Altered Cerebral Vessel Innervation in the Spontaneously Hypertensive Rat

Tony J-F. Lee and Akira Saito

From the Department of Pharmacology, Southern Illinois University, School of Medicine, Springfield, Illinois

SUMMARY. Autonomic innervation in the cerebral arterial walls of adult male spontaneously hypertensive rats and of normotensive Wistar-Kyoto rats was studied. When examined by fluorescence microscopy, dense catecholamine fluorescence was observed in anterior cerebral and middle cerebral arteries of both Wistar-Kyoto and spontaneously hypertensive rats. However, vertebral and basilar arteries and small pial arteries of Wistar-Kyoto rats received extremely sparse or no catecholamine fluorescence, whereas, in the respective regions of spontaneously hypertensive rats, catecholamine fluorescence was found to be significantly elevated. The endogenous norepinephrine content was also higher in cerebral arteries of spontaneously hypertensive than of Wistar-Kyoto rats. When examined ultrastructurally (potassium permanganate fixation), the incidence of granular vesicle-containing nerves, indicative of sympathetic nerves, was found to be significantly elevated in all cerebral arteries of spontaneously hypertensive rats examined. In contrast, the agranular vesicle-containing nerve, indicative of nonsympathetic nerves, with close synaptic cleft distance (<2 μm) was found to decrease or remain unchanged in the cerebral arteries of spontaneously hypertensive rats. These results suggest that cerebral sympathetic vasoconstriction may become more prominent than nonsympathetic vasodilation in spontaneously hypertensive rats. This finding lends further credence to the previous in vivo findings that cerebral sympathetic vasoconstrictor nerves become more functional and exhibit a protective effect against brain lesions during hypertension. The potential roles of neurogenic components involved in cerebral blood flow autoregulation are also discussed. (Circ Res 55: 392-403, 1984)

WHEN fixed in KMnO₄, two types of varicosities or nerve terminals have been identified in the adventitial layer of the brain arteries from several species: the granular vesicle-containing nerves (GVN) and the agranular vesicle-containing nerves (AVN). The GVN are adrenergic and originate from the superior cervical ganglia. The AVN are nonadrenergic and originate from the superior cervical ganglia. The AVN are nonadrenergic and nonsympathetic, although their origin is not positively determined (Iwayama et al., 1970; Owman et al., 1974; Lee et al., 1980; Lee, 1981).

The relative ratio of AVN and GVN terminals varies among species and among regions even within species. The large cerebral arterial walls at the base of the normal adult cat brain have been found to contain more AVN (60%) than GVN (40%) terminals (Lee, 1981). The rabbit basilar arterial wall, on the contrary, contains predominantly GVN (98%) and very few AVN (2%) terminals (Lee et al., 1980). Although not quantitatively determined, it is suggested (Iwayama et al., 1970) that the rat cerebral arterial wall at the base of the brain contains equal AVN and GVN terminals. In peripheral arteries such as the rabbit ear and saphenous arteries, only GVN are found (Lee et al., 1980).

Results from in vitro tissue bath studies have indicated that transmural stimulation of the intramural nerves results in vasodilation exclusively in the cat cerebral arteries, predominantly vasoconstriction in the rabbit basilar arteries, and vasoconstriction exclusively in the rabbit ear and saphenous arteries (Lee et al., 1976, 1978, 1980; Lee, 1982b; Medgett and Langer, 1983). This morphopharmacological correlation suggests that GVN (sympathetic) are the vasoconstrictor nerves and AVN (nonsympathetic) are the dilator nerves in the rabbit and cat cerebral arteries (Lee, 1981, 1982a). This observation also holds true for the rat cerebral artery (Hirst et al., 1982; Edvinsson, 1982).

Although the functional significance of cerebral vessel sympathetic innervation in controlling normal cerebral blood flow has been shown to be minimal (Heistad, 1982), the sympathetic nerves may become functionally more important in animals with acute or chronic hypertension (Nordborg and Johansson, 1979; Heistad, 1982; Mueller and Ertel, 1983). On the other hand, the functional significance of the nonsympathetic nerves (AVN) in controlling cerebral circulation during hypertension has not been examined. The main purpose of this study was therefore designed to examine, first, the frequency of AVN and GVN terminals in normotensive rat cerebral arteries and, second, whether the ratio of AVN and GVN terminals is changed in cerebral arteries of spontaneously hypertensive rats.

