Adrenal Catecholamine Content in the Spontaneously Hypertensive Rats

Masayori Ozaki**, Yasuhiro Suzuki*, Yukio Yamori* and Kozo Okamoto*

In 1895, Oliver and Šafer\(^1\) reported that the extracts of the adrenal medulla, when injected intravenously, demonstrated a big increase in the arterial blood pressure of experimental animals. Later the chemical nature and pharmacological activity of adrenaline (abbreviated AD, hereafter) were observed, since then there has been a great interest in the possible relationship between this substance and pathogenesis of essential hypertension. von Euler\(^2\) discovered in 1946, that the sympathetic transmitter is noradrenaline (NA), which is also a strong vasoactive substance, and together with adrenaline, as catecholamine (CA) it drew a lot of attention.

There are many factors\(^3\) which are involved in the pathogenesis of essential hypertension. Until recently, however, no concrete positive results on the urinary excretion of CA and their metabolites in hypertensive diseases have been obtained\(^4,5\) except in the case of pheochromocytoma, and it has not yet been clarified whether any defect or deviation of CA metabolism is concerned in the pathogenesis of essential hypertension, although detailed CA metabolism has been disclosed in experimentally induced DOCA hypertension in rats\(^6,7\).

Okamoto and Aoki\(^8,9\) have succeeded in the separation of a spontaneously hypertensive rat (SHR) colony through selective sib-breeding from the Wistar strain which developed hypertension after about two months of age without any treatment. As the hypertension in these rats seems to be the most adequate model for human essential hypertension because of its spontaneous development, accompanying cardiovascular lesions and others\(^8,6,10\), experimental investigations on the CA and other related substances in SHR are of great interest.

Ozaki\(^11\) studied the monoamine metabolism in SHR compared with normotensive rats and observed that the heart NA and serotonin, and also adrenal CA are slightly elevated in SHR. Okamoto's coworkers\(^12\) reported that adrenal NA content in SHR is increased nearly twice as much as in controls when the animals are sacrificed under strict conditions without unnecessary stress. Later Ozaki\(^13\) supported their data and according to his detailed studies, among the activities of aromatic amino acid decarboxylase, monoamine oxidase (MAO) and catechol-O-methyl transferase in various organs, only liver MAO activity showed a decrease in SHR at the age of 6–8 months, while a clear increase in heart MAO activity was observed histochemically by Tabei\(^14\).

Tabei\(^14\) tried NA reaction histochemically using glutaraldehyde osmium tetroxide technique on the adrenals in SHR and determined that the dimensional ratio of the NA storing cell islets to the whole adrenal medulla was about twice as great as that of controls. Moreover, Morisawa's detailed studies\(^15\) on the adrenal medulla in SHR showed that the ratio of NA storing cell islets was already increased even 6 days after birth and became twice as great as that of controls on the 18th day of life. His further observations on the NA storing cell islets under various experimental conditions suggested that both synthesis and secretion of the NA of the adrenal medulla in SHR are increased as compared with those of controls.

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The purpose of this study is to determine quantitatively the CA contents in adrenal gland in relation to the development of hypertension in SHR. Consequently, primarily in this study, we measured the blood pressure and NA, AD levels in the adrenals in SHR at various ages during the development of hypertension and thereafter, and compared the results with those on the age-matched normotensive Wistar rats.

**Materials and Methods**

Twenty four normotensive male Wistar rats supplied from the Animal Center Laboratory in Kyto University, from the ancestor of which SHR had been separated, were taken as the controls and 29 SHR of F1-17 in Okamoto’s lineage were used in this experiment. Both groups were classified into three stages according to the age, that is, 40-60 days, 4-6 months and over 1 year after birth. In SHR, the first stage, 40-60 days after birth, corresponded the prehypertensive stage, the second stage, 4-6 months of age, was the early stage of hypertension and the third stage, over 1 year of age, included the rats which had already maintained stable high blood pressure for more than 6-8 months. All these rats were kept in air conditioned rooms (23±2°C with humidity of about 60 per cent), fed on stock chow diet (CLEA Co., Japan) and drinking water was ad libitum.

