Genetic Factors Involved in Spontaneous Hypertension in Rats
An Analysis of F₂ Segregate Generation*

YUKIO YAMORI, AKIRA OSHIMA, AND KOZO OKAMOTO

Genetic factors involved in spontaneous hypertension were analyzed in the F₂ generation obtained between spontaneously hypertensive and normotensive rats. Positive correlations to blood pressure were observed in the heart, pituitary and adrenal weights, and in the thyroidal acid phosphatase activity. A weak inverse correlation was observed between blood pressure and aromatic L-amino acid decarboxylase activity of the brainstem. The F₂ with the hypertensive pattern of renal esterase isozymes showed a significantly higher blood pressure than the F₂ with the normotensive pattern. These findings correlated to blood pressure may be related to the genetic pathogenesis of spontaneous hypertension except for the secondary effect of hypertension.

Our former study¹ on the heredity of hypertension in spontaneously hypertensive rats (Okamoto and Aoki, SHR)²,³ clarified that; (1) the degree of the genetic determination of the blood pressure was as high as 86–96 per cent, indicating a great importance of genetic factors, (2) the mode of inheritance was additive, and (3) a relatively small number of major genes might be involved in this hypertension.

The heredity of this hypertension, although it is multigenic, is controlled by a small number of major genes. This result indicates the possibility of clarifying the genetic factors of hypertension controlled by these major genes. Up to the present various characteristics have been demonstrated in SHR³,⁴ and pathogenetic roles are ascribed to some of these characteristic findings indicating the dysfunction of autonomic nervous system⁵–⁷ and multiple endocrine glands⁸–¹²

In order to determine the pathogenetic relationship of these characteristics to hypertension, the F₂ segregate generation obtained between inbred SHR and inbred normotensive rats is the appropriate material, for the F₂ with various combinations of the genes from SHR and normotensive rats show the wide variation of blood pressure from normotensive to hypertensive level. Consequently, some quantitative indices of endocrine and nervous systems, which were characteristic in SHR, were examined in the individual rats of F₂ generation in relation to their blood pressure.

MATERIALS AND METHODS

Cross generations were produced between both inbred strains of rats, SHR (F₁) and Wistar-Mishima (F₅₈) (WM). Four SHR's (males; 217502, 217505, females; 217506, 217508, which were the offspring obtained by mating between a male 200030, and a female 200034) were mated with 4 WM to produce 41 F₁ hybrids. From the intercrossing of these F₁ hybrids, F₂ were obtained.

Key Words: Spontaneously Hypertensive Rats
Hypertension, Heredity
Brainstem Aromatic L-Amino Acid
Decarboxylase
Thyroidal Acid Phosphatase
Adrenal Glucose-6-Phosphate
Dehydrogenase
Renal Esterase Isozyme
Hypophysal Weight,
Heart Weight

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Among them 53 males, which showed a greater variation in blood pressure than the females, were used for the present analysis with the age-matched 22 male SHR (F₂) and 24 male WM (F₅₉). The blood pressures were measured weekly without anesthesia by a tail-plethysmographic method. The detailed data of the blood pressures as well as the conditions of housing and feeding of these animals were reported in the previous article.

They were sacrificed by decapitation at the age of 7 months. The heart, kidney and endocrine organs were dissected carefully, blotted on paper, weighed, frozen on dry ice and kept in the refrigerator at -20°C until the enzyme or isozyme assay was ready to be made. Brainstem were chilled after dissection and analyzed for aromatic L-amino acid decarboxylase activity as previously reported. Acid phosphatase (ACP) activity of the thyroid was assayed by the method of Bessey et al.¹³ using 0.1 ml of the supernatant obtained after centrifuging (at 15,000 g for 10 min) 3 ml-water homogenate of one thyroid. Glucose-6-phosphate dehydrogenase (G6PD) activity was assayed by the method of Kornberg and Horecker,¹⁴ using 0.1 ml of the supernatant obtained after centrifugation (15,000 g, for 10 min) of 2.5-ml-water homogenate of one adrenal. Protein concentration was determined by a modification of the phenol reagent method.¹⁵ Renal esterase isozymes were analyzed by an enzyme-histochemical technique for α-naphthyl acetate esterase after starch gel electrophoresis as previously reported.¹⁶ The samples from SHR, WM and F₂ were always handled under the same conditions. The numerical data was statistically analyzed by Student's t-test, and regression equation as well as coefficient of correlation between blood pressure and these data were obtained.