Methods

Male spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) rats, 24 weeks old, were
purchased from Taconic Farms. One day prior to sacrifice, systolic blood pressures were measured by tail-cuff plethysmography with an electrophysgmanometer and a pneumatic pulse transducer (Narco Biosystems) connected to a Grass polygraph. The average of five measurements per animal was taken as the blood pressure.

Animals were anesthetized with pentobarbital (Nembutal, 40 mg/kg, ip) and exsanguinated. The entire brain with blood vessels attached was rapidly removed.

6-Hydroxydopamine Incubation

The entire brains with blood vessels attached from eight SHR and eight WKY were initially placed in Krebs' bicarbonate solution equilibrated with 95% O₂ and 5% CO₂ at 37°C for 30 minutes. The composition of the Krebs' solution was (mM) Na⁺, 144.2; K⁺, 4.9; Ca²⁺, 1.6; Mg²⁺, 1.2; Cl⁻, 126.7; HCO₃⁻, 25.0; SO₄²⁻, 1.19; glucose, 11.1; and calcium disodium ethylenediamine tetraacetate (EDTA), 0.023. 6-Hydroxydopamine (6-OHDA) was then added to a final bath concentration of 8 x 10⁻⁴ M for five of the eight brains in each group. A similar procedure for longer incubation to obtain in vitro adrenergic denervation was described by Aprigliano and Hermmsmyer (1976). Five of the seven brains of each group incubated in the presence of 6-OHDA were removed 15 minutes later and immediately immersed in cold Krebs' (4°C). The arteries were then dissected and processed for electron microscopy (three brains from each group) and fluorescence microscopy (two brains from each group). The remaining two pairs of brains incubated in the presence of 6-OHDA and three pairs of brains incubated in the absence of 6-OHDA were removed 6 hours later. The arteries then were dissected and processed for catecholamine fluorescence microscopy.

Electron Microscopy

Basilar arteries (o.d. 0.2 mm) and middle cerebral arteries (o.d. 0.15 mm; adjacent to the circle of Willis), and the anterior cerebral arteries (o.d. 0.15 mm, portion of circle of Willis) were excised with the aid of a dissecting microscope. The arterial segments were fixed in ice-cold 2% KMnO₄ in Millonig phosphate buffer (pH 7.4, 490 mOsmol). Preparation of the fixative, fixation, dehydration, embedding of the specimens were carried out as previously described (Lee et al., 1980; Lee, 1981). All blocks were oriented for transverse sectioning of the arteries. Ultrathin cross-sections of whole arteries were obtained with an LKB microtome fitted with a diamond knife. The sections were mounted on slot grids coated with Formvar and were stained with 0.4% uranylacetate with glyoxylic acid to induce catecholamine fluorescence as described in a previous report (Araki et al., 1982). The whole-mount preparations of arteries were treated with glyoxylic acid to induce catecholamine fluorescence according to the method of Axelson et al. (1974), as described in a previous report (Araki et al., 1982).

Bilateral Superior Cervical Ganglionectomy

Both SHR and WKY were anesthetized with pentobarbital (40 mg/kg, ip). Both superior cervical ganglia were isolated and extirpated by cutting the sympathetic trunk at a point proximal to the ganglia, then removing them with short lengths of their other branches attached (Lee et al., 1976). Tissues were examined 7 days after ganglionectomy.

Tissue Wet and Dry Weight

Basilar arteries and the pooled anterior, middle, and internal carotid arteries were blotted on filter paper in a standard fashion and their net weight was determined with an autobalance (Perkin and Elmer AM-2). The dry weight of the basilar artery was obtained after each artery was heated at 100°C in a covered crucible in an oven for 30 minutes, then transferred to a desiccator overnight. The dry weight of the artery was determined by the difference between weights of the crucible (with cover) containing the artery and that without the artery.

