Genetic predisposition to stroke in spontaneously hypertensive rats

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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 Anoki (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 A1-sb. 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 had 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 F2x 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 x C and A3 x 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 x C and theoretical gene concentration are shown in Fig. 1. The same procedure was used in the mating of A3 x 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 x 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...
FIG. 1. Mating procedure between stroke-prone SHR (A3) and resistant SHR (C). Theoretical average gene constitution in each group also is represented, and ratio (%) of A3 and C genes is as follows. A3 (A3 : 100); C (C : 100); F1 (A3 : 50, C : 50); F2 (A3 : 50, C : 50) backcross to 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>Tap water</th>
<th>1% salt</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR A3</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>BC(F1 × A3)</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>F1(A3 × C)</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>F2(F1 × F1)</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>BC(F1 × C)</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>SHR C</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>BC(F1 × A3)</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>F1′(A3 × WK)</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>F2′(F1 × F1′)</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>Normal WK</td>
<td>12</td>
<td>16</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-
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, A, 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 blood pressure values are means ± SD. *P < 0.001. **P < 0.01.

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 ± 9, 160 ± 4, and 130 ± 4 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 206 ± 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. These differences were not observed in tap water intake.

The same kind of genetic analysis was performed 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.

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Incidence of cerebrovascular lesions. No mortal cases or 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 = F2 < BC(F1 x A3) < A3. 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 in the F1 x WK group was significantly higher than that of tap water (P < 0.05) at all times except the 5th wk in WK.

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Tap water</th>
<th>1% Salt Solution</th>
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<tbody>
<tr>
<td></td>
<td>1 wk</td>
<td>3 wk</td>
</tr>
<tr>
<td>WK</td>
<td>17 ± 2</td>
<td>17 ± 3</td>
</tr>
<tr>
<td>A3</td>
<td>18 ± 3</td>
<td>18 ± 2</td>
</tr>
<tr>
<td>F1 x A3</td>
<td>19 ± 3</td>
<td>19 ± 4</td>
</tr>
<tr>
<td>SHR C</td>
<td>20 ± 1</td>
<td>19 ± 3</td>
</tr>
<tr>
<td>SHR F1 x C</td>
<td>20 ± 2</td>
<td>19 ± 4</td>
</tr>
<tr>
<td>F1 x WK</td>
<td>17 ± 2</td>
<td>16 ± 3</td>
</tr>
<tr>
<td>F1 x A3 x WK</td>
<td>17 ± 2</td>
<td>16 ± 3</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.
dences were 92% (11/12) for A₃, 41% (9/22) for BC(F₁ × A₃), and 4% (1/24) for the F₂, respectively. The one rat in the 24 F₂ hybrids showed also the severe hypertension as high as the A₃ rats, as seen in Fig. 4.

Relationship between blood pressure and CVL. The highest blood pressure obtained 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 A₃, C, and their hybrid rats exhibited the severe hypertension over 230 mmHg. However, in a series of experiments using A₃, WK, and their hybrids, the blood pressures of F₁ ranged from 165 to 213 mmHg, and those in the F₂ 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 A₃, 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 A₃, 3.4 ± 1.1 (SD) wk, and longest in C, 5.0 ± 1.0 wk, and the time in the F₁, F₂, and BC hybrids were in the range of the parental strains, A₃ 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 A₃-gene concentration. The levels of blood pressure of five groups except A₃, which had the highest average pressure, were not different significantly (P > 0.05). Under these conditions, however, the incidence of CVL was highly associated with the theoretical concentration of A₃-gene. A similar relationship between the cumulative incidence of the CVL and the average blood pressure or the theoretical gene concentration in A₃, 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, hyporesponsiveness), 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 (A₃) 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 F₁ and F₂ in A₃ × C was roughly intermediate between both parental SHR's, and those of the backcross groups were also intermediate between F₁ and A₃ or C. In addition, the blood pressure in the F₂
distributed continuously between the ranges of those in $A_3$ and $C$, with some of the pressure extending into the range of the two parental strains. The same kind of genetic data was demonstrated in the hybrid groups between $A_3$ and WK. That is, the $F_1$ was intermediate in blood pressure between the parents, and the $F_2$ ranged from a blood pressure as high as $A_3$ to a blood pressure approaching that of WK, although the experimental conditions were under the salt loading. The findings from $A_3 \times C$ and $A_3 \times WK$ suggested a characteristic pattern for the results of crosses, involving traits dependent on a few genes. If many genes are involved, the extremes in the pressure represented by the parental strains would occur infrequently in the $F_1$ groups. These results seemed to be compatible with the findings by Louis et al. (11) and Tanase et al. (22) which indicated the mode of inheritance of the hypertension in the SHR was polygenic. However, when the small sample size, the large variation in the parental strains, and the fact that the mean pressure measurements for $F_1$ and $F_2$ were closer to $C$ or WK than $A_3$ were considered, the possibility that a single-gene mode of inheritance might be involved in the determination of blood pressure in the SHR could not be excluded by this experiment.

