Effects of Antihypertensive Treatment on the Cerebral Microvasculature of Spontaneously Hypertensive Rats

Scot L. Harper

Experiments were performed in anesthetized 18-19-week-old spontaneously hypertensive rats (SHR) to evaluate the effects of delayed antihypertensive treatment on cerebrovascular function. Animals were treated for 25 ± 1 days with an oral antihypertensive regimen consisting of hydralazine, reserpine, and chlorothiazide, resulting in normotension within 2 weeks. Cerebral arterioles were examined via a constantly suffused open cranial window and video microscopy. Resting cerebral blood flow was measured using radioactive microspheres and the reference organ method. While untreated SHR exhibited reductions in arteriolar diameter compared with normotensive Wistar Kyoto rats (WKY), treatment restored arteriolar dimensions to normal. Increments in microvascular wall area, associated with medial hypertrophy in untreated SHR, were completely reversed in treated SHR to a magnitude not different from control. Resting cerebral blood flow was, however, decreased in treated SHR compared with both untreated SHR and WKY; this was due to an increase in total cerebrovascular resistance compared with WKY. Additionally, microvascular pressure in the largest arterioles in treated SHR was reduced compared with both WKY and untreated SHR. There was a significant increase in the relative pressure drop accounted for by the arterial vessels upstream from the cerebral microcirculation in treated SHR. These results suggest that 1) cerebral microvascular abnormalities induced by chronic hypertension are reversed by delayed antihypertensive therapy, and 2) there is a persistent elevation in cerebrovascular resistance upstream from the microcirculation representing large vessel adaptations that may not be readily reversible with treatment. (Stroke 1987;18:450-456)

CHRONIC hypertension is associated with structural vascular adaptations in the cerebral vasculature that serve to prevent vascular dilatation and rupture of the blood–brain barrier. Among these are constriction of large arterioles in the cerebral cortex and arteriolar wall hypertrophy as well as large cerebral artery changes. These adaptations, taken as a whole, largely prevent elevations in microvascular wall stress and maintain cerebral blood flow (CBF) within normal limits while shifting the autoregulatory range for CBF to higher pressures in spontaneously hypertensive rats (SHR).1,6

Little is known concerning the influence of antihypertensive therapy on the cerebral microvasculature during hypertension. Acute hypotension in hypertensive rats is associated with decreases in CBF, presumably due to a reduction in cerebral perfusion pressure below the lower limit of autoregulation. By contrast, chronic antihypertensive treatment is associated with restoration of CBF autoregulation toward the normal range, presumably by the reversal of structural vascular adaptations that caused the original increment in cerebrovascular resistance. However, morphometric studies addressing this possibility have been aimed primarily at large arteries. Therefore, the primary goal of this study was to characterize the cerebral microvascular hemodynamic responses to antihypertensive therapy in SHR. Segmental resistance was measured at several points in the cerebral vasculature to determine the extent to which antihypertensive treatment reverses structural and functional adaptations to hypertension. This approach provides a comprehensive profile of the cerebral vasculature by combining simultaneous measurements of structure and function in both large and small cerebral vessels.

Materials and Methods

Surgical Preparation

SHR and Wistar Kyoto rats (WKY) were anesthetized with a saline solution of Inactin, a sodium salt of ethyl-(1-methylpropyl)-malonylthiourea (Byk Gulden Konstanz, West Germany; 10 mg/100 g body wt, i.p.), with supplemental injections (2 mg/100 g body wt) given as needed, though rarely necessary. Inactin was used because it provides more consistent maintenance of mean arterial blood pressure (MABP) than other anesthetics, such as chloralose and urethane, and pentobarbital. The trachea was intubated to ensure a patent airway, and both femoral arteries were cannulated for measurement of systemic arterial blood pressure and microsphere injection. The rectal temperature was maintained at 37 ± 1°C with a heating mat.

Surgical procedures consisted of securing the head in a stereotactic device followed by a midsagittal inci-
sion through the scalp, using thermocautery and topically applied ferric chloride solution to minimize cutaneous and fascial bleeding. An approximately 4 x 7-mm area of parietal calvarium was circumscribed by an air-cooled burr held in an electric drill (Dremel Moto-Tool). The bone plate was removed with a periosteal elevator and forceps. The dura mater was reflected, ideally along avascular lines, using a dural hook and small ophthalmic scissors. The exposed cortical surface microvasculature was then bathed with a bicarbonate-buffered physiologic solution having the following composition after gas equilibration: P O₂, 40–45 mm Hg; P CO₂, 38–43 mm Hg; pH, 7.35–7.45. The bathing fluid was suffused over the brain surface at 3–4 ml/min such that the pool of fluid was constantly replenished at least 2–3 times per minute and the fluid temperature remained at 37 ± 1°C. The aforementioned values of P O₂, P CO₂, and pH were found to be stable during pilot studies lasting 2–3 hours, the average duration of a typical experiment. P O₂ measurements were made with microelectrodes, and vasoconstriction ensued when the CSF pool was allowed to stagnate with attendant increases in P O₂ and pH, and decreases in P CO₂. Therefore, as long as gas and fluid delivery was maintained at levels consistent with those of the pilot studies, artificial CSF gas tensions at the level of the cerebral cortex were presumed to be fairly constant. 15