Assay for Catecholamine

Catecholamines in the vascular tissues were assayed by the modified radioenzymatic thin layer chromatographic method of DaPrada and Zuricher (1976) and as described in a previous report (Lee et al., 1980). A pool of at least six basilar arteries, or four sets of anterior cerebral, middle cerebral, and internal carotid arteries to give a total weight of at least 0.8 mg was used for one assay. All arteries were cut open and were cleaned of blood clots and surrounding arachnoid membrane.

Statistical Method

The data were evaluated statistically by Student's t-test for pair or unpaired samples, as appropriate. However, because each mean was used in two comparisons (AVN vs. GVN and WKY vs. SHR), a simple but conservative control for type I error rate was employed. Namely, the conventional level of statistical significance (α = 0.05) was divided by 2 (the number of tests involving each mean), and comparisons were considered significant if the com-
Results

The systolic blood pressure of the rats as measured indirectly using tail cuffs was 191.6 ± 3.3 mm Hg ($n = 25$) with a range of 167–207 mm Hg in SHR and 123.3 ± 2.1 ($n = 26$) with a range of 111–136 mm Hg. The systolic blood pressure in SHR was significantly higher ($P < 0.001$) than in the WKY.

Fluorescence Microscopy

WKY

The catecholamine-fluorescent fibers were most densely distributed in the internal carotid and anterior cerebral arteries, followed by the middle cerebral arteries and basilar arteries in decreasing order. The vertebral-basilar junctional area, the vertebral arteries, and the small branches of middle cerebral arteries (from the surface area of sylvian sulcus) were not innervated (Fig. 1).

SHR

The catecholamine-fluorescent fibers were also found most densely in the internal carotid and anterior cerebral arteries and followed by middle and basilar arteries in the same order as seen in WKY. However, the vertebral-basilar junctional area, the vertebral arteries, and the small branches of middle cerebral arteries were always found innervated (Fig. 1).

By 1 week after bilateral superior cervical ganglionectomy, the catecholamine fluorescence had completely disappeared in all pial arteries of WKY ($n = 5$) and in all except the vertebral artery of the SHR ($n = 5$) examined.

The catecholamine fluorescence was not appreciably decreased in cerebral arteries of WKY and SHR after incubation for 6 hours in Krebs solution without 6-hydroxydopamine. On the other hand, the catecholamine fluorescence in cerebral arteries drastically decreased to less than 20% of the control 15 minutes, and completely disappeared 6 hours after incubation in the presence of 6-hydroxydopamine.

Number of Muscle Layers

Table 1 indicates that the basilar, middle cerebral, and anterior cerebral arteries contain significantly more muscle layers in SHR than in WKY.

Tissue Weight

Table 2 indicates that both wet and dry weights of basilar arteries per length are significantly heavier in SHR than in WKY.

Catecholamine Content

The catecholamine contents expressed as μg per mg wet weight of the cerebral arteries from SHR and WKY are shown in Table 3. In both basilar and pooled anterior cerebral, middle cerebral, and internal carotid arteries, the NE content was significantly higher in SHR than in WKY.

Electron Microscopy

In cerebral arteries of both WKY and SHR, all nerve tissues were found only in the adventitial layer. Based on the vesicle inclusion in the nerve terminals or varicosities, two types of nerve profile could be identified in cerebral arteries pretreated with 6-OHDA (Fig. 2). The diameter of small dense granules in GVN vesicles was 350–450 Å, and that of small agranular vesicles in AVN was also 350–450 Å. Occasionally, large granular and agranular vesicles (diameter 900 Å) in respective GVN and AVN can be found (Fig. 2).

Total AVN and GVN Terminals Throughout the Adventitial Layer

In WKY, there were significantly more AVN terminals than GVN terminals throughout the adventitial layer of the anterior cerebral, middle cerebral, and basilar arteries per cross-section (Fig. 3). In SHR, the GVN terminals per section were consistently greater in all three arteries than the corresponding arteries in WKY. However, in SHR, the AVN were found to be significantly fewer in anterior cerebral arteries, not different in middle cerebral arteries, and more in basilar arteries, compared to the corresponding arteries in WKY. Thus, in SHR, the anterior cerebral arteries contained significantly more GVN than AVN terminals; the middle cerebral arteries contained an equal number of AVN and GVN terminals; the basilar arteries contained significantly more AVN than GVN terminals.

