Cerebral Circulation and the Initiation Mechanism of Stroke in Stroke-prone Spontaneously Hypertensive Rats (SHRSP)*

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As is well known during this past quarter of a century, highest values have been reported for Japanese death rate from vascular disease in the central nervous system (B 30 by the Japanese standard classification in death rate statistics).¹ Further, among all the countries of the world stroke has most frequently occurred in Japanese.² Now, such a tendency seems to be continuing, as population indexes for aged people have been showing acceleratingly increased values.³ Of course it has been continually emphasized that it is absolutely necessary to find drastic countermeasures for stroke. However, such studies on the pathogenesis of cerebrovascular lesions have been limited by the lack of an appropriate animal model.

Recently, Okamoto, Yamori and Nagaoka⁴ reported the establishment of the stroke-prone spontaneously hypertensive rat (SHRSP) in which stroke (cerebral hemorrhage and/or softening) developed spontaneously, and with a high incidence (more than 80% in males) (Fig. 1).

The establishment of SHRSP proved to be very beneficial. First it experimentally showed the surpassing importance of hypertension^{4,5} as had been guessed epidemiologically by many clinicians.⁶⁻¹¹ Second, based on the evidence

obtained by successive selective breeding of stroke-prone and -resistant SHR, it suggested the important role of genetic factor.¹² Finally, it threw light on studies for clarifying the pathogenetic mechanisms of stroke, as pathological findings of cerebrovascular lesions in SHRSP were verified to correspond well to those in humans.¹³⁻¹⁵ Especially, as Yamori reported,¹³ SHRSP develop stroke just like that in Japanese people, i.e., "arterionecro-thrombogenic stroke", in contrast to "atherothrombogenic stroke" in the people of USA and European countries. This should be noted as compared with Ooneda's "plasmatic arterionecrosis" in humans.¹⁵ Such facts promise a further prophylactic approach to stroke in "Japanese" people using this newly-found suitable model.

However, when SHRSP were established in 1974, two important problems about the suitability as a model for stroke in humans remained unsolved.

The first problem was the difference in predilection sites of stroke between SHRSP and humans. In SHRSP, the most predominant site for stroke was the cortical region, in contrast to the relatively low incidence of stroke in the human cortex. On the other hand, the common site for stroke in SHRSP and humans was the basal ganglia, where the incidence was the highest in man, and next to the highest in SHRSP.

The other problem was cerebrovascular atherogenesis considering the possibility of SHRSP as a suitable model for "atherothrombogenic stroke".

In the present studies, the first problem were

Key Words:

Stroke-prone SHR (SHRSP)
Recurrent branching
Cerebral circulation
Cerebrovascular permeability
Electroencephalography of rat
Cerebrovascular reactivity

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* This study was completed by Ryoichi Horie, M.D., as his thesis. It was reported by Yukio Yamori, M.D., direct director of this thesis work, at the 6th Conference on the Pathogenesis of Hypertension, November 21, 1976, Tokyo.

This study was supported by grants from NIH (HL 17754), the Science and Technology Agency of the Government of Japan, the Japanese Ministry of Education and the Japanese Society for the Promotion of Science.

Fig.1. Stroke-prone SHR (SHRSP)

(a) SHRSP developed stroke at the average age of 9 months in males. Examples were hemiparesis, paraparesis, urinary incontinence, sudden loss of body weight, and so on.

(b) Cerebral bleeding (left: hemorrhage in the left frontal region) and cerebral softening (right: the right hemisphere became swollen, pushing the left hemisphere to the left in the frontal region).

(c) Histological findings of cerebrovascular lesions in SHRSP which developed stroke (Thrombosis in a microaneurysm with perivascular hemorrhage).

(d) Coronal section of the brain (left: cerebral bleeding at the basal ganglia spread to the cerebral cortex in the frontal region, right: large cyst formation due to cerebral softening).

persued for clarifying the initiation mechanism of stroke in relation to cerebral circulation.

MATERIALS AND METHODS

SHRSP⁴ used or examined in this study were the F₂₃₋₃₆ generation of SHR maintained at the Department of Pathology, Faculty of Medicine, Kyoto University (Kyoto, Japan). Stroke (cerebral hemorrhage and/or infarction) developed in these rats spontaneously as shown in Fig. 1. The incidence of stroke in these SHRSP, which died a natural death, was 84% in males and 80% in females. Controls were stroke-resistant SHR (SHRSR)⁴ (The incidence of stroke about 7%), and normotensive control rats of Wistar-Kyoto (WK), from which the SHR had been derived.

Apoplectic gene-free renal infarction hypertensive rats (RHR) were experimentally produced¹⁶ from WK.

All numerical data were statistically analyzed by the Student t-test.

Part 1. Analysis of predilection sites of stroke in SHRSP as compared with those in humans.

(1) In order to determine the areas supplied by the main cerebral arteries in SHRSP, SHRSR and WK, physiological saline with 0.5% trypan blue was injected into the anterior, middle, and posterior cerebral arteries, selectively, by the microsurgical technique for the cannulation of each artery, using an ultra-small caliber capillary tube.

(2) Blood supply to the basal ganglia was investigated further by a microangiographical technique with the tetralin transparency method or by serial sectioning, and the detailed feeding area of each branch was determined.

(3) Autopsies were performed on 1,278 SHRSP, which died spontaneously, to confirm cerebral lesions. These lesions were histologically examined to analyze the precise predilection sites of stroke statistically, according to the feeding areas of the main cerebral arteries determined by

the above-mentioned method.

(4) The primary pre-stroke arterial lesions were detected by applying the aforementioned microangiographical technique to about 50 SHRSP which were suspected to be in the initial stage of stroke, after a long-term daily observation with the closest attention on their behavior, characteristic appearance, symptoms of stroke, and loss of body weight.

(5) Forty-six male SHRSP, 46 male SHRSR, and 50 male WK were operated on at 4 months of age under Nembutal anesthesia (40 mg/Kg, i.p.). Bilateral carotid arteries were simultaneously ligated with great caution to avoid vagal irritation or injury which might cause hypoxia due to respiratory disturbance. After the ligation, death rate and survival time were examined. When rats died, the brains were immediately fixed in 10% formalin for routine histological examination. The localization of lesions was examined macroscopically or microscopically according to the feeding areas of main cerebral arteries in rats.

Part 2. Angioarchitectural investigation on the common predilection sites of stroke between rats and humans.

(1) About 300 rats (SHRSP with SHRSR and WK as controls) were used for microangiographical studies by perfusing a contrast medium (10% gelatin and 0.1% trypan blue in 75% barium) under a constant temperature (38°C), and pressure (equal to the final pressure of the rats following the preperfusion of heparinized saline) through the aortic catheter in the rat under Nembutal anesthesia (30 mg/Kg, i.p.). The rats perfused with the contrast medium were immediately frozen in a refrigerator for one hour and later fixed in cold 10% formaline. The brains were sectioned coronally or sagittally for x-ray photography using a Softex Type CMB (Softex Co., Ltd., Tokyo, Japan). These brain slices were dehydrated for preparation of histological sections, or made more transparent with tetralin/tetrahydronaphthalene for three-dimensional study of the course of arteries.

(2) The frequency distribution of branching angles in the main cerebral arteries of rats and humans was statistically analyzed using microangiographical pictures. As the main stream of the anterior cerebral artery in both rats and humans follows the path of the corpus callosum in the cerebral longitudinal fissure, and that of the middle cerebral artery of rats extends around over the surface of the cerebral hemisphere, the

branching angle measurements in the lateral projection could be accepted as a grossly adequate method. However, such measurements of the middle cerebral artery in humans could have no significance. Branching angles were determined by two line: the interorifice line of branching vessels, and the line between the two points which were the midpoint of the interorifice line and the midpoint of the line connecting two points which were at the same distance as the radius of the parent vessel from the orifice of the branching vessel.