RESULTS
1. Relationship between Blood pressure and Organ Weight in F₂ Segregate Generation (Fig.1, Table I)
Heart weight of SHR (1.78 ± 0.23 g, M ± SD)
Fig. 2. Relationship between blood pressure and enzyme activity in the F₂ segregate generation (male) obtained between SHR and Wistar-Mishima (WM). SHR, WM and F₂ are shown as circles, squares and black dots, respectively. Two columns encircled with dotted lines in the lower graph show the blood pressure in the F₂ with the low decarboxylase activity corresponding to the SHR's range, and in those with the high activity equal to the WM's range, respectively.

was significantly (p<0.01) greater than that of normotensive WM (1.11 ± 0.14). The greatest correlation to blood pressure was observed in heart weight of F₂: Coefficient of correlation; \( r = 0.48 \pm 0.11 \), Regression equation; \( Y = 85.2 + 43.3X \) (Y = blood pressure in mmHg, X = weight)

in g) and this correlation was statistically significant.

Among the endocrine gland weights, pituitary showed the greatest and significant correlation to blood pressure: \( r = 0.31 \pm 0.13, Y = 114.8 + 3.2X \) (X = weight in mg). Pituitary weight of SHR (11 ± 1.1 mg) was significantly (p<0.01) greater than that of WM (9 ± 1.3). Although the adrenal weight of SHR (55 ± 8 mg) was not significantly greater than that of WM (53 ± 7), a weak but significant correlation between blood pressure and adrenal weight was observed in \( F_2: r = 0.29 \pm 0.13, Y = 115.0 + 0.61X \) (X = weight in mg). Thyroid weight was significantly (p<0.01) greater in SHR (28 ± 6) than in WM (20 ± 4), but no significant correlation to blood pressure was observed in \( F_2 \).

No other organ weight examined showed a significant correlation to blood pressure in \( F_2 \) generation.

2. Relationship between Blood Pressure and Enzyme Activity in \( F_2 \) Segregate Generation

(Fig.2, Table I)

G6PD of the adrenal and ACPase of thyroid showed a significantly greater activity in SHR than in WM as shown in Table I, and were assayed in \( F_2 \) as two representative enzymes of these endocrine organs. The specific activity of G6PD of adrenal glands showed no clear correlation to blood pressure in \( F_2 \). However, the total thyroidal ACPase activity showed a significant correlation to blood pressure: \( r = 0.27 \pm 0.13, Y = 113.3 + 19.4 \log X \) (X = total activity in \( \mu \)M/h).

As for the enzyme activity of the nervous system in SHR aromatic L-amino acid decarboxylase activity of the brainstem was significantly (p<0.001) lower in SHR than in WM as shown in Table I. A weak inverse correlation between blood pressure and the enzyme activity was observed in \( F_2: r = -0.24 \pm 0.14, Y = 162.7 - 1.4X \) (X = specific activity in \( \mu \)M/h mg protein). Although the significance of the correlation was not so clear, the \( F_2 \) with low decarboxylase activity corresponding to the range of SHR's activity (M ± 2SD) showed a significantly (p<0.05)
### TABLE I  RELATION OF VARIOUS FACTORS TO BLOOD PRESSURE IN F2 FROM SHR (F21-22) AND WISTAR-MISHIMA (F57-59)

