Regional cerebral blood flow (rCBF) was repeatedly measured by the hydrogen clearance method in the frontal cortex of stroke-prone spontaneously hypertensive rats (SHRSP) at the age of 50 days and thereafter. When SHRSP rats developed severe hypertension (over 200 mm Hg at the age of 60 days) rCBF began to decrease abruptly in the frontal cortex — one of the three predilection sites of stroke in these rats. In contrast, such a reduction in rCBF was not noted in either stroke-resistant spontaneously hypertensive rats (SHRSR) which developed moderate hypertension (under 200 mm Hg), or in Wistar-Kyoto rats (WK) with normal blood pressure (under 150 mm Hg).

A similar marked reduction of rCBF with severe hypertension (over 200 mm Hg) was also detected in apoplectic gene-free renal infarction hypertensive rats (RHR) experimentally produced from age-matched WK animals. Blood samples were obtained through an implanted femoral artery canula without disturbing the nonanesthetized SHRSP, SHRSR and WK rats. Arterial blood gas analysis (Paco2, Paco and pH) showed no significant differences at the age of 5 months in any of these rats.

Chemical cerebrovascular reactivity, that is, an increase in rCBF in response to CO2 inhalation, showed no significant difference among SHRSP rats from the age of 50 days to 5 months. However, it markedly decreased in SHRSR rats at the age of 9 months and thereafter (the average age of male SHRSP rats which develop stroke is 9 months).

The present study showed stroke did not occur in antihypertensive agent-treated SHRSP rats. In these SHRSP rats rCBF did not decrease as long as blood pressure was well-controlled.

RECENTLY, there has been increasing clinical interest in studies on cerebral blood flow (CBF) in cerebrovascular disease. Methods for CBF measurement have made rapid progress since the introduction of the nitrous oxide technique, first developed by Kety and Schmidt.1,2 In patients with stroke there have been reported changes in regional cerebral blood flow (rCBF) at various stages. Many investigators have observed rCBF reduction after patients developed stroke.3,4 However, the causal relation between CBF and stroke, and especially between CBF and hypertension, have never been elucidated.

To clarify this relation, a number of experimental approaches have been tried.5-8 Among these, some emphasize clinical data, others add new experimental data and still others have developed new theories, for example, "hypertension breakthrough".9,10 As previously reported, a stroke-prone spontaneously hypertensive rat (SHRSP) was successfully developed in our laboratory at Kyoto University.11 More than 80% of these SHRSP rats spontaneously develop stroke (cerebral hemorrhage and/or cerebral softening). In our previous report, we confirmed the pathogenetic similarity of stroke in SHRSP rats and humans.12 In the present studies, using our original SHRSP rats, we tried to elucidate the causal relation between rCBF and stroke.

Methods

SHRSP rats used in this study were of the A3 strain among the F12-14 generations of SHR rats maintained at the Department of Pathology, Faculty of Medicine, Kyoto University (Kyoto, Japan). These A3 rats were the newly obtained offspring from crosses between A3 and A5m rats, and nearly 90% of them developed stroke spontaneously.13 Controls were stroke-resistant spontaneously hypertensive rats (SHRSR) and normotensive Wistar-Kyoto rats (WK), from which SHR rats had been derived.

Rats treated with antihypertensive medication (hydralazine 80 mg/liter in the drinking water) or untreated male SHRSP, SHRSR, and WK rats, 50 to 60 in each group, were used at various ages to determine blood pressure and rCBF. Thirty SHRSP rats and 20 renal infarction hypertensive rats (RHR), experimentally produced from age-matched WK rats, were used for a long-term observation on blood pressure and rCBF at the age of 50 days and thereafter.

Under anesthesia (sodium pentobarbital 40 mg/kg, i.p.), rats were placed in a stereotaxic apparatus, and enamel insulated platinum electrodes (0.3 mm in diameter and 25 mm in length) were bilaterally implanted into the frontal cerebral cortex. The electrodes were placed 2 mm from mid-sagittal line, 2 mm anterior to the bregma and inserted 2.5 mm. These electrodes and a reference electrode on the mid-sagittal line, 4 mm anterior to the bregma, were connected to a miniature receptacle and the whole assembly fixed on the skull with dental cement. rCBF was measured in conscious rats in a small gas chamber by the Fujitani et al. modification of the hydrogen clearance method.14 In each rat the measurement was first made 2 weeks after the operation (to avoid variation due to the effect of the implantation) and then repeatedly thereafter. Less than 5% of the rats were excluded from the experimental groups during the observations because their outlying values of rCBF were statistically rejected according to Smirnoff-Grubbs' formula.