Distribution of Synaptic Cleft Distances

Anterior Cerebral Arteries

Figure 4A indicates that there are significantly more AVN than GVN terminals with synaptic cleft distance less than 2.0 μm and no difference between 2 and 10 μm in WKY. In contrast, there are significantly more GVN than AVN terminals in almost all ranges of synaptic distances up to 8 μm in SHR. There are significantly more GVN terminals in SHR than in WKY with a synaptic distance of between 3.0 and 7.0 μm. The GVN terminals per section within synaptic distance of 2 μm are not different between SHR and WKY (Fig. 5). However, the AVN with synaptic distance less than 2 μm are significantly fewer in SHR than in WKY (Figs. 4A and 5).

Middle Cerebral Artery

Figure 4B indicates that more AVN than GVN terminals are found in close synaptic distance (less than 4 μm) in WKY. In SHR, except for the synaptic distance of less than 1 μm, the distribution of AVN with various synaptic clefs is not different from that
FIGURE 1. Distribution of whole-mount catecholamine-fluorescence fibers in cerebral arteries of WKY (panels A–C) and SHR (panels a–c). In WKY, no fluorescence is found in the vertebral artery (panel A) and the small branch of middle cerebral artery (panel B and C). On the other hand, the vertebral artery (panel a) and small branches of middle cerebral arteries (panels b and c) of SHR receive significant amounts of catecholamine fluorescence. Vertebral arteries (panels A,a) were cut open before fixation for inducing catecholamine fluorescence. Bars represent 100 μm.


### TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>WKY</th>
<th>SHR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basilar</td>
<td>4.0 ± 0.3 (4)</td>
<td>5.1 ± 0.1* (4)</td>
<td>&lt;0.025</td>
</tr>
<tr>
<td>Middle cerebral</td>
<td>3.3 ± 0.2 (4)</td>
<td>4.3 ± 0.1* (4)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Anterior cerebral</td>
<td>3.6 ± 0.1 (8)</td>
<td>4.2 ± 0.2* (8)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Number in parentheses = number of arterial cross sections.  
* Significantly different from WKY.

### TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>Basilar Artery Weight (mg/cm length)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WKY</td>
<td>SHR</td>
</tr>
<tr>
<td>Wet</td>
<td>0.17 ± 0.00 (11)</td>
<td>0.26 ± 0.01* (16)</td>
</tr>
<tr>
<td>Dry</td>
<td>0.046 ± 0.001 (6)</td>
<td>0.076 ± 0.009* (6)</td>
</tr>
</tbody>
</table>

Number in parentheses = number of arteries.  
* Significantly different from WKY.

### TABLE 3

<table>
<thead>
<tr>
<th></th>
<th>Endogenous Norepinephrine Content (µg/g wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WKY</td>
</tr>
<tr>
<td>Basilar</td>
<td>0.20 ± 0.10 (3)</td>
</tr>
<tr>
<td>Anterior cerebral, middle cerebral, and internal carotid</td>
<td>1.34 ± 0.31 (3)</td>
</tr>
</tbody>
</table>

Number in parentheses = number of experiments.  
* Significantly different from WKY.

### Discussion

Results of the present study demonstrate a higher concentration of endogenous norepinephrine (NE), denser catecholamine fluorescence with greater GVN:AVN terminal ratio, and more layers of muscle cells in cerebral arteries of SHR than in WKY, suggesting that cerebral sympathetic vasoconstrictor nerves become more prominent than nonsympathetic vasodilator nerves in SHR. Thus, the sympathetic vasoconstriction may surpass the nonsympathetic vasodilation in some brain regions of SHR.