<table>
<thead>
<tr>
<th></th>
<th>Blood pressure (mmHg)</th>
<th>Heart weight (g)</th>
<th>Pituitary weight (mg)</th>
<th>Adrenal weight (mg)</th>
<th>G6PD* weight (mg)</th>
<th>Thyroid weight (mg)</th>
<th>Brainstem Aromatic aminoacid decarboxylase</th>
<th>Kidney Esterase isozyme pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SHR</strong></td>
<td>184 ± 14.7</td>
<td>1.78 ± 0.23</td>
<td>11 ± 1.1</td>
<td>55 ± 8</td>
<td>74 ± 17</td>
<td>28 ± 6</td>
<td>98 ± 70</td>
<td>6.7 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>(21)</td>
<td>(21)</td>
<td>(21)</td>
<td>(20)</td>
<td>(11)</td>
<td>(21)</td>
<td>(15)</td>
<td>(21)</td>
</tr>
<tr>
<td><strong>WM</strong></td>
<td>120 ± 3.4</td>
<td>1.11 ± 0.14</td>
<td>9 ± 1.3</td>
<td>53 ± 7</td>
<td>58 ± 18</td>
<td>20 ± 4</td>
<td>31 ± 44</td>
<td>13.3 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>(24)</td>
<td>(23)</td>
<td>(23)</td>
<td>(23)</td>
<td>(13)</td>
<td>(23)</td>
<td>(16)</td>
<td>(24)</td>
</tr>
<tr>
<td><strong>F2</strong></td>
<td>148 ± 14.4</td>
<td>1.45 ± 0.07</td>
<td>10 ± 1.4</td>
<td>55 ± 6</td>
<td>57 ± 16</td>
<td>26 ± 5</td>
<td>65 ± 42</td>
<td>10.1 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>(52)</td>
<td>(49)</td>
<td>(49)</td>
<td>(49)</td>
<td>(29)</td>
<td>(49)</td>
<td>(50)</td>
<td>(48)</td>
</tr>
</tbody>
</table>

| Significant difference between SHR and WM | + | + | + | - | + | + | + | + |
| **P < 0.01** | **P < 0.01** | **P < 0.01** | **P < 0.05** | **P < 0.01** | **P < 0.001** | **P < 0.001** |

<table>
<thead>
<tr>
<th>Correlation with</th>
<th><strong>r</strong></th>
<th>0.48 ± 0.11</th>
<th>0.31 ± 0.13</th>
<th>0.29 ± 0.13</th>
<th>-0.05 ± 0.19</th>
<th>0.09 ± 0.13</th>
<th>0.27 ± 0.13</th>
<th>-0.24 ± 0.14</th>
</tr>
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<tbody>
<tr>
<td>blood pressure</td>
<td>Significance</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>±</td>
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<tr>
<td></td>
<td><strong>P &lt; 0.01</strong></td>
<td><strong>0.01 &lt; P &lt; 0.05</strong></td>
<td><strong>0.01 &lt; P &lt; 0.05</strong></td>
<td><strong>P = 0.05</strong></td>
<td><strong>P = 0.05</strong></td>
<td><strong>0.05 &lt; P &lt; 0.1</strong></td>
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</table>

M ± SD ( ): Number of cases examined  
* Specific activity (ΔOD340/min, mg × 10³)  
** Total activity (μM/h, one thyroid)  
*** Specific activity (μM/h, mg)  
S, N, and H: Zymogram pattern in SHR, control Wistar and hybrid F1
higher blood pressure (157 ± 12) than that (146 ± 12) in the F₂ with high decarboxylase activity within the range of WM (M ± 2SD).

3. Relationship between Blood Pressure and Zymogram Patterns of Esterase Isozymes of the Kidney in F₂ Segregate Generation (Fig. 3, Table I)

As previously reported⁴ the slow moving main esterase isozyme components from SHR’s kidney migrated faster toward the anode than those from WM. All SHR examined showed this strain-specific esterase isozyme pattern, while none of the WM had such a specific isozyme pattern. F₁ hybrids had both the isozyme components characteristic of SHR and controls, and showed an intermediate blood pressure between two parental strains⁴.

