SUMMARY The cerebral vessels of spontaneously hypertensive rats (SHR) are reported to have an increased luminal size and decreased wall thickness after chronic sympathetic denervation. In order to evaluate a possible physiological significance of this observation, we studied blood-brain barrier protein transfer in two month old SHR and their normotensive controls, Wistar-Kyoto (WKY), during profound vasodilation stimuli one month after a unilateral superior cervical ganglionectomy. Our hypothesis was that during acute vasodilation, protein transfer, which is dependent on vessel tension (tension = pressure \times \text{radius}/\text{wall thickness}), would be greater in the vasculature of the chronically denervated (De) than in contralateral innervated hemisphere (In). Vasodilation was induced with acute hypertension (norepinephrine) and seizures (bicuculline). Immediately prior, the cervical trunk to the innervated hemisphere was sectioned to prevent acute sympathetic neuronal effects. Protein transfer was assessed qualitatively with Evans blue dye and quantitatively with radioiodinated albumin. Successful chronic denervation was demonstrated by the absence of histofluorescence in De. Evans blue dye staining of SHR (n = 3) cerebral hemispheres was greater in De than in In. Radioiodinated albumin protein transfer was elevated in SHR De compared to In in each of eight animals studied (De-In = 0.16 \pm 0.04\%*, *p < 0.01); WKY (n = 8) De protein transfer was not different from In (De-In = 0.07 \pm 0.05\%). These results suggest that the trophic influence of sympathetic nerves on SHR cerebral vessels contributes to protection of the blood-brain barrier during hypertension and seizures.

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chronic innervation served as a control for the contralateral denervated tissue.

**Methods**

**Animals**

A total of 40 rats (23 SHR and 17 WKY) were used in this study. A unilateral superior cervical ganglionectomy was performed on each of the rats at four weeks of age and they were studied one month later. The rats were denervated at 4 weeks of age so that the cerebral vasculature was sympathetically denervated as hypertension was developing. Evans blue dye was used to determine qualitative protein transfer in three SHR during experimental conditions (hypertension plus seizures) in preliminary experiments. Radioiodinated albumin was used to determine quantitative protein transfer during experimental conditions in 16 rats (8 WKY, 8 SHR) and during control conditions in 10 rats (6 WKY, 4 SHR). Successful sympathetic denervation was demonstrated in three each WKY and SHR by a histofluorescence technique. Five additional SHR were used to determine the success of the brain flush in the denervated compared to the innervated hemisphere by assessing residual hemoglobin in the hemispheres after perfusion of the brain with saline. All rats were fed standard rat chow.

**Denervation**

At one month of age, unilateral cervical ganglionectomies were performed. They were done alternately on the right and left side so that, in each group studied, half of the animals had a right superior cervical ganglionectomy and the other half had a left superior cervical ganglionectomy. The ganglionectomy produced ptosis and enophthalmous on the side ipsilateral to the ganglionectomy in all rats in the denervated compared to the innervated hemisphere by assessing residual hemoglobin in the hemispheres after perfusion of the brain with saline. All rats were fed standard rat chow.

**Assessment of Protein Transfer**

At two months of age, one month after unilateral superior cervical ganglionectomy, protein permeability was determined. The animals were anesthetized with alpha chlorolose (80 mg/kg) via the tail vein in order to induce light anesthesia and preserve reflexes. The rats were artificially ventilated with room air and supplemental oxygen via tracheal intubation. Polyethylene catheters (PE-50, thin-walled) were inserted into the femoral artery and vein for measurement of arterial pressure and drug injection respectively. The superior cervical trunk to the innervated side of the head was sectioned to prevent acute sympathetic neuronal effects. Heparin (1000 units/kg) was injected intravenously to prevent coagulation. Decamethonium bromide (.06 mg/kg) was injected intravenously to prevent movement of the extremities during seizures. Arterial blood gases and pH were determined during basal conditions and after injection of drugs in the experimental group or saline in the control group. In the experimental group of SHR and WKY, norepinephrine (250 ug) was injected intravenously to induce hypertension followed two minutes later by bicucilline (1 mg/kg) in a bolus to induce seizures. In the control group of SHR and WKY, saline was injected instead of drugs at equivalent times.

A qualitative estimate of permeability of the BBB to albumin was obtained in preliminary experiments by injection of Evans blue dye intravenously. Evans blue dye binds to albumin and normally does not pass the BBB. During acute hypertension, however, the brain becomes stained with blue dye when the barrier is disrupted. Evans blue dye (2.5%, 3 ml/kg) was injected intravenously at the beginning of the experimental protocol in three SHR. At the end of each of these studies, the brain was examined for superficial and deep staining of the dye.

