loading. It has been reported that vascular receptor affinity to angiotensin II is enhanced either in high sodium intake or in DOCA plus salt, and that the administration of renin or angiotensin into rats pretreated with DOCA or aldosterone plus salt can produce severe renal vascular damages. Thus the slight increase of renin activity in rats with DOCA plus high salt or with high salt alone may result in a vicious cycle between renin, hypertension and renal vascular damages. High PRC of SHRSR aged 10 months in addition to the results of KRA may indicate an intimate correlation between renin and renal vascular lesions. Laragh et al. postulated an important role of renin in vascular complications in patients with essential hypertension, although other investigators disagreed with their theory. Increased vascular permeability and

*Japanese Circulation Journal Vol. 39, May 1975*
<table>
<thead>
<tr>
<th></th>
<th>WK rat</th>
<th>SHR</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(a)</td>
<td>(b) SHRSR</td>
<td>(c) SHRSP</td>
</tr>
<tr>
<td></td>
<td>Mean Artery</td>
<td>91 ± 4 (8) mmHg</td>
<td>128 ± 5 (4)</td>
</tr>
<tr>
<td>7 weeks of age</td>
<td>Pressure</td>
<td></td>
<td>119 ± 11 (8) a : c****</td>
</tr>
<tr>
<td></td>
<td>Body Weight</td>
<td>146 ± 8 (8) g</td>
<td>125 ± 10 (8) a : b****</td>
</tr>
<tr>
<td></td>
<td>Plasma Renin</td>
<td>36.4 ± 11.6 (8) ng/ml/hr</td>
<td>51.3 ± 27.5 (8) b : c**</td>
</tr>
<tr>
<td></td>
<td>Concentration</td>
<td></td>
<td>27.2 ± 14.2 (8)</td>
</tr>
<tr>
<td></td>
<td>Mean Artery</td>
<td>97 ± 8 (7) mmHg</td>
<td>143 ± 13 (7) a : b****</td>
</tr>
<tr>
<td>12 weeks of age</td>
<td>Pressure</td>
<td></td>
<td>152 ± 18 (5)</td>
</tr>
<tr>
<td></td>
<td>Body Weight</td>
<td>234 ± 17 (7) g</td>
<td>221 ± 20 (7) a : c**</td>
</tr>
<tr>
<td></td>
<td>Plasma Renin</td>
<td>27.8 ± 12.2 (7) ng/ml/hr</td>
<td>20.1 ± 7.1 (7) a : c***</td>
</tr>
<tr>
<td></td>
<td>Concentration</td>
<td></td>
<td>30.7 ± 22.3 (5)</td>
</tr>
<tr>
<td></td>
<td>Mean Artery</td>
<td>99 ± 2 (5) mmHg</td>
<td>179 ± 47 (7) a : b****</td>
</tr>
<tr>
<td>10 months of age</td>
<td>Pressure</td>
<td></td>
<td>176 ± 32 (5)</td>
</tr>
<tr>
<td></td>
<td>Body Weight</td>
<td>370 ± 12 (5) g</td>
<td>380 ± 19 (7) a : c***</td>
</tr>
<tr>
<td></td>
<td>Plasma Renin</td>
<td>22.9 ± 1.7 (5) ng/ml/hr</td>
<td>33.6 ± 24.7 (7) a : c**</td>
</tr>
<tr>
<td></td>
<td>Concentration</td>
<td></td>
<td>63.5 ± 25.2 (5)</td>
</tr>
</tbody>
</table>

Means ± SD are given. The number of cases is shown in parenthesis.  
* p < 0.05,  ** p < 0.025,  *** p < 0.01,  **** p < 0.005

thirst after injection of kidney extracts, renin or angiotensin have been observed15-19 but the separation between permeability and thirst factors has been also reported20 Kira et al. reported the presence of both permeability factor and hemocoagulant substance in the lysosomal fraction of rat kidney and speculated the latter as renin21. Further study is necessary to determine whether or not renin has vasculotropic effect. We observed increased cathepsin D and β-glucuronidase activities in the kidney of rats treated with DOCA plus salt5 Wolinsky et al demonstrated increased lysosomes in the vessels of rats with renal clip hypertension6 and showed preventive effects on vascular lesions by estrogen or methylprednisolone, acting as stabilizer of lysosomal membrane22. It was suggested that enhanced activities of lysosomal enzymes, especially of cathepsin D and β-glucuronidase, were related to local accumulation of collagen or mucopolysaccharides in rat kidney. Lazarus et al. reported that both specific collagenase and non-specific protease were necessary for maximal collagen fibril degradation23. Furthermore it has been shown that cathepsin B1 or collagenolytic cathepsin caused degradation of collagen at acid pH values24,25. Increased activities of both cathepsin D and β-glucuronidase were also demonstrated in human atherosclerotic aorta26. It remains to be elucidated whether increased activities of cathepsin D, β-glucuronidase or DNase are specific to vascular lesions and/or related to tubular and glomerular damages.

