Effects of Long-term Antihypertensive Treatment on Cerebral, Thalamic and Cerebellar Blood Flow in Spontaneously Hypertensive Rats (SHR)

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SUMMARY Cerebral blood flows (CBF) were measured in the parietal cortex, the thalamus and the cerebellum by the hydrogen clearance technique in anesthetized spontaneously hypertensive rats, of which hypertension was treated for 16 weeks (long-term) or 8 weeks (short-term) with antihypertensive agents of hydralazine and guanethidine.

As compared to non-treated control animals, CBF in the three regions were significantly increased while the calculated cerebrovascular resistances (CVR) were decreased in hypertension-treated animals. Such CBF and CVR changes were greater in SHR with long-term than short-term therapy. Both an increase in CBF and a decrease in CVR were closely related to a fall in the blood pressure.

From the present results, it was concluded that earlier and longer treatment of hypertension could lessen or even prevent the increased CVR due to the hypertensive vascular changes, and increase CBF as a result.

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CEREBRAL BLOOD FLOW (CBF) is decreased and the lower limit of CBF autoregulation is shifted to a higher level in long-lasting severe hypertension with vascular changes.1-8 Antihypertensive treatment is possible to restore the upward shift of CBF autoregulation in hypertensives close to the level in normotensives,9-11 but it is not known whether the decreased CBF is reversible or not by the treatment.

In the present study, we measured CBF in the parietal cortex, the thalamus and the cerebellum in SHR, of which hypertension was treated with antihypertensive agents for either short- or long-term.

Materials and Methods

Young female SHR of paired siblings, aged 4 weeks, were divided at random into three groups, group 1 for long-term treatment (16 weeks), group 2 for short-term treatment (8 weeks), and group 3 for control non-treatment. The animals were fed ad libitum a regular diet (Oriental Co, Japan) and tap water alone in group 3, or with addition of both hydralazine (3.5 mg/dl) and guanethidine (15 mg/dl) dissolved in drinking water in group 1 (started at age of 4 weeks) and group 2 (at age of 12 weeks). Body weight was measured every week, and blood pressure was measured by a tail-cuff method without anesthesia at age of 12 or 16 weeks.

All animals at the age of 20 weeks were anesthetized with intraperitoneally administered amobarbital (10 mg/100g body weight). In each rat, both femoral arteries were cannulated, one for continuous recording of the blood pressure, and the other for anaerobic sampling of arterial blood. Arterial pH and pCO₂ were determined with an IL meter model 113, and duplicate hematocrit (Hct) was measured with the microhematocrit method by centrifuging at 11,000 rpm for 5 min. The animal's head was fixed in a head-holder, and small burr holes, 2 mm in diameter, were made in the frontal skull 2 mm lateral to the bregma on each side, and in the occipital bone 3.5 mm posterior to the confluence. Teflon-coated platinum electrodes with platinum black on the tips were placed in the parietal cortex (2 mm in depth from the brain surface), in the thalamus (7 mm in depth) and in the cerebellum (2 mm in depth) by using a stereotaxic apparatus. The reference electrode was an Ag-AgCl electrode inserted under the skin. Polyethylene tubing (PE 50) was inserted 3 mm into the nasal cavity for giving hydrogen gas mixture. CBF was measured using a hydrogen clearance method under spontaneous breathing. The body temperature, as measured in the rectum, was kept close to 37°C.

After allowing more than 30 min for a steady state to be achieved, at least five CBF were measured at intervals of 10-15 min. One arterial blood sample was obtained between the third and fourth CBF determinations for gas analysis and Hct determination.

After termination of the experiment, the heart was quickly removed and weighed for relative heart weight. In each rat, both femoral arteries were cannulated, one for continuous recording of the blood pressure, and the other for anaerobic sampling of arterial blood. Arterial pH and pCO₂ were determined with an IL meter model 113, and duplicate hematocrit (Hct) was measured with the microhematocrit method by centrifuging at 11,000 rpm for 5 min. The animal's head was fixed in a head-holder, and small burr holes, 2 mm in diameter, were made in the frontal skull 2 mm lateral to the bregma on each side, and in the occipital bone 3.5 mm posterior to the confluence. Teflon-coated platinum electrodes with platinum black on the tips were placed in the parietal cortex (2 mm in depth from the brain surface), in the thalamus (7 mm in depth) and in the cerebellum (2 mm in depth) by using a stereotaxic apparatus. The reference electrode was an Ag-AgCl electrode inserted under the skin. Polyethylene tubing (PE 50) was inserted 3 mm into the nasal cavity for giving hydrogen gas mixture. CBF was measured using a hydrogen clearance method under spontaneous breathing. The body temperature, as measured in the rectum, was kept close to 37°C.

After allowing more than 30 min for a steady state to be achieved, at least five CBF were measured at intervals of 10-15 min. One arterial blood sample was obtained between the third and fourth CBF determinations for gas analysis and Hct determination.

After termination of the experiment, the heart was quickly removed and weighed for relative heart weight or percentile ratio of heart/body weight, and the brain was grossly examined. When either an improper placement of the electrode in the brain or gross tissue damage by inserting the electrode was found, data were excluded from the present results.

