Sympathetic Nerves Protect Against Stroke in Stroke-Prone Hypertensive Rats

A Preliminary Report

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SUMMARY Studies were performed to determine whether sympathetic nerves protect against stroke in hypertensive rats. The superior cervical ganglion was removed on one side in 28 stroke-prone spontaneously hypertensive rats (SHRSP) when the rats were 4 weeks old. The rats were fed Japanese rat chow and 1% saline drinking water. When the rats were 19 weeks old, systolic pressure was 206 ± 4 mm Hg (mean ± SE). All rats died between 19 and 23 weeks of age. Microscopic and histological examination demonstrated cerebral hemorrhage in seven rats. All the hemorrhages occurred in the denervated hemispheres. Ischemic cerebral infarctions were found in 13 rats; in 10 rats, the infarcts were only in the denervated hemisphere. Pathological changes of cerebral arteries (hyalinosis, fibrinoid changes, and thrombus formation) were observed primarily in denervated hemispheres. Wall-to-lumen ratio was less in arteries of the denervated hemisphere than in arteries of the innervated hemisphere. These preliminary observations suggest that denervation of cerebral vessels increases susceptibility to stroke and inhibits development of cerebral vascular hypertrophy in SHRSP.


KEY WORDS • sympathetic nerves • stroke • SHRSP • hypertensive rats

CEREBRAL vessels have extensive sympathetic innervation, but it has been difficult to demonstrate an important role for this innervation. Recent studies, however, suggest that sympathetic nerves may have pronounced effects on cerebral vessels during acute increases in arterial pressure. Sympathetic stimulation attenuates increases in cerebral blood flow during acute hypertension and protects the blood-brain barrier. Bevan has proposed that sympathetic nerves have important effects on blood vessels in addition to affecting blood flow. She found that sympathetic denervation inhibits normal growth of the rabbit ear artery. This “trophic” effect was greater in young rabbits than in mature rabbits. Bevan and Bevan also obtained preliminary observations that sympathetic denervation inhibits growth of the posterior cerebral artery in normal rabbits.

Recently we extended Bevan’s concept of a trophic effect with the observation that sympathetic nerves contribute to the development of vascular hypertrophy during chronic hypertension. We obtained evidence of pronounced hypertrophy of cerebral vessels in stroke-prone spontaneously hypertensive rats (SHRSP) and found that sympathetic denervation attenuated development of vascular hypertrophy. We speculated that vascular hypertrophy protects the cerebral microcirculation during hypertension and that the trophic effect of sympathetic nerves may be protective.

In our earlier study of SHRSP, none of the rats developed stroke and all survived to 13 months of age. Okamoto et al. have reported that the incidence of stroke in SHRSP varies between 0 and 89% in groups fed different diets. SHRSP fed Japanese rat chow have a much higher incidence of stroke than SHRSP fed American rat chow. In addition, providing the rats with 1% saline for drinking water, instead of tap water, increases the incidence of stroke. It is likely that strokes did not occur in our earlier study because the rats were fed American rat chow and drank tap water. This study was performed to determine whether sympathetic nerves protect against stroke in SHRSP.
Methods

We studied 28 SHRSP (18 males, 10 females). The rats were housed at 25°C. When the rats were 4 weeks old, they were anesthetized with pentobarbital (Nembutal 3 mg/100 g, intraperitoneally) and the superior cervical ganglion was removed on one side. All rats had ptosis and enophthalmos on the side ipsilateral to ganglionectomy. The rats were fed Japanese rat chow (Funabashi Farm) and 1% saline drinking water. Blood pressure was measured with an electro-sphygmomanometer (LTT Inc.) at 2-week intervals.