(3) For comparative studies between human and rat brains, carotid angiographical pictures of 41 men without cerebral lesions were randomly taken at the Department of Neurosurgery, Faculty of Medicine, Kyoto University. The frequency distribution of branching angles was obtained by measuring the angles of 137 branches from the anterior cerebral arteries taken at random.

Part 3. Cerebral circulation in the predilection sites of stroke in SHRSP as compared with those in SHRSR and WK

(1) Regional cerebral blood flow (rCBF) was repeatedly measured by Fujitani et al's modification of the hydrogen clearance method¹⁷ in the frontal and tempotal regions of the cerebral cortex for antihypertensive agent-treated (Apre-soline; 80 mg/L in the drinking water) or -untreated male SHRSP, SHRSR, and WK, 50 to 60 in each group, at the age of 50 days and thereafter.

Under Nembutal anesthesia (40 mg/Kg, i.p.), rats were placed in a stereotaxic apparatus, and enamelinsulated platinum electrodes (0.3 mm in diameter and 25 mm in length) were bilaterally implanted into frontal and temporal regions of the cortex, 2 mm and 2.5 mm apart from the midsagittal line, 2 mm anterior and posterior to the bregma, and 2.5 mm in depth from the cortical surface. These electrodes and a reference electrode on the sagittal line, 4 mm anterior to the bregma, were connected to a miniature receptacle and the whole assembly was fixed with dental cement on the skull.

In conscious rats put in a small gas chamber, rCBF was measured 2 weeks after the operation and periodically thereafter.

(2) Chemical cerebrovascular reactivity in SHRSP was determined by detecting an increase in rCBF response to 10% CO₂ inhalation. This was done at the ages of 50, 67, 167, and 200

days.

(3) Without disturbing the nonanesthetized rats, arterial blood samples, 0.2 ml in total, were obtained through an implanted femoral artery canula in 5 month-old male SHRSP, SHRSR and WK, and PaCO₂ and pH were analyzed by a blood gas analyzer (Corning-Eel, Model 161).

(4) On the other hand, the remaining male rats from the same litter as these experimental groups, i.e., 67 SHRSP, 48 SHRSR, and 59 antihypertensive agent-treated SHRSP of the F₃₃ generation in SHR were autopsied after natural death.

(5) rCBF measurement by the hydrogen clearance method was also performed, using 20 RHR at the age of 50 days and thereafter. (In these rats, blood pressure in the developmental cause of hypertension was indirectly measured at the tail by the tail-pickup method⁵)

(6) Model experiments were performed to examine the effect of the branching angle on blood flow in the cerebral arteries. The mean flow velocity was adjusted by changing the flow control valve. At the same time, when necessary, the oscillating superimposed flow velocity was controlled by the stroke volume and frequency of the pulsatile unit. Water flowed through the model branching of rigid acrylic pipes, where the main pipe had an internal diameter of 5.4 mm. Three types of branching geometry were used: (A) recurrent branching, (B) current branching and (C) combined model of recurrent and current branchings. To keep water pressure at the end of this test sections constant, constant head cylinders were used. Finally, flow volume through the main pipe or branchings was collected to be measured. The present experiment emphasized steady flow, since the blood flow in small arteries was considered to be quasi-steady.

Part 4. Estimation of cerebrovascular permeability and electroencephalographical detection of cerebral involvement in the predilection sites of stroke in SHRSP.

(1) For topographical examination of increased cerebrovascular permeability, 5 and 10 month-old SHRSP were perfused through an aortic canula with 200 ml of heparinized physiological saline (perfusion pressure equal to the final systolic blood pressure) under Nembutal anesthesia (40 mg/Kg, i.p.) 2 or 3 hours after the intravenous injection of 1 ml 0.5% trypan blue or 400–100 mg/Kg horseradish peroxidase-type II (Sigma) in physiological saline, respectively. The

rats injected with peroxidase were further perfused with 5% glutaraldehyde in 1/15M phosphate buffer (pH 7.4) for 20 minutes. Brains were extirpated and cut coronally for the macroscopical observation of the extravascular leakage of trypan blue, 50 μ thick sections prepared by a vibratom (Oxford) were stained by diaminobenzidine reaction¹⁸ to demonstrate the localization of peroxidase activity.

(2) Further, to quantitatively detect increased permeability, SHRSP were injected intravenously with 40 mg/Kg of horseradish peroxidase, 24 hours prior to the intensive perfusion as described above. The L-shaped cortical regions fed by anterior and posterior cerebral arteries, the other part of the cortex fed by middle cerebral arteries and the basal ganglia were all extirpated, weighted, and homogenized for the extraction of peroxidase, the activity of which was quantitatively assayed by spectrophotometrical determination of purpurogallin formation.

(3) Thirty WK (420 $\pm$ 30 gm) and 25 SHR (313 $\pm$ 12 gm) were used for EEG recording. Rats, anesthetized with intraperitoneal injection of Nembutal (30 mg/Kg), were placed in a stereotaxic frame, for exposing the skull, and 6 small pores of 1 mm in diameter were made by a dental drill at frontal, parietal, and occipital areas on both sides. Then, stainless steel electrodes (220 μ in diameter) insulated except for a small loop formed at the tip to avoid any injury to the dura and cortex, were inserted through the pores of the skull and settled gently on the dura. The frontal (F), parietal (P), temporal (T), and occipital (O) electrodes on both sides were located 2 mm, 2 mm, 5 mm, and 2 mm lateral to the sagittal line and 5 mm, 2.5 mm, and 0 mm anterior to the inter auricular line, respectively. The indifferent electrode (I) was placed on the mid-sagittal line, 4 mm anterior to the bregma. All electrodes were connected with insulated copper wires to a miniature receptacle, and the whole assembly was embedded in dental cement. Two weeks after the operation EEG was recorded and then repeated for several months under various conditions. When EEG was recorded, rats were in a clear plastic cage (30 $\times$ 40 $\times$ 40 cm) and could move freely. EEG was recorded by a ME-92D (Nihon-Koden), connected to an autoanalyzer MAF-5 (Nihon-Koden), and a photic stimulator was MS-2PS (Nihon-Koden). Monopolar (I-F, I-P, I-O), and bipolar (F-P, P-O, O-F) leading were recorded. Photic stimulation was carried out at the rate of 3, 6, 9, 12, 18, and 24

cps. EEG was recorded during wakefulness or sleep, and under barbiturate anesthesia. The recording calibration was 3 cm/sec and 100 μ V/4 mm.

(4) Around 50 SHRSP and SHRSR (A1-sb,

Cortical Blood Supply in Rat Brain

Fig. 2. Cortical blood supply by the main cerebral arteries in rat.

A3, A4, B1, and B2 strain, male, 4 months of age) were operated on for chronic EEG recording precisely as described above. They were followed up, by repeating EEG records, until their natural death. Then they were autopsied for further histological studies on the cerebral lesions.

RESULTS

Part 1. Analysis of predilection sites of stroke in SHRSP as compared with those in humans

(1) SHRSP, SHRSR, and WK showed no differences in basic angioarchitectural pattern for cerebral blood supply.

Feeding areas of the main cerebral arteries in the rat brain were demonstrated by coloring the brain with trypan blue injected into the anterior, middle, and posterior cerebral arteries. Cortical blood supply demonstrated by this study is illustrated in Fig. 2.

The internal carotid artery took a horizontal path forward (comparatively longer than in man)

Fig. 3. Analysis of 1740 cerebral lesions in 1278 autopsy cases of SHRSP.

(a) Differences in the predilection sites of stroke between rats and humans.

(b) Localization of cerebrovascular lesions in SHRSP.

