Cerebral Artery Mass in the Rabbit is Reduced by Chronic Sympathetic Denervation

ROSEMARY D. BEVAN, M.D., HIROMICHI TSURU, M.D., AND JOHN A. BEVAN, M.D.

SUMMARY Weights of matching right and left middle or posterior cerebral arteries and their main branches from the same animal were compared 8–10 weeks after unilateral denervation by superior cervical ganglionectomy. When compared in pairs, the denervated arterial systems weighed significantly less (mean 85%) than their innervated counterparts. This suggests that the sympathetic innervation exerts a trophic influence on extracerebral arteries.

THERE IS NOW CONSIDERABLE EVIDENCE that the sympathetic nervous system influences not only the tone of vascular smooth muscle and its sensitivity to vasoactive agents, but also the structure and constituents of the blood vessel wall. It may be of particular importance in influencing the structure and reactivity of the vasculature during its development. A number of studies based on experiments in which the effects of pre- or postganglionic interruption of the sympathetic supply to the rabbit ear artery have been analyzed, indicate a complex age-dependent effect on the blood vessel wall which weighs less, is thinner and stiffer, and because of a diminution in muscle mass is less capable of developing tone. The sympathetic nervous system modulates the metabolic activity of cells and therefore might be expected to affect the balance of synthesis and degradation not only of smooth muscle constituents but the elaboration of extracellular collagen, elastin and glucosaminoglycans.

Diseases of cerebral blood vessels in man are a common cause of morbidity and mortality. For this reason it was considered of interest to determine if the sympathetic innervation influenced cerebral blood vessels in a manner similar to that established in peripheral vessels. Because of the extreme variability of the pial circulation and the difficulty in demonstrating the small differences that result from denervation, measurements were restricted to weights of comparable innervated and denervated vascular segments. Our measurements show that the net weights of two cerebral arteries and their main branches are significantly reduced by this procedure, a finding that suggests that cerebral arterial structure is influenced in a trophic manner by the sympathetic nervous system.

A preliminary report has been previously published.

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Methods

Both growing, young adult and mature New Zealand white male rabbits fed on an unlimited standard pellet were used. A total of 15 rabbits were used in this study.

Superior Cervical Ganglionectomy

Following sedation with chlorpromazine (10 mg/kg IM) the animals were anesthetized by intraperitoneal injection of an anesthetic mixture containing per 100 ml; chloral hydrate, 4.25 g; pentobarbital sodium, 0.972 g; magnesium sulfate, 2.126 g; propylene glycol, 42.8%; and alcohol, 11.5%. The dose used was 2 ml per kg. One superior cervical ganglion, alternatively the right and left, was exposed and removed under sterile conditions.

Catecholamine Histofluorescence

The effectiveness of chronic denervation was assessed by processing a whole mount of a segment of ear artery from both denervated and control side for catecholamine histofluorescence. Either the formaldehyde method of Falck et al., or the glyoxylic acid method of Lindvall and Bjorklund was used.

Tissue Preparation

Eight to ten weeks after denervation animals were stunned by a blow on the head and rapidly exsanguinated. The skin over the head was rapidly removed and the cerebral cavity opened with a sagittal section just anterior to the frontal lobes. After rapid removal of the bony vault, the brain with its pial circulation was detached from the spinal cord and submerged in cold Krebs bicarbonate solution. Composition of the Krebs solution was (mM): Na, 144.2; K, 4.9; Ca, 1.6; Mg, 1.2; Cl, 126.7; HCO3, 25.0; SO4, 1.19; glucose, 11.1; and calcium disodium ethylenediaminetetraacetate (EDTA), 0.023 equilibrated with 95% O2 and 5% CO2 at room temperature. The courses of the right and left middle and posterior cerebral arteries and their main branches were inspected. Only if their general arterial patterns were similar on both sides were the vessels admitted to the experimental series for comparison and then only that part of the arterial tree showing greatest bilateral similarity was selected.