An Aus Jena microscope with Nikon water immersion lenses (10 x, n. a. = 0.22, 20 x, n. a. = 0.33) was used to provide images for a Cohu Electronics 4400 video camera and Panasonic TV monitor. All images were recorded on video cassettes with a Panasonic NV-8320 video recorder so that measurements could be made after the experiment. A timer superimposed on the video screen allowed synchronization of video tape and chart recorder data. Water immersion lenses were used to avoid resolution problems caused by the curved air-water interface of the suffusion solution. Illumination of the brain surface was provided by a ½-in. fiber-optic bundle oriented at 20–30° to the optical plane of the microscope and connected to a Martin Instruments light source. Infrared light was filtered out such that light from the fiber-optic bundle had no thermal effect on the stationary fluid pool.

In a typical experiment, the animal was allowed to recover for a minimum of 30 minutes after all surgery was completed. Evidence of damage (e.g., petechial hemorrhage) to 5% or more of the tissue resulted in termination of the experiment before data acquisition. If none of the microvessels dilated by a minimum of 50% when adenosine (10⁻³ M) was applied topically, the preparation was discarded. Although some vessels might not normally dilate following adenosine application, the absence of dilation in all vessels clearly indicated that the autoregulatory mechanism was disrupted and that the vasculature was pressure-passive.

Categorization of cerebral vessels by hierarchical branching pattern was difficult because of the extensive collateralization of the proximal vasculature. In spite of these conditions, first-order arterioles (1A) were defined as the largest vessels that enter the operative field from directly beneath the skull margin. Second-order arterioles (2A) were defined as those vessels that branch from 1A vessels at nearly right angles and, likewise, third-order arterioles (3A) branched at nearly right angles from 2A vessels. Fourth-order arterioles (4A) branched similarly from the 3A and either directly perfused surface capillaries or dove into the cortical parenchyma. Although there were clearly exceptions to this method of ordering (e.g., vessels of 3A diameter branching directly from large 1As) it was nevertheless useful for grouping the vessels into approximately equal categories of diameter and branch order. In most cases, 4A vessels were the immediate precursors to either capillaries or short precapillary vessels. This procedure has been described in detail previously. 1

**Measurements of Microvascular Pressures, Diameter, and Blood Flow**

Microvascular pressures were measured in all orders of arterioles and venules using a servo-nulling pressure measurement system employing glass pipette microelectrodes with sharpened tip diameters of 1–1.5 μm. Electrodes were attached to a fluid-filled tubing system containing 2 M NaCl. When electrode tips were immersed in physiologic solutions, an interface developed between the 2 M NaCl and the 0.15 M NaCl. When a pressure greater than "zero" (atmospheric) was applied to the tip, the saline interface was displaced, thereby altering pipette resistance. The system generated a precise counterpressure to restore the saline interface, and this counterpressure appeared on the chart record as the equivalent of microvascular pressure. This system was calibrated before and after each experiment.

Vessel diameters were measured using a video-splitting device (IPM Model 907) that employed digitized images of videotaped experimental records. This system was calibrated at a constant magnification with a Bausch and Lomb stage micrometer. The range of measurement and linearity of output were confirmed with a stage micrometer. Repeated measurements of a particular steady-state microvessel were made using an analog-to-digital conversion program that sampled diameter measurements every second and computed average diameter over 1 minute. The average of these determinations was recorded as the diameter for that particular vessel and perturbation. Since the largest variability exists between vessels and/or animals, repeated measurements of a single vessel improved the reliability of that particular measurement without adversely affecting the variability of the entire sample. Both internal and external vessel diameters were measured for calculation of wall thickness and wall area. External vessel wall boundaries were particularly easy to discern in the brain given the high contrast between vessel wall substance and underlying brain tissue. Internal wall boundaries were evident from the rough endothelial surface of the vessels. Passive diameters were measured after topical application of adenosine (10⁻³ M). All vessel measurements were made single-
blot these samples against lung tissue other than that of the contralateral, unoperated cortex. It therefore appears that surgical preparation does not adversely affect blood flow in the area of cortex under study.