F₂ produced by the intercrossing among F₁ hybrids showed 3 zymogram patterns of esterase isozymes; They were SHR (S), hybrid (H) and normotensive control (N) patterns. Incidence of these patterns was 15S, 24H and 13N, and the ratio was not statistically different from the expected proportion of 1 : 2 : 1. The distribution of blood pressure in F₂ with these 3 patterns is shown in Fig. 3. The means of the blood pressure in F₂ with S, H, and N patterns were 152 ± 15.2, 149 ± 14.8 and 140 ± 12.3 mmHg, respectively, and the blood pressure of F₂ with S pattern was significantly (p<0.05) higher than that of F₂ with N pattern. By the backcrossing between F₁ hybrid and SHR or WM, the offspring with two zymogram patterns, H and S or N, were obtained, respectively. The backcross generation with S pattern showed a significantly (p<0.01) higher blood pressure (177 ± 9.8) than that with H pattern (162 ± 7.8), but there was no significant difference in blood pressure between the offspring with H pattern and that with N pattern.

Discussion

An analysis of the correlation between blood pressure and various characteristics in F₂ segregate generation obtained by the cross breeding between SHR and normotensive rats is supposed to be the appropriate method to prove the pathogenetic relationship of these characteristics to hypertension. In this analysis also the secondary effect of hypertension on these indices must be carefully eliminated. Among the various quantitative indices heart weight in F₂ showed the highest correlation to blood pressure. However, this is thought to be due to the secondary effect of hypertension, i.e., functional hypertrophy against the increased total peripheral vascular resistance, because cardiac output¹⁶ and catecholamine turnover of the heart¹⁷ in SHR are decreased after the development of hypertension. It is also because such a cardiac hypertrophy is observed in other forms of experimental hypertension.

The other indices, especially which show some difference in SHR even before the development of hypertension, may be concerning the pathogenesis of hypertension. The correlation of these indices to blood pressure, however, was relatively small. It is mainly because not only the small number of major genes but also multiple minor genes and the interaction among these genes are involved in the genetic pathogenesis of this hypertension. Rather clear correlation between pituitary weight and blood pressure indicates that the mild hyperfunction observed in the target endocrine organs in SHR is due to the central dysregulation involving pituitary gland⁸ and possibly, higher control center in the hypothalamus, too.¹⁰ Increased ACPase activity of the thyroid in SHR was observed histochemically⁹ and confirmed quantitatively in this study. As the functional significance of the thyroidal ACPase is speculated⁵ the significant correlation of the activity to blood pressure observed in F₂ indicates that thyroidal function participates in the pathogenesis of SHR and substantiates the former functional study on the thyroid in SHR¹² However, some F₂ with the thyroidal ACPase activity within control range showed hypertension. Consequently, it can be deduced that hypertension of SHR may develop even without marked activation of thyroidal function.

The reduction of aromatic L-amino acid decarboxylase activity in the brainstem⁷ and a concomitant decrease in norepinephrine level⁷ as well as a delay in norepinephrine synthesis from tyrosine¹⁸ might possibly be related to the insufficiency of the central noradrenergic sympathoinhibitory mechanism¹⁹,²⁰ and, therefore, to the pathogenesis of hypertension in SHR. As the inverse correlation between the brainstem decarboxylase activity and blood pressure was not so marked, it is obvious that the hypertension can not be ascribed to the abnormality of this enzyme activity, alone.

As for the renal esterase isozyme patterns, effect of the difference in the patterns on blood pressure level was rather marked in the backcross generation between F₁ hybrids and SHR, but not so marked in F₂ generation and could not be
observed in the backcross generation between F₁ and normotensive controls. Therefore, one gene controlling the SHR-specific esterase isozymes may affect the blood pressure through the interaction with other major genes of hypertension, or may be merely linked with one of the major genes.

As observed in the previous study on blood pressure in the F₂ segregate generation, the onset of hypertension was delayed even in the F₂ with hypertension in comparison with the parent SHR. It means that the combination of whole genes is necessary for the early onset and the establishment of severe hypertension. These results in F₂ indicate that it is possible to establish several sorts of mild to severe hypertensive rats with different combinations of hypertensive genes involved in SHR.

