Genetic predisposition to stroke in spontaneously hypertensive rats

AKINOBU NAGAOKA, HISASHI IWATSUKA, ZIRO SUZUOKI, AND KOZO OKAMOTO
Biological Research Laboratories, Central Research Division, Takeda Chemical Industries, Osaka 532, and Faculty of Medicine, Kyoto University, Kyoto, Japan

Hypertension and stroke in spontaneously hypertensive rats (SHR) were investigated genetically using stroke-prone SHR (A3), stroke-resistant SHR (C) and their hybrids, hybrid of A3 and C (F1), offspring of F1 × F1, and those of backcrossing of F1, to the respective parental strains, BC(F1 × A3) and BC(F1 × C). The average blood pressure measured without anesthesia increased in the following order during the experimental period: C < BC(F1 × C) < F1 < BC(F1 × A3) < A3. The F2 represented a wider spread of variation than the F1, with some of the pressure extending into the range of both parental strains. When the drinking water was replaced with a 1% salt solution, the blood pressure increased and the onset of stroke markedly accelerated in all groups of SHR. Under the hypertensive conditions, the incidence of stroke was associated with A3-gene concentration rather than with the level of blood pressure. Similar but less dramatic effects of salt were observed in another series of hybrid groups derived from A3 and normal Wistar-Kyoto rats. These findings suggest that the genetic factors are of great importance in the development of stroke as well as hypertension in the SHR.

Cerebral hemorrhage; cerebral infarction; genetic factors in stroke; salt sensitivity

In 1963, spontaneously hypertensive rats (SHR) regarded as a useful animal model for essential hypertension were isolated from the Wistar-Kyoto rat colony by Okamoto and Anki (15). Later, the SHR colony was further separated by Okamoto and his colleagues into several sublines (16), some of which had a tendency to develop cerebrovascular lesions (cerebral hemorrhage and/or infarction), otherwise known as a stroke. Recently, "stroke-prone SHR" was established by Okamoto et al. (18) from the sublines of A3 and A3s. The stroke-prone SHR showed a spontaneously high incidence of the cerebrovascular lesion (CVL). In contrast, a subline C could hardly develop the CVL in spite of the presence of hypertension and was called "stroke-resistant SHR."

It is well known that high blood pressure is one of the causative factors of stroke in the human (2, 8-10, 19, 21) and that abnormally high salt intake results in an elevation of the blood pressure in both men and experimental animals (3, 4, 6, 23). As expected, it was recently demonstrated that increased salt intake accelerated the onset of CVL in the stroke-prone SHR (7). In the present studies, effects of increased salt intake on the CVL were examined in the SHR with different genetic constitutions in order to validate a predisposition to the CVL in the stroke-prone SHR, A3.

METHODS

The offspring (F27- F28) of SHR sublines were introduced to our laboratories from Kyoto University in 1972 and have since been bred by selective sibmatings. The two kinds of SHR used in the present study were of the F29 generation in the stroke-prone SHR (A3) and F28 generation in the stroke-resistant SHR (C). These SHR and Wistar-Kyoto rats (WK), used as normotensive control rats, originated from the same ancestors. The degree of inbreeding in these strains was the same as the previous report (18). Animals of F1, F2, and backcrossing (BC) hybrids were produced by reciprocal matings of A3 × C and A3 × WK. The males were used in the experiments, because it had been reported that the incidence of stroke was much higher in the males than in the females of the stroke-prone SHR (18). The procedure in the mating of A3 × C and theoretical gene concentrations are shown in Fig. 1. The same procedure was used in the mating of A3 × WK. However, backcrossing of the F1 to WK was not done because the F1 rats as well as WK showed no incidence of stroke as described in the item of results. Rats were kept five or six in a cage until 7 wk of age under the controlled conditions of temperature (23-24°C), humidity (50-60%), and light (7:30 A.M.-6:00 P.M.) and were given a laboratory chow, CA-1, Japan CLEA, containing 0.4% salt and drinking water ad libitum.