**Antihypertensive Treatment Regimen**

The treatment consisted of the following doses added to the rats' drinking water: hydralazine, 10 mg/kg/day; reserpine, 0.20 mg/kg/day; and chlorothiazide, 200 mg/kg/day. These doses produced normotension within 2 weeks in pilot studies. Solutions were prepared fresh and added to the rats' drinking water every evening in the desired doses. Treatment was initiated at age 15–16 weeks, and lasted for 25 ± 1 days. Thus, rats were 18–19 weeks old at the time of study. All rats (treated and untreated) were housed in air-conditioned quarters with light time control and were fed standard rat chow. The amount of food and water consumed were measured daily, and the body weight and blood pressure were measured 3 times weekly. Blood pressure responses to treatment were made using a tail cuff occlusion apparatus (Narco Biosystems).

**Statistical Analysis**

Data are reported as mean ± SEM. Two means were compared using the appropriate two-tailed t test. Comparisons between more than 2 means were evaluated initially by analysis of variance to detect significant differences among the group means. Multiple comparisons were then executed as necessary using Duncan's multiple range test. Probability values <0.05 were considered to indicate a significant difference between means.

**Results**

Table 1 shows the mean values for MABP, heart rate, and total CBF in the 3 treatment groups. Treatment of the SHR resulted in a significant decrease in MABP to a level not different from that seen in normotensive WKY. This decrease in MABP in treated SHR was accompanied by a significant reduction in heart rate compared with untreated SHR. Also evident in treated SHR was a significant decrease in CBF compared with that seen in untreated animals.

Figure 1 depicts resting microvascular pressures in cerebral microvessels. Significant microvascular hypertension was evident in all classes of arterioles in untreated SHR, consistent with previous observations. In treated SHR, there was normalization of microvascular pressures with the exception of the 1A, the largest arterioles, in which a significant reduction in microvascular pressure was evident. This suggests that...
Table 1. Resting Hemodynamics in WKY, Untreated SHR, and Treated SHR

<table>
<thead>
<tr>
<th></th>
<th>WKY</th>
<th>SHR-U</th>
<th>SHR-T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial blood pressure (mm Hg)</td>
<td>111±4 (16)</td>
<td>171±6 (16)</td>
<td>106±3* (17)</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>292±14</td>
<td>343±11</td>
<td>275±11*</td>
</tr>
<tr>
<td>Cerebral blood flow (ml/min/100 g)</td>
<td>74.1±2.8 (8)</td>
<td>77.3±3.9 (8)</td>
<td>69.3±4.8*† (8)</td>
</tr>
<tr>
<td>Cerebrovascular resistance (mm Hg/ml/min/100 g)</td>
<td>1.44±0.06</td>
<td>2.26±0.21</td>
<td>1.59±0.10*†</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; number in parentheses. WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats; U, untreated; T, treated.

*p < 0.05 vs. SHR-U.
†p < 0.05 vs. WKY.

resistance upstream from the 1A in treated SHR was greater, on a relative basis, than in normotensive or hypertensive rats. Indeed, as Figure 2 demonstrates, the percent of the mean arterial pressure head dissipated in treated SHR was greater than that in either WKY or untreated SHR. Approximately 58.5% of the arterial pressure head was dissipated before reaching the 1A in treated SHR, compared with 53.2% in WKY, and only 43% in untreated SHR. Thus, a greater fraction of total cerebrovascular resistance existed upstream from the 1A in treated SHR than in WKY or untreated SHR. Also, 36.8% of the arterial pressure head was dissipated from the 1A to the 4A in untreated SHR, compared with 21.6% in WKY and 16.0% in treated SHR. The arterioles in treated SHR therefore appear to account for a smaller fraction of total cerebrovascular resistance than in either WKY or untreated SHR.

There was evidence of significant regression of structural microvascular adaptations in treated SHR. Table 2 shows that treatment decreased arteriolar wall cross-sectional area in the 1A and 4A to normal levels, implying that the mechanisms accounting for microvascular hypertrophy in untreated SHR have been prevented or reversed. Data for resting microvascular diameter are shown in Table 3. While untreated hypertension was associated with reductions in the diameter of 1A, 2A, and 4A, treatment restores vessel diameter to the normal range. In fact, 1A diameters are even slightly increased above control (WKY) levels. Inasmuch as the reduction in arteriole diameter in untreated SHR is likely due to a combination of constriction and hypertrophy (see Table 2), treatment probably involves a reversal of both hypertrophic and constrictor influences.