(c) Analysis of lesions in the basal ganglia of rat brain.

(d) Laterality of the incidence of stroke in SHRSP, analyzed by the types of cerebral lesions (softening, bleeding and hemorrhagic infarction) and in the 3 predilection sites of stroke (anteromedial cortex, occipital cortex and basal ganglia).

after giving off the posterior cerebral artery, and terminated in two main branches (anterior and middle cerebral arteries) which formed a "carotid fork" (more sharply than in man). The anterior cerebral arteries formed an "azygos" vessel which supplied the cortex and medullary substance of the anterior two-thirds of the cerebral hemisphere in the sagittal section. The middle cerebral arteries extended upward over the lateral and dorsal surface of the hemisphere, dividing into multiple branches. The posterior cerebral arteries originated from the "internal" carotid artery, curved and extended upward to supply the tentorial surface of the hemisphere including the occipital cortex and medullary substance (5 mm-wide zone along the rear edge of the occipital lobe in adult rats).

(2) The anterior part of the caudate-putamen nucleus (corresponding to the head of the caudate nucleus in man) was supplied medially by Heubner's arteries from the anterior cerebral arteries and laterally by the lateral group of lenticulostriate arteries. While the posterior part was supplied by the medial group of lenticulostriate arteries (Fig. 9-a).

(3) Statistical analysis of the predilection sites of stroke, totaling 1,740 lesions in 1,278 autopsy cases of SHRSP, is summarized in Fig. 3.

In SHRSP the predilection site with highest incidence was the cortical region (Fig. 3-a). Cortical lesions were classified into three types according to the areas supplied by main cerebral arteries: (1) anteromedial lesion (33.7%), supplied by the anterior cerebral artery. (2) lateral lesion (6%), supplied by the middle cerebral artery, and (3) occipital lesion (30.1%), supplied by the posterior cerebral artery (Fig. 3-b). Frequency of anteromedial and occipital lesions was significantly higher than that of lateral lesions. The site of second greatest frequency in rats was the basal ganglia (24.5%) (Fig. 3-a), especially in the anterior part (88.8%) (Fig. 3-c). This area was supplied by the lateral group of lenticulostriate arteries and Heubner's arteries (Fig. 16-a). The most frequent site of infarction was in the left hemisphere; the occipital cortex and basal ganglia were involved but not the anteromedial cortex, which was supplied by the "azygos" anterior cerebral artery (Fig. 3-d).

(4) Studies on the predilection sites of stroke in numerous autopsy cases of SHRSP suggested that primary pre-stroke arterial lesions were localized in the "boundary zone" of the main cerebral arteries. Minute lesions in the antero-

medial cortex, between the anterior and middle cerebral arteries, were found medially in the area supplied by the anterior cerebral arteries. However, starting points in the occipital cortex between posterior and middle cerebral arteries were localized posterolaterally in the area covered by posterior cerebral arteries. These findings indicated that both anterior and posterior cerebral arteries were responsible for the development of cerebral lesions.

(5) Blood pressures in SHRSP (210 ± 3 mmHg, $M \pm SE$) and SHRSR (183 ± 2) were significantly higher than those in WK (128 ± 1), and they were also significantly different between SHRSP and SHRSR at the age of 4 months. Body weights in both SHRSP (300.9 ± 7.7 gm, $M \pm SE$) and SHRSR (310 ± 7.7) were similar, but significantly less than in WK (345.9 ± 9.4).

Lethality after simultaneous carotid ligation was markedly different between SHR and WK. Although all WK survived after the operation, none of 46 SHRSP survived, and 82%, (38 out of 46) of SHRSR died.

In the cases which died, the mean survival times for SHRSP (35.3 ± 7.8 hours, $M \pm SE$) and SHRSR (32.8 ± 7.6) were not different.

Pathological examination of lesions in 46 SHRSP and 38 SHRSR revealed that incidences of softening and hemorrhage were respectively 58% and 42% in SHRSP and 52% and 48% in SHRSR.

The histologically-determined localization of these lesions was consistent with the predilection sites of cerebral lesions observed in SHRSP at autopsy, this is, anteromedial cortex, occipital cortex, and basal ganglia. The incidence of these sites were respectively 20, 17, 56% in SHRSP and 20, 19, 53% in SHRSR. For SHRSP and SHRSR there was no significant difference in these incidences.

Cerebrovascular lesions in SHRSP and SHRSR after the simultaneous carotid ligation were preponderantly located in the basal ganglia, especially in the anteromedial and anterolateral parts fed by lateral lenticulostriate and Heubner's arteries. The percentage of lesions in these parts were respectively 31 and 18 in SHRSP, and 22 and 20 in SHRSR.

Lesions were more frequently observed on the left side, and such a laterality was noted in the occipital cortex and basal ganglia.

Part 2. Angioarchitectural investigation on the common predilection sites of stroke between rats

Fig.4. Microangiographical manifestation of recurrent and current branches from the main cerebral arteries.

1(a): Current branches of the anterior cerebral artery in human brain. (A modified reproduction of a micro-angiographical picture from reference 39)

1(b): Recurrent branches of the anterior cerebral artery in rat brain (medial part of the brain in a sagittal section.)

2(a): The lenticulostriate arteries and their recurrence in human brain (A modified reproduction of a microangiographical picture from reference 40).

2(b): The lenticulostriate arteries and their recurrence in rat brain. (anterior part of the brain in a frontal section).

Fig.5. Developmental course of hypertension and cortical cerebral blood flow in SHRSP.

and humans.

(1) Angioarchitectural characteristics common to these sites of predilection were examined. Anterior and posterior cerebral arteries supplied the anteromedial and occipital areas (Fig. 2), and showed a high incidence of "recurrent branching" from the main stem. However, the middle cerebral arteries, which did not supply areas commonly infarcted, had few recurrent branchings. These recurrent branchings from the anterior and posterior cerebral arteries were commonly observed in the brains of SHRSP, SHRSR, and WK; such typical recurrent branchings were rare in the cortical area of the human brain. Heubner's and lenticulostriate arteries also had recurrent branchings. Such branchings were commonly observed in SHRSP, SHRSR, WK, and humans (Fig. 4).

(2) A summary of statistical comparisons in frequency distribution of branching angles in the main cerebral arteries of rats was as follows: Main branches of the anterior cerebral arteries had a high frequency of branching angles less 90° both in SHRSP ($89.3 \pm 1.6^\circ$, $M \pm SE$) and in WK ($88.9 \pm 1.9^\circ$). I.e., the area supplied by the anterior cerebral arteries was mainly through recurrent branchings. However, such recurrent branchings were not noted in the main branches of the middle cerebral arteries in SHRSP ($137 \pm 1.4^\circ$) and WK rats ($128 \pm 1.9^\circ$).

(3) There was a significant difference in frequency distribution between rats and humans. The mean of the branching angles of main branches from the anterior cerebral artery was $114.6 \pm 1.9^\circ$ in humans. Therefore, it was concluded that, in rats, the area supplied by anterior cerebral arteries was mainly through recurrent branching, but such was not true in humans.

Part 3. Cerebral circulation in the predilection sites of stroke in SHRSP as compared with that in SHRSR and WK.

(1) rCBF at the frontal cortex in SHRSP was markedly decreased at the ages of 5 and 10 months (63.6 ± 5.8 and 57.3 ± 5.2 ml/min/100 gm, $M \pm SE$, respectively), in comparison with rather high values in SHRSR (5-month-old; 110.9 ± 6.4 , 10-month-old; 102 ± 6.0) and normal values in WK (101.0 $\pm$ 7.4, 93.8 $\pm$ 4.4). Blood pressure in SHRSP at the ages of 5 and 10 months was over 200 mmHg (230 ± 4 and 215 ± 4 , respectively), in contrast to moderate hypertension in SHRSR (5-month-old: 173 ± 8 , 10-month-old; 190 ± 5) and a normotensive level in WK (5-month-old; 132 ± 3 , 10-month-old; $130 \pm$

5).

