Effect of Carotid Artery Ligation on Regional Cerebral Blood Flow in Normotensive and Spontaneously Hypertensive Rats

Jun-ichiro Choki, M.D., Takenori Yamaguchi, M.D., Yo Takeya, M.D., Yasuyuki Morotomi, M.D., and Teruo Omae, M.D.

SUMMARY Regional cerebral blood flow (rCBF) was measured in normotensive rats (NTR) and spontaneously hypertensive rats (SHR), in a lightly anesthetized state and with control of 

\[ \text{Paco}_2 \] by artificial ventilation. Without carotid artery ligation, NTR and SHR showed almost identical rCBF values and distribution, despite significantly elevated levels of blood pressure in SHR. Bilateral carotid artery ligation, however, caused much more pronounced decreases of rCBF (ischemia) in SHR than NTR, in regions supplied by the carotid artery. The reduction of rCBF in SHR was rather homogeneous and symmetrical.

Mechanisms causing the differences between NTR and SHR are discussed.

CEREBRAL BLOOD FLOW (CBF) in patients with benign arterial hypertension is known to be not much different from that in normotensives.¹ In animals, Strandgaard et al.² observed no significant differences in CBF between baboons with normotension and Goldblatt hypertension. On the other hand, bilateral ligation of the common carotid arteries in spontaneously hypertensive rats (SHR) is known to cause a much greater increase in anaerobic metabolites in the brain³ and a more severely reduced reactivity of the cortical arterioles to vasodilating agents⁴ than in normotensive Wistar rats (NTR), as well as more extensive infarcts in the regions supplied by the carotid artery.⁵ These findings suggest that bilateral carotid ligation causes more severe ischemic damages to the brain in SHR. Actual values of cerebral blood flow, however, were not measured in these experiments.

The present study was designed to compare the regional cerebral blood flow (rCBF) values in SHR with those in NTR, before and after bilateral ligation of the common carotid arteries.

Methods

Sixteen female NTR and 18 SHR ranging in weight from 180 to 330g and in age from seven to 12 months were used. The animals were lightly anesthetized with amobarbital (100 mg/kg) injected intraperitoneally and a tracheostomy was made. A short polyethylene catheter was placed in the aorta through the right iliac artery for sampling arterial blood and for monitoring blood pressure with a strain gauge and Goldblatt hypertension. Two venous catheters were passed trans-femorally, one into the inferior vena cava for the injection of a diffusible radioactive indicator, antipyrine labelled with carbon-14, and the other into the right iliac vein for an arterial-venous shunt, for determination of the arterial concentration of the diffusible radioactive indicator. The circulation then was stopped by the rapid intravenous injection of a saturated solution of potassium chloride. The skull was opened and the brain was removed from the animal quickly (within ten minutes) and frozen at -100 to -150°C in 2-methylbutane cooled with liquid nitrogen. After warming to -20°C in a freezer, the frozen brain was cut coronally into four slices, the anterior three including the cerebral hemispheres and the diencephalon, and the posterior one including the brainstem and cerebellum. From each slice ten serial sections (20μ thick) were made in a cryostat at -20°C, the sections were placed on glass slides and dried within several seconds on a hot plate. The slides then were put in a cassette with the sections of brains facing the emulsion of X-ray film. Small pieces of plastic, containing known amounts of ¹⁴C incorporated in the polymer of the plastic, also were put in the cassette as standards. After exposure for approximately three weeks the film was developed and the optical densities of the autoradiographic images were measured with a densitometer (Sakura PD 11). The concentrations of antipyrine ¹⁴C in the various regions of the brain were determined from a curve which was plotted using the values of the optical densities and the concentrations of the plastic standards. Values for rCBF were calculated by a digital computer from the equation:

\[ C(T) = \frac{\lambda k}{5} C^{e}_{at} e^{-\frac{T}{\lambda}} dt \]
TABLE 1 Mean Arterial Blood Pressure, \( P_{aco_2} \), \( P_{ao_2} \), and Arterial pH

<table>
<thead>
<tr>
<th>Group</th>
<th>MABP (mm Hg)</th>
<th>( P_{aco_2} ) (mm Hg)</th>
<th>( P_{ao_2} ) (mm Hg)</th>
<th>arterial pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NTR (10)*</td>
<td>115 ± 15</td>
<td>36.2 ± 2.7</td>
<td>132 ± 27</td>
<td>7.413 ± 0.037</td>
</tr>
<tr>
<td>SHR (8)*</td>
<td>147 ± 18**</td>
<td>35.5 ± 2.1</td>
<td>134 ± 27</td>
<td>7.423 ± 0.061</td>
</tr>
<tr>
<td>Experimental</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NTR (6)*</td>
<td>111 ± 11</td>
<td>34.4 ± 2.6</td>
<td>128 ± 52</td>
<td>7.594 ± 0.077</td>
</tr>
<tr>
<td>SHR (10)*</td>
<td>151 ± 31**</td>
<td>34.7 ± 3.0</td>
<td>134 ± 41</td>
<td>7.378 ± 0.092</td>
</tr>
</tbody>
</table>

*Number of animals, values are mean ± S.D. **Significant differences from NTR (P < 0.01).

