GENETIC PREDISPOSITION TO STROKE IN SPONTANEOUSLY HYPERTENSIVE RATS

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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 (F2, F20) 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 F20 generation in the stroke-prone SHR (A3) and F20 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-resistant 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 : 15).

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>Tap water</td>
<td>1% salt</td>
</tr>
<tr>
<td>SHR A3</td>
<td>12</td>
</tr>
<tr>
<td>BC(F1 x A3)</td>
<td>5</td>
</tr>
<tr>
<td>F1                      (A3 x C)</td>
<td>12</td>
</tr>
<tr>
<td>F2                      (F1 x F1)</td>
<td>12</td>
</tr>
<tr>
<td>BC(F1 x C)</td>
<td>5</td>
</tr>
<tr>
<td>SHR C</td>
<td>12</td>
</tr>
<tr>
<td>BC(F1 x A3)</td>
<td>0</td>
</tr>
<tr>
<td>F1' (A3 x WK)</td>
<td>12</td>
</tr>
<tr>
<td>F2(F1' x 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 \( \mu \)m 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\(^\circ\)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 x C) < F1 = F2 < BC(F1 x A3) < A3. Blood pressure above 200 mmHg developed in A3, BC(F1 x 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 x 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 F2 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-
TABLE 2. Statistical analysis of blood pressures in A,, C, and their F, and F, hybrids

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Time After Start of Experiment, wk</th>
</tr>
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<tbody>
<tr>
<td>A,</td>
<td>19</td>
<td>2</td>
</tr>
<tr>
<td>C</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>F,</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>Ff,</td>
<td>12</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>t-value</th>
<th></th>
<th>A, vs. C</th>
<th></th>
<th>A, vs. F,</th>
<th></th>
<th>F, vs. Ff,</th>
</tr>
</thead>
<tbody>
<tr>
<td>A, vs. C</td>
<td></td>
<td>8.93*</td>
<td>1.94*</td>
<td>2.97*</td>
<td>2.27*</td>
<td>8.30*</td>
</tr>
<tr>
<td>A, vs. F,</td>
<td></td>
<td>5.14*</td>
<td>6.65*</td>
<td>4.91*</td>
<td>5.12*</td>
<td>5.94*</td>
</tr>
<tr>
<td>F, vs. Ff,</td>
<td></td>
<td>6.11*</td>
<td>9.67*</td>
<td>8.67*</td>
<td>6.69*</td>
<td>5.78*</td>
</tr>
</tbody>
</table>

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

FIG. 3. Cumulative incidence of cerebrovascular lesions (CVL) in A,, C, and their F, and BC hybrid groups.
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ences were 92% (11/12) for A, 41% (9/22) for BC(A' × A), and 4% (1/24) for the F, respectively. The one rat in the 24 F, hybrids showed also the severe hypertension as high as the A, 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 A, C, and their hybrid rats exhibited the severe hypertension over 230 mmHg. However, in a series of experiments using A, WK, and their hybrids, the blood pressures of F, ranged from 165 to 213 mmHg, and those in the F, 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, 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, 3.4 ± 1.1 (SD) wk, and longest in C, 5.0 ± 1.0 wk, and the time in the F, and BC hybrids were in the range of the parental strains, A, 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,-gene concentration. The levels of blood pressure of five groups except Azs, 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,-gene. A similar relationship between the cumulative incidence of the CVL and the average blood pressure or the theoretical gene concentration in A, 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 (A,) 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, and F, in A, × C was roughly intermediate between both parental SHR's, and those of the backcross groups were also intermediate between F, and A, or C. In addition, the blood pressure in the F,
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 $F_3$ 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 hypertension-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 $F_1$ ($A_3 \times WK$) 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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REFERENCES


