Sympathetic Nerves Protect the Blood-Brain Barrier in Stroke-Prone Spontaneously Hypertensive Rats

SEIZO SADOSHIMA, M.D., AND DONALD HEISTAD, M.D.

SUMMARY Disruption of the blood-brain barrier may play a major role in the pathogenesis of hypertensive encephalopathy. In this study we determined whether sympathetic nerves to cerebral vessels protect the blood-brain barrier during chronic hypertension. We removed the cervical sympathetic ganglion on one side in 24 stroke-prone hypertensive rats when they were 1 month old. After signs of cerebral dysfunction developed at the mean age of 160 ± 5 days (se), we injected $^{125}$I-albumin and Evans blue dye intravenously to evaluate the permeability of the blood-brain barrier. Twelve rats showed disruption of the barrier without histological evidence of cerebral hemorrhage or infarction. The barrier was disrupted in the denervated hemisphere in all 12 rats and in the innervated hemisphere in only three rats (p < 0.05). Permeability to $^{125}$I-albumin was 3.53 ± 0.83 (brain albumin x 100/blood albumin) in areas of the cerebrum stained with blue dye and 0.24 ± 0.02 in unstained areas (p < 0.05). We conclude that sympathetic nerves protect the blood-brain barrier against disruption during chronic hypertension and thereby may protect against hypertensive encephalopathy.

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KEY WORDS • blood-brain barrier • sympathetic nervous system • spontaneously hypertensive rats • albumin • chronic hypertension • stroke

MAJOR cerebral vascular complications of chronic hypertension are stroke and hypertensive encephalopathy. We have reported that interruption of sympathetic nerves to cerebral vessels of stroke-prone spontaneously hypertensive rats (SHRSP) attenuates development of cerebral vascular hypertrophy and leads to a high incidence of stroke. Thus sympathetic nerves contribute to development of cerebral vascular hypertrophy and protect against stroke in chronic hypertension.

In contrast to the concept that "spasm" of cerebral vessels produces ischemia and hypertensive encephalopathy, other studies suggest that hypertensive encephalopathy is produced by passive dilatation of cerebral vessels with disruption of the blood-brain barrier. Several studies indicate that intense electrical stimulation of sympathetic nerves attenuates increases in cerebral blood flow and protects the blood-brain barrier during sudden increases in arterial pressure. However, it has not been demonstrated that endogenous sympathetic tone protects the blood-brain barrier, and protection of the barrier by sympathetic nerves has not been demonstrated during chronic hypertension.

We have obtained evidence that the blood-brain barrier is more resistant to disruption by acute increases in arterial pressure in hypertensive rats than in normotensive rats. We suggested that vascular hypertrophy induced by chronic hypertension may protect the blood-brain barrier to albumin. Because sympathetic denervation inhibits the development of cerebral vascular hypertrophy and because sympathetic stimulation protects the blood-brain barrier to albumin, we speculated that denervation would increase the incidence of disruption of the blood-brain barrier in chronic hypertension.

The purpose of this study was to determine whether sympathetic nerves protect against disruption of the blood-brain barrier in SHRSP. The rats were studied when they developed signs of brain dysfunction. Our hypothesis was that when the rats developed brain dysfunction, we would find disruption of the barrier, with increased permeability to albumin, and disruption of the barrier would be present primarily in the denervated hemisphere. This finding would provide the first evidence that sympathetic nerves to cerebral vessels protect against hypertensive encephalopathy.
Methods

We anesthetized 24 SHRSP with pentobarbital at 4 weeks of age and removed the superior cervical ganglion on one side; we exposed the ganglion on the other side but did not interrupt it. All rats had piosis and enophthalmos on the side ipsilateral to ganglionectomy but not on the sham-operated side. The rats were fed Japanese rat chow (Funabashi Farm) and 1% saline drinking water.

Permeability of the blood-brain barrier was examined when the rats developed signs of brain dysfunction, such as neurological deficits, weight loss, aggressiveness, or hyperkinesis. The average age of the rats was 160 ± 5 (SE) days, and the mean arterial pressure was 203 ± 6 mm Hg. The rats were anesthetized with pentobarbital (4 mg/100 g i.p.) and intubated for artificial ventilation with room air and supplemental oxygen. Arterial blood pH was 7.36 ± 0.02; PaCO₂ was 33 ± 0.9 mm Hg; and PaO₂ was 198 ± 17 mm Hg.