The present study showed that the analysis of the F₂ segregate generation was so far the best method to evaluate the genetic relation of each characteristic finding in SHR to the pathogenesis of this hypertension. It is expected that this kind of analysis will clarify the biochemical lesions controlled by the major genes of this genetic hypertension.

REFERENCES


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DISCUSSION:

Chairman: KOICHI OGINO, Kyoto Univ.

Dr. MOTOMURA: Is there sexual dependency in cross-breeding between SHR and WM rat in development of hypertension in F₁ or F₂?

Dr. YAMORI: No.

Dr. KANEKO: How greatly are the blood pressure levels affected by the extirpation of endocrine glands in the SHR or the rats with renal hypertension?

Dr. SOKABE: There might be many studies on the influence of endocrine organ on the experimental hypertension of renal or adrenal origin. However, how do you think about the correlation between hypophysis and brain stem on the pathogenesis of SHR’s hypertension?

Dr. EBIIHARA: I would like to refer our studies on this subject. Influences of hypophysectomy, thyroidectomy, adrenalectomy and gonadectomy on the development or maintenance of hypertension were examined on the SHR of 4 weeks age (prehypertensive stage) or 20 weeks age (stage of established hypertension). Hypophysectomy alone among these procedures suppressed the development or maintenance of hypertension. This suggests that hypophysis may play an essential part on the pathogenesis of hypertension of SHR. In answer to Dr. Kaneko, I make mention of the results suggesting that neither thyroid nor adrenal gland is the essential on the development of hypertension from the endocrine extirpation experiments on the rat with hypertension of Goldblatt type.

Dr. YAMORI: Similar results as Dr. Ebihara’s study have been reported by many investigators since Dr. Aoki’s first experiment. It is a fact that separate extirpation of target endocrine organ does not so much influence as hypophysectomy but brings only a modification on the hypertension level. However, it cannot hastily be concluded only from these results that hypophysis would play a main pathogenetic role on the development or maintenance of hypertension, considering severe emaciation of the animals induced by hypophysectomy. Therefore, it is a cogent study to investigate the correlation between the blood pressure level and the weight of hypophysis in F₂ of SHR. It is very interesting in connection with Dr. Ebihara’s experiment that the blood pressure level in F₂ has a best correlation with the weight of hypophysis among other various endocrine organs.

I also answer to Dr. Kaneko as follows. As reported by many investigators, the blood pressure levels in the rats of renal hypertension is not so greatly influenced by extirpation of various endocrine organs as in SHR. Especially, it is noticed that the weight of hypophysis in the rat with renal hypertension is not so much changed as in SHR.

In answer to Dr. Sokabe, we anticipate two possible systems, autonomous system and endocrine system, as centrifugal pathway connecting the central dysfunction to the peripheral function. The former possibility is that genetic insufficiency in noradrenergic system of brain stem, functioning as sympatho-inhibitory mechanism, would form a relatively sympathotonic basis in the peripheral sympathetic nervous system. (Science, 170, 1970, J. Pharmacol. Pharmac. 1972). As an evidence of the latter possibility, a slight hypersecretion of anterior pituitary hormones and also the posterior pituitary hormone is adduced.

Dr. KIRA: We observed that adrenalectomy inhibited the development of experimental renal hypertension by the injection of rat’s kidney extract and that blood pressure in the hypertensive rat with endocrine kidney was depressed by the adrenalectomy. Therefore, I would like to stress the importance of the hypophysis-adrenocortical system on the pathogenesis of hypertension.

Dr. SHIGIYA: I would like to present our research on morphological features of rat’s coronary arteries by the coronary angiograms. Septal main coronary artery was branched off from left coronary artery in 83% of SHR, on the contrary, this septal artery in Wistar rat from left coronary artery in 49% and from right one in 39%. Further study is needed whether this difference between SHR and WM rat would be due to adaptation or natural selection.