SUMMARY  The ultrastructural distribution of the autonomic nerves of brain arteries was investigated in renal (one-kidney, one clip) hypertensive and normotensive Wistar-Kyoto rats. Sympathetic and nonsympathetic nerve terminals were found only in the adventitial layer of brain arteries of renal hypertensive and normotensive rats. In both normotensive and renal hypertensive rats the total nerve endings were dense in anterior cerebral artery, moderately dense in middle cerebral artery, and sparse in basilar artery. In normotensive rats, nonsympathetic nerves outnumbered sympathetic nerves in anterior cerebral, middle cerebral, and basilar arteries. In renal hypertensive rats these two types of nerve terminals in close apposition to smooth muscle decreased in anterior cerebral and basilar arteries, while those in middle cerebral arteries remained unchanged. These results suggest that the potential neurogenic control of cerebral blood vessels as well as the trophic effect of sympathetic nerves on brain blood vessels may decrease in renal hypertensive rats. As this finding contrasts with that in spontaneously hypertensive rats, the pattern of innervation in brain arteries may differ in different types of hypertension. (Hypertension 7: 514-518, 1985)

RESULTS  from morphological studies have demonstrated that there are two types of nerve endings in the adventitial layer of brain arteries: granular vesicle-containing nerves (GVN) and agranular vesicle-containing nerves (AVN). The GVN are of sympathetic origin, and the AVN are of nonsympathetic origin.1-5 Results from in vitro pharmacological studies suggest that the GVN mediate vasoconstriction and the AVN mediate vasodilation3-7; however, the functional importance of cerebral vessel innervation in controlling brain blood flow in vivo remains controversial.5,9 Tissue concentrations of norepinephrine are reported to be decreased in mesenteric artery and heart in renal hypertensive animals.10,11 On the other hand, the density of sympathetic innervation has been shown to increase in brain arteries of the spontaneously hypertensive rats (SHR).5,12 Furthermore, autoregulation of lower and higher limits of cerebral blood flow has been reported to change in hypertensive animals.13-15 These results suggest that autonomic nerves undergo some changes when the animal becomes hypertensive. Alterations in the pattern of autonomic nerves may be different depending on the type of hypertensive model. In this study, we therefore examined and compared the ultrastructural distribution of sympathetic and nonsympathetic nerve terminals and their relationship to smooth muscle cells in the cerebral arterial walls of renal (one-kidney, one clip) hypertensive rats (RHR) and normotensive rats (NR).

Methods  The experiments were performed on normotensive male Wistar-Kyoto rats. Chronic renal hypertension was induced in 7-week-old rats by placing a silver clip (internal diameter, 0.25 mm) over the left renal artery through a left lumbar incision and followed 1 week later by contralateral nephrectomy. The control animals were sham-operated. The left kidney was exposed after a lumbar incision and was manipulated without compressing the artery. The operating time was approximately the same as that of the procedure for artery clipping. One week later the contralateral kidney was also exposed but not removed. All of the procedures were accomplished with the rats under sodium pentobarbital (Nembutal, 50 mg/ml). The systol-
ic blood pressure of the nonanesthetized animal was measured by tail cuff technique.

Fourteen rats underwent operation for hypertension. Eight rats survived 10 weeks after the operation and had a systolic blood pressure of at least 158 mm Hg. The arbitrarily chosen animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and exsanguinated when they were 21 to 24 weeks old. The anterior cerebral artery (a portion of the circle of Willis), middle cerebral artery, and basilar artery (adjacent to the circle of Willis) were dissected and incubated in Krebs solution containing 6-hydroxydopamine (10⁻⁴ M) at 37°C for 15 minutes to enhance the granulation of adrenergic nerve endings. The composition of Krebs solution was as follows (mM): NaCl 113, KCl 4.8, CaCl₂ 2.5, MgSO₄ 1.2, NaHCO₃ 25.0, glucose 11.1, and disodium ethylenediamine tetraacetate 0.023. The tissues were fixed in ice-cold KMnO₄ (2%) in Millonig’s buffer (pH 7.4) for 30 minutes. After the initial fixation, each sample was dissected into five small pieces 1 mm in length and fixed for another 30 minutes. Samples were dehydrated and embedded in epoxy resin. At least three blocks from each arterial preparation (anterior cerebral, middle cerebral, and basilar arteries) were selected from each of the three animals examined. Transverse sections of the vessels were obtained using a Reichert ultramicrotome (American Optical, Buffalo, NY, USA) fitted with a diamond knife and mounted on formvar-coated slot grids. The sections were stained with uranyl acetate and lead citrate and examined by an electron microscope (JEOL 100B). A total of 4654 AVN and GVN terminals were photographed together with the closest smooth muscle cells. The nerve terminals or varicosities were defined as regions of axonal swelling containing at least six vesicles. Neuromuscular distance was measured from each micrograph. Each tissue block was coded before it was examined, and the code was broken only after completion of all measurements. The data were evaluated statistically by paired or unpaired t-test. The 0.05 level of probability was accepted as significant.

