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

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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 Paco2 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, Stranγgaard 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 polygraph. Two venous catheters were passed transfemorally, 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. Rectal temperature was kept between 36 and 37°C with a heat lamp. Each rat was paralyzed with 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 polygraph. Two venous catheters were passed transfemorally, 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. Rectal temperature was kept between 36 and 37°C with a heat lamp. Each rat was paralyzed with a minimal dose of decamethonium (initially 10 mg/kg, injected intravenously, with 5 mg/kg added when needed) and ventilated artificially with a respirator (Rodent Respirator model 680, Harvard). The ventilating mixture consisted of room air to which small amounts of oxygen were added. Paco2 was maintained constant between 30 and 40 mmHg; Paco2 was maintained above 100 mmHg. In 6 NTR and 10 SHR (experimental group), both common carotid arteries were doubly ligated after careful dissection and separation of the cervical vago-sympathetic trunks. Approximately two hours after the carotid ligation, at the desired level of Paco2, regional cerebral blood flow was measured. In the remaining 10 NTR and 8 SHR (control group), the surgical preparation was performed in a similar manner as in the experimental group except that the carotids were not ligated.

For measurement of rCBF 30 μCi of antipyrine-14C dissolved in 0.6 ml of distilled water was injected into the inferior vena cava at a constant rate for one minute.6 During the injection approximately 0.1 ml of blood was withdrawn every ten seconds through the three way stopcock in the 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:

\[ C(t) = \frac{1}{\lambda} \int \left( C_a e^{-\lambda t} - C_t e^{-\lambda T} \right) dt \]
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TABLE 1 Mean Arterial Blood Pressure, Paco2, Pa02, and Arterial pH

<table>
<thead>
<tr>
<th>Group</th>
<th>MABP (mm Hg)</th>
<th>Paco2 (mm Hg)</th>
<th>Pa02 (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>7.394 ± 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).

Results

Paco2, Pa02, Arterial pH and Blood Pressure

The blood gas measurements and the blood pressure in NTR and SHR of both experimental and control groups are shown in table 1. The mean arterial Paco2 ranged from 34.4 to 36.2 mmHg and there were no significant differences among those values. Arterial Pa02 was 133 mmHg and 134 mmHg in NTR and SHR of the control group, and 128 mmHg and 134 mmHg in the experimental group, respectively. In the control group, the mean arterial blood pressure (MABP) at the time of rCBF measurement was 115 mmHg in NTR and 147 mmHg in SHR. In the experimental group, MABP before carotid ligation was 111 mmHg in NTR and 151 mmHg in SHR. In both control and experimental groups, the mean MABP of SHR was higher than that of NTR, and the differences were statistically significant.

Regional Cerebral Blood Flow

Mean rCBF values in the various sites of the brain in NTR and SHR of the control and the experimental groups are summarized in table 2. In the control group of both NTR and SHR, the mean rCBF in the convexity cortex of the parietal and occipital regions ranged from 1.02 to 1.10 ml/g/min. The frontal and basal cortex in both NTR and SHR showed somewhat lower values than the convexity cortex. There were no differences between NTR and SHR in the rCBF of any of the cortical sites. In deep structures, such as the caudate nucleus, putamen, thalamus and hypothalamus, mean rCBF ranged from 0.79 to 1.34 ml and the differences between NTR and SHR were not significant, except for the medial thalamus. The hippocampus showed somewhat lower values than the overlying cortex. The rCBF of the brainstem and cerebellum were measured in the coronal section at the pontine level. Values of about 1.0 ml were obtained in the brainstem. The cerebellum showed the highest mean rCBF in both NTR and SHR (NTR: 1.27 ml; SHR: 1.25 ml).

In the experimental group, bilateral ligation of the common carotid arteries produced significant decreases of rCBF.

<table>
<thead>
<tr>
<th>Group</th>
<th>% of control</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal</td>
<td>0.93 ± 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>Oecipital</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>Oecipital</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.

in the regions supplied by the carotid arteries (anterior circulation) in both NTR and SHR. In the anterior circulation of NTR, individual rCBF values ranged from 0.40 to 0.92 ml in the convexity cortex and 0.41 to 0.92 ml in the putamen. The mean rCBF values of the convexity cortex were approximately 0.7 ml (66 to 73% of the values in the control group).

In SHR of the experimental group, carotid ligation produced a much greater decrease of rCBF in the anterior circulation than that in NTR. Individual rCBF values ranged from 0.01 to 0.99 ml in the convexity cortex and from 0.01 to 0.82 ml in the putamen. The mean rCBF values of the convexity cortex were approximately 0.2 ml (21 to 27% of control values). The thalamus and the hypothalamus of the experimental SHR also showed significantly lower rCBF than control animals (75% of control in the thalamus and 63% in the hypothalamus), but the magnitude of rCBF decrease was smaller than in the cerebral cortex.

In the posterior circulation, rCBF tended to decrease after the carotid ligations in both NTR and SHR. The differences in rCBF between the control and experimental group reached statistically significant levels in the cerebellum but not in the brainstem. The decreases of rCBF were similar in NTR and SHR. Typical autoradiograms of NTR and SHR of the experimental group are depicted in figures 1 and 2.

The relationships between rCBF values of corresponding regions in the right and left hemispheres after carotid ligation are shown in figure 3. Although the degree of rCBF reduction varies from animal to animal, rCBF values in both hemispheres were almost similar in individual animals.

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 14C-antipyrine. It has been stated that the method using 14C-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 Paco2 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

![Figure 2. Autoradiograms of the brain of one SHR with bilateral carotid ligations. Mean CBF was 27% of the control. MABP was 172 mmHg before ligation and 214 mmHg at the time of rCBF measurement. A: Section through the frontal region; B: parieto-temporal; C: occipital; D: cerebellum and brainstem.](image)

![Figure 3. Relationships of rCBF values in the right and left hemispheres of the experimental group.](image)
FIGURE 4. Course of MABP in 5 NTR and 8 SHR for 2 hours after carotid ligation. MABP of SHR dropped rapidly about 90 minutes after the ligation, while that of NTR increased continuously up to 2 hours.

FIGURE 5. Course of MABP after carotid ligation in 2 subgroups of SHR. Four animals with mean CBF of over 25% of control had a lower MABP before the ligation than the other 4 animals. The course of MABP after carotid ligation in the former 4 animals was similar to that of NTR.

Spontaneously hypertensive rats 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.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.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.15 Although it has been reported that the reactivity of the cortical vasculature to vasodilators in SHR is not different from that of NTR,16 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. Eklof and Siesjo10 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 Eklof and Siesjo. 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.,7 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 ligation 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 higher level of 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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References

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