The developmental course of hypertension and cortical cerebral blood flow in SHRSP is shown in Fig. 5. When SHRSP developed severe hypertension over 200 mmHg around the age of 60 days, rCBF in both frontal and temporal cortices began to decrease. Under these circumstances, it was considered to be noteworthy that the beginning of rCBF reduction in the frontal region was earlier and more abrupt than that in the temporal region. Also, during the developmental course of hypertension and at the hypertensive steady state, the frontal region constantly showed lower rCBF values than the temporal region. When severe hypertension was established and stabilized at the age of 90 days, an anti-hypertensive agent (DU-717, 100 mg/day) reported to have no diuretic effect¹⁹ was administered. This therapy effectively decreased blood pressure below 200 mmHg and inversely increased rCBF in proportion to the lowering systemic blood pressure. During this therapy, the maximum increment of rCBF was statistically significant. Also observed was that suspension of anti-hypertensive therapy resulted in redevelopment

Fig. 6. Reduction of rCBF and cerebrovascular reactivity in SHRSP.

Fig. 7. Developmental course of renal hypertension and cortical cerebral blood flow in rats.

of severe hypertension and a marked rCBF decrease.

The effect of long-term antihypertensive therapy (chronic administration of Apresoline in drinking water (80 mg/l) at the age of 40 days and thereafter) on rCBF at the frontal cortex in male 10-month-old SHRSP, SHRSR and WK was as follows: rCBF in treated SHRSP with moderate hypertension (185 ± 3 mmHg, 86.7% of control SHRSP) was maintained within normal range (85.7 ± 4.3 ml/min/100 gm). As for SHRSR, the treated group showed lower levels in blood pressure (158 ± 3 , 81.9% of control group) than those in control SHRSR, and rCBF in this group decreased but was within normal range (77.6 ± 31 , 75.9% of control group). Treated and untreated WK showed no statistically significant differences in either blood pressure or rCBF.

(2) For SHRSP, rCBF increase in the frontal region in response to 10% CO₂ inhalation only showed significant differences in both absolute values and percent increase at the advanced stage. At the early stage of severe hypertension no reduction of cerebrovascular reactivity was observed, in spite of the marked rCBF reduction (Fig. 6).

(3) In arterial blood gas analysis, no significant differences were noted in 5-month-old SHRSP (PaCO₂: 33.6 ± 2.0 mmHg, PaO₂: 85.4 ± 10.8 mmHg, pH: 7.43 ± 0.01 , M $\pm$ SE, respectively) SHRSR (32.1 ± 2.3 , 107.0 ± 17.8 , $7.42 \pm$

0.04) and WK (32.1 ± 2.9 , 85.4 ± 6.2 , 7.40 ± 0.02).

(4) Treated SHRSP showed no stroke, in contrast to a high percentage (89.3%) of stroke (cerebral bleeding and/or softening) among untreated SHRSP who suffered a natural death. SHRSP showed low incidence of stroke (10.7%). In regard to average life spans, untreated SHRSP had the shortest (276 ± 16 days, M $\pm$ SE) and SHRSR had the longest (413 ± 23). Treated SHRSP showed a significantly longer life span (353 ± 23) than that of untreated SHRSP, but shorter than that of SHRSR.

(5) The developmental course of renal infarction hypertension and cortical cerebral blood flow in RHR is shown in Fig. 7. 2 weeks after the operation, blood pressure developed over 200 mmHg. At this time, rCBF in both frontal and temporal cortices showed about 50% of the normal values in WK with normal blood pressure before the operation. rCBF decreased gradually approximately in an inverse proportion to the rise in blood pressure.

In regard to the developmental course of renal infarction hypertension and rCBF, too, during the developmental course of hypertension and at the hypertensive steady state, the frontal region constantly showed lower rCBF values than the temporal region (Fig. 7).

The incidence of stroke in RHR (their maximum blood pressure: 238 ± 5 mmHg) was 25.3%.

(6) In model experiments, flow rate through the main pipe and branchings depended on both the total flow rate and the pressure at the end of the test section. Thus in the present experiment, water pressure at the ends of main pipe branchings were carefully controlled so as to be the same (within an accuracy of about 0.1 mm H₂O). Reynold's number (Re) was also taken into sufficient consideration.

$$\text{Re} = \frac{UD}{\nu}, \quad U = \frac{Q}{\pi \left(\frac{D}{2}\right)^2}$$

(When U is the mean flow velocity, D = 0.85 cm is the internal diameter of the main pipe, and $\nu = 0.014$ is the kinematic water viscosity.) When the total flow rate was small, the main current and branching current were noted to increase linearly with the total flow rate. When total flow rate became larger than a certain value, deviation from this linear relation was noted. Increase rate in the branching flow became less, while such rate in the main flow became greater. As total flow became larger the recurrent branching flow became less than the current flow.

Part 4. Estimation of cerebrovascular permeability and electroencephalographical detection of cerebral involvement in the predilection sites of stroke in SHRSP.

(1) Anatomical distribution of increased cerebrovascular permeability was detected by the leakage of trypan blue or peroxidase.

In the brain of SHR, especially SHRSP over the age of 8 months, leakage of trypan blue and peroxidase was clearly demonstrated macroscopically and histochemically. Such leakage was often noted not only surrounding the petechial hemorrhage or small softening, but also in the anteromedial and occipital areas of the subcortex and cortex or basal ganglia without apparent cerebrovascular lesions. None of WK, even over the age of 8 months, showed such a leakage in the brain.

(2) By peroxidase activity assayed by spectrophotometrical determination of purpurogallin formation quantitatively, increased permeability of the intracerebravascular system was reassured in SHRSP at the advanced stage of hypertension. Such was especially true in the predilection sites of the areas fed by arteries with recurrent branching from the main stream.

(3) During wakefulness, EEG showed predominantly generalized 20–50 cps waves with an

TABLE I NORMAL PATTERN OF CORTICAL ELECTROENCEPHALOGRAPHY IN RATS AND HUMANS

<i>Normal Pattern of Rat Cortical Electroencephalography</i>
<i>During wakefulness:</i>
1) <i>Predominant generalized β activity</i> (20–50 cps, 10–100 μ V)
2) <i>Intermittent slow α and θ waves</i> (8–4 cps, 50–150 μ V)
<i>During drowsiness:</i>
1) <i>Attenuation of fast activity</i>
2) <i>Appearance of high voltage activity and spindle-like activity</i> (6–9 or 14–16 cps, 100–500 or 50–300 μ V)
<i>During sleep:</i>
1) <i>Loss of fast activity</i>
2) <i>Predominant generalized θ and δ waves</i> (6–3 cps, 100–500 μ V)
<i>Normal Pattern of Human Cortical Electroencephalography</i>
<i>During wakefulness:</i>
1) <i>Occipital dominant α activity</i> (8–12 cps, 10–100 μ V)
2) <i>Intermittent fast and rare θ waves</i>
<i>During drowsiness:</i>
1) <i>Attenuation of α activity</i>
2) <i>Appearance of low voltage fast waves and θ waves (ripple phase)</i>
3) <i>Appearance of hump</i> (3–10 cps, 100–300 μ V), <i>and sleep spindle</i> (14 cps) (hump and spindle phase)
<i>During sleep:</i>
1) <i>Loss of fast activity</i>
2) <i>Predominant generalized θ and δ waves</i> (0.5–6 cps, 100–500 μ V) (hill wave phase)

amplitude of 10 to 100 μ V, and also intermittent slow α and θ waves (8–4 cps, 50–150 μ V) (Fig. 8:a).