### Granular and Agranular Vesicle-Containing Nerves

When fixed in KMnO₄, the adrenergic nerve terminals in a variety of preparations are characterized by the presence of many small and a few large dense core granular vesicles (Hökfelt and Jonsson, 1968; Lee, 1981). The KMnO₄-fixed nerve terminals containing exclusively agranular vesicles, not affected by sympathetic denervation or 6-hydroxydopamine (6-OHDA) pretreatment, are nonadrenergic, nonsympathetic nerves (Iwayama et al., 1970; Owman et al., 1974; Lee et al., 1980; Lee, 1981). In those studies, 6-OHDA was given to animals, iv or ip, shortly before the experiments. Presumably, 6-OHDA is taken up specifically by the adrenergic nerve terminals to improve the granulation of the dense core vesicles (Iwayama et al., 1970; Lee et al., 1980; Lee, 1981). Accordingly, based on the vesicle inclusions, the adrenergic nerve (GVN) terminals and the nonadrenergic nerve (AVN) terminals can be differentiated (Fig. 2). In the present study, 6-OHDA was not given to animals by iv or ip routes, since potential differences may exist in cerebral arterial wall (such as endothelial cells or blood brain barrier) between SHR and WKY which may affect the transport of 6-OHDA to the nerve terminals. The isolated brain arteries were therefore incubated with 6-OHDA in vitro as described by Aprigliano and Herrmsmeyer (1976). Since the catecholamine fluorescence in all cerebral arteries of SHR and WKY drastically decreased in 15 minutes and disappeared completely 6 hours after incubation in the Krebs solution containing 6-OHDA, it was suggested that 6-OHDA was taken up by the adrenergic nerves during incubation. In all WKY and SHR cerebral arteries examined, AVN and GVN were found only in the adventitial layer. This is consistent with that of GVN. The distribution of AVN with synaptic cleft distance of any range is not different between WKY and SHR. However, there are significantly more GVN with synaptic distances between 2.0 and 7.0 µm in SHR than in WKY.

### Basilar Artery

Figure 4C indicates that there are significantly more AVN than GVN with a synaptic cleft distance between 3.0 and 6.0 µm in WKY. The distribution of AVN terminals with a synaptic cleft distance of any range is not different between WKY and SHR. However, the number of GVN terminals is significantly higher in SHR than in WKY in almost all synaptic distance ranges up to 8 µm.

### Relative Incidence of AVN and GVN within Unit Synaptic Cleft Distance

In WKY, within each micrometer range of synaptic cleft distance per section of the anterior and middle cerebral arteries, the AVN terminals are greater than or at least equal to GVN terminals (Fig. 6). On the other hand, in SHR, there are always more GVN than AVN at various synaptic distances in anterior cerebral artery. Although the AVN still outnumber the GVN with close synaptic distance in middle cerebral artery of SHR, the ratio of GVN over AVN becomes greater than that at any respective range in WKY (Fig. 6).
FIGURE 2. Electron micrographs of dual innervation of anterior cerebral arteries of WKY (panel a) and SHR (panel b) fixed in potassium permanganate. Two types of varicosities can be identified based on the vesicle inclusion. One contains many small dense-core granular vesicles (GVN), as indicated by G, and the other contains many small agranular vesicles (AVN), as indicated by A. Occasionally, large size vesicles (arrow head) can be seen. Tissues were preincubated in 6-hydroxydopamine (10^{-6} M) in vitro for 15 minutes before fixation. Smooth muscle cell (SMC).
FIGURE 3. Granular vesicle-containing nerves (GVN) and agranular vesicle-containing nerves (AVN) in the adventitial layer of anterior cerebral (panel A), middle cerebral (panel B), and basilar (panel C) arteries in spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY). Left panel shows absolute number (mean ± SEM) of nerve terminals observed in each transverse section of the blood vessel wall. Right panel shows the percentage (mean ± SEM) of AVN and GVN of total nerve endings per transverse section. Panel A: anterior cerebral artery; there are significantly more AVN (60%) than GVN (40%) in WKY. The number of GVN (70%) is significantly higher than that of AVN (30%) in SHR. Panel B: middle cerebral artery; there are significantly more AVN (75%) than GVN (25%) in WKY. In SHR, AVN, and GVN become equal in number. Panel C: basilar artery; there are significantly more AVN (80%) than GVN (20%) in WKY. In SHR, both AVN and GVN increase. However, the percent difference between AVN (60%) and GVN (40%) becomes smaller than that in WKY. * indicates significantly different (P < 0.025) from GVN (paired t-test); b indicates significantly different (P < 0.025) from the respective type of nerve endings in WKY (unpaired t-test), n (number of sections).

found in cerebral arteries of other species (Lee et al., 1980; Lee, 1981).