Utilizing a dissection microscope, the previously selected paired middle and/or posterior cerebral arteries were removed usually from close to their origin from the circle of Willis, distally to a level of convenient dissection. This was carried out by observers who were unaware of the side of the superior cervical ganglionectomy. A considerable effort was made to remove equivalent arterial systems on the two sides. Each arterial system was placed in a separate Petri dish. Ten minutes after the dissection of the second vessel, each arterial complex was wiped in a standard manner on a dry, flat glass plate. Then using dry forceps, the vessel system was placed on a planchette and weighed using a Kahn electrobalance and then replaced in the cold Krebs solution. Vessels were subsequently reweighed in alternative sequence. Mean values of the two measurements of each vessel were used for comparison.

Statistical Analysis

Paired observations on control and denervated vessels were compared using the paired t-test. A statistical inference of significance was made.

Results

The findings of this study of 15 rabbits are summarized in table 1 and figure 1. Vascular specimens from middle and posterior cerebral systems were studied. Denervated middle and posterior arteries weighed on the average 80 and 88% respectively of their innervated counterparts. The success of denervation was confirmed at the time of sacrifice by fluorescence microscopy of segments of both ear arteries. Since each pair of vascular specimens was probably anatomically unique, a paired comparison of their weights was made when p < 0.002 and p < 0.01 for the middle and posterior cerebral arterial systems respectively.

Discussion

This study shows that 8–10 weeks after superior cervical ganglionectomy selected portions of the ipsilateral pial arterial system weighed less than corre-

### Table Middle Cerebral Arteries

<table>
<thead>
<tr>
<th>Side of denervation</th>
<th>Tissue weight (grams)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Denervated</td>
</tr>
<tr>
<td>L</td>
<td>1.45</td>
</tr>
<tr>
<td>R</td>
<td>0.78</td>
</tr>
<tr>
<td>L</td>
<td>1.85</td>
</tr>
<tr>
<td>L</td>
<td>1.91</td>
</tr>
<tr>
<td>L</td>
<td>0.50</td>
</tr>
<tr>
<td>R</td>
<td>2.03</td>
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<tr>
<td>R</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>1.33 ±0.24*</td>
</tr>
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</table>

*p ≤ 0.02.

### Table Posterior Cerebral Arteries

<table>
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<th>Side of denervation</th>
<th>Tissue weight (grams)</th>
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<tbody>
<tr>
<td></td>
<td>Denervated</td>
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<tr>
<td>R</td>
<td>1.80</td>
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<tr>
<td>L</td>
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<td>R</td>
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<td>L</td>
<td>1.20</td>
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<tr>
<td>R</td>
<td>1.91</td>
</tr>
<tr>
<td></td>
<td>1.82 ±0.14*</td>
</tr>
</tbody>
</table>

*p ≤ 0.01 (*paired t comparison).
One difficulty that had to be considered in this study was the well-known variability in the detailed pattern of pial vasculature. This occurs not only between individual animals but between corresponding left and right-sided vessels from the same animal. Our response to this was to select only pial arterial systems in which the pattern on the right and left sides were similar. Furthermore, arterial systems were selected and removed by investigators who were unaware of the side of the chronic denervation. These investigators made an effort to remove corresponding vascular systems on the two sides. The high significance of the changes measured and their consistency in both middle and posterior cerebral vessels supports our general conclusion.

The variability of the detailed pattern of the cerebral vasculature precludes an analysis of the basis of the observed changes in vessel weight. If exactly anatomically corresponding segments of, for example, the middle cerebral artery are not identical before sympathetic denervation the precise effect of this procedure cannot be determined. Denervation of the ear artery of the young adult rabbit results in a decrease in tissue weight. This is associated with a decrease in wall thickness, in the cross sectional area of the tunica media and a proportionate diminution in the ability of this vessel to develop tension to a maximum concentration of agonist. Therefore, changes in vessel wall mass may reflect a possible trophic effect of the sympathetic innervation. The mean change in weight of the ear artery after the same period of denervation was 13%: this compares to the 15% diminution found in the cerebral arterial system. It may be relevant that in a separate study the posterior cerebral artery was sometimes and partially innervated by neurons from the contralateral superior cervical ganglion after 8 weeks of ipsilateral denervation. This may account for the smaller change.