During drowsiness, attenuation of the fast activity (20–50 cps) was noted. Then high voltage (100–500 μ V), 6–9 cps spindle-like activity of one to 10 sec duration, appeared over all cortical areas-especially over frontal areas.

During sleep, loss of fast activity (20–50 cps) and predominant generalized high voltage θ and δ waves (6–3 cps, 100–500 μ V) were observed. During all sleep phases, EEG of the animal could be rapidly altered with reappearance of the pre-

Fig.8. Electroencephalographical detection of cerebral involvement of severe hypertension in SHRSP.

(a) Normal EEG during wakefulness (WK rat:5M)

(b) EEG during wakefulness, appearance of diffuse α pattern-like sequence (SHRSP: 10M)

(c) EEG during drowsiness, absence of spindle-like activity (16 cps) in the left side (SHRSP: 10M).

(d) EEG recorded during wakefulness 1 day before death from stroke (SHRSP: 10M).

dominant generalized β activity (20–50 cps) and intermittent slow α and θ waves (8–4 cps), as summarized in Table I.

Sequential EEG changes under hexobarbital (30 mg/Kg, i.v.) anesthesia were as follows. thirty sec after injection: High-amplitude (50–100 μ V) fast wave (20–30 cps) of spiky appearance, varying with fast waves of smaller amplitude, seemed to be similar to barbiturate fast waves in human EEG. 1'30'' after injection: Slower wave forms of irregular contour and random occurrence appeared, and spiky waves of irregular amplitude were superimposed on and between these slow waves. Predominant high voltage θ activity, so called spindle-like activity (6–9 cps, 100–150 μ V), was noted as the characteristic pattern of rat EEG. 3' after injection: Characteristic spindle-like activities appeared in all areas of the cortex. 5' after injection: Predominant generalized θ

and δ waves (6–3 cps, 100–500 μ V) and loss of fast activity were noted as anesthesia deepened. 25' after injection: Predominant generalized β activity (20–50 cps) and intermittent slow α and θ waves (8–4 cps) reappeared. Righting reflex of the animal also appeared at this time.

(4) When SHRSP developed signs and symptoms of cerebral stroke EEG showed marked abnormalities i.e., gradual or especially abrupt (more than 20 gm/300–400 gm rat/1 week) weight loss, increased irritability, aggressiveness, white eyes (due to retinal edema), weakness of extremities, mono- or paraparesis, urinary incontinence, selfdestructiveness, enlargement of skull (especially, in the occipital region) decrease in respiratory rate, and circling (it is necessary to differentiate cerebellar ataxia and otitis media).

In over 8-month-old SHRSP (less frequently and delayed in SHRSR), electroencephalographi-

cal abnormalities began to be remarkable as follows: (1) Attenuation of predominant generalized β activity (20–50 cps, 10–100 μ V), (2) Dominance of slow α and θ waves (8–4 cps, 50–150 μ V), (3) diffuse slow α pattern (Fig. 8:b), (4) Diffuse θ pattern, (5) Polymorphousness in predominant slow α and θ waves, (6) Appearance of δ waves (3–2 cps, 100–500 μ V), (7) High voltage polymorphous θ and δ waves (6–3 cps, 200–500 μ V) (Fig. 8:d), (8) Flat EEG in a wide sense, (9) Laterality.

Although such findings were obtained from EEG during wakefulness, it was often difficult to decide the foci in the brain.

During drowsiness, EEG frequently demonstrated more marked laterality as follows: (1) Appearance of spiky components, (2) Lazy activity or asymmetry of spindle-like activity (6–9 cps, 100–500 μ V) (Fig. 8:c), (3) Less frequency, lazy activity of 6 and 14 positive spikes on the pathological side of the cerebral hemisphere.

The above-mentioned abnormalities less frequently appeared in the temporal region than in the frontocentral region.

These findings corresponding to cerebral lesions were reassured histologically at autopsy.

It was of greatest importance that lazy activity or asymmetry of spindle-like activity was noted by EEG during drowsiness in 5-month-old SHRSP which showed severe hypertension over 200–220 mmHg, although neither asymmetry nor abnormal waves were detected during wakefulness.

DISCUSSION

The successful establishment of SHRSP by Okamoto and colleagues⁴ suggested the existence of genetic factors for stroke,¹² and also made it possible to confirm the great importance of hypertension in the development of cerebral stroke^{4,5}. Further evidence that SHRSP are models for human stroke was obtained in the present studies by analyzing local factors for stroke in SHRSP and comparing them with the same factors in humans.

SHRSP and WK showed no differences in their cerebral blood supply patterns; there were no specific angioarchitectural characteristics in SHRSP that could explain their strokeproneness.

In SHRSP the most preponderant site for stroke was the cortical region (69.8%), in contrast to the relatively low incidence of stroke in the human cortex (13.1% according to the mean of the clinical statistics by Stehbens²⁰ and others).

In SHRSP, massive cortical lesions were seen, as compared to small asymptomatic cortical lesions in man. Further statistical analysis of the three types of cortical lesions in SHRSP revealed that anteromedial and occipital lesions occurred much more than lateral lesions.

On the other hand, the common site of stroke in rats and humans is the basal ganglia, where the incidence is next to the highest in SHRSP (24.5%) compared with the highest in man (66.5%), according to the aforementioned statistics. Consequently, there are three predilection sites of stroke in SHRSP, i.e., anteromedial cortex, occipital cortex, and basal ganglia, in contrast to humans, where the most frequent lesions occur in the basal ganglia.

After simultaneous bilateral carotid ligation, young SHRSP and SHRSR were exposed to an experimental system to accelerate induction of cerebrovascular lesions. Here, for these SHR, the predilection sites of lesions were consistent with those observed in SHRSP after natural death. Such facts suggested local factors which were common to these predilection sites of stroke in SHRSP and humans.

In the present studies, it was confirmed that angioarchitectural characteristics common to these predilection sites in both rats and humans were recurrent branchings. Anterior and posterior cerebral arteries, which seemed to be responsible for anteromedial and occipital lesions in SHRSP, have recurrent branchings. Lenticulostriate arteries (responsible for stroke in basal ganglia in both SHRSP and humans) branch off recurrently from the middle cerebral arteries. However, the four main arteries (seemingly not so responsible for cortical lesions), i.e., the middle cerebral arteries in rats and the three main cerebral arteries in humans, had few recurrent branchings. In both SHRSP and humans, statistical comparison of the frequency distribution of branching angles in the main cerebral arteries confirmed the importance of “recurrence” in the predilection sites of stroke. It is generally recognized that in man, few cortical branchings of the middle and posterior cerebral arteries show such a recurrence.^{21,22}

The persistence of such “recurrent branching” of the anterior and posterior cerebral arteries in rats is understandable when phylogenetic difference in degree of developmental convolution of the brain between rats and humans is considered, referring to the arterial development following ontogenic developmental convolution of human

Fig.9. (a) Similarity of blood supply to the basal ganglia in human and rat brains.
 (b) Persistence of recurrent branching from anterior and posterior cerebral arteries in rats, in phylogenic comparison with human brain.

brain, as described by Padget⁴¹ (Fig. 9:b).