Blood pressure (B.P.) was measured by the tail-water plethysmographic method9 once a week from the 5th week of life.

Each rat was placed in a cage at least one day before sacrifice and not disturbed all through the night until the next early morning, when the rat was sacrificed by decapitation as quickly as possible with great caution to avoid unnecessary stress.

Catecholamine contents were measured spectrofluorometrically according to the method of Crout.10 Immediately after the excision of the right adreanal gland, it was weighed, homogenized and deproteinized with cold 5 per cent trichloracetic acid in N/10 HCl and centrifuged, while the weight of left adrenal gland was checked. Each aliquot of 0.5ml was adjusted to pH 8.4 by the dropwise addition of NH4OH with the acetate buffer containing disodium ethylenediamine tetraacetate, and CA was adsorbed by the alumina oxide. For the measurement of NA and AD in the effluent from the alumina column, the trihydroxyindole reaction was carried out at pH 6.5 and pH 3.5, and then fluorescence was read by the Amino-Bowman or Farrand spectrofluorometer (activation peak at 395 mλ and fluorescence peak at 505 mλ for the pH 6.5 series; 410 mλ and 520 mλ for pH 3.5 series). In this series of the experiment internal CA recoveries were not less than 85 per cent.

**Results**

1. Blood Pressure and Body Weight

Average blood pressure and mean body weight are shown in Table I.

In the first stage, B.P. of SHR was a little higher than that of the controls, and some of them had already reached up to 150 mmHg. In the second stage, the difference in B.P. between two groups became much greater than in the first stage and the B.P. of SHR were over 150 mmHg in all of them. B.P. of SHR in the se-

<table>
<thead>
<tr>
<th>Age</th>
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<tbody>
<tr>
<td>40-60 days</td>
</tr>
<tr>
<td>4-6 months</td>
</tr>
<tr>
<td>Over 1 year</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Experimental Group (No. of Animals)</th>
<th>Blood Pressure (mm Hg)</th>
<th>Body Weight (g)</th>
<th>Weight of Adrenal Gland (mg)</th>
<th>Noradrenaline (ng/rg-Adrenal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40-60</td>
<td>NR (12)</td>
<td>121.3 ± 8.4</td>
<td>46.1 ± 45.8</td>
<td>16.6 ± 5.8</td>
</tr>
<tr>
<td>days</td>
<td>SHR (10)</td>
<td>137.9 ± 15.3</td>
<td>152.2 ± 35.3</td>
<td>18.3 ± 4.7</td>
</tr>
<tr>
<td>4-6</td>
<td>NR (6)</td>
<td>119.3 ± 8.3</td>
<td>261.2 ± 42.8</td>
<td>18.7 ± 2.9</td>
</tr>
<tr>
<td>Months</td>
<td>SHR (13)</td>
<td>188.3 ± 23.7</td>
<td>274.6 ± 45.0</td>
<td>22.5 ± 2.9</td>
</tr>
<tr>
<td>Over 1 year</td>
<td>NR (6)</td>
<td>120.2 ± 7.0</td>
<td>415.8 ± 37.0</td>
<td>22.5 ± 2.3</td>
</tr>
<tr>
<td>SHR (6)</td>
<td>185.7 ± 7.0</td>
<td>381.5 ± 24.2</td>
<td>28.5 ± 3.2</td>
<td>3.22 ± 1.9</td>
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M±SD
Significant difference

\*
\*0.01 < p < 0.05

\**0.001 < p < 0.01

\***0.001 > p

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cond and third stages was kept approximately at the same level. On the other hand, B.P. of the controls remained around 120 mmHg throughout these three stages.

The mean body weight in SHR group was not significantly different from that of the control and was slightly decreased in the third stage, when the cardiovascular pathological lesions were noted.

2. Weight of Adrenal Glands

The left adrenals were slightly heavier than the right ones but there were no significant differences between right and left adrenals both in SHR and control groups. Significant differences were noted in weight of the adrenal glands between the controls and SHR in the second and the third stages, especially in the later stage. In SHR adrenal enlargement was observed after 4 months to over a year of age.