Results

The systolic blood pressure of all animals used in this study before the operation was lower than 135 mm Hg. One week after renal artery clipping, the systolic blood pressure was 150.4 ± 7.8 mm Hg (n = 14). These animals were then subjected to contralateral nephrectomy. The systolic blood pressure of four out of eight rats that survived after nephrectomy (measured 1 day before they were killed) was 178.5 ± 10.1 mm Hg (range 158–206 mm Hg; n = 4, p < 0.01), while the systolic blood pressure of four sham-operated animals was 126.3 ± 6.7 mm Hg (range 110–135 mm Hg). Figure 1 is an electron micrograph of AVN and GVN in the adventitial layer of the anterior cerebral artery. Figure 2 shows the number and percentage of AVN and GVN terminals per section of the vessels were 200.7 ± 7.4 (n = 6) in the anterior cerebral artery, 51.3 ± 7.2 (n = 6) in the middle cerebral artery, and 36.8 ± 8.0 (n = 6) in the basilar artery. In RHR, there was a significant reduction in the total number of nerve endings per section in the anterior cerebral artery (113.5 ± 9.0; n = 10, p < 0.001) but not in the middle cerebral (59.5 ± 11.2; n = 6) or the basilar artery (31.1 ± 3.8; n = 8).

Figure 3 shows the distribution of neuromuscular distances with various widths between the nerve endings and the membrane of the nearest smooth muscle cells in anterior cerebral, middle cerebral, and basilar arteries.

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![Electron micrograph of the AVN (A) and GVN (G) in the adventitial layer of the cerebral artery. The GVN are of sympathetic nerves, and the AVN are of nonsympathetic nerves. The GVN may mediate the constrictor response, and the AVN may mediate the dilator response of brain blood vessels. The GVN and AVN were observed only in the adventitial layer of anterior cerebral, middle cerebral, and basilar arteries in both NR and RHR. SM = smooth muscle. (Bar = 1.0 μm.)](http://hyper.ahajournals.org/doi/fig/10.1161/HYPERTENSIONAHA.113.120227)
FIGURE 2. The number of GVN and AVN in the adventitial layer of anterior cerebral, middle cerebral, and basilar arteries in NR and RHR. The two left-most columns show the number of nerve endings per section observed in the transverse section of the blood vessel wall. The two right-most columns show the percentage of AVN and GVN of total nerve endings per section.

* = significantly different from GVN (paired t test): *1 = p < 0.05, *2 = p < 0.01, *3 = p < 0.001; δ = significantly different from the respective type of nerve in NR: δ1 = p < 0.05, δ2 = p < 0.01, δ3 = p < 0.001; n = number of sections.

FIGURE 3 (right). Distribution of synaptic distances between nerve endings and the membrane of the nearest smooth muscle cells in anterior cerebral (A), middle cerebral (B), and basilar arteries (C) of NR and RHR. The left and right panels show histograms of AVN and GVN respectively. There were more AVN than GVN in the anterior cerebral artery, with a synaptic cleft of less than 3.0 μm in NR. There were significantly fewer AVN, with neuromuscular distances less than 7.0 μm, and GVN, with neuromuscular distances less than 2.0 μm, in RHR than in NR. In the middle cerebral artery, there was no difference in the distribution of AVN and GVN between NR and RHR. In the basilar artery, there were significantly fewer AVN and GVN in RHR than in NR in close neuromuscular distances. * = significantly different from GVN with same synaptic cleft range (paired t test): *1 = p < 0.05, *2 = p < 0.01, *3 = p < 0.001; δ = significantly different from the respective type of nerve in NR with the same cleft range: δ1 = p < 0.05, δ2 = p < 0.01, δ3 = p < 0.001.
arteries. There were more AVN than GVN in the anterior cerebral artery, with a synaptic cleft of less than 3.0 \( \mu \)m in NR. Both the terminals of AVN with neuromuscular distances less than 7 \( \mu \)m and the terminals of GVN with neuromuscular distances less than 2 \( \mu \)m were significantly fewer in RHR than NR (\( p < 0.05 \)). There was not a significant difference between the distribution of AVN and GVN in the anterior cerebral artery of RHR. The distribution of AVN in the basilar artery of NR was not significantly different from that of GVN among entire ranges of neuromuscular distances. Both AVN with neuromuscular distances less than 4 \( \mu \)m and GVN with neuromuscular distances less than 1 \( \mu \)m decreased in the basilar artery of RHR. There was no significant difference in the distribution of AVN and GVN in the basilar artery of RHR.

