The Effect of Long-Term Antihypertensive Treatment on Medial Hypertrophy of Cerebral Arteries in Spontaneously Hypertensive Rats

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SUMMARY The effects of antihypertensive treatment on the structural changes of middle cerebral arteries (MCA) were studied quantitatively and morphometrically in young spontaneously hypertensive rats (SHR). Fifteen male SHR, 10 weeks of age, were divided into control and experimental groups. In the experimental group, the animals were administered hydralazine and guanethidine for the following 10 weeks. At the age of 20 weeks, mean arterial blood pressure of experimental animals was 177 ± 9 mm Hg (mean ± SD), being significantly lower than that of 195 ± 12 mm Hg in control ones. Media thickness of large (external diameter ≥ 200 μm) and medium sized MCA (150-200 μm) in treated SHR was 12.3 ± 2.8 and 6.3 ± 1.1 μm, respectively, being significantly smaller than that of 14.0 ± 2.2 and 8.5 ± 2.6 μm, respectively, in control SHR. The media cross-sectional area and the ratio of media thickness to external diameter were also significantly reduced by antihypertensive treatment. In the smaller vessels (75-150 μm), however, vascular morphometry revealed no difference between the two groups. Long-term antihypertensive treatment during the early phase of hypertension attenuates the development of medial hypertrophy in large cerebral arteries.

VASCULAR STRUCTURAL CHANGES and their relevance for the development, maintenance and implications of hypertension are still controversial in spontaneously hypertensive rats (SHR). Nordborg and Johansson1 found that the media to radius ratio of medium and large sized cerebral arteries was greater in untreated SHR.2 Very recently, we have reported that cerebral vascular hypertrophy during the development of hypertension in SHR is associated with a rise in basal blood pressure during developmental hypertension in SHR.3

In this study, we examined whether or how effectively antihypertensive treatment prevents cerebral vascular hypertrophy during the development of hypertension. We measured the media thickness of middle cerebral arteries (MCA), of which external diameter ranged from 75-250 μm, in SHR with or without receiving 10 weeks of antihypertensive treatment.

Method

Fifteen male SHR, 10 weeks of age, were used. SHR of the same siblings were divided at random into two groups; control and experimental groups. In the experimental group, the animals received antihypertensive agents over a period of 10 weeks. The control animals were fed stock chow diet (Oriental Co. Ltd., Tokyo, Japan) and tap water ad libitum, while the experimental ones were administered both hydralazine (3.5 mg/dl; 0.7 mg/day/animal) and guanethidine (15 mg/dl; 3 mg/day/animal) in drinking water. Systolic arterial blood pressure (SABP) was measured weekly by a tail-cuff method without anesthesia. At the age of 20 weeks, the animals were anaesthesitized with intraperitoneal injection of amobarbital (100 mg/kg body weight). One femoral artery was cannulated for recording heart rate, arterial blood pressure, and sampling the blood for acid/base balance (IL-meter, model 113). The animals breathed room air spontaneously and the rectal temperature was maintained close to 37°C throughout the experiment.

The brain and cerebral vessels were fixed by the perfusion method through a 16-gauge needle punctured into the left ventricle of the heart after thoracotomy. Prior to perfusion, an incision was made in the bilateral jugular veins for drainage of the blood and perfusate, and the descending aorta was clamped. The perfusion was executed with an initial fixative of 2% formaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3) for 5 minutes followed by a more concentrated fixative of 4% formaldehyde and 5% glutaraldehyde (Karnovsky, 1965) for 20 minutes. The fixative was infused manually with a plastic syringe which was connected to a pressure gauge with a T-tube, and the perfusion pressure was maintained constantly close to mean arterial blood pressure (MABP) in each animal. The brain was removed carefully and immersed in the same fixative at 4°C for 12 hours.

Three portions of bilateral MCA — proximal part, intermediate and distal branches (cylindrical segments of 1 mm in length) — were taken for morphometrical examination (fig. 1). They were postfixed in 1% osmium tetroxide at 4°C, dehydrated in graded ethanol, and embedded in Epon resin (Araldite, TAAB). One μm thick cross sections of each vessels were stained with toluidine blue, and the external diameter (D) and media thickness (t) were microscopically measured by an eye piece micrometer at the magnification of ×1000. Round or elliptic vessels with an external diameter more than 75 μm were investigated (fig. 1).