When the rats died, macroscopic and histological examination was performed on each animal. The brain was fixed in 10% neutral formalin for 7 days, and seven coronal sections of the brain were made for histological examination. Maximum dimensions of cerebral lesions were measured with an ocular micrometer. The heart, lungs, and kidney were studied histologically. Wall-to-lumen ratio of the cerebral arteries was calculated using a modification of the method of Nordborg and Johansson.11 Pial and intraparenchymal arteries were magnified, traced on paper, and the length of the internal elastic membrane and the area of media were calculated using a digitizer. The formula used was:

\[
\text{wall/lumen ratio} = \frac{\sqrt{4\pi S} + L^2}{2L} - 1/2
\]

where \(S\) is the surface area of media and \(L\) is the length of internal elastic membrane. External diameter (\(D\)) of the arteries was also calculated using the following formula:

\[
D = \frac{\sqrt{4\pi S} + L^2}{2}
\]

Histofluorescence examination for catecholamines in cerebral arteries was performed on five SHRSP which showed signs of stroke. Small tissues from parietal lobes and sections of internal carotid arteries were quickly removed, preserved in liquid nitrogen, and examined with a modification14 of the histofluorescence technique of Bjorklund et al.18

Results

Twenty-one rats developed definite behavioral changes. They became aggressive, irritable, and hyperkinetic, with a propensity to jump, attack, and bite. The rats ultimately became drowsy and lethargic, and died within 1 to 5 days of the onset of behavioral changes. Focal neurological signs, either monoparesis or hemiparesis, were observed in three of these rats. Seven rats died unexpectedly without prior evidence of illness. All 28 rats died between 19 and 23 weeks of age (average, 151 ± 2 st days of age) (fig. 1).

We have compared survival of these rats with survival of our breeder colony of SHRSP. Rats are kept in the breeder colony for approximately 10 months. The rats eat American rat chow, drink tap water, and their sympathetic nerves are not interrupted. Of the last 144 rats in the breeder colony, all except one survived for 10 months without signs of stroke.

Foci of cerebral hemorrhage were observed in seven rats (figs. 2 and 3). All of the hemorrhages occurred in the denervated hemisphere (\(p < 0.05\) by the sign test).14 Hemorrhage was observed in the parietal and frontal cortex and was not observed in the occipital cortex, basal ganglia, or cerebellum.

Ischemic cerebral infarction was characterized by local areas of eosinophilic neural cells with swollen cytoplasm, shrinkage, and pyknosis of cell nuclei, reactive astrocytosis, and infiltration by mononuclear cells. Ischemic cerebral infarction was found in 13 rats; in 10 rats, the infarcts were only in the denervated hemisphere. One ischemic infarct occurred in the occipital cortex; all others were located in the frontoparietal or temporal cortex. Thus, among 20
SHRSP with cerebral hemorrhage or ischemic infarcts, 17 occurred only on the denervated side ($p < 0.01$, fig. 3). The number of hemorrhagic lesions per involved animal was $1.3 \pm 0.2$ (mean $\pm$ se), and their maximum dimension was $1.1 \pm 0.2$ mm. The number of lesions per involved animal for ischemic infarction was $1.8 \pm 0.4$ in denervated hemisphere, $1 \pm 0$ in innervated hemisphere, and $8$ in both hemispheres. Maximum dimension of the lesions was $1.0 \pm 0.2$ mm, $1.1 \pm 0.1$ mm, and $0.6 \pm 0.1$ mm respectively.

Pathological changes were observed in the cerebral arteries of 22 SHRSP. We found hyalinosis, fibrinoid necrosis, proliferation of adventitial cells, and thrombi in areas adjacent to areas of stroke. These changes occurred primarily in the denervated hemisphere (fig. 3) ($p < 0.05$). Renal vascular, glomerular, and tubular changes were common in these rats, and the abnormalities often were severe. Changes in the lungs were not prominent. Small foci of myocardial fibrosis were frequent, and three rats had myocardial infarction. Wall-to-lumen ratio was calculated in 395 pial and parenchymal arteries (188 arteries in denervated hemisphere and 207 arteries in innervated hemisphere). The wall-to-lumen ratio was $0.14 \pm 0.03$ in arteries of denervated hemisphere and $0.16 \pm 0.04$ in arteries of innervated hemisphere ($p < 0.05$). Wall-to-lumen ratio was significantly less in small arteries (external diameter $< 80 \mu m$) of denervated hemisphere than that in innervated hemisphere (fig. 4). Wall-to-lumen ratio in arteries with diameters larger than $80 \mu m$ showed no significant difference in the denervated and innervated hemispheres.

Specific fluorescence for catecholamines was not detected in cerebral arteries or the internal carotid arteries on the denervated side. Fluorescence was detected frequently on the innervated side. Absence of fluorescence on the denervated side does not provide quantitative evidence concerning the effectiveness of denervation but nevertheless indicates marked depletion of catecholamines.