Discussion

The results of the present study document structural and functional alterations in the cerebral microvascula-
ture following treatment in SHR. Among the major findings in treated SHR were 1) a shift in total cerebrovascular resistance toward arteries upstream from the arterioles under study, 2) a regression of microvascular hypertrophy with treatment, and 3) a reduction in total CBF associated with an increment in total cerebrovascular resistance compared with control.

The large arteries upstream from the cerebral microcirculation have been shown to provide a significant fraction of overall cerebrovascular resistance in normotensive rats\(^1\) as well as in hypertensive strains.\(^1,3\) Further, these large arteries are able to vary their resistance with changes in MABP in both normotensive and hypertensive strains.\(^15\) These data. Direct measurement of microvascular pressure cannot infer the location(s) of elevated resistance from such results. That significant increases in large artery resistance are due to structural vascular adaptations is supported by work in SHR\(^14\) and renal hypertensive rats.\(^16\)

While the relative resistance in large cerebral arteries in SHR is not as great as in WKY (Figure 2), the large pressure drop between the aorta and the largest arterioles implies an increment in large artery absolute resistance in SHR compared with WKY. The existence of lower than normal pressure in the 1A of treated SHR (Figure 1) confirms this elevation in upstream resistance and suggests that it may not be totally reversed by treatment. There may be irreversible damage to connective tissue components of the arterial wall, leading to an increase in wall stiffness and vascular resistance. It is also conceivable that absolute wall mass is increased, even in treated SHR, and this impedes blood flow at the lower arterial pressure resulting from treatment (Table 1).

The reduction in CBF at normotension in the treated SHR (Table 1) suggests that cerebrovascular resistance is increased relative to WKY normotensive controls, which is indeed the case (Table 1). However, one cannot infer the location(s) of elevated resistance from these data. Direct measurement of microvascular pressures, diameters, and wall cross-sectional area are helpful in elucidating the distribution of resistance. The absence of significant microvascular hypertrophy (Table 2) and constriction (Table 3) in treated SHR suggests that the arterioles under study are not making a significantly larger contribution to overall resistance. This evidence, coupled with the finding of significantly lower microvascular pressure in the largest arterioles (Figure 1), suggests that the increment in resistance must occur upstream from the microcirculation. Whereas 53.2 and 43% of mean arterial pressure is dissipated above the level of the largest arterioles in WKY and untreated SHR, respectively, 58.5% is dissipated in the treated SHR (Figure 2). In addition, only 16% of the arterial pressure head is dissipated between 1A and 4A in treated SHR, compared with about 22% in WKY and 37% in untreated SHR. Thus there appears to be a shift in vascular resistance in treated SHR from the arterioles to the large arteries, combined with an elevation in large artery absolute resistance compared with WKY.

Autoregulation of CBF has been shown to improve significantly following antihypertensive treatment, though results seem to vary with duration of hypertension. For example, complete restoration of CBF autoregulation to normal was shown in treated renal hypertensive rats following 5–6 weeks of severe hypertension, presumably due to reversal of structural vascular adaptations.\(^11\) Indeed, recent preliminary results suggest that treatment initiated before the onset of hypertension results in prevention of a vast majority of structural adaptations in both SHR and renal hypertensive rats, leading to virtually normal CBF autoregulation.\(^13\) However, results obtained in 24-month-old SHR treated for 10 weeks\(^19\) indicate that while CBF autoregulation is improved, there are decreases in CBF compared with control during moderate hypotension. This implies that some cerebrovascular abnormalities persist in treated animals and result in decreases in CBF at lower than normal arterial pressure. These results are supported by findings in the present study utilizing 4–5-month-old SHR treated for approximately 4 weeks. It therefore appears that the duration of hypertension is a significant factor affecting the reversibility of cerebrovascular abnormalities with treatment. The results of the present study suggest that structural abnormalities in the microcirculation may be more readily reversible than those occurring in larger

### Table 2. Resting Arteriolar Cross-Sectional Wall Area in WKY, Untreated SHR, and Treated SHR

<table>
<thead>
<tr>
<th>Vessel Type</th>
<th>WKY (8)</th>
<th>SHR-U (8)</th>
<th>SHR-T (9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>1162 ± 110</td>
<td>1421 ± 151*</td>
<td>1090 ± 116*</td>
</tr>
<tr>
<td>2A</td>
<td>672 ± 21</td>
<td>668 ± 43</td>
<td>640 ± 63</td>
</tr>
<tr>
<td>3A</td>
<td>301 ± 40</td>
<td>375 ± 56</td>
<td>317 ± 51</td>
</tr>
<tr>
<td>4A</td>
<td>199 ± 36</td>
<td>297 ± 12</td>
<td>181 ± 30*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM in μm²; number in parentheses. WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats; U, untreated; T, treated. \(^*p < 0.05\) vs. WKY.