Two to four specimens were collected from each part of MCA vessels which were sized small (75 < D <
FIGURE 1. Three portions of bilateral middle cerebral arteries (MCA) — proximal, intermediate and distal branches — for morphometrical examination (A), and one μm thick cross section stained with toluidine blue (×165, B). D: external diameter, t: media thickness.

150 μm), medium (150 ≤ D < 200 μm) and large (D ≥ 200 μm) according to the external diameter. In total, 200 MCA specimens were examined. The media thickness, the media to external diameter ratio (t/D ratio), and the media cross-sectional area (S) (calculated as S = π × t × (D - t)) were compared between control and experimental groups.

The heart and body weight were weighed and relative heart/weight ratio was estimated in each animal. The statistical differences between the two groups were evaluated with an unpaired t-test.

Results

The SABP at the age of 10 weeks was 184 ± 3 mm Hg (mean ± SD) in the control group (n = 7) and 183 ± 16 mm Hg in the experimental group (n = 8), the difference being insignificant. During the next 10 weeks, the SABP in control animals rose progressively and reached the level of 238 ± 3 mm Hg at the age of 20 weeks. In experimental animals, on the other hand, SABP fell slightly for the first five weeks, and then showed a slight rise to the level of 194 ± 10 mm Hg (fig. 2). The difference of SABP measured by a tail-cuff method or directly from femoral artery at 20 weeks of age was highly significant between the two groups (p < 0.001). MABP of experimental SHR under anesthesia was 177 ± 9 mm Hg, being also significantly lower than 195 ± 12 mm Hg in control SHR (p < 0.01). Heart rate was smaller in the treated group than the untreated one (p < 0.05). However, there were no essential differences in arterial acid base parameters between the groups (table 1). Neither heart weight, body weight nor relative heart/weight ratio was actually altered by antihypertensive treatment (table 2).

In experimental SHR, both t and t/D ratio of the medium and large sized vessels were significantly smaller than those of the corresponding vessels in control animals (p < 0.001–0.01, table 3, fig. 3 and 4). Furthermore, the media cross-sectional area of the experimental animals was also smaller than that of the control ones (p < 0.01) as shown in figure 5. In the small arteries, on the other hand, there were no significant differences of mean values for t, t/D ratio and S between the two groups.

FIGURE 2. Changes in systolic arterial blood pressure (SABP) of control and experimental groups. Antihypertensive treatment was started at the age of 10 weeks in the experimental group. SABP in control animals (open circle) rose progressively and reached the mean level of 238 mm Hg at the age of 20 weeks. In experimental animals (closed circle), SABP fell slightly for the first five weeks and then showed a slight rise to the level of 194 mm Hg. Each circle with bar indicates mean ± SD.
CEREBRAL ARTERIES IN ANTIHYPERTENSIVE TREATMENT

Discussion

Both morphometric and hemodynamic studies have suggested that cerebral vessels undergo structural changes in hypertensive humans and animals. Hemodynamic studies on SHR showed that the total peripheral resistance is abnormally high even when the vessels are maximally relaxed. Folkow et al have reported that these increased vascular resistance is caused by an adaptive hypertrophy of the vascular wall exposed to the increased strain of the intraluminal blood pressure.

The grade of medial hypertrophy of cerebral arteries seems to be considerably variable depending on the

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<th>TABLE 1</th>
<th>Arterial Blood Pressure and Acid Base Parameters in Control and Experimental Animals at the Age of 20 Weeks</th>
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<tr>
<td>Group</td>
<td>No. of rats (7)</td>
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<td>Control</td>
<td>(7)</td>
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<td>Experimental</td>
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<tr>
<td>Significance (p value)</td>
<td>&lt;0.001</td>
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Values are mean ± SD. Statistical differences were evaluated by an unpaired t test. NS = not significant (p > 0.05); SABP* = systolic arterial blood pressure measured by a tail-cuff method; SABP = systolic arterial blood pressure measured directly from femoral artery; MABP = mean arterial blood pressure measured directly from femoral artery.