Animals in each hybrid group were divided into two groups at 8 wk of age, which were given tap water and 1% salt solution as the drinking fluid for 12 wk (Table 1). In the process for allocating rats into the two groups, it was considered that rats from the same litter were divided into the two groups in about the same ratio, and especially in the series of A3 × WK the rats were assigned more to the salt-loaded group than to the control group, as it was anticipated that the CVL would hardly develop in the latter group for this experimental period. Some of the rats died spontaneously during the experimental period. The organs from these rats and survivors, sacrificed at the beginning of the 13th wk, were fixed with 10% Formalin. The brains were cut in four or five frontal sections for the macroscopic observation to...
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FIG. 1. Mating procedure between stroke-prone SHR (A₃) and resistant SHR (C). Theoretical average gene constitution in each group is represented, and ratio (%) of A₃ and C genes is as follows: A₃ (A₃ : 100); C (C : 100); F₁ (A₃ : 50, C : 50); BC(F₁ × A₃) (A₃ : 75, C : 25); and backcross to C (A₃ : 25, C : 75).

TABLE 1. Number of rats in parental strains and their hybrid groups used

<table>
<thead>
<tr>
<th>Parental Strains and Types of Hybrid</th>
<th>No. of rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHRA₃</td>
<td>12</td>
</tr>
<tr>
<td>BC(F₁ × A₃)</td>
<td>5</td>
</tr>
<tr>
<td>F₁(A₃ × C)</td>
<td>12</td>
</tr>
<tr>
<td>BC(F₁ × C)</td>
<td>5</td>
</tr>
<tr>
<td>SHRC</td>
<td>12</td>
</tr>
<tr>
<td>BC(F₁ × A₃)</td>
<td>0</td>
</tr>
<tr>
<td>F₁′(A₃ × WK)</td>
<td>12</td>
</tr>
<tr>
<td>F₂(F₁ × F₁′)</td>
<td>0</td>
</tr>
<tr>
<td>Normal WK</td>
<td>12</td>
</tr>
</tbody>
</table>

detect the CVL, and then embedded in paraffin to prepare the histological sections, 5 µm in thickness, which were stained with hematoxylin-eosin staining for microscopic observation. Blood pressure was measured weekly using a tail-pulse-pickup method (13) in unanesthetized rats. The animals were previously warmed for 10 min in a chamber which was maintained at 37–38°C, and then they were placed into a rat holder. The average of three recordings of blood pressure taken at approximately 30-s intervals at their rest condition was recorded as systolic blood pressure of the rat. The variation in the three recordings was less than 5%. Volume intake of the drinking water was also measured weekly. Rats were observed every day for clinical symptoms suggestive of stroke or hypertensive encephalopathy, which could be detected visually.

Arithmetical means, standard deviation and standard error of the mean were calculated. The difference between the means from two independent samples was analyzed by the Student unpaired-t-test.

RESULTS

Blood pressure. On tap water, the systolic blood pressure differed among the six groups in the series of crosses between A₃ and C (Fig. 2). Throughout the experimental period, the pressure level was higher in the following order: C < BC(F₁ × C) < F₁ = F₂ < BC(F₁ × A₃) < A₃. Blood pressure above 200 mmHg developed in A₃, BC(F₁ × A₃), F₁, and F₂ at 2.5, 4.5, 6, and 7 wk after the start of the experiment, respectively, although only the pressure of A₃ elevated to 230 mmHg by 7 wk. On the other hand, the blood pressure of BC(F₁ × C) and C did not reach this level (200 mmHg).

The blood pressures in A₃, C, and their F₁, and F₂ hybrid groups are shown in Table 2 in order to determine whether differences between the means indicate different populations sampled. A₃ had a larger variation than C, but a highly significant difference was observed between the parental strains. The blood pressure of the F₁ was intermediate between the parents and was significantly lower than that of A₃ and higher than that of C, although the mean appeared to be closer to C than A₃. The F₂ was also generally intermediate but represented a wider range than the F₁. Moreover, the blood pressure in the F₂ tended to distribute continuously between the parents, and some of their pressure mea-

FIG. 2. Time course of systolic blood pressure (BP) in rats receiving a tap water (○—○) or 1% salt solution (●—●). Elevation of blood pressure was accelerated by salt loading in all groups except normal WK group. Values are means ± SD.
As, C, and their F₁ and F₂ hybrids

tion than the control WK. The F₁ in A, and the F₂ in BC(F₁ x A₃) showed a much higher intake of salt solution in each group of animals than that of the other five groups. There was no significant difference in the intake of either solution between SHR and WK in the salt solution intake in all six groups of SHR. The intake of salt solution was increased with the experimental period. These differences were not observed in tap water intake.