TABLE 2 Regional Cerebral Blood Flow in NTR and SHR with and without Bilateral Carotid Artery Ligation

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Control Group</th>
<th>Experimental Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NTR</td>
<td>SHR</td>
</tr>
<tr>
<td>Frontal</td>
<td>0.83 ± 0.14†</td>
<td>0.85 ± 0.16</td>
</tr>
<tr>
<td>Parieto-temporal</td>
<td>1.10 ± 0.09</td>
<td>1.09 ± 0.11</td>
</tr>
<tr>
<td>Occipital</td>
<td>1.06 ± 0.10</td>
<td>1.02 ± 0.12</td>
</tr>
<tr>
<td>Frontal</td>
<td>0.82 ± 0.18</td>
<td>0.81 ± 0.23</td>
</tr>
<tr>
<td>Parieto-temporal</td>
<td>0.85 ± 0.15</td>
<td>0.87 ± 0.11</td>
</tr>
<tr>
<td>Occipital</td>
<td>0.89 ± 0.15</td>
<td>0.74 ± 0.15</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>0.87 ± 0.15</td>
<td>0.79 ± 0.18</td>
</tr>
<tr>
<td>Putamen</td>
<td>1.08 ± 0.11</td>
<td>1.09 ± 0.13</td>
</tr>
<tr>
<td>Thalamus, medial</td>
<td>1.34 ± 0.14</td>
<td>1.19 ± 0.07*</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>1.08 ± 0.15</td>
<td>1.00 ± 0.10</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.98 ± 0.11</td>
<td>0.85 ± 0.10</td>
</tr>
<tr>
<td>Brainstem</td>
<td>1.04 ± 0.07</td>
<td>0.98 ± 0.08</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>1.27 ± 0.15</td>
<td>1.25 ± 0.11</td>
</tr>
</tbody>
</table>

\( rCBF \), ml/g/min, Mean ± S.D.; *Significant differences from control NTR and **from control SHR (P < 0.01).
FIGURE 1. Autoradiograms of the brain of one NTR with bilateral carotid ligations. Mean CBF was 74% of the control. MABP was 121 mmHg before ligation and 134 mmHg at the time of rCBF measurement. A: Section through the frontal region; B: parieto-temporal; C: occipital; D: cerebellum and brainstem.

Changes in Blood Pressure after Carotid Ligation

Bilateral ligation of the carotid arteries produced a marked elevation of systemic blood pressure in both NTR and SHR. In 5 NTR and 8 SHR, MABP was monitored for at least two hours after ligation. An early peak of elevated MABP was observed after ligation; its percentage of the initial value was 120% in NTR and 128% in SHR (figure 4). After the initial peak, MABP of NTR continued to show a constant increase for the following two hours, but in SHR, MABP started to drop rapidly around one and a half hours after the ligation. The pulse rate also tended to increase after carotid ligation in both NTR and SHR. The mean pulse rate before ligation was 333 and 353 per minute in NTR and SHR, respectively, and it increased up to 130% of the initial value in both types of animals after ligation. At the time of blood flow measurement, MABP of NTR was above the initial value in all animals. In SHR, however, three of the ten animals had lower MABP than initially; two of those were less than one-half of the initial values, and the animals had almost no CBF in the anterior circulation. In contrast, rCBF in the anterior circulation was maintained at approximately 90% of control in one SHR in which MABP at the time of...
blood flow measurement was 180% of the initial value. The average value for MABP at the time of rCBF measurement was 136 mmHg (122% of the initial MABP) in NTR and 150 mmHg (100% of the initial) in SHR.

To express the extent and magnitude of cerebral ischemia in each animal, mean CBF in the anterior circulation was calculated as the average of rCBF values from twenty-six regions of the brain: 18 regions from the cerebral cortex in three slices and the rest from the caudate, putamen, and hippocampus. Of eight SHR in which MABP was monitored for at least two hours, four animals showed a mean CBF of more than 25% of the nonischemic animals, while the other 4 animals had a mean CBF of less than 10% of the control. The first 4 animals had an initial MABP of 129 mmHg, and the latter 155 mmHg. The course of MABP after ligation in the first 4 animals resembled that of NTR (figure 5).

Discussion

In the present study rCBF of NTR and SHR was measured by the autoradiographic method using $^{14}$C-antipyrine. It has been stated that the method using $^{14}$C-antipyrine underestimates blood flow, especially at high perfusion rates, because of a limitation of the diffusion of antipyrine from the capillary net to the brain tissue. At the present time, however, there is no better indicator for the autoradiographic method. Autoradiography appears to be the most suitable method for the measurement of true regional blood flow, because this method allows measurements in almost as many, and as small regions in the brain at the same time. As long as the measurement is performed at a normal Paco$_2$ level, and flow values are used for comparing different conditions (control with experimental conditions), this method is suitable for use in the study of the cerebral circulation.