It has been reported that the incidence of CVL in the SHR is increased by salt loading (7, 17). In the present experiments, it was found that SHR $A_3$, $C$, and their hybrids were very sensitive to salt, showing a further increase of blood pressure in addition to the spontaneous increase of their blood pressure. The response to salt was not markedly different among all six groups of SHR, although WK did not respond to salt. The $F_1$ between $A_3$ and WK showed an intermediate response. Therefore, it may be concluded that the extreme response to salt is one of genetic characteristics of SHR. However, the genes participating in this response seem to be different from those determining the vulnerability to stroke, because the incidence of stroke under the salt-loading conditions is obviously different among the six groups of SHR in contrast to the salt sensitivity. The magnitude of the blood pressure response to salt was much less in the SHR as compared with a strain of hypertensive-prone rats produced by Dahl et al. (5), but an increased turnover of $^{22}$Na was observed in both strains of rats (14, 20). The SHR (1, 12, 14) but not Dahl's strain (25) showed a high preference to salt. These facts may suggest a possible importance of salt metabolism and/or salt sensitivity to genetically mediated hypertension as well as some types of experimental hypertension.

Hypertension is an important risk factor to the development of stroke in the human as indicated from epidemiological studies (8–10). Also in the present study, it was demonstrated that 1) the onsets of both hypertension and CVL were accelerated by the salt loading, 2) all of the rats that developed the CVL showed a severe hypertension irrespective of type of hybrids, and 3) SHR $A_3$ has the highest blood pressure and susceptibility to CVL among all of the groups with different genetic constitutions. 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($F_1 \times A_3$), $F_1$, $F_2$, BC($F_1 \times C$), and $C$ in the series of crosses of $A_3$ and $C$, the incidences of CVL in these groups increased in association with the theoretical concentration of $A_3$-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 $A_3$, $C$, and their hybrid groups seemed to correlate positively to the $A_3$-gene concentration. Similar but less prominent correlation was observed in the hybrid groups between $A_3$ and WK. Moreover, the incidence of CVL in the $F_1$ in $A_3 \times C$ was intermediate between the parents during the experimental period except the late period (10–12 wk). The $F_2$ was also intermediate in the incidence, although the final incidence was lower than that in the $F_1$. On the other hand, the $F_1$ in $A_3 \times WK$ did not show the CVL, but in the $F_2$, 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 incidences of CVL in the $F_1$ ($A_3 \times C$) and $F_2$ ($A_3 \times WK$) would be closer to that in $A_3$. Alternatively, if it were dependent on a recessive gene, the incidence in the $F_1(A_3 \times C)$ would be closer to that in $C$, and the incidence (4%) in the $F_2(A_3 \times WK)$ would approach the expectation value (25%). At the late period, however, the relationship between the onset of CVL and $A_3$-gene concentration in the cross of $A_3$ and $C$ became unclear, that is, at the 12th wk the final incidence of the CVL was 84–92% in the hybrid groups except $F_2$ 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.

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