Incidence of cerebrovascular lesions. No mortal cases of cerebral lesions were observed in any group of rats drinking tap water except for one rat in the A₃ group. The blood pressure of this exceptional rat reached 230 mmHg at the 5th wk and it died at the 7th wk. The other A₃ rats, though hypertensive in similar severity, did not develop any abnormal symptoms or the CVL.

Figure 3 illustrates the cumulative incidence of the CVL in A₃, C, and their hybrid groups. Many of the salt-loaded SHR developed the CVL as well as the severe hypertension. At the 6th–10th wk, the incidence of CVL was increased in association with the concentration of SHR A₃-gene in a manner similar to the spontaneous changes in the blood pressure; C < BC(F₁ x C) < F₁, thus, the F₁ and F₂ were intermediate between the parental strains, but the incidence in the F₂ was lower than that in the F₁ at the 10th–12th wk. It is also remarkable from Fig. 3 that the incidence of CVL is increased with the salt-loading period in all six groups of SHR. At the end of the experiments, the incidences were 92% for A₃, BC(F₁ x A₃), and F₁, 70% for F₂, 83% for BC(F₁ x C), and 50% for C, respectively.

The effect of the genetic constitution on the onset of CVL was observed in the salt-loaded hybrid groups of WK and A₃. None of WK and the F₁ showed the CVL until the termination of experiment. The final incidence increased with the experimental period. These differences were not observed in tap water intake.

### TABLE 2. Statistical analysis of blood pressures in A₃, C, and their F₁ and F₂ hybrids

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Time After Start of Experiment, wk</th>
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<tbody>
<tr>
<td>A₃</td>
<td>19</td>
<td>2</td>
</tr>
<tr>
<td>C</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>F₁</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>F₂</td>
<td>12</td>
<td>5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>t value</th>
<th></th>
<th>A₁ vs. C</th>
<th>8.03*</th>
<th>9.46*</th>
<th>12.04*</th>
<th>9.27*</th>
<th>8.91*</th>
<th>8.92*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A₁ vs. F₁</td>
<td>5.14*</td>
<td>5.27*</td>
<td>4.65*</td>
<td>5.12*</td>
<td>3.84*</td>
<td>5.94*</td>
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<tr>
<td></td>
<td></td>
<td>C vs. F₁</td>
<td>6.11*</td>
<td>9.671*</td>
<td>9.671*</td>
<td>3.601*</td>
<td>5.78*</td>
<td>3.601*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F₁ vs. F₂</td>
<td>1.65</td>
<td>0.60</td>
<td>1.26</td>
<td>0.72</td>
<td>1.90</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Blood pressure values are means ± SD, n, number of animals. The number in A₃ group was 11 after 8 wk, because one of them died of stroke at the 7th wk. *P < 0.001. †P < 0.01.

The blood pressure was more steeply elevated in the SHR on the salt solution, as compared with the water controls (Fig. 2). All groups showed a severe hypertension (over 230 mmHg) by 6 wk after the start of salt loading. Even under this condition, A₃ was the most hypertensive and took only 2.5 wk to develop the severe hypertension. However, the blood pressure differences between the other five groups were less, and the time required for the development of severe hypertension was 4, 5, 5.5, 5, and 6 wk in BC(F₁ x A₃), F₁, F₂, BC(F₁ x C), and C, respectively.

The same kind of genetic analysis was performed on hybrid groups of A₃ and normotensive control WK (Fig. 2). The blood pressure of their F₁' was almost intermediate between those of the parents under the nonsalt-loading conditions. For example, the blood pressure in A₃, F₁', and WK was 220 ± 19 (SD), 160 ± 9, and 129 ± 5 mmHg at the 5th wk, and 234 ± 14, 173 ± 11, and 130 ± 4 mmHg at the 10th wk, respectively. In contrast to A₃, the blood pressure in WK did not respond to salt, namely, the pressure level in the salt-loaded group did not differ significantly at any week compared with that in the control group (P > 0.05). The F₁' showed only a slight elevation in the pressure in response to salt. The salt-stimulated blood pressure in the F₁' and F₂(F₁' x F₁') was almost the same, but the variation in the pressure was larger in the F₂ than the F₁'. The F₂ covered the entire range between the pressure of the parental strains. BC(F₁' x A₃) showed a much higher blood pressure than the F₁' and F₂. That is, the blood pressure in the F₁', F₂, and BC(F₁' x A₃) was 171 ± 10 (SD), 171 ± 28, and 205 ± 33 mmHg at the 5th wk, and 185 ± 15, 180 ± 30, and 206 ± 30 mmHg at the 10th wk, respectively.