Results

An increase in body weight with age, ranging from 4 to 20 weeks, was of no difference among three groups. Systolic blood pressure in the conscious state was averaged 134 ± 18 (± SD) mmHg in 5 animals of group 1 at age 12 weeks, 148 ± 3 mmHg in 5 of group 2, and 203 ± 8 mmHg in 5 of group 3 at age 16 weeks, respectively.

Average values for mean arterial pressure (MAP),
TABLE 1  Mean Arterial Pressure (MAP), Arterial Acid-base Parameters and Hematocrit (Hct) in Spontaneously Hypertensive Rats (SHR) with or without Antihypertensive Treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>Duration of Treatment</th>
<th>MAP (mmHg)</th>
<th>pH</th>
<th>pCO2 (mmHg)</th>
<th>pO2 (mmHg)</th>
<th>Hct (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16 weeks</td>
<td>133 ± 6</td>
<td>7.351 ± 0.030</td>
<td>40.2 ± 4.8</td>
<td>67.2 ± 7.2</td>
<td>45.4 ± 2.2</td>
</tr>
<tr>
<td>2</td>
<td>8 weeks</td>
<td>158 ± 5</td>
<td>7.399 ± 0.028</td>
<td>39.7 ± 4.7</td>
<td>79.4 ± 26.1</td>
<td>43.2 ± 2.6</td>
</tr>
<tr>
<td>3</td>
<td>none</td>
<td>169 ± 6</td>
<td>7.379 ± 0.022</td>
<td>39.4 ± 4.6</td>
<td>75.8 ± 8.5</td>
<td>43.8 ± 1.5</td>
</tr>
</tbody>
</table>

Values are mean ± SD. Each group consists of 5 animals. Group 1 vs 2; *p < 0.05, †p < 0.001. Group 1 vs 3; ‡p < 0.001. Group 2 vs 3; §p < 0.02.

arterial acid-base parameters and Hct at the resting state of 20-week aged rats under anesthesia are summarized in table 1. MAP was significantly lower in group 1 (133 ± 6 mmHg) than group 2 (158 ± 5), or group 3 (169 ± 6), between the latter two of which MAP was also significantly different. There were no differences in mean values for pH, pCO2, pO2 and Hct among three groups except for a slight but significant decrease in pH in group 1, as compared with that in group 2.

The last three values of five CBF determinations in each region, and the calculated cerebrovascular resistance (CVR) were used for statistical analysis. Mean values for CBF of 15 determinations in 5 animals of each group are summarized in table 2. Cortical CBF was 64.0 ± 9.7 ml/100g/min in group 1, being significantly higher than 53.2 ± 9.1 in group 2 or 43.2 ± 7.7 in group 3. Cortical CBF was increased by antihypertensive treatment, its increase being significantly greater in the long-term (48% of non-treatment) than the short-term treatment (23%). Cortical CVR was reduced in the two treated groups, but its change was greater in group 1 through group 2. In a similar manner, both thalamic and cerebellar CBF were significantly increased while CVR in these regions was decreased in treated groups 1 and 2, as compared with those in non-treated groups.

Table 1 demonstrates the relationship between CBF in each region and MAP in all animals. There were highly significant inverse correlations present in the cortex (r = -0.58, p < 0.001), the thalamus (r = -0.51, p < 0.001) and the cerebellum (r = -0.64, p < 0.001). The slope of the regression line was steeper in the cerebellum (-0.48, ΔCBF/ΔMAP), the cortex (-0.37) and the thalamus (-0.29), in that order. Relations of CVR to MAP (fig. 2) were also significant in the cortex (r = 0.75, p < 0.001), the thalamus (r = 0.80, p < 0.001) and the cerebellum (r = 0.81, p < 0.001). Lowering of blood pressure by the treatment reduced the vascular resistance.

As shown in table 2, there were regional differences of CBF and CVR in non-treated animals, namely a slightly but significantly higher flow and lower resistance in the thalamus than the others. However, such regional differences were not observed in both treated groups of animals.

Heart weight was 0.80 ± 0.09 g or 0.40 ± 0.02% of body weight in long-term treated SHR, 0.81 ± 0.07 g or 0.41 ± 0.03% in short-term treated ones, and 0.84 ± 0.05 g or 0.43 ± 0.02% in non-treated ones, respectively. Only a significant difference was found in the percentile ratio of heart and body weight between long-term treatment and control groups (p < 0.05).