3. Catecholamine Contents in Adrenal Glands

(Table I, Fig. 1)

As the right and left adrenal weights were not significantly different from each other and the CA contents in both adrenals were confirmed to be approximately the same by our preliminary studies, the CA contents of right adrenals were measured in this experiment.

The levels of NA (both µg per one adrenal and mg per g of adrenal weight) in SHR were markedly higher and almost twice as much as those in the controls. The amount of NA per one rat, which should be about twice as much as the content per one adrenal, was consequently supposed to be prominently increased in SHR compared with the controls. On the other hand, AD contents per adrenal in SHR were significantly greater than the controls only in the second and the third stages, and the AD level per g of adrenal weight in SHR was not significantly increased throughout these three stages. There was not so much difference in total catecholamine contents (NA + AD) per g of adrenal between SHR and the controls except in the second stage. Although NA and AD contents per one adrenal in SHR were elevated gradually from the first to the third stage, this tendency was not noted in NA and AD levels per g of adrenal weight, especially in the last stage because of the adrenal hypertrophy due to cortical hyperplasia usually found in old SHR.

The evidently increased level of adrenal NA in SHR appeared to be correlated with the increase in blood pressure, especially from the first toward the second stage.

**DISCUSSION**

As shown in our results, the levels of adrenal NA in SHR were almost twice as high as those of controls and appeared to be correlated with the development of the hypertension in these rats.

Tabei's and Morisawa's histochemical studies on the adrenal medulla of SHR already revealed an evident increase in the dimension

<table>
<thead>
<tr>
<th>Adrenaline (µg/r-Adrenal)</th>
<th>Noradrenaline (mg/g)</th>
<th>Adrenaline (µg/g)</th>
<th>Noradrenaline + Adrenaline (mg/g)</th>
<th>Noradrenaline (µg/rat)</th>
<th>Adrenaline (µg/rat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.09</td>
<td>0.133</td>
<td>0.458</td>
<td>0.592</td>
<td>4.38</td>
<td>14.31</td>
</tr>
<tr>
<td>± 1.40</td>
<td>± 0.020</td>
<td>± 0.133</td>
<td>± 0.137</td>
<td>± 1.31</td>
<td>± 3.42</td>
</tr>
<tr>
<td>7.48</td>
<td>0.235***</td>
<td>0.423</td>
<td>0.660</td>
<td>8.74***</td>
<td>15.42</td>
</tr>
<tr>
<td>± 1.07</td>
<td>± 0.039</td>
<td>± 0.088</td>
<td>± 0.116</td>
<td>± 2.19</td>
<td>± 2.70</td>
</tr>
<tr>
<td>10.47</td>
<td>0.135</td>
<td>0.880</td>
<td>1.015</td>
<td>5.13</td>
<td>33.8</td>
</tr>
<tr>
<td>± 4.09</td>
<td>± 0.026</td>
<td>± 0.137</td>
<td>± 0.160</td>
<td>± 0.80</td>
<td>± 6.93</td>
</tr>
<tr>
<td>21.58*</td>
<td>0.292***</td>
<td>0.965</td>
<td>1.257**</td>
<td>13.24***</td>
<td>43.21**</td>
</tr>
<tr>
<td>± 3.85</td>
<td>± 0.066</td>
<td>± 0.162</td>
<td>± 0.177</td>
<td>± 3.09</td>
<td>± 7.75</td>
</tr>
<tr>
<td>13.77</td>
<td>0.160</td>
<td>0.655</td>
<td>0.815</td>
<td>7.22</td>
<td>28.9</td>
</tr>
<tr>
<td>± 5.53</td>
<td>± 0.030</td>
<td>± 0.209</td>
<td>± 0.324</td>
<td>± 0.76</td>
<td>± 10.75</td>
</tr>
<tr>
<td>24.95**</td>
<td>0.263*</td>
<td>0.895</td>
<td>1.158</td>
<td>15.90**</td>
<td>53.55**</td>
</tr>
<tr>
<td>± 5.11</td>
<td>± 0.101</td>
<td>± 0.224</td>
<td>± 0.252</td>
<td>± 5.07</td>
<td>± 10.02</td>
</tr>
</tbody>
</table>

NR : Normotensive control rats
SHR: Spontaneously hypertensive rats
r : right

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of NA storing cell islets even in quite young rats and their histochemical findings were confirmed by our quantitative data.