Water or salt solution intake in the parental strains and F₁ hybrid group is shown in Table 3. There was no significant difference in the intake of either solution among the six groups of SHR. The intake of salt solution in each group of animals was higher than that of tap water. The SHR including A₃ took more salt solution than the control WK. The F₁ in A₃ x WK was roughly intermediate in this respect. The difference between SHR and WK in the salt solution intake increased with the experimental period.

### TABLE 3. Intake of tap water or 1% salt solution

<table>
<thead>
<tr>
<th>Group</th>
<th>1 wk</th>
<th>3 wk</th>
<th>5 wk</th>
<th>1% Salt Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHRs A₃</td>
<td>18 ± 3</td>
<td>18 ± 2</td>
<td>18 ± 3</td>
<td>26 ± 8</td>
</tr>
<tr>
<td>F₁(A₃ x C)</td>
<td>18 ± 3</td>
<td>20 ± 2</td>
<td>19 ± 4</td>
<td>25 ± 16</td>
</tr>
<tr>
<td>SHR C</td>
<td>20 ± 1</td>
<td>20 ± 3</td>
<td>18 ± 1</td>
<td>25 ± 6</td>
</tr>
<tr>
<td>F₁'(A₃ x WK)</td>
<td>19 ± 2</td>
<td>19 ± 3</td>
<td>18 ± 4</td>
<td>24 ± 12</td>
</tr>
<tr>
<td>WK</td>
<td>17 ± 3</td>
<td>17 ± 2</td>
<td>16 ± 4</td>
<td>27 ± 7</td>
</tr>
</tbody>
</table>

Values are means ± SD. The intake of salt-solution in each group was significantly higher than that of tap water (P < 0.05) at all times except the 5th wk in WK.

### FIG. 3. Cumulative incidence of cerebrovascular lesions (CVL) in A₃, C, and their F₁, F₂, and BC hybrid groups.
dences were 92% (11/12) for A_3, 41% (9/22) for BC(F_1' × A_3), and 4% (1/24) for the F_2, respectively. The one rat in the 24 F_2 hybrids showed also the severe hypertension as high as the A_3 rats, as seen in Fig. 4.

**Relationship between blood pressure and CVL.** The highest blood pressure observed in the individual rats of each salt-loaded group is plotted in Fig. 4. These values were recorded before the onset of the clinical symptoms, which closely related to the cerebral lesions, in order to exclude the changes of blood pressure after the onset of the symptoms. Most of A_3, C, and their hybrid rats exhibited the severe hypertension over 230 mmHg. However, in a series of experiments using A_3, WK, and their hybrids, the blood pressures of F_1' ranged from 165 to 213 mmHg, and those in the F_2 and BC hybrids were distributed over a wider range, reflecting the difference in their response to salt. The animals with the CVL, irrespective of type of hybrids, showed the severe hypertension. However, some of the animals with the severe hypertension did not develop the lesions. Such animals were frequently observed in the C group, 50% of which did not show both the CVL and clinical symptoms, although they showed sustained hypertension over 230 mmHg.

In A_3, C, and their hybrid rats, which developed the cerebral lesions, the average elapsed time from the onset of the severe hypertension to that of the CVL (i.e., the average duration of the severe hypertension) was shortest in A_3, 3.4 ± 1.1 (SD) wk, and longest in C, 5.0 ± 1.0 wk, and the time in the F_1, F_2, and BC hybrids were in the range of the parental strains, A_3 and C. Moreover, the half of C rats without the CVL attained a blood pressure of 230 mmHg at 5.8 ± 1.8 wk.

Figure 5 shows the average blood pressure at the 6th wk and the incidence of CVL at the 7th wk after the salt-loading in relation of the A_3-gene concentration. The levels of blood pressure of five groups except A_3, which had the highest average pressure, were not differ-ent significantly (P > 0.05). Under these conditions, however, the incidence of CVL was highly associated with the theoretical concentration of A_3-gene. A similar relationship between the cumulative incidence of the CVL and the average blood pressure or the theoretical gene concentration in A_3, C, and their hybrid groups was observed at the 6th-10th wk.