Chemical cerebrovascular reactivity in 25 SHRSP rats was determined by detecting an increase in rCBF response to 10% CO2 inhalation for 10 minutes. This condition was found to be enough to produce maximum vasodilatation...
based on preliminary attempts using a heat clearance method to measure CBF. The CO2 reactivity was measured at the ages of 50, 67, 167, and 200 days.

Blood pressure during the course of hypertension was indirectly measured at the tail by the tail-pickup method. Arterial blood samples, 0.2 ml, were obtained through an implanted femoral artery cannula in 5-month-old male SHRSP's, SHRSR and WK rats without disturbing the nonanesthetized rats. Paco2, Pao2 and pH were analyzed by a blood gas analyzer (Corning-Eel, Model 161).

The remaining male rats from the same litter as those in the experimental groups, i.e., 67 SHRSP, 48 SHRSR and 59 antihypertensive agent-treated SHRSP rats of F3 generation in SHR were autopsied after natural death. Fifty-three RHR rats loaded with 1% NaCl in the drinking water were examined after natural death or after sacrifice at the age of 9 months. After macroscopic observation all the brains of these rats were histologically examined to determine the incidence of stroke, and their average life spans were calculated.

All numerical data were expressed as mean ± standard error and statistical differences were calculated by Student's small sample t test.

Results

As shown in figure 1, rCBF in the frontal cortex in SHRSP rats is markedly decreased at the age of 5 and 10 months (63.6 ± 5.8 and 57.3 ± 5.2 ml/min/100 gm, respectively) in comparison with rather high values in SHRSR rats (5-month-old; 110.9 ± 6.4, 10-month-old; 102 ± 6.0) and normal values in WK rats (101.0 ± 7.4, 93.8 ± 4.4). On average, the blood pressure of SHRSP rats at the age of 5 and 10 months is over 200 mm Hg (230 ± 4 and 215 ± 4, respectively) in contrast to moderate hypertension in SHRSR rats (5-month-old; 173 ± 8, 10-month-old; 190 ± 5) and normotensive level in WK rats (5-month-old; 132 ± 3, 10-month-old; 130 ± 5).

The development course of hypertension and cortical cerebral blood flow in SHRSP rats is shown in figure 2. When SHRSP rats developed severe hypertension over 200 mm Hg around the age of 60 days, rCBF in the frontal cortex began to decrease. When severe hypertension was established and stabilized at the age of 90 days, an antihypertensive agent (DU-717, 100 mg/day), which was reported to have no diuretic effect, was administered. This therapy effectively decreased the blood pressure below 200 mm Hg and inversely increased rCBF in direct proportion to the lowering of systemic blood pressure. During this therapy, the maximum increment of rCBF was statistically significant. It was also observed that the suspension of antihypertensive therapy resulted in redevelopment of severe hypertension and a marked decrease of rCBF.

The effect of long-term antihypertensive therapy (chronic administration of hydralazine in drinking water [(80 mg/liter) at the age of 40 days and thereafter] on rCBF in male 10-month-old SHRSP, SHRSR and WK rats is shown in figure 3. rCBF in treated SHRSP rats with moderate hypertension (185 ± 3 mm Hg, 87.7% of control SHRSP rats) was maintained within the normal range (85.7 ± 4.3 ml/min/100 gm). The treated group of SHRSR rats showed lower levels in blood pressure (158 ± 3, 81.9% of control group) than those in control SHRSR rats. rCBF in this group decreased but was within the normal range (77.6 ± 31, 75.9% of control group). Treated and untreated WK rats had no statistically significant differences in either blood pressure or rCBF.