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<td>Significance (p value)</td>
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Values are mean ± SD. Statistical differences were evaluated by an unpaired t test. NS = not significant (p > 0.05); HR = heart rate, beats per minute; HW = heart weight; BW = body weight; H/B = heart weight/body weight ratio.

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<th>TABLE 3</th>
<th>Morphometries of Middle Cerebral Artery (MCA) in Control and Experimental Animals</th>
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<td>MCA</td>
<td>Parameters</td>
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<td>Large</td>
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<td>(D ≥ 200 μm)</td>
<td>t</td>
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<tr>
<td>Medium</td>
<td>D</td>
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<td>(150 ≤ D &lt; 200)</td>
<td>t</td>
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<tr>
<td>Small</td>
<td>D</td>
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<td>(75 ≤ D &lt; 150)</td>
<td>t</td>
</tr>
<tr>
<td>S</td>
<td>1645 ± 900</td>
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</tbody>
</table>

Values are mean ± SD. Statistical differences were evaluated by an unpaired t test. NS = not significant (p > 0.05); () = number of rats; N = number of MCA specimens evaluated; D = external diameter (μm); t = media thickness (μm); t/D = media to external diameter ratio (%); S = media cross-sectional area (μm²).
size of the vessels. Nordborg and Johansson\(^1\) reported that there was a significant increase in the media to radius ratio among the medium and large sized vessels in 15-day-old SHR compared to Wistar Kyoto Rats (WKR). In 200-day-old SHR, however, such ratio was increased only in arterioles smaller than 20 μm of the radius. In contrast, Schrempf et al\(^7\) showed that the increased media thickness in 12-month-old SHR was evident not only in intracerebral arterioles with a radius above 10 μm but also in all leptomeningeal arteries, as compared with WKR. Similarly, recent study demonstrated in SHR that the media to radius ratio as well as the media cross-sectional area of proximal MCA (about 120 μm of radius) in SHR was significantly higher than that in WKR.\(^8\)

Takeuchi et al\(^9\) observed in hypertensive rabbits of the Goldblatt type that the media thickening in cerebral arterioles of some 100 μm in diameter was completely reversed by antihypertensive drug treatment. Cervós-Navarro et al\(^7,10\) also reported in SHR that under antihypertensive treatment, the development of an abnormally thick media was clearly reduced in the leptomeningeal and basal arteries, but not in intracerebral arterioles (radius of 5–20 μm).

In this study, we examined the effect of antihypertensive treatment on the media thickness of MCA during developing hypertension. In a non-treated control SHR, blood pressure rose progressively until 20 weeks of age and reached the plateau of 230 mm Hg or over in SABP. In a treated SHR, on the other hand, SABP showed a less gradual rise but did not exceed 200 mm Hg at the age of 20 weeks, suggesting the efficacy of long-term antihypertensive treatment. The medial hypertrophy in experimental SHR was markedly diminished in medium sized vessels as well as large vessels, compared to control SHR. In the small arteries, on the other hand, there were no differences in the media thickness, the ratio or the media cross-sectional area between the two groups. Folkow\(^11\) has described that a rapid and considerable regression of the hypertrophic vascular adaptation to hypertension takes place when the pressure load is kept reduced to a normal level for only eight weeks or less. Our present findings showed that the pronounced increase in the media thickness of MCA vessels in SHR was clearly reduced by antihypertensive drug treatment even though their blood pressure was not lowered to the normotensive level.

Under antihypertensive treatment in this study, medial hypertrophy was decreased mainly in the medium and large sized MCA. It is well known that cerebral vascular resistance is regulated primarily by arterioles and small arteries,\(^12,13\) but an emerging concept suggests that large arteries are also important determinants of cerebral vascular resistance.\(^14\) Under resting conditions, large arteries contribute about 25–50% of total cerebral vascular resistance in rabbits, cats and dogs.\(^12,14-16\) Kontos et al\(^17\) reported in cats that when
From the present results, it is concluded that hypertension-related structural alterations such as medial hypertrophy of large cerebral arteries could be reversed by long-term and satisfactory antihypertensive treatment even in sustained hypertension as found in developmental hypertension.

References

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