Studies on Adrenocortical Hormone, Electrolytes and Ultrastructural Features of the Adrenal Cortex in Spontaneously Hypertensive Rat

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Recently, some morphological evidences of hyperfunction of the adrenal cortex have become apparent in spontaneously hypertensive rat (SHR). The present study gave higher fluorimetric assay for either corticosterone and cortisol in SHR at the advanced stage of development of hypertension. Electron microscopically, the mitochondria was closely proliferated, and the smooth surfaced endoplasmic reticulum of the zona fasciculata was well developed. In the zona glomerulosa, the Golgi complex was more prominent in SHR. These findings would suggest that hyperfunctional stage of the adrenal cortex still remained in the zona glomerulosa and zona fasciculata in SHR. The relationship between corticosteroids and their property of inducing hypertension was discussed.

INTRODUCTION

In 1963, the spontaneously hypertensive rat (SHR) with 100% incidence of heredity has been separated (16). They develop hypertension spontaneously around four months after birth without any artificial procedure, just similarly to essential hypertension in man. Many studies on these rats have been made up to the present time. Among these studies, the following seem to be concerned with the pathogenesis of hypertension in the rat, particularly in respect of the endocrine system: An evidence on hyperfunction of adenohypophysis-adrenocortical and adenohypophysis-thyroid system, and further, on increased area of noradrenalin storing cell islets in the adrenal medulla (21). Recently, a zymohistochemical study and electron microscopic observations on the adrenal cortex of SHR were
performed by Tsuchiya, Sugihara and Kawai (9) (24), and they found out some positive findings of hyperfunction of the zona glomerulosa and the zona fasciculata of the adrenal cortex in SHR. In this study a determination of plasma glucocorticoid and serum electrolytes and electron microscopic observations were made on SHR at the advanced stage of hypertension.

**MATERIAL AND METHODS**

SHR F15 at the advanced stage of hypertension (17) that had been produced at the Department of pathology, Faculty of Medicine, Kyoto University were prepared for the examination (16). Another Wister strain rats of the same age distributions supplied by the Animal Center Laboratory, Faculty of Medicine, Kyoto University were used as the control. They were fed Oriental stock chow diet (MF and NMF) (Oriental Yeast Co., Japan) and were given tap water. Table 1.

<table>
<thead>
<tr>
<th>Table 1. Age Distribution and Body Weight of SHR and Control Examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>------</td>
</tr>
<tr>
<td>Maximum</td>
</tr>
<tr>
<td>Minimum</td>
</tr>
<tr>
<td>Mean</td>
</tr>
</tbody>
</table>

* Mean standard error

shows age distribution ranging from 16 to 20 months of SHRs and from 9 to 20 months of control Wister rats, and average body weight of 363 gm. ranging from 240 to 700 gm. of SHRs and of 382 gm. ranging from 250 to 570 gm. of control Wister rats. They included 3 cases out of 22 normotensive and 2 cases of unknown F15 SHRs in the former group. Among 24 control rats, 5 rats were found to be hypertensive, which were not excluded from the latter group. These materials were decapitated exactly 5 minutes after intraperitoneal injection of 30 mg. per kg body weight of Nembutal (Abbott Lab.). The rats had been trained in the manner of pricking before their sacrifice. And the whole amount of peripheral blood obtained was heparinized separately for biological analysis, and was partially nonheparinized to estimate serum electrolytes.

The blood pressure was measured indirectly without anesthesia using a modified tail-water plethysmographic method (16). Plasma corticosterone and cortisol were analysed by means of simultaneous fluorimetric determination (7), a modification of Silber, Busch and
Osnapas' method (19), which depends on the fact that fluorimetric intensity ratio of corticosterone to cortisol in 6:4 (v/v) sulfuric acid-ethanol reagent is much higher than that in 9:1 (v/v) reagent (7). Each stock solution of corticosterone and cortisol which contained 2 \( \mu \text{g/ml} \) of steroid was diluted with ethanol for working standards. Each working standard contained 0.04 \( \mu \text{g/ml} \) of corticosterone and cortisol. Serum electrolytes were detected by Technicon Flame-photometer type 3. The data thus obtained were statistically evaluated by t-test.

For electron microscopic examination the adrenal glands were obtained from 3 SHRs and 4 control rats aged 12 to 16 months. Small pieces of the adrenal tissue were fixed in 0.1 M phosphate-buffered 1.0 per cent glutaraldehyde with perfusion method followed by 1.3 per cent osmium tetraoxide. After rapid dehydration in increasing concentration of ethanol, the material was embedded in Epon 812 according to the method of Luft. Thin sections were made with JUM-5 microtome and were stained with uranyl acetate and lead. JEM-P 5 electron microscope was used.

