The Chronic Influence of Sympathetic Nerves on Cerebral Vessels is Age-Related

SHIRLEY M. MUELLER, M.D., M.S., PHILLIP J. ERTEL, B.S., DAVID L. FELTEN, M.D., PH.D.,
AND J. MARC OVERHAGE, B.S.

SUMMARY The purpose of this study was to determine the effect of chronic sympathetic denervation on cerebral vessels of the spontaneously hypertensive rat when superior cervical ganglionectomy was performed in adulthood. In a previous study, we have demonstrated increased protein transfer across the cerebral vessels of the chronically denervated hemisphere when superior cervical ganglionectomy was performed in adolescent spontaneously hypertensive rats. After four weeks of sympathetic denervation, the adult rats in this study did not demonstrate increased protein transfer in the denervated compared to the innervated cerebral hemisphere. Thus, the “trophic” effect of sympathetic nerves on cerebral vessels appears to be age-related.

WE HAVE PREVIOUSLY demonstrated a “trophic” influence of sympathetic nerves on the cerebral vasculature of spontaneously hypertensive rats (SHR) during the development of hypertension. A possible “trophic” influence of sympathetic nerves on cerebral vessels of adult SHR with established hypertension has not been examined. Thus, the purpose of this study was to determine the effect of chronic sympathetic denervation on cerebral vessels of SHR when superior cervical ganglionectomy was performed in adulthood rather than adolescence. We used the same experimental protocol that we used in our previous study. Prolonged cerebral vasodilation was induced with acute hypertension (norepinephrine) and seizures (bicuculline). We hypothesized that if a reduction in SHR intraparenchymal cerebral vessel wall thickness was present after chronic denervation, it would lead to a significantly enhanced wall tension (tension = pressure × radius/wall thickness) as blood pressure and vessel radius were increased. Enhanced blood-brain barrier (BBB) permeability, proportional to vessel wall tension, would be expected to occur. We determined protein permeability in the cerebral hemispheres and temporals muscle of SHR four weeks after unilateral superior cervical ganglionectomy performed in nine to 19 week old young adult animals. The tissue with normal chronic innervation served as a control for the contralateral denervated tissue. Successful sympathetic denervation was demonstrated by using a histofluorescence technique.

Methods

Animals

Fourteen SHR were used in this study. A unilateral superior cervical ganglionectomy was performed between nine and 19 weeks of age. One month later radioiodinated albumin was used to determine quantitative protein transfer in 11 of these rats. Successful sympathetic denervation was demonstrated in another three rats by a histofluorescence technique.

Denervation

Unilateral cervical ganglionectomies were performed alternately on the right and left side so that 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 indicating a functionally successful sympathetic denervation.

In order to objectively demonstrate the presence or absence of vascular sympathetic innervation, we used the sucrose-phosphate-glyoxylic acid (SPG) histofluorescence method of de la Toree. This method permits for the consistent visualization of fine noradrenergic varicosities when present. The absence of fluorescent varicosities on the chronically ganglionectomized side in the presence of fluorescent varicosities on the contralateral side was viewed as evidence of successful sympathetic denervation. Analysis of sympathetic varicosities was carried out by single blind examination of the middle cerebral artery and its branches in coronal sections taken at the level of the rostral boundary of the optic chiasm of the cerebral hemispheres. Sections were viewed and photographed on a Leitz Orthoplan microscope equipped with Ploem epillumination accessories.

Assessment of Protein Transfer

Protein transfer was determined one month after the unilateral superior cervical ganglionectomy. The animals were anesthetized with alpha chloralose (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 an acute effect of sympathetic nerve traffic on the inner-
Heparin (1000 units/kg) was injected intravenously to prevent coagulation. Decamethonium bromide (0.06 mg/kg) was injected intravenously to prevent movement of the extremities during seizures. Norepinephrine (250 μg) was injected intravenously to induce hypertension followed two minutes later by bicuculline (4 mg/kg) in a bolus to induce seizures.

A quantitative determination of protein permeability was obtained by using I\(^{125}\) labeled human serum albumin (RISA, Mallinckrodt Nuclear) as previously described. Briefly, 10 μCi 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 induction of hypertension a second reference sample was withdrawn in order to again assess radioactivity.

The animals were killed with an intravenous KCl injection. The ascending aorta was cannulated through the left ventricle and the descending aorta was 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. Wet brain, muscle and blood samples were all weighed 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 \times 100. A one-tailed paired t-test was used to evaluate protein transfer in the denervated compared to the innervated cerebral hemisphere so that the results could be compared with our previous study.

**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. 1, left). 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. 1, right). 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.

**Quantitative Regional Protein Transfer**

During norepinephrine-induced acute hypertension, the increase in mean arterial pressure was 59 ± 7 mm Hg (173 ± 5 to 232 ± 7, \( p < 0.001 \)). The maximum arterial blood pressure associated with bicuculline-induced seizures was 234 ± 8 mm Hg. The total protein transfers, 0.78 ± 0.12% for the cerebral hemispheres and 1.27 ± 0.26% for the temporalis muscle, were both significantly elevated over previous values obtained during control conditions in SHR (0.09 ± 0.01, \( p < 0.001 \) and 0.46 ± 0.12, \( p < 0.005 \)).