To examine permeability of the blood-brain barrier to albumin, we used a method similar to that which we have used previously. Approximately 10 μCi 125I-labeled serum albumin was injected i.v. and allowed to circulate for 1 hour. Arterial blood samples were obtained 15 and 45 minutes after injection. Evans blue dye 20 mg/kg was injected i.v. 30 minutes before the rats were killed. The rats were given heparin i.v. and then killed with KCl i.v. The ascending aorta was cannulated through the left ventricle, and the descending aorta was ligated. To remove 125I-albumin from the lumen of cerebral vessels, the upper body was perfused through the aortic cannula with 200 to 300 ml of 0.9% saline. To assess efficacy of perfusion in removal of 125I-albumin from the lumen of cerebral vessels, samples of saline effluent from the right atrium were examined for 125I-albumin radioactivity. Radioactivity in the last sample of effluent always was less than 0.2% of 125I-albumin activity in arterial blood.

After macroscopic examination, the brain was fixed in 10% neutral formalin for 1 day, and 8–10 coronal sections were made. The sections were examined for extravasation of Evans blue dye. Areas that were stained with dye were cut into two specimens: one for histological study and one for estimation of permeability. The rest of the brain was used for histological study and for estimation of permeability. Cerebral infarction was defined as focal necrotic lesions with disappearance or ischemic change of neural cells and reactive proliferation of glial cells often with extravasation of erythrocytes. When infarction was present, fibrinoid vascular changes and thrombus formation were frequent and edema was usually marked.

We estimated the fraction of blue-stained areas of cerebrum that had histological evidence of edema or eosinophilic changes. The region of cerebrum that had blue staining was defined by macroscopic examination of coronal sections. The areas of edema and eosinophilic changes were measured in histological sections with an eye-piece micrometer.

Radioactivity in arterial blood samples and brain samples were determined with a gamma counter. An index of permeability of the blood-brain barrier to albumin was determined from the equation:

\[
\text{permeability index (\%)} = \left( \frac{\text{counts in tissue/g tissue}}{\text{counts in blood/g blood}} \right) \times 100.
\]

Permeability to albumin also was determined in a control group. We studied five age-matched SHRSP in which one superior cervical ganglion had been removed at 4 weeks of age. The rats appeared to be healthy at the time of the study. The average age of the rats was 158 ± 5 days, and mean arterial pressure was 195 ± 8 mm Hg. They were examined in the same way as the other rats.

Statistical comparison of the incidence of blue staining of the cerebrum was made by sign test: staining only in the denervated hemisphere was considered positive, staining only in the innervated hemisphere was negative, and bilateral staining was neutral. Paired t tests were used to compare two values obtained with 125I-albumin.

Results

Among 24 rats that had signs of brain dysfunction, 12 rats had disruption of the blood-brain barrier (with staining by blue dye) without histological evidence of cerebral infarction or hemorrhage (fig. 1). The 125I-albumin was 3.53% ± 0.83% in blue-stained areas and 0.24% ± 0.02% in unstained areas (p < 0.05). Histological examination showed interstitial edema, with slight eosinophilic changes of some nerve cells, in blue-stained areas. We estimated that 66% ± 6% of blue-stained cerebrum had histological evidence of edema, and 24% ± 3% of blue-stained cerebrum had slight eosinophilic changes. In rats without evidence of infarction or hemorrhage, vascular changes were not detected: specifically, we did not observe fibrinoid necrosis, hyalinosis, or thrombi in blue-stained or unstained areas of the cerebrum.

Among 24 rats with signs of brain dysfunction, 12 had already developed histological evidence of stroke: six had cerebral hemorrhage, and six had ischemic infarction. Five rats had lesions only in the denervated hemisphere, two only in the innervated hemisphere, and five had bilateral lesions.

The major finding in the study was that, among the 12 rats with brain dysfunction without cerebral infarction or hemorrhage, there was staining with blue dye in the denervated cerebrum of all 12 rats, but the innervated cerebrum was stained in only three of these rats (p < 0.05) (fig. 2). Most of the areas with disruption of the blood-brain barrier were in the frontoparietal region. In the denervated hemisphere of the 12 rats, 15 areas were observed with blue staining: 11 in the frontoparietal region, two occipital, one temporal, and one basal ganglia. The total amount of 125I-albumin that entered the cerebral parenchyma in the blue-stained areas was almost five times greater in denervated cere-
Brain of a stroke-prone spontaneously hypertensive rat that developed signs of brain dysfunction. The left superior cervical ganglion was removed at 4 weeks of age. The blood-brain barrier was disrupted in the left cerebrum, as indicated by staining with Evans blue dye. Histological examination demonstrated no evidence of hemorrhage or ischemic infarction.