RESULTS

Both of the adrenal glands were weighed immediately after the necropsy. It averaged 65.4 mg. in SHR and 50.7 mg. in control with standard error of 4.4 and 3.3 respectively. The adrenal weighed significantly more in SHR than in control as observed previously (Table II) (1).

The systolic blood pressure was 162.0 ± 3.7 mmHg in SHR and was 137.4 ± 3.7 mmHg in control. There were 3 cases of normotensive and 2 cases of unknown among 22 SHRs, and they included 5 hypertensive cases out of 24 control rats.

The fluorescence intensity of plasma corticosterone and cortisol was measured using Farrand Fluorometer with an exciting wave length of 470 m\( \mu \) and a fluorescence wave length of 530 m\( \mu \). The results were listed on Table II and III. The recoveries of corticosterone were as follows: in 6:4 (v/v) sulfuric acid-ethanol reagent 99.4 per cent ranging from 92.2 to 110 per cent, and in 9:1 reagent 107.8 per cent ranging from 99.6 to 120 per cent. That of cortisol ranged from 112.0 to 130 per cent with 119.9 per cent on average in 6:4 sulfuric acid-ethanol reagent and averaged 110.8 per cent ranging from 98.1 to 117.7 per cent in 9:1 reagent. The plasma corticosterone content was 7.6 ± 1.0 \( \mu \text{g/dl} \) in SHR, and was 5.1 ± 0.5 \( \mu \text{g/dl} \) in control rat. The plasma cortisol was not be demonstrated in almost all of both SHR and control. A very small quantity of plasma cortisol, however, was detected in some individuals of both groups.
Table II. Blood Pressure, Plasma Corticosterone and Plasma Cortisol Content of SHR and Control Examined