**Classification of CVL and clinical symptoms.** The CVL which developed in 71 rats with the salt loading were detected macroscopically in most cases. These lesions were classified simply into hemorrhage (28%), hemorrhagic infarction (58%), and infarction (14%). Initial clinical symptoms were observed in 94% of these 71 rats, and their incidences were as follows: 1) excitement (piloerection, exophthalmus, increased locomotion), 27%; 2) stereotyped lifting of arms, 22%; 3) hyperirritability (jumping, rapid escaping, etc.), 14%; 4) behavioral and psychological depression (decreased locomotion, hyperresponsiveness), 12%; 5) epileptic symptoms (sudden and abnormal hyperkinesia, convulsion of extremities), 6%; 6) hemiplegia, 3%; and 7) sudden death, 10%. After showing the symptoms, the rats lost their weight gradually and died within about 2 wk. Predilection sites of the lesions were the cortex or subcortex of frontal, medial, and occipital areas of telencephalon.

**DISCUSSION**

A possible involvement of genetic factors in the development of CVL has been suggested from the fact that stroke-prone SHR is established by successive selective inbreeding among the sublines of SHR (18). The present studies were carried out in order to clarify the relationship between hypertension and stroke and also to validate the genetic predisposition to stroke in the stroke-prone SHR.

The blood pressure of stroke-prone SHR (A_3) was significantly higher than that of stroke-resistant SHR (C) under the nonsalt-loading conditions, which was suggestive of the importance of high blood pressure in the CVL, as described previously (18). The average blood pressure of the F_1 and F_2 in A_3 × C was roughly intermediate between both parental SHR's, and those of the backcross groups were also intermediate between F_1 and A_3 or C. In addition, the blood pressure in the F_2...
Hypertension irrespective of type of hybrids, and 3) SHR CVL among all of the groups with different genetic constitution. Thus, there seems to be no doubt that hypertension is also one of the great risk factors to the occurrence of CVL in the stroke-prone SHR. This is supported by the observation that the treatment of hypertension with reserpine or hydralazine is associated with a decrease in the incidence of CVL in the SHR under the same salt-loaded conditions (unpublished data) as well as in man (24). However, the presence of the other factors involved in the development of CVL was suggested from the evidence that 1) although the level of blood pressure which was accelerated with salt was almost the same between BC(F1 × A3), F1, F2, BC(F1 × C), and C in the series of crosses of A3 and C, the incidences of CVL in these groups increased in association with the theoretical concentration of A3-gene; 2) in spite of the same magnitude of the highest blood pressure level in these rats, the final incidence of CVL was markedly lower in C group than in the other groups; and 3) the elapsed time from the onset of severe hypertension to the onset of CVL was the shortest in stroke-prone SHR and the longest in stroke-resistant SHR. From these findings, it was suggested that the onset of stroke in SHR was dependent not only on the severity of hypertension but also on the genetic predisposition.

The incidence of CVL in SHR A3, C, and their hybrid groups seemed to correlate positively to the A3-gene concentration. Similar but less prominent correlation was observed in the hybrid groups between A3 and WK. Moreover, the incidence of CVL in the F1 in A3 × C was intermediate between the parents during the experimental period except the late period (10–12 wk). The F2 was also intermediate in the incidence, although the final incidence was lower than that in the F1. On the other hand, the F1 in A3 × WK did not show the CVL, but in the F2, the CVL was observed in one rat out of 24 rats. These results might be insufficient in the sample size to analyze an exact mode of inheritance of stroke in the SHR. However, if it is allowed for us to speculate on the mode of inheritance, it would not be dominant or recessive but polygenic. If it were dependent on a dominant single gene, the incidence of CVL in the F1 (A3 × C) and F1′ (A3 × WK) would be closer to that in A3. Alternatively, if it were dependent on a recessive gene, the incidence in the F1(A3 × C) would be closer to that in C, and the incidence (4%) in the F2(A3 × WK) would approach the expectation value (25%). At the late period, however, the relationship between the onset of CVL and A3-gene concentration in the cross of A3 and C became unclear, that is, at the 12th wk the final incidence of the CVL was 84–92% in the hybrid groups except F2 and C. This could be explained by the experimental procedure that a salt was loaded as one of the environmental factors accelerating the development of CVL. The salt loading proved of great benefit to narrowing the distribution in age of the development of CVL in the stroke-prone SHR.
REFERENCES