Analysis of lesions in the rat basal ganglia revealed that the anterior part, supplied by the lateral group of lenticulostriate arteries and Heubner's arteries, showed a high incidence of

stroke in SHRSP (88.8%). Such was in contrast to the highest incidence of putamen with the body of caudate nuclei in man.²¹ The head of the caudate nucleus in man is proved to be supplied by Heubner's arteries, and the body of the

caudate nucleus with putamen is supplied by the lateral group of lenticulostriate arteries.²¹⁻²³ Such fact indicates the significance of the lateral group of lenticulostriate arteries (and Hubner's arteries which are markedly developed in the rat brain) for the development of stroke in both rats and humans. The microangiographical manifestation of lenticulostriate arteries (and Heubner's arteries) in the basal ganglia common to rat and human brains is illustrated in Fig. 9:a. Heubner's arteries and lenticulostriate arteries in rats and humans also have "recurrent branchings" from their main streams, that is, anterior and middle cerebral arteries, respectively. Double feeding of the anterior part of the striatum by Heubner's and lateral lenticulostriate arteries in rats also may be explained by a phylogenetic difference in the degree of developmental convolution of the brain between rats and humans. These comparative studies between rat and human brains confirmed the significance of "recurrent branchings" as one of the local factors for stroke in the cortical region in rats and in the basal ganglia of both rat and human brains.

Statistically significant differences in the incidence of stroke between right and left hemispheres and between right and left basal ganglia might also be caused by right and left differences in the main cerebral blood supply. The left common carotid artery shows "recurrent branching" from the aortic arch, in contrast to "current branching" of the right brachiocephalic artery from the aorta in rats. On the other hand, no laterality was observed in the incidence of stroke in the hemispheres supplied by the anterior cerebral arteries which form an "azygos" artery.

Shellshear (1921)²⁴ was the first to find the existence of "recurrent branching" in the lateral group of lenticulostriate arteries. Matsuoka^{25,26} was the first to suggest the significance of "recurrent branchings" of the lenticulostriate arteries for the pathogenesis of stroke. Katsuki^{27,28} stressed its importance in stroke by examining the brain by a plastic injection method. Finally, the establishment of SHRSP made it possible to actually clarify the responsibility of "recurrent branching" for the pathogenesis of stroke.

The present study indicates not only "recurrent branching", but also a "boundary zone" as the local factors for stroke. The importance of a "boundary zone" in the pathogenesis of stroke in humans was suggested by Van Den Bergh,²⁹ but was confirmed in the present study

on the primary prestroke arterial lesions in SHRSP killed at the initial stage of stroke.

The difference of predilection sites of stroke between rat and human brains is very important for clarifying the local factors of stroke such as "recurrent branching" and "boundary zone". SHR were clearly vulnerable to ischemic hypoxia induced by simultaneous bilateral carotid ligation, all SHRSP and 80% of SHRSR died in average 35 and 32 hours after ligation, while all WK survived. The predilection sites of lesions in these SHR were consistent with those observed in SHRSP after natural death. However, incidences of lesions in the basal ganglia, especially in the anteromedial and anterolateral parts, were markedly increased by acute induction in comparison with spontaneous lesions observed in SHRSP at autopsy. These findings confirmed that cerebral lesions were acceleratedly induced by simultaneous bilateral carotid ligation, even in young SHRSP and SHRSR. Further, these lesions developed at the "minor loci" of cerebral circulation fed by "recurrent" branches, especially at the basal ganglia fed by lateral lenticulostriate arteries and Heubner's arteries which branched off with a sharp angle from the middle and anterior cerebral arteries, respectively. (The preponderant occurrence of lesions in the basal ganglia and the increased incidence of cerebral hemorrhage indicate that the acute induction of lesions by simultaneous bilateral carotid ligation makes SHRSP and SHRSR even better models with lesions very similar to those in man.)

There is some experimental evidence that "recurrent branching" causes a flow decrease in the distal portion of branching when flow pressure is controlled at a constant level, and that laminar flow causes a decrease in particles contained in the distal portion of "recurrent branchings".^{30,31} These phenomena might result in circulatory disturbances such as a decrease in blood flow and anemic blood flow in the distal portion of "recurrent branching". In particular, circulatory disturbances (ischemic hypoxia) might be intensified in regions supplied by the anterior cerebral artery, which is the "azygos" artery, and by the posterior cerebral arteries, which also "recurrently" branch off from the internal carotid arteries in rats.

In order to reassure the importance of recurrent branching as a local factor for stroke, especially under severe hypertension, rCBF was measured in the frontal and temporal regions. In other words, for the region fed by recurrent

and current branches from the main cerebral arteries, rCBF was compared in SHRSP and apoplectic gene-free RHR. Also, the effect of branching angles on the stream, i.e., flow ratio between recurrent and current branchings from the main stream, was analyzed by an experimental model system of minute tubes (constructed after due consideration of their likeness to the actual branching patterns).

Recently, there has been an increasing clinical emphasis on the significance of studies on rCBF in cerebrovascular disease. Further, since methods for rCBF measurement made rapid progress after the nitrous oxide technique first developed by Kety and Schmit^{32,33} many results have been reported. For stroke cases, various changes in rCBF at various stages have been reported, where many researchers observed rCBF reduction by their own methods at least after patients developed stroke.³⁴⁻³⁶ However, the causal relation between rCBF and stroke, especially between the initiation mechanisms, has never been elucidated. To clarify such a causal relation, many experimental approaches using various experimental system and methods have been tried.³⁷⁻³⁹ Among these reports, some coincided with clinical data, others added new experimental findings, and still others advocated new theory, for example, "hypertension breakthrough"^{40,41} The experimental models mainly used here were, renal infarction hypertensive models or animals with carotid artery ligated to observe cerebral ischemia. However, these models were not truly suitable, and hence all of these experimental system had some inevitable limitations.

Here we used our original SHRSP (A₄), which were considered to have the most concentrated genetic disposition of stroke. In regard to the relationship between hypertension and rCBF, Gotoh⁴² concluded that hypertension caused a temporary rCBF increase, but that long-termed hypertension resulted in cerebral arteriosclerosis followed by rCBF decrease. In other words, he suggested that functionally rCBF reduction might not result from hypertension itself. Rather, it might result from cerebrovascular organic changes due to hypertension or the aging process. Moreover, Tazaki⁴³ reported that in essential hypertension patients 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-termed hypertension caused organic cerebrovascular changes followed

by rCBF reduction. Further, the same results were reported by Shenkin⁴⁴; While aging, hypertension, and systemic arteriosclerosis had no effect on cerebral circulation, hypertension with cerebral arteriosclerosis caused cerebral circulatory disturbance. In contrast to these reports, Terashi et al.⁴⁵ reported that, in comparing elderly hypertensive patients and elderly normotensive people, there was no CBF difference, although in the former there were markedly higher values for cerebrovascular resistance (CVR). Also reported was that, after a marked CVR increase, hypertension with cerebral arteriosclerosis caused a decrease in both CBF and cerebral metabolic rate of oxygen (CMRO₂). Furthermore, by long-term prospective investigation of 135 people for 10 years, a most noteworthy result was that CBF reduction was recognized before the occurrence of stroke (especially in the case of cerebral bleeding).