**SUMMARY**

1) In rats treated with DOCA plus high salt or with high salt alone, hypertensive rats with renal vascular lesions showed an incomplete suppression of KRA. Cathepsin activity of rat kidney was higher under high salt loading than in the control. Beta-glucuronidase activity was greatest in rats with renal vascular lesions and smallest in rats fed on normal chow. RNase and DNase activities were greater in rats with renal vascular lesions than in rats without renal vascular lesions.

*Japanese Circulation Journal Vol. 39, May 1975*
under high salt loading.
2) In rats of both sexes SHR showed greater KRA and cathepsin activities than WK rat under high salt loading. In female rats DNase, RNase and β-GPase activities were greater in SHR than in WK rat under high salt loading.
3) KRA was higher in SHRSP aged 10 months than in SHRSR, though KRA of SHR was smaller than KRA of WK rat. Cathepsin activity was greater in SHRSP than in SHRSR. DNase and β-NAGase activities were greater in SHR than in WK rat.
4) In 7 weeks of age SHR showed more PRC than SHRS. At the age of 10 months SHRSP showed higher PRC than WK rat. The roles of renin and lysosomal enzymes in hypertensive renal vascular lesions were discussed to some extent.

(Research is sponsored by the Science and Technology Agency of Japanese Government.)

The authors are most grateful to Dr. Professor Kozo Okamoto of the Department of Pathology, Kinki University, Emeritus Professor of Kyoto University for his invaluable advice and his kind supplies of SHR, especially SHRSP.

REFERENCES

Discussion:

*Chairman:* MASAO IKEDA, Tokyo Univ.

S. FUKUCHI (Tohoku Univ.): You mentioned that kidney renin activity in SHR group was decreased compared to the normal group and plasma renin concentration was elevated especially in the animals with vascular lesions. Will you let me know why there was a discrepancy between levels of kidney renin activity and plasma renin concentration?

N. SAITO (Kyoto Univ. Nutrition Dept.): Kidney renin activity was lower at the sustained hypertensive phase 3 months after birth compared to those in the control: Wister Kyoto rat. Kidney renin activity in stroke-prone SHR at the age of 9 to 10 months after birth was lower than that in the control at the same age, but higher than that in stroke resistant SHR. Plasma renin concentration in stroke-prone SHR at the same age was higher than in the control group. Plasma renin concentration and kidney renin activity were not measured simultaneously in our experiments. So that we can not explain the discrepancy of both values. However, renin activities in stroke-prone SHR were tended to elevate. The reason of it seems to be due to vascular lesions in the kidney which may be induced in stroke-prone SHR at the age of 10 months.

Y. KANEFKO (Yokohama City Univ.): This experimental result seems to support the concept in which renin is responsible for the development of hypertensive vascular lesions.

T. KOKUBU (Ehime Univ.): May I ask two questions?

1) Is there any relationship between vascular lesions and the lysosomal enzymes except renin which are not specific for kidneys and may exist in other organs.

2) Will you tell me about the decrease in body weight of SHR with high salt intake? How was the amount of food intake of animals? Was there any difference of it compared to that in the control group?

N. SAITO: I think a certain lysosomal enzyme may act vasculotoxically at the site of arterial wall. However, it is still under study and not clear yet that the lysosomal enzyme liberated from the kidney into the blood stream may produce the vascular lesion of other organs. Fibrinoid necrosis of arterioles and small arteries may occur in SHR with high salt intake. In these cases, cathepsin activity was high on an average in the kidney as well as in the blood plasma. So that I would suggest elevation of plasma cathepsin may induce the vascular lesion without elevation of blood pressure.

2) The body weight in SHR with high salt intake became to decrease when the blood pressure elevated more than 200 mmHg. Although the amount of food intake did not change so much.