Figure 4 shows the incidence of stroke and the life span in SHRSP, SHRSR, and antihypertensive agent-treated SHRSP rats. Treated SHRSP showed no stroke in contrast to the high percentage (89.3%) of strokes (cerebral bleeding and/or softening) among untreated SHRSP rats who suffered a natural death. SHRSR rats showed a low incidence of stroke (10.7%). The average life spans of untreated

![Figure 1](http://stroke.ahajournals.org/)

**FIGURE 1.** Blood pressure and cortical cerebral blood flow in the frontal region of male stroke-prone (SP) or -resistant (SR) SHR and Wistar-Kyoto rats (WK).
SHRSP rats was the shortest (276 ± 16 days) and SHRSP rats was the longest (413 ± 23). Treated SHRSP rats showed a significantly longer life span (353 ± 23) than did untreated SHRSP rats, but shorter than that of SHRSR rats.

Arterial blood gas analysis showed no significant differences in 5-month-old SHRSP, SHRSR, and WK rats. PaCO₂: 33.6 ± 2.0, 32.1 ± 2.3, 32.1 ± 2.9, PaO₂: 85.4 ± 10.8, 107.0 ± 17.8, 85.4 ± 6.2, pH: 7.43 ± 0.01, 7.42 ± 0.04, 7.40 ± 0.02, respectively.

In SHRSP rats rCBF increase in response to 10% CO₂ inhalation only showed significant differences in both absolute values and percent increase after long periods of hypertension. Early severe hypertension caused no reduction of cerebrovascular reactivity, in spite of the marked rCBF reduction (table 1).

The developmental course of hypertension in rats produced by renal infarction and cortical cerebral blood flow are shown in figure 5. When blood pressure rose over 200 mm Hg 2 weeks after the operation, rCBF in the frontal cortex was about 50% of normal in WK rats who had normal blood pressure before the operation. rCBF decreased gradually approximately in an inverse proportion to the elevation in blood pressure.

The incidence of stroke in RHR rats (their maximum blood pressure: 238 ± 5 mm Hg) was 25.3%.
TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>No. of Rats</th>
<th>Incidence of Stroke</th>
<th>Average Life Span</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stroke-prone SHR</td>
<td>67</td>
<td>(89.3)</td>
<td>(276 ± 16)</td>
</tr>
<tr>
<td>Stroke-resistant SHR</td>
<td>48</td>
<td>(10.7)</td>
<td>(413 ± 23)</td>
</tr>
<tr>
<td>Treated Stroke-prone SHR</td>
<td>59</td>
<td>(0)</td>
<td>(353 ± 11)</td>
</tr>
</tbody>
</table>

*Inter-group difference: significant (P < 0.05)*

FIGURE 4. Incidence of stroke (cerebral hemorrhage and/or softening) and life span in stroke-prone, resistant SHR and treated stroke-prone SHR (F<sub>3</sub> generation of SHR).

Discussion

Clinicians have considered the maintenance of CBF to be essential to the proper treatment of patients with cerebrovascular diseases.<sup>18, 19</sup> A number of investigators have conducted experiments to clarify the relationship between rCBF and stroke.<sup>20</sup> These studies have largely been based on rapid methodological progress in rCBF measurements, e.g., the methods of pial artery diameter measuring,<sup>21</sup> microsphere,<sup>22</sup> autoradiography,<sup>23</sup> inert gas inhalation,<sup>1, 2</sup> intraarterial <sup>133</sup>Xe injection,<sup>24</sup> <sup>133</sup>Xe inhalation-external counting,<sup>25</sup> and hydrogen clearance.<sup>26</sup> Unfortunately, many of these studies have been unconvincing and fragmented, and many problems remain unsolved.

Two problems are of special importance. The first is the absence of conclusively reliable data about cerebral blood flow in patients before they develop stroke, and the second involves the inevitable limitations in the experimental systems due to lack of truly suitable experimental models for stroke in humans.

With the successful establishment of SHRSP rats<sup>11</sup> which...
spontaneously develop cerebral stroke (hemorrhage and/or softening) with an incidence over 80%, it is possible to study CBF before and after stroke. The stroke suffered by these SHRSP rats has been shown to be pathogenically similar to stroke in humans. Using this animal model, we conducted our present studies to determine the causal relation between rCBF and stroke, especially the initiation mechanisms. The animals used were the original SHRSP rats (A4), which were considered to have the greatest predisposition for stroke, and are regarded as models for stroke observed in severe hypertension, that is, "arterio-necro-thrombogenic stroke." 