Using the hydrogen clearance method for long-term studies on rCBF in conscious SHRSP, rCBF reduction, without PaCO₂ changes and chemical cerebrovascular reactivity, was detected as early at ages 50 days to 5 months. Male SHRSP generally develop stroke at 9 months, and before this time there is rCBF reduction. Thus, in SHRSP at 10 months, this reduction, with decreased cerebrovascular reactivity, was greatly marked. Such facts suggest that severe hypertension results in functional rCBF decrease. Further, long-term moderate ischemic hypoxia induces an increase in vascular permeability⁴⁶ and then causes cerebrovascular organic changes such as arterionecrosis-the basic lesions leading to stroke.^{47,48}

This probability that severe hypertension caused functional rCBF decrease was supported by the other similar results, i.e., a relation between the developmental cause of hypertension and rCBF reduction was obtained in RHR. Also, it was observed that administering an antihypertensive agent caused an rCBF increase in nearly inverse proportion to the lowering of systemic blood pressure. The effect of aging on rCBF is a problem. I.e., is such an abrupt rCBF decrease-as seen when SHRSP developed severe hypertension around age 60 days-related mainly to the aging process? For humans, Tazaki⁴⁵ speculated such to be true. However, for age-matched moderate hypertensive SHRSR and normotensive WK, there were statistically significantly higher rCBF levels (about twice as high as SHRSP). Thus, perhaps this aging question can be neglected. In the

present study, two other important problems were also elucidated. The first-the prophylactic effect of moderate blood pressure control on stroke, was initially reported experimentally by Yamori and Horie⁴⁹. Now, this fact was again confirmed, where the mechanism of stroke prophylaxis by controlling severe hypertension under a certain level (about 200 mmHg) appeared to be due to maintaining rCBF within normal range. So in this sense, blood pressure control and rCBF control are equivalent. Thus, Such a close relation between these two was first suggested experimentally in the present study. The second problem is that in antihypertensive agent-treated SHRSP and-untreated SHRSR there was a marked difference in rCBF but no significant difference in blood pressure. This fact 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 SHRSP and RHR is different, even though there is a similar rCBF reduction in inverse proportion to the development of severe hypertension. All of these experimental results suggested the importance of the apoplectic genetic factor in SHRSP. I.e., they suggested the possibility that a certain genetic factor in the cerebrovascular system itself is involved in stroke-proneness, with or without relating to severe hypertension.

In addition, also confirmed was the effect of recurrent branching of the main cerebral arteries. That is; age-matched SHRSP and RHR produced experimentally from WK showed similar decreases in rCBF, when they developed severe hypertension over 200 mmHg 60 days after birth or 2 weeks after the operation, respectively. It is note worthy that there was regional difference in rCBF reduction, i.e., reduction in the frontal cortex fed by recurrent branches was greater than that in the temporal cortex fed by current branches from the main cerebral arteries. Incidence of stroke was higher in the former than in the latter.

On the other hand, model experiments proved that the flow ratio in recurrent branches was lower than that in current branches. These results seemed to support the importance of recurrent branching in the main cerebral arteries, i.e., the significance of hemodynamics as one of the local factors for stroke. rCBF reduction, caused by severe hypertension, -especially in the region fed by recurrent branches, probably results

Fig.10. Initiation mechanism of stroke.

in long-term moderate ischemic hypoxia followed by initiation of cerebrovascular damage.

In fact, in areas supplied by recurrent arteries, there is an increase in vascular permeability before the development of stroke. By topographical study of increased cerebrovascular permeability, it was shown that the anteromedial or occipital area of the cortex or subcortex, and basal ganglia often with leakage of trypan blue and peroxidase, corresponded well to the predilection sites of cerebral hemorrhage and softening in SHRSP. In other words, cerebrovascular permeability was increased in SHRSP, especially at the sites fed by recurrent arteries. Further, qualitatively and quantitatively, increased permeability of the intracerebrovascular system was reassured in the predilection sites of the areas fed by arteries with recurrent branching from the main stream.

However, such an increased permeability was manifested at the later stage of hypertensive involvement of the cerebrovascular system, rather than at an early stage, when the vascular involvement should be detected. Assuming that increased permeability of the cerebrovascular system might partly result from the long-term moderate hypoxic state (ischemic hypoxia) caused by severe hypertension, an electroencephalographical approach was tried. Such was done in the hope

that EEG would detect initial functional brain alteration due to hypoxia. Then, such a detection might be useful for predicting stroke in the future. SHRSP with signs and symptoms of cerebral stroke showed marked abnormalities in cortical EEG and during drowsiness; laterality in EEG was manifested more remarkably. Especially, lateralities in spindle-like activity (lazy activity), which were frequently observed in over 8-month-old SHRSP (during wakefulness, they often showed diffuse slow α or diffuse θ pattern EEG), were detected in 5-month-old SHRSP with blood pressure over 200–220 mmHg. These findings suggested that it is possible that any functional change in the brain prior to increased cerebrovascular permeability might occur. Further, it is possible to conduct further electroencephalographical studies to throw light on how to predictively detect cerebrovascular involvement in hypertension.

Based on the above-mentioned experimental facts, the following initiation mechanism of stroke was suggested.

Around age 60 days, SHRSP developed severe hypertension followed by functional rCBF reduction, especially rCBF in the region fed by recurrent branches from the main cerebral arteries. Long-termed moderate ischemic hypoxia due to rCBF reduction caused gradual cerebrovascular organic damage followed by increased permeability of the cerebrovascular system. Such results in arterionecrosis of cerebral vessels, following cerebral hemorrhage and/or softening, i.e., "arterionecrothrombotic stroke", as Yamori, reported.¹³

SUMMARY

The predilection sites of cerebrovascular lesions (cerebral hemorrhage and/or softening) were studied in 1,278 SHRSP. The precise supply to the main cerebral arteries was determined by trypan blue injections and microangiography. The three major territories were the anteromedial cortex, the occipital cortex, and the basal ganglia. A common angioarchitectural characteristic of these areas was the blood supply through "recurrent branching" from the main stream. In the basal ganglia, where there is a preponderance of lesions, the arteries responsible for these lesions belonged to the lateral group of lenticulostriate arteries. The primary prestroke arterial lesions were further studied microangiographically in SHRSP killed at the time the initial symptoms of stroke were detected. These points were located

at the "boundary zone" of the main cerebral arteries. Such findings indicated the importance of these two angioarchitectural minor loci as the basis for functional or organic circulatory disturbances that might cause stroke. Since these local factors for stroke are common in the cortex and basal ganglia of rats and basal ganglia of humans, these SHRSP are regarded as good pathogenetic models for studies on stroke in humans. After simultaneous bilateral carotid ligation, accelerated induction of cerebrovascular lesions in young SHRSP and stroke-resistant SHR (SHRSR) showed predilection sites of stroke approximately consistent with those in SHRSP after natural death. (and rather consistent with those in humans). Such findings confirmed that these lesions developed at the "minor loci" of cerebral circulation fed by "recurrent branches" from the main cerebral arteries.

Regional cerebral blood flow (rCBF) was repeatedly measured by the hydrogen clearance method in both frontal and temporal cortices of SHRSP at the age of 50 days and thereafter. When SHRSP developed severe hypertension over 200 mmHg at the age of 60 days, rCBF in both frontal and temporal cortices began to decrease abruptly, especially in the frontal region of the cortex—one of the three predilection sites of stroke in SHRSP. Adding to that, during the developmental course of hypertension and at the hypertensive steady state, the frontal region constantly showed lower rCBF values than the temporal region. In contrast, such a reduction in rCBF was not noted in either SHRSR which developed moderate hypertension under 200 mmHg, or in Wistar-Kyoto rats (WK) with normal blood pressure under 150 mmHg.

A similar marked reduction of rCBF under severe hypertension over 200 mmHg was also detected in apoplectic gene-free renal infarction hypertensive rats (SHR) experimentally produced from age-matched WK. Blood samples were obtained through an implanted femoral artery canula without disturbing the nonanesthetized SHRSP, SHRSR and WK. Among these rats, arterial blood gas analysis (PaCO_2 , PaO_2 and pH) showed no significant differences at the age of 5 months.

Chemical cerebrovascular reactivity, that is, an increase in rCBF in response to CO_2 inhalation, showed no significant difference among SHRSP from the age of 50 days to 5 months. However, it markedly decreased in SHRSP at the age of 9 months and thereafter (the average age of male

SHRSP which develop stroke is 9 months).

The present study reassured that no stroke occurred in antihypertensive agent-treated SHRSP. Moreover, in these SHRSP as long as blood pressures were well-controlled by antihypertensive agents to within moderate hypertensive range below 200 mmHg, rCBF did not decrease. On the other hand, the effect of branching angles on the stream, i.e., flow ratio between recurrent and current branchings from the main stream, were analyzed by an experimental model system of minute tubes.