The change in protein transfer in the denervated SHR cerebral hemisphere (−0.09 ± 0.03%) and in the denervated temporalis muscle (+0.08 ± 0.12%) was not significantly elevated over the innervated (one-tailed test) (table 1). Thus, although the increase in arterial pressure was significant and the elevation in

---

**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 left demonstrates numerous noradrenergic varicosities indicated by arrows (×600) while the denervated on the right does not demonstrate histofluorescence (×600) from the same animal.
Table 1  Denervated Versus Innervated Regional Protein Transfer

<table>
<thead>
<tr>
<th></th>
<th>Denervated</th>
<th>Innervated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral hemisphere</td>
<td>0.73 ± 0.16</td>
<td>0.82 ± 0.17</td>
</tr>
<tr>
<td>Temporalis muscle</td>
<td>1.31 ± 0.20</td>
<td>1.23 ± 0.23</td>
</tr>
</tbody>
</table>

*Values were obtained in 11 spontaneously hypertensive rats one month after a unilateral superior cervical ganglionectomy was performed between 9 and 19 weeks of age. Values are presented as mean ± SE. The denervated was not significantly larger than the innervated.

The major finding in this study is that chronic cervical sympathectomy did not alter BBB protein transfer during acute cerebral vasodilation as it did when sympathetic denervation was initiated in adolescent SHR. When unilateral sympathetic denervation was performed in SHR at four weeks of age in our previous study, the protein transfer for the denervated cerebral hemispheres and temporalis muscle was significantly elevated over the innervated (fig. 2). When unilateral sympathetic denervation was performed in SHR at nine to 19 weeks of age in this study, the protein transfer for the denervated cerebral hemispheres and temporalis muscle did not differ (one-tailed test) (fig. 2). Thus, the "trophic" effect of sympathetic nerves on cerebral vessels appears to be age-related.

Discussion

The major finding in this study is that chronic cervical sympathectomy disruption in SHR adults did not alter BBB protein transfer during acute cerebral vasodilation as it did when sympathetic denervation was initiated in adolescent SHR. When unilateral sympathetic denervation was performed in SHR at four weeks of age in our previous study, the protein transfer for the denervated cerebral hemispheres and temporalis muscle was significantly elevated over the innervated (fig. 2). When unilateral sympathetic denervation was performed in SHR at nine to 19 weeks of age this study, the protein transfer for the denervated cerebral hemispheres and temporalis muscle did not differ (one-tailed test) (fig. 2). Thus, the "trophic" effect of sympathetic nerves on cerebral vessels appears to be age-related.

There are three comments that should be made concerning the findings in this study. First, a profound vasodilatory stimulus was provided during acute hypertension (increase in mean arterial pressure significant at 0.001) and seizures (neuronal discharge providing a metabolic stimulus) so that any effect of chronic sympathetic denervation during acute vasodilation would be unmasked. Second, the protein transfer across the BBB and temporalis muscle was significant and markedly elevated over previously obtained control values (p < 0.001 and 0.005 respectively). Therefore, disruption of the BBB and increased permeability of the temporalis muscle vasculature were present so that an altered vasodilatory capacity would be expected; protein transfer has been demonstrated to correlate directly with vessel wall tension. Third, the denervated minus innervated protein transfer ratio across the cerebral vasculature was markedly weighted toward the innervated side (two-tailed test, p < 0.01). This may be due to denervation hypersensitivity of the chronically denervated vessels leading to enhanced constriction after BBB disruption and less protein transfer. In our earlier study in which increased BBB protein transfer was present in the denervated hemisphere, the effect of denervation hypersensitivity was apparently less important than the "trophic" effect of sympathetic nerves on vascular smooth muscle. The effect of denervation hypersensitivity may not have been as pronounced in the temporalis muscle because norepinephrine freely crosses the endothelium of the vasculature in skeletal muscle (as opposed to the BBB) and an "acute" effect of circulating norepinephrine could not be expected during hypertension and seizures.

A similar age-related "trophic" influence of sympathetic nerves on organs from normotensive animals has recently been demonstrated. Bloom and colleagues showed a decrease in parotid gland weight as well as acinar cell hypotrophy after early superior cervical ganglionectomy that was not found in adult animals. Rusterholz and Mueller demonstrated a "trophic" influence of sympathetic nerves on rabbit ear vasculature in growing animals that was not present in adults.

In summary, the "trophic" influence of sympathetic nerves on the cerebrovascular bed that was previously found in young spontaneously hypertensive rats was not observed in adult animals. Thus, the "trophic" influence of sympathetic nerves on the cerebrovascular bed appears to be age-related. We speculate that surgical or chemical cervical sympathectomy in adults with established hypertension is not likely to lead to vascular complications that might result from the absence of the "trophic" effect during the development of hypertension.
Acknowledgments
The authors wish to thank Mrs. Sue Frazee for typing this manuscript.

References
The chronic influence of sympathetic nerves on cerebral vessels is age-related.
S M Mueller, P J Ertel, D L Felten and J M Overhage

*Stroke*. 1983;14:286-289
doi: 10.1161/01.STR.14.2.286

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1983 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/14/2/286

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Stroke* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Stroke* is online at:
http://stroke.ahajournals.org//subscriptions/