A quantitative determination of protein permeability was obtained by using [125I] labeled human serum albumin (RISA, Mallinckrodt Nuclear) as previously described. Briefly, 10 uCi of RISA was administered intravenously and allowed to circulate for ten minutes prior to withdrawing the first reference arterial blood sample for determination of radioactivity. Six minutes after treatment with drugs or saline, a second reference sample was withdrawn in order to again assess radioactivity.

Animals were killed with an intravenous KCl injection. In those animals injected with RISA, the ascending aorta of the animal was cannulated through the left ventricle and the descending aorta ligated immediately after death. The brain was then perfused through the cannula with 0.9% saline for three minutes in order to remove RISA from the lumen of the cerebral vessels. At the end of the perfusion, the cerebral hemispheres and temporalis muscle were removed and divided into left and right samples. Brain, muscle, and blood samples were all weighed wet and their radioactivity measured in a gamma counter. The counts per gram of blood from the two reference arterial samples were averaged and this value was used to calculate permeability to albumin expressed as percent protein transfer by using this formula: % protein transfer = counts/g in tissue × 100. Results for the control and experimental SHR and WKY groups were com-
pared using an unpaired t-test. A paired t-test was used for comparison of the denervated and innervated sides of the brain and muscle from the control and experimental SHR and WKY.

Results

Vascular Innervation

A normal pattern of vascular innervation was observed in the large middle cerebral artery and its smaller penetrating branches in the innervated hemisphere. The large vessels on the surface of the brain were innervated by abundant plexi of fluorescent varicosities found within the outer third of the muscular layer (fig. 1a). The plexi followed penetrating branches which radiated into the substance of the brain. Some plexi were visible for a distance of one millimeter or more. On the denervated side, the plexi of fluorescent varicosities were absent or severely diminished as observed in the same section (fig. 1b). If the varicosities were present on the denervated side they were sparse, found adjacent only to large vessels, and the intensity of the fluorescence was dull. On the denervated side, no varicosities were found surrounding the penetrating branches of the middle cerebral artery.

Qualitative Protein Transfer in Denervated Versus Innervated Hemisphere During Experimental Conditions

In three SHR in which Evans blue dye was used to determine protein transfer, the denervated cerebral hemisphere visibly demonstrated more blue staining than the innervated hemisphere. In one of these animals, intraparenchymal hemorrhage was observed in the denervated cerebral hemisphere.

Quantitative Regional Protein Transfer During Control and Experimental Conditions

Arterial Pressure

For the control group prior to injection of saline, the mean arterial pressure of the WKY was 93 ± 5 mm Hg while that of the SHR was 143 ± 6 mm Hg (mean ± se). After injection of saline at the two specified times, there was little change in mean arterial pressure of either WKY or SHR [WKY 113 ± 6 and 114 ± 7 mm Hg; SHR 137 ± 10 and 128 ± 15 mm Hg (mean ± se)]. In the experimental group prior to treatment, the mean arterial pressure of the WKY was 98 ± 7 mm Hg and that of the SHR was 126 ± 6 mm Hg. During norepinephrine-induced acute hypertension, the increase in mean arterial pressure for the SHR and WKY was not significantly different. In WKY the pressure increased 78 ± 9 mm Hg (98 ± 7 to 176 ± 3), and in SHR the increase was 72 ± 5 (126 ± 6 to 198 ± 5). The maximum arterial blood pressures associated with bicuculline-induced seizures were similar to those achieved during acute hypertension (WKY 170 ± 4; SHR 198 ± 4 mm Hg). No appreciable differences in the mean values for blood gases or pH were detected between the groups either before or after treatment (table 1).

Total Regional Protein Transfer

The protein transfer for the two sides of the cerebral hemispheres and temporalis muscle were averaged to obtain a value for regional protein transfer (table 2). After treatment, the total protein transfer measured in the SHR and WKY experimental group was significantly increased in all averaged brain regions and in the temporalis muscle (p < 0.05).

Denervated Versus Innervated Regional Protein Transfer

During experimental conditions, there was a significant difference between the chronically denervated and contralateral innervated vasculature of the SHR cerebral hemispheres (+0.16 ± 0.04%, p < 0.01) plus both the SHR (+0.97 ± 0.37%, p < 0.05) and WKY.