Cerebral Vessel Innervation in Normotensive and Chronically Hypertensive Rats

In normotensive WKY, the total AVN terminals per cross-section throughout the adventitial layer outnumbered the GVN terminals in all three strategically selected arteries (Fig. 3). The density of the GVN per cross-section, indicative of adrenergic innervation (Lee, 1981), varied from region to region and was found to be highest in the anterior cerebral artery and lowest in the basilar artery. Results from frequency-nerve terminal distribution with varying synaptic cleft distances (Figs. 4 and 5) reveal that all WKY cerebral arteries examined contain more AVN than GVN terminals in close synaptic cleft distance. This is similar to that found in the respective regions of the normal cat cerebral arteries (Lee, 1981).

On the other hand, the total GVN terminals per cross-section in all cerebral arteries examined are consistently higher in cerebral arteries of the SHR than those in the respective arteries of the WKY (Fig. 3). Meanwhile, the AVN terminals per arterial cross-section are found to be significantly fewer in the anterior cerebral artery, not different in the middle cerebral artery, or slightly elevated in the basilar artery in SHR, compared to those in WKY (Fig. 3). Therefore, in the SHR, the GVN terminals per cross-section throughout the adventitial layers absolutely outnumber the AVN terminals in the anterior cerebral artery, and the ratio of total GVN over AVN in middle cerebral and basilar arteries also becomes greater in SHR than in WKY (Fig. 3).

The density of the GVN per cross-section in cerebral arteries of WKY seems to parallel the catecholamine fluorescence observed in the respective arteries. The anterior cerebral arteries receive the densest and the basilar arteries receive the sparsest catecholamine fluorescence. These results are somewhat similar to those found in rat cerebral arteries by Kobayashi et al. (1981), except that these authors found denser sympathetic innervation in the proximal (vertebral) than the distal (circle of Willis) end of the basilar artery, and this is different from our present finding. Unfortunately, in the report by Kobayashi et al. (1981), the strain of the rat was not indicated. In WKY (present study), the proximal end of basilar arteries is found to receive extremely sparse or no sympathetic innervation, and the small branches of pial arteries receive no sympathetic innervation. These two arteries in SHR, however, receive significantly denser sympathetic innervation (Fig. 1). These results, together with the general elevation of GVN in all arteries examined, suggest that the vascular sympathetic innervation is denser in most of the brain regions in SHR than in WKY.

Synaptic Cleft Distance

The synaptic cleft distance of varicosity from the smooth muscle cells is another determinant for functional significance of a given innervation (Bevan and Su, 1974; Su and Lee, 1975; Lee et al., 1980; Lee, 1981). Presumably, the more intimate the synaptic cleft, the higher the concentration of the transmitter at the postsynaptic receptors would be and, therefore, the larger the response for a given amount of transmitter release. However, the synaptic concentration of the transmitter would be expected to recede with the cube of the distance from nerve terminals (Bevan and Su, 1974). For this reason, the mean synaptic cleft distance estimated by averaging
FIGURE 4. Distribution of granular vesicle-containing nerve (GVN) and agranular vesicle-containing nerve (AVN) terminals with various synaptic cleft distances in anterior cerebral artery, middle cerebral artery, and basilar artery of WKY and SHR. The ordinate indicates frequency of nerve terminals per transverse section (mean ± SEM), and the abscissa represents synaptic or neuromuscular distances in μm. * indicates significantly different (P < 0.025, paired t-test) from the GVN terminals within the same synaptic cleft range. δ indicates significantly different (P < 0.025, unpaired t-test) from their respective type of nerve terminals within the same synaptic cleft range in WKY, n (number of sections).
Endogenous Norepinephrine Content in Chronic Hypertension