Dr. K. TANAKA (Miyasaki Med. School): Did you measure plasma renin activity or plasma renin concentration?

Dr. N. SAITO: We measured plasma renin concentration by radio-immunoassay and kidney renin activity by bioassay.

Dr. I. NISHIMORI (Nagasaki Univ.): You mentioned that a certain lysosomal enzyme may act locally at the arterial wall as a vasculotoxic substance. May it act within cells or from outside of cells? If it act from outside of cells, what is the target site for it? We have demonstrated a vasculotoxic substance other than pressor substance in eluted fraction from kidney extract. So that, we supposed a vasculotoxic substance might be liberated into the blood and act from outside of cells. Will you tell me more details on this problem?

Dr. N. SAITO: Lysosomal enzymes seem to be liberated to the outside of cells by exocytosis and then act vasculotoxically to ground substances or cells. It is supposed that the increase in cathepsin may cause the damage of collagen fibril around the cells in arterial wall.

Dr. Y. YAMORI (Kyoto Univ.): It has been postulated that renin played a role related with development of high blood pressure and also with hypertensive vascular lesions. According to our studies in SHR, renin seems to have no role as a cause of hypertension. It seems, however, that there is some correlation between kidney renin activity and occurrence of fibrinoid necrosis of small arteries. Plasma renin activities decrease usually in SHR with high salt intake. Among these animals, those with increase in renin activity showed the acute vascular lesion. At the present time, however, there has been no proof whether increase of renin activity was the cause of vascular lesions or the result due to vascular lesions. So that, I think Laragh's concept in which levels of plasma renin activity influence

to cardiovascular complication of essential hypertension, is not conclusive. We would like to study further on this problem by using stroke-prone SHR.

K. OGINO (Kyoto Univ.): Vasculotoxic substances other than renin has been studied by several investigators. We have studied on this subject by means of lysosomal enzymes. According to our experiments, activities of both renin and lysosomal enzymes in the kidney were correlated with the development of fibrinoid necrosis of renal small arteries.

We suppose that lysosomal enzymes may act within the kidney as vasculotoxic substances. It is not conclusive at the present time that these enzymes are liberated into the blood stream from the kidney and then cause acute vascular lesions in other organs. Several problems are remained to be confirmed further. Whether one of these enzymes for example cathepsin liberated from the kidney into the blood stream may or may not have activity to induce the acute vascular lesion at the optimal pH? Whether the plasma concentration of these enzymes may be or may not be enough to induce the acute vascular lesion?

Dr. M. IKEDA (Tokyo Univ.): I would like to add some comments as the chairman of this subject.

First of all, I should say that strokes may be resulted from several types of vascular lesion in the brain as follows. The vascular lesions of small cerebral arteries which may induce strokes, consist of acute destructive lesion (fibrinoid necrosis) and of chronic proliferative and/or degenerative lesions (arteriolosclerosis). The former is the fundamental vascular lesion for massive cerebral hemorrhages in hypertension and also may induce small cerebral infarction when occlusion of vessels occurs form it. The chronic proliferative and/or degenerative vascular lesions induce the narrowing or occlusion of vessels which may result in small cerebral infarction. The grade of narrowing in the latter type of vascular lesions is closely related to the grade and sustained duration of hypertension. The larger cerebral infarctions develop from the occlusion of thrombo-atherosclerotic lesions of the larger cerebral arteries. Keeping these background in one’s mind, one should analyse vascular lesions responsible for strokes.

Secondly, as far as studies on enzymes in the kidney are concerned, I would suggest that the synthetic enzymes which may relate to proliferative vascular lesions should be studied as well as the degrading enzymes which may relate to destructive vascular lesions. And also I would like to know the change of these enzymes in arterial wall of organs other than the kidney.

Thirdly, one should be careful when one evaluate sodium loading for vascular lesions in hypertension. It is quite clear that high salt intake accelerates the development of hypertension. High blood pressure itself which is accelerated by sodium loading may influence to development of vascular lesions. However, high salt intake alone without high blood pressure may influence to development of vascular lesions although it’s mechanism is not uncertain.

Fibrinoid necrosis of small arteries is often found in malignant phase of essential hypertension. It is not, however, the specific vascular lesion for malignant hypertension. It may occur in essential hypertension with cerebral hemorrhage without clinical manifestation of malignant hypertension. Malignant hypertension and malignant phase of essential hypertension are the term due to clinical definition.