Figure 1. Sucrose-phosphate-glyoxylic acid (SPG) histofluorescence of the denervated compared to the innervated cerebral vessels one month after unilateral superior cervical ganglionectomy. The innervated cerebral vessel on the right (1a) demonstrates numerous noradrenergic varicosities indicated by arrows (X600) while the denervated on the left (1b) does not demonstrate histofluorescence (X600) from the same animal (Wistar-Kyoto). The endothelium is visible in both the denervated and innervated vessel due to non-specific uptake of SPG.
(0.52 ± 0.20%, p < 0.25) temporalis muscle (table 3, fig. 2). There was no significant difference in protein transfer between the denervated and innervated cerebral hemispheres of WKY (+0.07 ± 0.05%, NS) (table 3, fig. 2). Thus, although both SHR and WKY showed a significantly elevated protein transfer compared to controls during experimental conditions, only the chronically denervated SHR cerebral hemisphere and temporalis muscle as well as the WKY temporalis muscle showed a significantly increased protein transfer compared to the contralateral control. There was no difference between the chronically denervated and contralateral innervated tissue in the SHR and WKY control groups (table 3).

Effectiveness of Brain Flush

We have previously determined that there was no difference in effectiveness of brain flush between SHR and WKY when using a similar perfusion technique. In this study, we determined whether efficacy of perfusion of the denervated compared to the contralateral innervated cerebral hemisphere from the SHR experimental group was similar to assess whether disparate perfusion of one hemisphere compared to the other could have contributed to our results. We assessed residual hemoglobin content in aliquots of hemisphere homogenates from SHR (n = 5) exposed to experimental conditions one month after a unilateral superior cerebral ganglionectomy. The ascending aorta was perfused with saline as described above. Each hemisphere was homogenized with 3 ml of diluant and 40% of this mixture was added to an additional 20 ml of diluant. Six drops of zapoglobin were added, the mixture was shaken and readings were taken with a hemoglobinometer. There was no significant difference in hemoglobin content between the chronically denervated (1.25 ± 0.04) and contralateral innervated (1.29 ± 0.04) hemisphere in this experimental group of SHR.

Discussion

The major finding in this study is that intact sympathetic nerves provide a protective effect to the cerebral hemisphere vasculature of SHR during episodes of hypertension and seizures, apart from an acute effect on vasomotor tone. Such an influence of sympathetic nerves on blood vessels has been termed "trophic," that is, not directly related to sympathetic neuronal discharge. A similar protective effect was observed in the vasculature of the temporalis muscle in both SHR and WKY rats. It should be noted that protein transfer across the temporalis muscle was greater than that across the BBB because muscle vascular beds are more permeable to protein than the cerebrovascular bed. In addition, the protein transfer across the SHR muscle vasculature was greater than in WKY due to a probable enhanced transcapillary albumin escape in hypertensive compared to normotensive muscle. The protective effect of sympathetic nerves on SHR cerebral vessels may be due to enhancement of vascular muscle mass secondary to a trophic influence of sympathetic nerves. Thus, precipillary tension would be less in the innervated compared to denervated vasculature during pronounced vasodilation because wall thickness is greater (tension = pressure × radius/wall thickness). Since protein transfer appears to correlate directly with vessel wall tension, the denervated vasculature with the greater vessel tension would be expected to demonstrate elevated protein transfer compared to the innervated. The possibility that chronic sympathetic innervation influences the physiological properties of WKY cerebral vessels during vasodilation cannot be ex-
TABLE 3  Denervated Versus Innervated Regional Protein Transfer

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th></th>
<th>Experimental</th>
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<tr>
<td></td>
<td>Denervated</td>
<td>Innervated</td>
<td>Denervated</td>
<td>Innervated</td>
</tr>
<tr>
<td>Normotensive*</td>
<td>Cerebral hemisphere</td>
<td>0.08 ± 0.01</td>
<td>0.09 ± 0.02</td>
<td>1.51 ± 0.32</td>
</tr>
<tr>
<td></td>
<td>Temporalis muscle</td>
<td>0.22 ± 0.02</td>
<td>0.33 ± 0.05</td>
<td>2.16 ± 0.29†</td>
</tr>
<tr>
<td>Hypertensive†</td>
<td>Cerebral hemisphere</td>
<td>0.08 ± 0.01</td>
<td>0.09 ± 0.01</td>
<td>1.77 ± 0.24§</td>
</tr>
</tbody>
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|                | Temporalis muscle                 | 0.38 ± 0.17         | 0.54 ± 0.18                   | 3.09 ± 0.51†        | 2.12 ± 0.19