The endogenous NE content of cerebral arteries examined is also greater in SHR than in WKY. The NE content is about 2.5 times higher in basilar arteries of SHR than in those of WKY based on content per tissue wet weight (see Table 2). However, the wet weight per unit length of the basilar artery is heavier (1.5X) in SHR than in WKY. This is also true for the dry weight of the basilar artery, suggesting that the increase in tissue weight in SHR arteries probably is not due to increased water content. Thus, expression of NE concentration by μg content per tissue wet weight may underestimate the NE content of the tissue. Accordingly, the true total content of NE per unit length of the basilar artery should be 1.5 times higher than the estimated concentration based on content per wet weight (2.5 μg/g). Thus, the corrected NE content per unit length will be 3.75 times (2.5 X 1.5) higher in SHR than in WKY. This value parallels the differences found for the NE content between basilar arteries of SHR and WKY. In the pooled anterior cerebral, middle cerebral, and internal carotid arteries, the NE content per tissue wet weight is also found to be higher in SHR than in WKY by 1.8 times. If the weight per unit length of these arteries is also 1.5 times higher in SHR than in WKY, as it is in the basilar artery, the corrected content of NE should be 2.75 times higher in SHR than in WKY. These latter values are also equivalent to the ratio of NE content in combined anterior cerebral and middle cerebral arteries of SHR over WKY. These results suggest that the elevated NE content in cerebral arteries of SHR is most likely due to elevated GVN terminals, although several factors, such as an accelerated biosynthesis or increased reuptake of NE or the presence of more storage vesicles per varicosity (Graham et al., 1970; Burnstock et al., 1970), cannot be ruled out.
be proved until the diverse transmitter mechanisms in cerebral arteries are determined. However, evidence exists to indicate that release of NE from sympathetic nerve terminals in cerebral arteries is inhibited by ACh (Edvinsson et al., 1977; Duckles and Kennedy, 1982). This suggests that release of transmitters from sympathetic nerve terminals may be inhibited by substance(s) released from the neighboring AVN terminals. In the present study, the decreasing ratio of AVN:GVN terminals, especially those with close synaptic distances, in most SHR cerebral arteries, suggests a decreased presynaptic inhibition of transmitter release from GVN in the SHR cerebral artery. Therefore, the release of transmitter from sympathetic nerve terminals will be facilitated and a greater synaptic transmitter concentration may be achieved. Together with the findings that the SHR cerebral arteries contain elevated muscle layers (present study) and may become more sensitive to vasoactive agent (Winquist and Bohr, 1983), it is possible that the cerebral neurogenic vasoconstriction enhances in SHR. Indeed, results from several in vivo physiological studies have suggested that the sympathetic nerves of superior cervical ganglionic origin become more active and more functional in controlling brain circulation and serve an important protective role against stroke in genetically hypertensive rats (Hart et al., 1980; Sadoshima and Heistad, 1982; Heistad, 1982; Mueller and Ertel, 1983).

It is interesting to note that GVN increase in all cerebral arteries examined, and AVN decrease or remain unchanged in cerebral arteries of SHR (present study), whereas both GVN and AVN terminals decrease in cerebral arteries of renal hypertensive rats (RHR) (Saito and Lee, 1983). These results suggest that the active neurogenic vasodilation for increasing cerebral blood flow may be weakened or impaired in both RHR and SHR, and the active cerebral vasoconstriction may be improved in SHR but weakened in RHR. Indeed, both RHR and SHR have been shown to be more susceptible to ischemic brain damage than normotensive WKY, after acute hypotension (Barry et al., 1982). On the other hand, RHR suffer brain ischemia and edema following a sudden increase in blood pressure, presumably due to rupture of the blood brain barrier induced by high blood pressure; this does not occur in normotensive rats (NR) or SHR (Fujishima et al., 1978; Johansson and Linder, 1981). This is also consistent with the observations that the lower limit of cerebral blood flow (CBF) autoregulation are reset to higher blood pressures in chronic hypertensive rats (Fujishima and Omae, 1976; Barry et al., 1982; Sadoshima and Heistad, 1983), in man (Strandgaard et al., 1973) and in the baboon (Jones et al., 1976). Although the hypertensive adaptation of the upper limit of autoregulation has not been reported in the renal hypertensive rat, it has been reported to reset to higher blood pressure in hypertensive baboons (Strand-
gaard et al., 1974) and, possibly, in genetically hypertensive rats (Sadoshima and Heistad, 1982). Although sympathetic nerves have little or no effect on the lower limit of CBF autoregulation (Jones et al., 1976; Sadoshima and Heistad, 1983), the above results suggest that sympathetic vasoconstrictor nerves (AVN) are probably involved in setting the upper limit and the nonsympathetic vasodilator nerves (GVN) the lower limits of the autoregulatory maintenance of cerebral blood flow. Thus, alterations of the pattern and function of cerebral vessel innervation during hypertension may differ with the hypertensive model.