Thus, the autoradiographic method has been applied by
Spontaneously hypertensive rats\textsuperscript{11} are known to develop high arterial blood pressure after several weeks of age. The mechanisms of the development and maintenance of this raised blood pressure have been investigated intensively in recent years. Hypertension is usually associated with an increased resistance of the peripheral vascular bed in both humans and animals. The increase in vascular resistance may be caused by functional or structural changes which occur in the vascular wall. Folkow et al.\textsuperscript{12, 13} considered the increased vascular resistance in SHR as due to an adaptive hypertrophy of the vascular wall exposed to the increased strain of high intraluminal blood pressure. Thus, in chronic hypertension, an elevated arterial blood pressure (increased perfusion pressure to the organs) does not directly result in an increased tissue blood flow. In patients with essential hypertension, Kety et al.\textsuperscript{14} reported values of total CBF similar to those of normal subjects, using the nitrous oxide method. In addition, cerebral blood flow of normal brain remains relatively constant despite acute rises of systemic blood pressure, except at abnormally high pressures.\textsuperscript{15} Although it has been reported that the reactivity of the cortical vasculature to vasodilators in SHR is not different from that of NTR,\textsuperscript{4} the actual values of cerebral blood flow in SHR have not been reported in the literature. In this study, it was found that rCBF and its distribution in SHR was almost identical with that of NTR, despite significantly increased levels of MABP.

The main purpose of these experiments was to determine if bilateral carotid ligation causes any difference in the degree and extent of cerebral ischemia in NTR and SHR. Eklöf and Siesjö\textsuperscript{8} measured CBF 30 minutes after bilateral carotid ligation in normal Wistar rats. They reported that CBF in the fronto-parietal cortex decreased to about 50% of the control value, and that additive arterial hypotension resulted in further decreases of CBF and increases of its inter- or intra-hemispherical inhomogeneities. In the present study, rCBF in the cerebral cortex of NTR decreased to 60 to 80% of the control values after bilateral carotid ligation. The degree of ischemia was somewhat less in our study than that of Eklöf and Siesjö. Furthermore, there were no obvious differences in the degree of ischemia between the two hemispheres. The different results can be attributed partly to the blood pressure, which showed marked and longstanding elevation after carotid ligation, and to differences of the interval from carotid ligation to measurement of blood flow.

According to Ogata et al.,\textsuperscript{6} the posterior cerebral artery branches off from the internal carotid artery in rats and the posterior communicating artery connects the posterior cerebral artery with the superior cerebellar artery or the basilar artery. Thus, bilateral carotid ligation in rats produces ischemia in the regions supplied by the anterior, middle and posterior cerebral arteries.

There are several potential channels for collateral circulation to the regions normally supplied by the carotid artery; one of the most important is the anastomosis via the posterior communicating arteries of the circle of Willis. Others are extracranial anastomoses between the vertebral artery and branches of the carotid arteries, and cortical and deep-seated anastomotic channels from the posterior circulation.

The results of the present study in NTR suggest that these
anastomotic channels function effectively to perfuse the forebrain when the carotid arteries are ligated bilaterally. After careful observation of the cerebral vessels in rats, Brown stated that no major vessels in the circle of Willis are incomplete or string-like, including the posterior communicating artery. This fact again is in agreement with the absence of severe ischemia after bilateral carotid ligation in NTR.

In contrast, rCBF in the distribution of the anterior circulation in SHR showed much lower values than in NTR, when the carotid arteries were ligated. This drastic reduction in CBF was observed diffusely throughout the cerebral cortex, including the frontal, parieto-temporal and occipital regions, and in the putamen and the lateral thalamus. There was no obvious asymmetry of rCBF from side to side, and no inhomogeneity in the ischemic area. There were rough correlations of the degree of CBF reduction with the initial MABP before ligation and with the course of MABP after ligation in SHR. Animals which showed relatively high values of mean CBF in the anterior circulation had lower values of initial MABP, and the courses of MABP after ligation in those animals were rather similar to those of NTR.

Ischemia may be much more pronounced if there is absence or hypoplasia of the posterior communicating artery, which is thought to be the most important collateral channel between the posterior and anterior circulation. Ogata et al., however, found that the posterior communicating artery of SHR was neither absent nor hypoplastic and that the size of the posterior communicating artery was not much different from that of NTR. The failure to detect morphological differences in the posterior communicating arteries, the homogenous and symmetrical nature of ischemia and the correlation of the degree of ischemia with systemic blood pressure suggest that functional mechanisms, rather than morphological ones, may account for differences in the degree of ischemia between NTR and SHR.

Strandgaard et al. reported that in hypertensive subjects the lower limit of blood pressure for cerebral autoregulation is shifted to higher levels than in normotensive subjects, and that CBF starts to decrease at higher levels in hypertensive subjects when the systemic blood pressure is lowered. If the lower limit of autoregulation is shifted upwards in SHR, a much higher perfusion pressure is needed through the collateral channels to maintain adequate CBF when the carotid arteries are ligated bilaterally. In the present study, the perfusion pressure in the anterior circulation through the collateral channels might have been much lower than the lower pressure level for autoregulation in SHR, probably due to increased vascular resistance and to an inability to maintain the elevated level of systemic blood pressure.

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