<table>
<thead>
<tr>
<th>No.</th>
<th>SHR Blood Pressure</th>
<th>SHR Plasma Corticosterone</th>
<th>SHR Plasma Cortisol</th>
<th>Control Blood Pressure</th>
<th>Control Plasma Corticosterone</th>
<th>Control Plasma Cortisol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>152 mmHg</td>
<td>3.2 µg/dl</td>
<td>3.6 µg/dl</td>
<td>130 mmHg</td>
<td>12.6 µg/dl</td>
<td>0 µg/dl</td>
</tr>
<tr>
<td>2</td>
<td>?</td>
<td>6.0 µg/dl</td>
<td>0 µg/dl</td>
<td>134 mmHg</td>
<td>3.4 µg/dl</td>
<td>0.4 µg/dl</td>
</tr>
<tr>
<td>3</td>
<td>180 mmHg</td>
<td>4.0 µg/dl</td>
<td>2.0 µg/dl</td>
<td>124 mmHg</td>
<td>3.6 µg/dl</td>
<td>1.6 µg/dl</td>
</tr>
<tr>
<td>4</td>
<td>160 mmHg</td>
<td>9.4 µg/dl</td>
<td>0 µg/dl</td>
<td>152 mmHg</td>
<td>3.6 µg/dl</td>
<td>0 µg/dl</td>
</tr>
<tr>
<td>5</td>
<td>172 mmHg</td>
<td>6.6 µg/dl</td>
<td>3.0 µg/dl</td>
<td>138 mmHg</td>
<td>10.0 µg/dl</td>
<td>0 µg/dl</td>
</tr>
<tr>
<td>6</td>
<td>154 mmHg</td>
<td>4.2 µg/dl</td>
<td>3.0 µg/dl</td>
<td>126 mmHg</td>
<td>6.0 µg/dl</td>
<td>0 µg/dl</td>
</tr>
<tr>
<td>7</td>
<td>?</td>
<td>2.4 µg/dl</td>
<td>0.6 µg/dl</td>
<td>152 mmHg</td>
<td>5.2 µg/dl</td>
<td>0 µg/dl</td>
</tr>
<tr>
<td>8</td>
<td>158 mmHg</td>
<td>4.2 µg/dl</td>
<td>6.0 µg/dl</td>
<td>134 mmHg</td>
<td>2.0 µg/dl</td>
<td>2.0 µg/dl</td>
</tr>
<tr>
<td>9</td>
<td>142 mmHg</td>
<td>9.0 µg/dl</td>
<td>4.2 µg/dl</td>
<td>174 mmHg</td>
<td>4.0 µg/dl</td>
<td>0 µg/dl</td>
</tr>
<tr>
<td>10</td>
<td>172 mmHg</td>
<td>10.2 µg/dl</td>
<td>1.8 µg/dl</td>
<td>120 mmHg</td>
<td>2.0 µg/dl</td>
<td>2.0 µg/dl</td>
</tr>
<tr>
<td>11</td>
<td>170 mmHg</td>
<td>6.6 µg/dl</td>
<td>0 µg/dl</td>
<td>140 mmHg</td>
<td>6.0 µg/dl</td>
<td>0 µg/dl</td>
</tr>
<tr>
<td>12</td>
<td>164 mmHg</td>
<td>10.2 µg/dl</td>
<td>0 µg/dl</td>
<td>144 mmHg</td>
<td>4.0 µg/dl</td>
<td>0 µg/dl</td>
</tr>
<tr>
<td>13</td>
<td>154 mmHg</td>
<td>8.4 µg/dl</td>
<td>2.4 µg/dl</td>
<td>144 mmHg</td>
<td>4.4 µg/dl</td>
<td>0 µg/dl</td>
</tr>
<tr>
<td>14</td>
<td>176 mmHg</td>
<td>26.4 µg/dl</td>
<td>0 µg/dl</td>
<td>164 mmHg</td>
<td>4.8 µg/dl</td>
<td>0 µg/dl</td>
</tr>
<tr>
<td>15</td>
<td>174 mmHg</td>
<td>6.6 µg/dl</td>
<td>0 µg/dl</td>
<td>142 mmHg</td>
<td>8.4 µg/dl</td>
<td>0 µg/dl</td>
</tr>
<tr>
<td>16</td>
<td>?</td>
<td>8.0 µg/dl</td>
<td>4.0 µg/dl</td>
<td>144 mmHg</td>
<td>4.8 µg/dl</td>
<td>0 µg/dl</td>
</tr>
<tr>
<td>17</td>
<td>?</td>
<td>4.8 µg/dl</td>
<td>2.4 µg/dl</td>
<td>128 mmHg</td>
<td>2.8 µg/dl</td>
<td>3.2 µg/dl</td>
</tr>
<tr>
<td>18</td>
<td>198 mmHg</td>
<td>5.4 µg/dl</td>
<td>0.6 µg/dl</td>
<td>108 mmHg</td>
<td>3.6 µg/dl</td>
<td>2.0 µg/dl</td>
</tr>
<tr>
<td>19</td>
<td>170 mmHg</td>
<td>12.6 µg/dl</td>
<td>0 µg/dl</td>
<td>? mmHg</td>
<td>6.0 µg/dl</td>
<td>0 µg/dl</td>
</tr>
<tr>
<td>20</td>
<td>168 mmHg</td>
<td>6.0 µg/dl</td>
<td>2.0 µg/dl</td>
<td>176 mmHg</td>
<td>4.2 µg/dl</td>
<td>0 µg/dl</td>
</tr>
<tr>
<td>21</td>
<td>176 mmHg</td>
<td>8.3 µg/dl</td>
<td>0.8 µg/dl</td>
<td>148 mmHg</td>
<td>6.0 µg/dl</td>
<td>0 µg/dl</td>
</tr>
<tr>
<td>22</td>
<td>150 mmHg</td>
<td>6.0 µg/dl</td>
<td>2.0 µg/dl</td>
<td>110 mmHg</td>
<td>5.4 µg/dl</td>
<td>0 µg/dl</td>
</tr>
<tr>
<td>23</td>
<td>?</td>
<td>?</td>
<td>? µg/dl</td>
<td>? mmHg</td>
<td>4.2 µg/dl</td>
<td>0 µg/dl</td>
</tr>
<tr>
<td>24</td>
<td>120 mmHg</td>
<td>7.2 µg/dl</td>
<td>0 µg/dl</td>
<td>? mmHg</td>
<td>? µg/dl</td>
<td>? µg/dl</td>
</tr>
</tbody>
</table>

Table III. Blood Pressure, Weight of the Adrenal, Plasma Corticosterone, Plasma Cortisol and Electrolytes Estimating the Difference between SHR and Control

<table>
<thead>
<tr>
<th>SHR Blood Pressure</th>
<th>Control Blood Pressure</th>
<th>Estimation</th>
</tr>
</thead>
<tbody>
<tr>
<td>162±3.7 mmHg</td>
<td>137±3.7 mmHg</td>
<td>Significant*</td>
</tr>
<tr>
<td>Weight of adrenal</td>
<td>65.4±4.4 mg</td>
<td>Significant**</td>
</tr>
<tr>
<td>Plasma corticosterone</td>
<td>7.6±1.0 µg/dl</td>
<td>Non-significant**</td>
</tr>
<tr>
<td>Plasma cortisol</td>
<td>1.8±0.3 µg/dl</td>
<td>Significant*</td>
</tr>
<tr>
<td>Electrolytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na</td>
<td>142±0.5 mEq/l</td>
<td>Significant**</td>
</tr>
<tr>
<td>K</td>
<td>5.3±0.2</td>
<td>Non-significant**</td>
</tr>
<tr>
<td>Cl</td>
<td>100±1.0</td>
<td>Non-significant**</td>
</tr>
</tbody>
</table>

*: P 0.01 **: P 0.05
They were calculated as $1.8 \pm 0.3 \mu g/dl$ in SHR and $0.4 \pm 0.2 \mu g/dl$ in control (Table I).