*Values were obtained in the same six control and eight experimental Wistar-Kyoto rats in Table 2 one month after unilateral superior cervical ganglionectomy and are presented as mean ± SE.
†Values were obtained in the same four control and eight experimental spontaneously hypertensive rats in Table 2 one month after unilateral superior cervical ganglionectomy and are presented as mean ± SE.
§Significantly different than innervated at p < 0.05 (paired t).
†Significantly different than innervated at p < 0.025 (paired t).
‡Significantly different than innervated at p < 0.01 (paired t).

eluded with this study. However, such an influence, if present, would probably be minor compared to that observed in SHR because vascular hypertrophy is not required as a protective mechanism in WKY. During control conditions, we did not find an effect of chronic sympathetic denervation on cerebrovascular protein transfer. This was expected since chronic sympathetic denervation does not alter other parameters of cerebral vessel function during basal conditions.6

The chronic absence of sympathetic nerve influence on SHR cerebral vessels led to a small but significant difference in protein transfer during extreme vasodilatory stimuli in this study. That such an influence could have physiological significance in humans is uncertain. It is possible that treatment of hypertensive patients with sympathetic outflow inhibitors may lead to a functional sympathetic denervation. Thus, during repeated period­ic hypertensive insults, blood-brain barrier function may be compromised. In addition, those epileptics who have hypertension plus a Horner’s syndrome (with associated lack of sympathetic influence to the cerebral vasculature) may be similarly affected, as well as patients who have had a sympathetic block for therapy of stroke17 and also have hypertension and/or seizures. In all of these examples, the cumulative effects of repeated insults could be important.

Sadoshima et al.18 reported that chronic cerebrovascular sympathetic denervation of one hemisphere increased susceptibility to stroke on the denervated side in stroke-prone SHR. This study agrees with our own. However, in contrast to the present study, sympathetic fibers to the innervated hemisphere of the stroke-prone SHR were not acutely divided, and any difference between the two hemispheres could have been due to the vasoconstrictor effect of acute sympathetic neuronal discharge to the innervated hemisphere during blood pressure elevations,8,9 rather than due to the effect of chronic sympathectomy on the denervated cerebrovascular bed. Thus, our study demonstrated that the chronic absence of sympathetic nerves led to greater damage.

![Figure 2](image_url)  Denervated-Innervated protein transfer (%) during experimental conditions in the cerebral hemispheres and temporalis muscle of WKY and SHR. The denervated SHR cerebral hemisphere protein transfer was elevated over the innervated in 8 out of 8 animals studied. Both the WKY and SHR denervated temporalis muscle protein transfer were elevated over innervated (denervated > innervated) in 6 out of 8 WKY and 8 out of 8 SHR.)
in denervated SHR cerebral vessels during pathologic conditions in the absence of an acute neuronal influence to the innervated vasculature.

A previous study from this laboratory has demonstrated that the properties of another intact vascular bed are also altered after chronic sympathetic denervation. In that study, the ear vasculature from rabbits with a chronic unilateral sympathectomy demonstrated a lower flow-pressure curve at maximal dilation compared to the innervated vascular bed. These results suggested that the cross-sectional area of the chronically denervated vasculature was increased compared to the innervated vasculature and could be due to reduced vascular smooth muscle, leading to greater maximal dilation. The present finding that sympathetic nerves chronically influence physiologic properties of the vasculature of the temporalis muscle and cerebral hemispheres is in agreement with our previous finding of a vascular alteration following chronic sympathectomy.

In the current study we used norepinephrine to induce hypertension. Because adrenergic agonists have a minimal, if any, effect on cerebral vessels in the absence of BBB disruption, any direct effect of norepinephrine on cerebral vessels prior to disruption of the BBB is unlikely. After BBB disruption, the effect of norepinephrine on vascular smooth muscle would be constrictive, and in the presence of denervation hypersensitivity, the chronically denervated vasculature would be expected to constrict more than the innervated vasculature. If so, less BBB disruption would be expected on the denervated side. Since this was not the case, it is improbable that the direct constrictor effect of norepinephrine on cerebral vessels is related to our findings.

In summary, during hypertension and seizures in SHR, the chronic influence of sympathetic nerves protects the blood-brain barrier apart from any acute effect on vasomotor tone. This effect may be secondary to a sympathetic influence on vascular hypertrophy. We speculate that a surgical or chemical sympathectomy may predispose to enhance BBB permeability, cerebral edema, and stroke in hypertensive individuals.

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