**Discussion**

This study provides preliminary evidence that sympathetic denervation increases susceptibility to stroke in hypertensive rats. The mechanism of the protective effect of sympathetic nerves is not established by these experiments, but we speculate that three factors may be important. First, stimulation of sympathetic nerves attenuates increases in cerebral blood flow during acute hypertension. Thus, sympathetic neural discharge may protect cerebral vessels. Second, a trophic effect of sympathetic nerves may accentuate cerebral vascular hypertrophy during chronic hypertension. Vascular hypertrophy may increase cerebral resistance, reduce pressure in small cerebral vessels, and thereby protect the cerebral microcirculation. Third, a trophic effect of sympathetic denervation may involve electrogenic mechanisms. Denervation induces partial depolarization of vascular muscle in the portal vein. If sympathetic denervation produces partial depolarization of cerebral vascular muscle, and if electrogenic mechanisms contribute to autoregulatory changes in blood flow, denervation may impair autoregulatory responses by electrophysiological effects.

The percentage of arterioles that have sympathetic innervation apparently varies in different regions of cerebral. Between 55% and 80% of the arterioles are innervated in the frontal, temporal, and parietal cortex, and only about 28% in the occipital cortex. We found that hemorrhage or ischemic infarction was rare in the occipital cortex. Thus, neural effects on cerebral vessels appear to be relatively unimportant in occipital cortex but, in densely innervated frontoparietal and temporal regions, sympathetic nerves have an important protective effect against stroke.

The finding that sympathetic nerves protect against stroke seems rational with respect to hemorrhage. Mechanisms by which sympathetic nerves might protect against ischemic stroke are less clear. We recently have obtained preliminary evidence (unpublished observations) that disruption of the blood-brain barrier to albumin occurs in the denervated hemisphere of SHRSP without evidence of stroke. We
speculate that disruption of the barrier may pre-
dispose rats to cerebral ischemia by allowing access of
vasoactive substances to cerebral vessels and cerebral
tissue. Denervation hypersensitivity also may contrib-
ute to ischemia by augmentation of vasoconstrictor
responses to catecholamines. An alternative possi-
bility is that reduction of vascular hypertrophy by
sympathetic denervation may increase the cerebral
vascular diameter and wall stress, lead to fibrinoid
vascular changes and thrombosis, and thereby pro-
duce ischemic stroke.

We recently have studied a group of SHRSP that
underwent sham excision of one superior cervical
ganglion. In these rats, in contrast to our finding that
stroke is increased in the denervated hemisphere,
hemorrhage was observed in the hemisphere ipsi-
lateral to sham surgery in two rats and in the contra-
lateral hemisphere in two rats (unpublished observa-
tions). The incidence of ischemic infarction has not yet
been determined.

We have used two different methods to determine
the effects of sympathetic denervation on the wall-to-
lumen ratio of cerebral vessels in SHRSP. In a
previous study, the brain was perfused with formalin
at 80% of the rat's systolic pressure.10 In this study, the
wall-to-lumen ratio was determined in vessels that
were fixed in their collapsed state. We found, in both
studies, that denervation inhibited the development of
vascular hypertrophy in small cerebral arteries. Our
measurements may not represent the in vivo wall-to-
lumen ratio, particularly in light of other measure-
ments10 that suggest that the wall-to-lumen ratio in
vivo is less than our measurements. Although each ap-
proach has important limitations to the measurement
of the wall-to-lumen ratio, the conclusion that denerv-
ation inhibits the development of cerebral vascular
hypertrophy is strengthened by similar findings using
two different methods.

Yamori et al.21 studied the effects of bilateral
superior cervical ganglionectomy on the cerebral
blood flow in SHRSP. They performed ganglion-
cectomy in SHR at 3 months or 6 months of age. The
authors did not describe the incidence of stroke after
ganglionectomy.

In summary, it has been difficult to demonstrate an
important role for sympathetic nerves in the cerebral
circulation.2-3 These experiments demonstrate that
sympathetic denervation increases the incidence of
cerebral vascular changes and strokes in SHRSP.
Thus, we suggest that sympathetic nerves protect
against stroke in the presence of severe hypertension.

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