This model experiment proved that the flow ratio in recurrent branches was lower than that in current branches.

Using trypan blue or peroxidase injected intravenously 2 to 3 hours before sacrifice, increased cerebrovascular permeability was detected. The intracerebral localization of increased permeability corresponded with the predilection sites of stroke in SHRSP, qualitatively and quantitatively.

Electroencephalographic studies proved that any functional changes in the brain prior to increased cerebrovascular permeability might occur.

From these experiments, initiation mechanism of stroke was suggested as follows.

Severe hypertension in SHRSP causes functional reduction of rCBF, especially in the areas fed by recurrent branches from main cerebral arteries. Long-termed duration of moderate ischemic hypoxia due to rCBF reduction results in increased cerebrovascular permeability. This then causes arterionecrosis, which is regarded as a pathological finding common to cerebral bleeding and cerebral infarction.

Aknowledgments

The author wishes to express his cordial thanks to Professor Hajime Handa (Department of Neurosurgery, Faculty of Medicine, Kyoto University) for his proper advice and hearty encouragement, and to Associate professor Yukio Yamori (Department of Pathology, Faculty of Medicine, Kyoto University) for his excellent guidance with consistent enthusiasms, without which the present studies could not have been completed. Also invaluable were the help of Dr. Masayasu Sato, Mr. Hideyuki Niimi, PH. D., Dr. Ichiro Akiguchi, as co-workers, and Miss Yasu Okuyama who did not spare herself for the illustrations.

Further, the author should never forget the warm-hearted spiritual support of Dr. Kozo Okamoto, Emeritus Professor of the Department of Pathology, Faculty of Medicine, Kyoto University, and of his parents.

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Discussion:

Chairman: K. Ogino M.D. (Kyoto Univ.)

Dr. K. OGINO: Yamori succeeded to produce two types of cerebral arterial lesions—hypertensive fibrinoid degeneration and arteriolipidosis—

in SHRSP. Please remark to pathophysiological significance of rCBF reduction and cerebrovascular reactivity to CO₂ inhalation with reference to two types of cerebrovascular lesion.

Did you succeed to produce atherothrombotic stroke in SHRSP?

Which antihypertensive agent is most effective to prevent stroke in SHRSP?

Dr. Y. YAMORI (*Kyoto Univ.*): rCBF reduction in SHRSP begins when their blood pressure surpasses 200 mmHg. After a long-lasting rCBF reduction, a significant decrease in cerebrovascular reactivity to CO₂ inhalation is noted. In contrast to these findings in SHRSP, rCBF is well maintained at the initial stage in arteriolipidosis-prone rats with ring-like fat deposits in cerebrobasal arteries. However, the cerebrovascular reactivity itself is already decreased at the initial stage. Therefore, rCBF reduction in the former model is initially a functional and reversible change. In the final stage, it becomes an organic and irreversible change.

SHRSP is regarded as being the best model for cerebrovascular diseases (cerebral hemorrhage and infarction) due to arterionecrosis caused by severe hypertension. That is, SHRSP is a model for "arterionecro-thrombogenic" stroke. We have been using SHR fed on a high fat cholesterol diet to try to obtain a model for "athero-thrombogenic stroke" (cerebral infarction). However, we have not yet been successful.

By giving SHRSP long-lasting treatment with anti-hypertensive agents the incidence of stroke decreased. However, for these same SHRSP, it is now necessary to evaluate different effects of various kinds of hypertensive agents on cerebral vessels themselves.

Dr. Y. KANEKO (*Yokohama City Univ.*): You showed that there was a decrease in local cerebral blood flow (CBF) in stroke-prone (SP) SHR. However, more evidence appears to be needed before you conclude that the occurrence of stroke depended on the decrease in local CBF. You reported that levels of arterial pressure were higher in SP-SHR than in stroke-resistant (SR) SHR. Besides, PRA might have been higher in SP-SHR. The occurrence of stroke might have been dependent on these factors other than the decrease in CBF. Furthermore, from a clinical point of view, symptoms suggesting a decrease in local CBF do not usually precede the occurrence of stroke.

Dr. Y. YAMORI: A cause-effect relationship between rCBF reduction and cerebrovascular

diseases was noted not only in SHRSP but also in renal hypertensive rats. Two facts showed that rCBF reduction is of causal importance in the development of cerebrovascular diseases. First, the predilection sites of stroke-lesions corresponded to the area fed by recurrent branchings where rCBF was more markedly decreased. Second, there was strong evidence indicating that no stroke occurred as long as rCBF was maintained within a normal range by long-lasting anti-hypertensive treatments. By detailed observation on the process of the development of cerebrovascular permeability due to hypoxia. This increase finally resulted in arterionecrosis, which was the common basic lesions for both hemorrhage and infarction.

There is an unavailability of rCBF detection in the basal ganglia, the predilection site of stroke in man. Also, there is difficulty involved in prospective studies on rCBF in cases with severe hypertension. Therefore, it is hard to clinically prove such a cause-effect relationship between rCBF reduction and stroke in man.

Dr. A. EBIHARA (*Jichi Med. Coll.*): It is very interesting that high cholesterol diet reduced the blood pressure in SHR, because recent data of epidemiological studies have shown the low incidence of cerebral hemorrhage in people with low level of serum cholesterol.

Framingham study disclosed that control of hypertension was effective to reduce the incidence of the cerebral stroke but not myocardial infarction. Did you get the similar results in SHR?

Dr. Y. YAMORI: Epidemiological studies by Dr. Komachi et al. proved that in Japan there was an inverse correlation between plasma cholesterol level and the incidence of stroke, either hemorrhage or infarction. This data is completely consistent with our finding that, in SHRSP fed on a high-fat-cholesterol diet, hypertensive cerebrovascular disease (hemorrhage and infarction) due to arterionecrosis was decreased. This consistency between epidemiological data and our findings on SHRSP indicates that stroke in Japanese people is "arterionecro-thrombogenic stroke", similar to that in SHRSP. However, it is mainly different from "athero-thrombogenic" stroke observed in the U.S.A. or European countries.

Our experimental observation also proved that the heart is rather immune to hypertensive vascular lesions in contrast to the brain or kidneys. In the heart, the incidence of arterio-

necrosis is low. Further, coronary arteries are rather resistant to acute arterial fat deposition induced by hypertension. I believe, that this is because coronary arteries are mainly perfused with diastolic blood pressure.

Dr. T. OZAWA (*Tokyo Univ.*): How do you think about autoregulation in SHRSP?

Dr. Y. YAMORI: The concept of "autoregulation" of cerebral circulation is based on the findings obtained mainly by acute experiments. Our chronic observation on rCBF reduction under severe hypertensive state may be essentially different from acute observation on rCBF alteration in animals with extensive blood pressure fluctuation.

Dr. M. MATSUNAGA (*Kyoto Univ.*): Is blood flow reduction always prerequisite to development of angionecrosis in any organ? We found that the reduction of renal blood flow in SHRSP closely correlated with plasma renin level, i.e. renal blood flow was not reduced as long as

plasma renin was within normal range. On the other hand, elevated plasma renin level was always associated with angionecrosis in the kidney. Therefore, it seems unlikely that there is much time lag between blood flow reduction and development of angionecrosis in the kidney.

Dr. Y. YAMORI: In the kidney also, arterionecrosis is often observed as in the brain. It is possible that renal blood flow reduction precedes the development of renal vascular lesions, but a causal relationship between blood flow reduction and vascular lesions is not yet proven for the kidney. There is, of course, organ difference in hypertensive vascular lesions. For example, an increase in vascular permeability is different, depending on the structural difference in various organs.

The reaction of arterial walls in response to such an increase in permeability is also different, and it is influenced by the amount of connective tissue around the arteries in various organs.