Many investigators have recognized a reduction of rCBF after patients developed stroke. Gotz et al. concluded that hypertension caused a temporary rCBF increase, but that long-term hypertension resulted in cerebral arteriosclerosis followed by a decrease in rCBF. Tazaki et al. reported that patients with essential hypertension under age 50 showed high rCBF levels, while hypertensive patients over age 50 showed decreased rCBF levels. He concluded that the aging process and long-term hypertension caused structural cerebrovascular changes followed by rCBF reduction. Similar results were reported by Shenkin et al. who found that aging, hypertension, and systemic arteriosclerosis had no effect on cerebral circulation; hypertension with cerebral arteriosclerosis caused cerebral circulatory disturbance. Terashi et al. found no CBF difference between elderly hypertensive patients and elderly normotensive people, although the former showed markedly higher values for cerebrovascular resistance (CVR). With a marked CVR increase, hypertension with cerebral arteriosclerosis caused a decrease in both CBF and cerebral metabolic rate of oxygen. Their long-term prospective investigation of 135 people for 10 years (fig. 6) clearly showed CBF reduction before the occurrence of stroke (especially for intracerebral bleeding).

In our long term observation of rCBF in conscious SHRSP rats, we found rCBF reduction without any change in PaCO2 and chemical cerebrovascular reactivity prior to the development of stroke. Male SHRSP rats generally develop stroke at 9 months (as previously reported), and before this occurs there is a rCBF reduction. In our SHRSP rats at 10 months, this reduction was further intensified and cerebrovascular reactivity also decreased. These facts suggest that severe hypertension initially results in functional rCBF reduction, probably due to reversible arterial constriction which we observed ophthalmoscopically or with a vital microscope in these rats. Sustained hypertension accelerates vascular collagenous and noncollagenous protein synthesis in arterial systems as well as cerebral ar-
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High blood pressure. Such an explanation is supported by the difference in rCBF but no significant difference in blood pressure control on stroke, initially reported experimentally by Yamori and Horie.4 This was again confirmed by controlling severe hypertension (under about 200 mm Hg) rCBF remained within a normal range.

The second is that in antihypertensive agent-treated SHRSPrats and untreated SHRSrats there is a significant difference in rCBF but no significant difference in blood pressure. This may result from the difference in the degree that cerebral vessels structurally adapt to similar long-term high blood pressure. Such an explanation is supported by the fact that the incidence of stroke in SHRSPrats and SHR rats is different, even though there is a similar rCBF reduction in nearly inverse proportion to the lowering of systemic blood pressure.

In the present study, two other important points were elucidated. The first is the prophylactic effect of moderate blood pressure control on stroke, initially reported experimentally by Yamori and Horie.4 This was again confirmed.

The probability that severe hypertension causes a functional rCBF decrease was also supported by our observation on a relation between the cause of hypertension and rCBF decrease using rats made hypertensive from renal infarction, as well as by our finding of the effect of antihypertensive agents on the prevention of rCBF reduction in nearly inverse proportion to the lowering of systemic blood pressure.

In the present study, two other important points were elucidated. The first is the prophylactic effect of moderate blood pressure control on stroke, initially reported experimentally by Yamori and Horie.4 This was again confirmed. By controlling severe hypertension (under about 200 mm Hg) rCBF remained within a normal range.

The second is that in antihypertensive agent-treated SHRSPrats and untreated SHRSrats there is a significant difference in rCBF but no significant difference in blood pressure. This may result from the difference in the degree that cerebral vessels structurally adapt to similar long-term high blood pressure. Such an explanation is supported by the fact that the incidence of stroke in SHRSPrats and SHR rats is different, even though there is a similar rCBF reduction in nearly inverse proportion to the development of severe hypertension. All of these experimental results suggested the importance of the "apoplectic genetic factor" in SHRSPrats. They suggested the possibility that genetic factors in the cerebrovascular system itself are involved with the stroke-proneness and not necessarily related to severe hypertension.

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