In the middle cerebral artery of NR, the number of AVN with neuromuscular distance between 1.1 and 2.0 \( \mu \)m was higher than that of GVN. There was a significant difference in the distribution of AVN and GVN between NR and RHR (\( p < 0.05 \)). There were more AVN than GVN with a neuromuscular distance less than 2 \( \mu \)m in the middle cerebral artery of RHR.

**Discussion**

Morphological and in vitro pharmacological studies have shown that brain arteries of several species receive sympathetic vasoconstrictor (GVN) and nonsympathetic vasodilator (AVN) nerves. The distribution of these two types of nerves sharply varies among species and regions. For example, rabbit basilar artery is mainly (98%) innervated by sympathetic nerves (GVN). On the other hand, 60% of nerve endings are of AVN in large cerebral arteries and 80% are of GVN in small cerebral arteries of the cat. The present study found this variation in regional distribution of AVN and GVN in cerebral arteries of the rat (see Figure 2). In both NR and RHR the total nerve endings were found to be dense in anterior cerebral artery, moderately dense in middle cerebral artery, and sparse in basilar artery. Similar to findings in the cat, all the large arteries examined at the base of the brain in NR contained more AVN than GVN terminals. In RHR, however, the AVN and GVN terminals were found to be significantly decreased in some arteries. This finding in RHR contrasts to that found in SHR in that the GVN terminals were significantly increased in all cerebral arteries examined, while the AVN terminals were decreased in some regions of the SHR. As neurotransmitter concentration in the synaptic cleft is expected to fall off with the cube of the distance from the nerve endings, the nerve endings with closer synaptic distance will be more effective in controlling muscle activity than those with a wider synaptic distance. Thus, the reduction of AVN and GVN with close neuromuscular distances in cerebral arteries of RHR suggests that the functional innervation in cerebral blood vessels in these animals may be weakened or impaired. This finding corresponds to the reported impaired function of sympathetic nerves in peripheral tissues of the renal hypertensive animals. As neither vasoconstrictor nor vasodilator neurotransmitter has been positively identified in cerebral arteries, the sensitivity change of cerebral arteries to neurotransmitters has not been determined.

Results from in vivo studies on the functional importance of cerebral vessel innervation in controlling brain blood flow remain controversial. This may be due in part to marked variation in pattern of cerebral vessel innervation. It has been shown, however, that sympathetic nerves exert protective effects on cerebral circulation in acute (cat) and chronic (rat) hypertension. Stimulation of sympathetic nerves attenuates the passive dilation of cerebral vessels and protects the integrity of blood-brain barriers during an acute increase in blood pressure. Sympathetic nerves also exert a trophic effect to increase the thickness of the blood vessel wall in chronically hypertensive rats. A decrease in sympathetic nerve density in RHR cerebral arteries therefore suggests that animals with renal hypertension are prone to cerebrovascular lesions. Indeed, it has been demonstrated that RHR, but not NR or SHR, suffer brain ischemia and edema following a sudden increase in blood pressure. These lesions are thought to result from the rupture of the blood-brain barrier induced by high blood pressure.

It has been suggested that the neurogenic mechanism is involved in the autoregulation of cerebral blood flow. Results from in vitro pharmacological studies have shown that nonsympathetic nerves (AVN) mediate vasodilatation of brain arteries. A decrease in the number of AVN in RHR (present study) and SHR cerebral arteries, therefore suggests that the active neurogenic vasodilation may be impaired in RHR and SHR. It has been reported that the lower limit of autoregulation of cerebral blood flow shifts to a higher level in both RHR and SHR and that both RHR and SHR are more susceptible to ischemic brain damage than NR following acute hypotension. Together these results favor the presence of a functional vasodilator innervation in rat cerebral blood vessels.

The mechanism of the decrease in nerve endings is not yet clear. It has been suggested that high blood pressure extrudes nerves from the arterial wall in the sheep carotid artery. In cerebral arteries, however, high blood pressure is unlikely to be the primary factor in the reduction of nerve endings as we found no appreciable difference in the distribution of AVN and GVN in the middle cerebral artery between RHR and NR. Furthermore, our previous study found that the density of sympathetic nerves (GVN) in brain arteries was higher in SHR than in NR.

Although the exact role of AVN and GVN in controlling brain circulation in vivo is not completely known, results of the present and previous studies suggest that the functional importance of cerebral vessel innervation may vary in different types of hypertension. Hypertension has been reported to be frequently associated with cerebrovascular diseases such as stroke. Further investigation of the mechanism(s) involved in the decrease or increase of the vasoconstrictor and dilator nerve endings in brain arteries (of RHR...
and SHR) may provide valuable information for designing rational therapeutic approaches in controlling various types of hypertension as well as derangement of brain circulation.

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