**Table 2**  Mean Values for Cerebral Blood Flow (CBF) and Calculated Cerebrovascular Resistance (CVR) in 3 Different Regions of the Brain in SHR with or without Antihypertensive Treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>CBF (ml/100 g/min)</th>
<th>CVR (mm Hg/ml/100 g/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16 weeks</td>
<td>64.0 ± 9.7</td>
<td>2.12 ± 0.36</td>
</tr>
<tr>
<td>2</td>
<td>8 weeks</td>
<td>53.2 ± 9.1</td>
<td>3.08 ± 0.72</td>
</tr>
<tr>
<td>3</td>
<td>none</td>
<td>43.2 ± 7.7</td>
<td>3.99 ± 0.06</td>
</tr>
<tr>
<td>4</td>
<td>16 weeks</td>
<td>61.2 ± 10.1</td>
<td>2.26 ± 0.54</td>
</tr>
<tr>
<td>5</td>
<td>8 weeks</td>
<td>60.2 ± 11.1</td>
<td>2.71 ± 0.55</td>
</tr>
<tr>
<td>6</td>
<td>none</td>
<td>48.2 ± 4.8</td>
<td>3.53 ± 0.45</td>
</tr>
<tr>
<td>7</td>
<td>16 weeks</td>
<td>67.0 ± 11.0</td>
<td>2.04 ± 0.37</td>
</tr>
<tr>
<td>8</td>
<td>8 weeks</td>
<td>58.1 ± 8.1</td>
<td>2.76 ± 0.35</td>
</tr>
<tr>
<td>9</td>
<td>none</td>
<td>40.2 ± 6.4</td>
<td>4.33 ± 0.94</td>
</tr>
</tbody>
</table>

Values are mean ± SD. Each group consists of 15 measurements in 5 animals. Group 1 vs 2; *p < 0.05, †p < 0.02. Group 1 vs 3; ‡p < 0.001. Group 2 vs 3; §p < 0.001.
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FIGURE 1. Relation of mean arterial pressure (MAP) to cerebral blood flow (CBF) in the parietal cortex, the thalamus and the cerebellum in all SHR with or without antihypertensive treatment.

hand, in non-treated SHR, thalamic flow was slightly higher than the others by unknown reason.

Because of no differences in physiological parameters such as arterial pCO₂, pO₂, and Hct, which are major factors affecting CBF, such post-treatment flow increases seem to be due to decreases in the cerebral vascular resistance itself. In fact, our morphometric study of the middle cerebral arteries in SHR, of which hypertension was treated for 10 weeks with same antihypertensive agents as this study, demonstrated a significant reduction in both vascular wall thickness and wall to lumen ratio of the arteries 150–250μ in diame-
ter, compared with those in non-treated SHR (unpublished data). These findings together with the others obtained by Schrempf et al,12 who found leptomeningeal arteries in SHR to be affected by the treatment, strongly suggest that long-term antihypertensive treatment reduces the vascular resistance of cerebral arteries not only hemodynamically but also morphometrically.

It is known that CBF in hypertensive patients is the same as in normotensive subjects, of the order of 50 ml/100g/min.1 CBF is reduced when cerebral arteriosclerosis, hypertensive vascular disease or aging is superimposed upon hypertension.1 Structural adaptations to long-lasting hypertension, such as hypertrophy of the elastic tissue and muscular media, are completed within 3 weeks after experimentally induced renovascular hypertension in rats12 or at least 2 months after the

FIGURE 2. Relation of mean arterial pressure (MAP) to cerebrovascular resistance (CVR) in the parietal cortex, the thalamus and the cerebellum in all SHR with or without antihypertensive treatment. CVR was calculated by MAP/CBF.
establishment of hypertension in baboons. Such vascular changes reduce the arterial lumen and blood flow, but thickened walls with lessened distensibility will act on preventing overperfusion of CBF against a rapid rise in the blood pressure.4

There have been many observations describing the changes in cerebral autoregulation in hypertension1,4 and in treated hypertension,3,11 although the changes in baseline CBF after long-term antihypertensive treatment are little mentioned in animal experiment as well as human study. In hypertensive patients with stable symptoms of occlusive cerebrovascular disease, however, Meyer et al14 have demonstrated a significant increase in CBF, measured by the nitrous oxide method, with significant decreases in MAP and CVR after the administration of 500-750 mg daily of alpha-methyldopa for two weeks. In contrast, Lavy et al15 found no change in regional CBF, measured by the 133Xe inhalation method, after 12 weeks of methyldopa therapy in subjects with essential hypertension.

Hydralazine is known as a vasodilator, of which intravenous administration causes a fall in blood pressure with a concomitant increase in CBF16 and intracranial pressure.17 Through the closed cranial window, Auer et al18 observed a significant dilatation of pial arteries immediately after intravenous dihydralazine in SHR and normotensive rats. Such arterial response to the drug far exceeds hypotensive-induced autoregulatory dilatation, indicating a direct effect of hydralazine on the cerebral vessels. By long-term oral administration of hydralazine alone or by a combination with reserpine, however, the arterial pressure was moderately lowered but CBF remained unchanged with decreased CVR in hypertensive arteriosclerotic disease.19, 20

Cerebral vascular reactivities to changes in arterial CO2 tension and blood pressure are diminished before,4 and possibly recovered after, treatment of hypertension. The present study indicates, in addition to the vasoreactivity alteration, that resting CBF per se increases by the treatment and its increase is directly related to lowering of the blood pressure. Such hypotensive effects on CBF increase are also related to the duration and initiation of the treatment. Long-term antihypertensive therapy is undoubtedly preventing the development of cerebral vascular disease, as previously demonstrated in SHR with experimentally induced cerebral ischemia.21

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References

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