Among five control SHRSP, none had staining with blue dye. The $^{125}$I-albumin index in these rats was $0.13\% \pm 0.02\%$, with no difference between innervated and denervated hemispheres. Thus, the $^{125}$I-albumin index of blue-stained areas in rats with signs of brain dysfunction was almost 30 times greater than the $^{125}$I-albumin index of control rats.

Discussion

We conclude from this study that sympathetic denervation increases susceptibility to disruption of the blood-brain barrier in stroke-prone spontaneously hypertensive rats. Thus, sympathetic nerves protect the blood-brain barrier during chronic hypertension. The mechanism of this protective effect was not established in this study, but we speculate that several factors may be important. First, sympathetic stimulation during acute hypertension attenuates increases in cerebral blood flow and protects the blood-brain barrier. Thus, sympathetic nerves may buffer increases in blood flow in SHRSP and protect the blood-brain barrier. Second, sympathetic nerves are necessary for normal development of arteries and for development of cerebral vascular hypertrophy. Hypertrophy of upstream vessels may reduce pressure in small distal vessels and thereby protect the cerebral microcirculation. Third, vascular stress is inversely proportional to wall thickness. Augmentation of vascular hypertrophy by sympathetic nerves may reduce vascular stress during chronic hypertension and protect the blood-brain barrier.

The extent of innervation of cerebral vessels varies in different regions of cerebrum. A larger fraction of vessels has been reported to be innervated in frontoparietal and temporal cortex than in occipital cortex. We found in this study that disruption of the blood-brain barrier was frequent in denervated frontoparietal cortex and rare in occipital cortex. Thus, sympathetic nerves appear to have important protective effects against disruption of the blood-brain barrier in the densely innervated anterior part of the cerebrum.

Permeability of the blood-brain barrier to albumin has been reported to be increased in SHRSP, and it has been suggested that increased permeability may contribute to cerebral vascular lesions. Other investigators have found increased permeability in spontaneously hypertensive rats, without vascular or parenchymal changes except for edema. We have not attempted to determine, in these experiments, whether permeability of the blood-brain barrier is altered in SHRSP without evidence of brain dysfunction. Our experiments are in accord with the previous observations and hypotheses, however, that disruption of the barrier may occur prior to other vascular changes and in the absence of cerebral infarction, and that disruption of the barrier may produce brain dysfunction.

Raichle et al. have examined neural effects on permeability of the blood-brain barrier to water. They have reported that the barrier is not freely permeable to
water, and that stimulation of central noradrenergic pathways (which arise from the locus coeruleus) produces an increase in permeability to water. Our study has addressed effects of cervical sympathetic nerves on permeability to albumin, and it is unlikely that central noradrenergic pathways are involved in this effect.

Byrom examined permeability of the blood-brain barrier in renal hypertensive rats that developed signs of brain dysfunction. Areas of disruption of the blood-brain barrier were demonstrated after injection of trypan blue i.v. Water content was similar in the brain of normal rats, hypertensive rats without signs of brain dysfunction, and in unstained areas of brain in rats with signs of dysfunction. In blue-stained areas of brain in rats with signs of dysfunction, however, water content was increased significantly. Thus, in hypertensive rats with brain dysfunction, there are focal areas of disruption of the blood-brain barrier and brain edema.

Conclusions
This study indicates that sympathetic nerves protect against disruption of the blood-brain barrier during chronic hypertension. The finding may be important for two reasons. First, it has been proposed that disruption of the blood-brain barrier plays a major role in the pathogenesis of hypertensive encephalopathy. Thus, in chronic hypertension sympathetic innervation of cerebral vessels may protect against stroke and hypertensive encephalopathy. Second, we speculate that disruption of the blood-brain barrier may play a role in the pathogenesis of cerebral vessel permeability. Thus, protection against ischemic infarction by sympathetic nerves may be mediated in part by protection against disruption of the blood brain barrier.

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S Sadoshima and D Heistad

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