### Table 3. Resting and Passive Arteriolar Diameters in WKY, Untreated SHR, and Treated SHR

<table>
<thead>
<tr>
<th>Vessel Type</th>
<th>WKY</th>
<th>SHR-U</th>
<th>SHR-T</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A Resting</td>
<td>56 ± 4</td>
<td>46 ± 2t</td>
<td>59 ± 4*</td>
</tr>
<tr>
<td>Passive</td>
<td>78 ± 4</td>
<td>73 ± 7</td>
<td>74 ± 3</td>
</tr>
<tr>
<td>2A Resting</td>
<td>37 ± 3</td>
<td>30 ± 3t</td>
<td>34 ± 4*</td>
</tr>
<tr>
<td>Passive</td>
<td>51 ± 3</td>
<td>51 ± 5</td>
<td>49 ± 3</td>
</tr>
<tr>
<td>3A Resting</td>
<td>24 ± 2</td>
<td>23 ± 2</td>
<td>24 ± 3</td>
</tr>
<tr>
<td>Passive</td>
<td>39 ± 4</td>
<td>41 ± 6</td>
<td>38 ± 3</td>
</tr>
<tr>
<td>4A Resting</td>
<td>14 ± 1</td>
<td>11 ± 1t</td>
<td>13 ± 1*</td>
</tr>
<tr>
<td>Passive</td>
<td>19 ± 1</td>
<td>17 ± 2</td>
<td>21 ± 2*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM in μm. WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats; U, untreated; T, treated. \(^*p < 0.05\) vs. SHR-U. \(t p < 0.05\) vs. WKY.
arteries upstream. One might also speculate that irreversible structural adaptations may extend into the microcirculation with longstanding hypertension, implying a gradual decrease with time in the efficacy of antihypertensive therapy in the cerebral circulation. Interestingly, studies of CBF in hypertensive human subjects indicate an increase in gray matter blood flow following antihypertensive treatment.17 An earlier study in hypertensive patients indicated a decreased CBF before antihypertensive therapy, following which CBF was restored to normal.18 While these results are at variance with the present study and several others in both human and animal models, it is clear in any case that antihypertensive therapy is effective in the reversal of hemodynamic abnormalities associated with hypertension.

That antihypertensive treatment reverses structural adaptations was implied in previous studies on renal hypertensive rats11 and confirmed by recent preliminary findings in SHR.19 Treatment reverses hypertrophy, but not hyperplasia, in large arteries in SHR.4 Increases in vessel wall collagen and elastin are, however, unaffected by treatment.20,21 It appears that elevations in large artery resistance in treated SHR (present study) may be due to a combination of hyperplasia and increased stiffness. The degree to which these abnormalities could be reduced by extended treatment is probably minimal.

The sympathetic nervous system has been suggested to have a trophic effect on blood vessels in the brain, which may account for some of the hypertrophy associated with hypertension.2,22-24 Supporting this contention are studies demonstrating a decrease in vessel hypertrophy following denervation in stroke-prone SHR.25 The failure of reserpine, a sympathetic outflow blocker, to completely reverse structural changes in large arteries of SHR or in cerebral arteries of SHR (present study) suggests that, while the trophic effect of the sympathetic nervous system may be important, there may also be nonspecific, irreversible vascular alterations that occur in concert with any trophic influences or may even precede hypertension.26 Moreover, reductions in mean arterial pressure following treatment would prevent elevations in arteriolar blood pressure, thereby eliminating a speculated stimulus for hypertrophy. Inasmuch as increments in arteriolar wall stress are largely absent in SHR,1 one could hypothesize that microvascular hypertension triggers a combination of arteriolar constriction and hypertrophy to normalize wall stress and prevent rupture of the vessel wall. It is conceivable that this protective mechanism is somehow altered in the stroke-prone SHR.

The primary implication of the present study is that antihypertensive treatment can be of significant value in reversing structural and functional adaptations in the cerebral microvasculature. There appears to come a point, however, when sustained hypertension causes irreversible damage to the cerebral vasculature, primarily to those vessels upstream from the microvasculature. While this may not result in a serious functional deficit, the existence of vascular damage nonetheless increases the risk of cerebrovascular pathology unrelated to an increment in blood pressure, namely, the formation of thrombi leading to cerebral ischemia and potential infarction.

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References


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