According to Table I, the serum sodium level is statistically higher in SHR than in control.

Electron microscopically, the cells of the zona glomerulosa in SHR showed prominent Golgi complex adjacent to the nuclei. The number of Golgi complex was increased and the dense bodies were found not only within the Golgi region, but were scattered throughout the cytoplasm. In some areas large spherical vacuoles containing membranebound dense osmiophilic material were seen. The mitochondria of the zona fasciculata in SHR were closely packed and tended to be round with tubular cristae (Figure 1 and 2). The smooth-surfaced endoplasmic reticulum of the zona fasciculata were well developed and occasionally dilated. Many cells of the inner zona fasciculata and reticularis appeared to be increased in number of lysosomes in SHR (Figure 3).

**DISCUSSION**

In the present study the total amount of plasma corticosterone was generally smaller than in other results (12) (19) (25) (26). The level of corticosterone per 100 ml of plasma was found to be higher in
SHR than in control, but not to be significant statistically. It is generally accepted that there is no appreciable amount of cortisol in the rat plasma fluoroscopically. Almost no case, particularly of the control group contained any cortisol in the peripheral blood. A small number of SHR, however, showed fluorimetrically the significantly higher content on average of plasma cortisol.

Both cortisol, the major glucocorticoid of the human adrenal and corticosterone, and also the major glucocorticoid of the rat adrenal have a slightly salt-retaining activity. A high level of serum sodium in SHR may be considered to be influenced and, even partially, maintained by the higher plasma concentration of these glucocorticoid. BROWNIE and SKELTON made several suggestions in viewpoint of adrenalregenerating hypertension and pointed out there was an increased secretion of glucocorticoid, presumably of corticosterone (3).

Recently, a relationship between adrenocortical steroids other than aldosterone and essential hypertension is keenly discussed. It has been shown in the literatures that in essential hypertension both renin and angiotensin have the capability to stimulate cortisol and corticosterone secretion through the adrenal (4) (8). Furthermore, these steroids have the property of inducing hypertension (10) (22). There are also some observations which confirmed excessive, constant, and prolonged secretion of cortisol in Cushing's disease (5) (11) and in essential hypertension (27).

These observations have given rise to the speculation that cortisol oversecretion can induce high blood pressure in Cushing's syndrome, presumably by direct or indirect effect on the walls of blood vessels (5) (11).

Many works concerning the relationship between experimental hypertension and sympatico-adrenomedullary system and hypothalamo-hypophyseal neurosecretory system in SHR (6) (13) (14) appeared. On the other hand, it is considered that there is also adrenohypophysial-adrenocortical hyperfunction in these rats (1) (2).

Tsuchiyama, Sugihara and Kawai demonstrated some interesting findings of the adrenal cortex in SHR at the early stage of development of hypertension (9) (20) (24). These findings disclosed: a) The cell cord of zona fasciculata showed acidophilic and trabecular appearances. b) These cells contained only minute fine droplets of lipid. c) The secondary alcohol dehydrogenase activity was generally reduced throughout the zona fasciculata. d) Electron microscopically the mitochondria was closely and actively proliferated, and the smooth endoplasmic reticulum of the zona fasciculata was also well developed. Moreover, the Golgi complex was prominent in the zona glomerulosa.

From these above-mentioned results they concluded that the morphological changes of the adrenal cortex in SHR at the early hypertensive stage suggest a hyperactive phase in the glomerular and
fascicular zones of the adrenal gland.

In the present study, the author pointed out a few characteristic ultrastructural findings of the adrenal cortex in SHR at the advanced stage of hypertension. These findings would suggest that hyperfunctional state of the adrenal cortex still remains in the zona glomerulosa and fasciculata of SHR but is partially accompanied by an exhaustive tendency of these adrenocortical cells.

High content of corticosterone and cortisol in the plasma and morphologically hyperactive appearances of the adrenal cortex may occur not primarily but secondarily at the advanced stage of development of hypertension in SHR. It is now debatable whether corticosteroids could play a role in inducing such type of hypertension as in SHR. It is expected further to study the plasma concentration of these corticosteroids during the pre- or early stage of development of hypertension in the rat.

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REFERENCES

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