It was demonstrated that the releasable CA of the adrenal medulla and the heart in SHR was increased by the injection of tyramin\textsuperscript{13}, and the dimensional ratio of NA storing cell islets in SHR decreased easily under various stress-loading and recovered faster thereafter than in the controls\textsuperscript{15}. Consequently, the increased NA in the adrenal medulla of SHR is conceived to be not the only retained one but also easily releasable one and closely related with sympathetic function.

![Graph](image)

NA has been shown to cause an elevation of B. P. by bringing about an increase in total peripheral resistance, often accompanied by a decrease in cardiac output; AD however causes an elevation of systolic B. P. by increasing the cardiac output. According to DEnCKLA\textsuperscript{17}, the total peripheral vascular resistance in SHR appeared to be increased in comparison with that of the controls, while the cardiac output was decreased. These data were quite similar to the pharmacodynamic action of NA as mentioned above, and it was suggested that the increased NA level of the adrenal medulla in SHR may partly participate in the maintenance or development of the spontaneous hypertension.

On the other hand, OKAMOTO et al.'s electrophysiological observations\textsuperscript{18} on the spontaneous splanchnic discharge and their transection experiments at the various levels of the brainstem in SHR offered the decisive evidences that the increased sympathetic vasoconstrictor discharge originating from the lower brainstem constitutes one important factor in the pathogenesis of, or at least, in the maintenance of hypertension in SHR. Moreover, MATSUMOTO's histometrical and histochemical studies\textsuperscript{19} on the superior cervical ganglion in SHR revealed that the nuclear and cellular sizes of the nerve cells and some enzyme activities are increased in comparison with those of controls. Recent histochemical observations on the NA fluorescence of the peripheral vascular system in SHR showed an increase in intensity of the fluorescence\textsuperscript{20}. As both adrenal medulla and sympathetic ganglia are innervated by sympathetic preganglionic fibers and regulated by the sympathetic nervous system as well as the peripheral vascular system by sympathetic vasmotor nerve, our findings are evidently consistent with the above-mentioned observations suggestive of the sympathetic hyperfunction in SHR. We can not here jump to the conclusion that only humoral catecholamine released from adrenal medulla is an initiating or maintaining factor of spontaneous hypertension. An increase in adrenal NA content in SHR might be only one of the numerous neural and endocrine factors of spontaneous hypertension already proved in SHR\textsuperscript{10} and could be taken as an indicator of the sympathetic hyperfunc-

*Fig. 1. Blood pressure and adrenal catecholamine contents in spontaneously hypertensive rats and normotensive control rats at their three stages of life.*
tion in this animal.

As for the practical measurements of adrenal CA which is easily releasable, proper care should be taken to keep the rats under minimum stress before sacrifice as described in this article, for especially SHR appears to be more responsive to various kinds of stress than the controls\textsuperscript{15,21}.

So far as investigated up to the present, there is a bulk of evidences for concluding that the hypertension in SHR is more relevant to essential hypertension than any other experimentally induced hypertension\textsuperscript{10}. However, adrenal CA content in the patient with essential hypertension can not be determined under undebatable conditions and urinary CA excretion never gives exact information about the CA secretion from adrenal medulla, although the data on the urinary CA excretion in essential hypertension has been accumulated. Therefore, our present findings indicate the necessity of further investigations on detailed catecholamine metabolism in SHR in order to find any clue to clarify the role of catecholamine in essential hypertension.

**Summary**

Adrenal catecholamine contents were measured spectrofluorometrically by Croult's method in 29 male spontaneously hypertensive rats at the prehypertensive, early and advanced hypertensive stages and in 24 age-matched male normotensive Wistar rats. Adrenal noradrenaline contents per adrenal, per animal, and per gram of adrenal weight in spontaneously hypertensive rats were almost twice as high as those in the controls throughout these stages. Adrenaline contents per adrenal were larger in the early hypertensive stage in the former rats, especially in the advanced one, but no great differences in adrenaline content per gram of adrenal weight were noted between the former and latter groups.

**Acknowledgement**

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