Recently Hart et al. found that sympathetic denervation attenuated the development of vascular hypertrophy in stroke-prone spontaneously hypertensive rats. This effect was found in the parenchymal but not pial vessels. Their results are consistent with the concept that sympathetic nerves may exert a trophic influence on blood vessels — the consideration that led to this present study. The changes in weight found in this study of pial vessels were relatively small and might be beyond the resolution of the morphometric method used by Hart and colleagues. However there may be other considerations of importance, for example, species, strain, age, duration of denervation etc. Both studies different as they are in context and method lead to the same suggestion: namely, that the fabric of the vascular wall seems to depend on the integrity of its adrenergic innervation.

The explanation for the difference in weight between the innervated and denervated arteries remains to be elucidated. It may be due to a change in cell size as a result of reduced "activation" of the smooth muscle cells, somewhat analogous to "disuse atrophy of skeletal muscle." Vascular smooth muscle cells of major arteries are however always under tension. In addition circulating factors may increase vascular tone. These would tend to counteract the effect of denervation. Some of the effects of denervation of voluntary muscle such as impaired amino acid transport and reduced protein synthesis may be overcome by stretching the fibers.

References

Unusual Clinical Signs in Left Subclavian Artery Occlusion: Clinical and Angiographic Correlation

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SUMMARY A case of left subclavian steal syndrome with transient ischemic attacks of left carotid artery distribution is presented. An attempt to explain this uncommon symptomatology is based on a rare patent cervical arterial network, stealing blood from the left common carotid artery and supplying the distal portion of the obstructed left subclavian artery.

THE OCCURRENCE OF CEREBRAL INSUFFICIENCY in the subclavian steal syndrome is well known, and was described by several authors as mainly regarding the vertebrobasilar system.1-4 Later, Lord et al5 proved statistically that discontinuity of the circle of Willis is the principal factor activating these symptoms in this syndrome. On the other hand, the numerous clinical signs of the subclavian steal syndrome described up till now are thought to be due to additional coexistent atherosclerotic extra- and intracranial lesions in the same patient.6

The importance and variety of collaterals and their task in the hemodynamics of the subclavian steal syndrome was stressed in some reports.5-7 The role of the carotid system, which is included among these collaterals, has always been overshadowed by the well known vertebro-vertebral shunt.7 Thus, little is known about probable clinical signs that might occur when the carotid system acts as an unusual collateral pathway in that syndrome.

We therefore intend to discuss a case of left subclavian steal syndrome with presenting signs of transient ischemic attacks of the region supplied by the left internal carotid artery. We found it interesting to relate these symptoms to the internal carotid system as an unusual collateral network in this case, as well as with the absence of coexistent demonstrable atherosclerotic lesions of that artery and its branches.

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Case Report
A right handed 52 year old man was examined in the emergency room because of difficulties of speech and subjective weakness and tingling sensations involving the right side of his face and right extremities. As reported by his family, the onset was abrupt, about two hours prior to hospitalization.

The neurological examination revealed a fully alert athletic man, with hypotonic mild weakness of the right arm and leg, a positive Chaddock sign in the right, and an obvious right central facial weakness. On examining his speech, a nominal aphasia was noted which caused him tension and anxiety. The examination of the optic discs was essentially normal, except for slight arteriosclerotic changes; spontaneous venous pulsations were observed bilaterally. The remainder of the cranial nerves were normal and meningeal signs were absent. A perceptible difference was felt on palpation between the carotid pulses (Rt > Lt) without bruits. The arterial blood pressure was 160/80 mm Hg in the right arm and 110/70 mm Hg on left. A distinct systolic murmur was heard in the left supraclavicular fossa. No murmurs were audible over the precordial area. Otherwise, the physical examination was normal. At this stage the patient was given intravenous Rheomacrodex (Dextran 40) and transferred from the emergency room to the ward. On neurological examination three hours later, these findings disappeared with a complete recovery of the right hemiparesis and aphasia.

The past history of our patient revealed a mild diabetes of four years duration and a previous transient episode of right unilateral weakness and speech distur-
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