Is Altered Cerebral Vessel Innervation in Chronic Hypertension Caused by High Blood Pressure?

It is intriguing that the decrease of AVN terminals in anterior cerebral artery occurs only in close synaptic cleft distance (<2 μm), whereas the elevated GVN terminals occur primarily in wider synaptic cleft distance (between 3 and 8 μm) in SHR cerebral arteries. It has been suggested that high blood pressure may extrude nerves from the arterial wall in the sheep carotid artery (Keatinge and Torrie, 1976). However, high blood pressure is unlikely to be the primary factor in the reduction of AVN terminals with close synaptic cleft distance in cerebral arteries, since there was no appreciable difference in the distribution of AVN terminals in both middle cerebral and basilar arteries between WKY and SHR. The increase in GVN terminals in SHR cerebral arteries is probably not due to high blood pressure either. In renal hypertensive rats, both the AVN and GVN terminals per cross-section decrease in the anterior cerebral and basilar arteries (Saito and Lee, 1983). These results suggest that alteration of AVN and GVN in hypertensive animals may be due to factors other than the high blood pressure itself.

Conclusion

It has been postulated that the increased sympathetic adrenergic activity in peripheral vascular beds causes and maintains hypertension (Abboud, 1982; Brody et al., 1983; Zimmerman, 1983). In early hypertension, there are signs of a net increase of adrenergic influence on the heart (Julius et al., 1971) but, at least in established hypertension, this seems to be due more to a withdrawal of parasympathetic rather than to an increase of sympathetic tone (Kornber et al., 1973). The increased sympathetic activity in brain arteries of adult SHR (Sadoshima and Heistad, 1982; Mueller and Ertel, 1983) is, however, accompanied by an increase of sympathetic vasoconstrictor nerves in most, and a greater decrease of nonsympathetic vasodilator nerves in some, brain regions. The increased sympathetic activity in cerebral arteries of SHR is further thought to have a beneficial protective effect against lesions such as stroke (Hart et al., 1980; Beausang-Linder and Bill, 1981; Sadoshima and Heistad, 1982; Mueller and Ertel, 1983). The hypertension-related changes in the pattern and function of innervation in cerebral vasculature seem to vary with the hypertensive model.

We thank Sue Sarunski for technical assistance, Dr. Jerry A. Colliver for assistance in statistical analysis, Linda Ragel for artwork, and Salie Fluckiger for preparation of the manuscript.

This work was supported by Grant HL 27763 from the National Institutes of Health, by Grant-in-Aid 83-1040 from the American Heart Association, with funds contributed in part by the American Heart Association/Illinois Affiliate, and funds from Southern Illinois University School of Medicine.

Address for reprints: Tony J-F. Lee, Department of Pharmacology, Southern Illinois University, School of Medicine, P.O. Box 3926, Springfield, Illinois 62708.

Received September 2, 1983; accepted for publication June 28, 1984.

References

Da Prada M, Zurcher G (1976) Simultaneous radioenzymatic determination of plasma and tissue adrenaline, noradrenaline, and dopamine within the femtomole range. Life Sci 19: 1161–1174

Circulation Research/Vol. 55, No. 3, September 1984
Lee and Saito: Innervation of Brain Arteries in Hypertension

Hökfelt T, Jonsson G (1968) Studies on reaction and binding of monoamines after fixation and process for electron microscopy with special reference to fixation with potassium permanganate. Histochemie 16: 45-67
Nordborg C, Johansson BB (1979) The ratio between media thickness and radius in cerebral arteries and arterioles of SHR and NR. Jpn Heart J 20 (suppl I): 290-292

INDEX TERMS: Cerebral vessel innervation • Sympathetic nerves • Nonsympathetic nerves • Spontaneously hypertensive rats • Ultrastructure • Neuromuscular relationship
Altered cerebral vessel innervation in the spontaneously hypertensive rat.

T J Lee and A Saito

Circ Res. 1984;55:392-403
doi: 10.1161/01.RES.55.3.392

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1984 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/55/3/392