Comparison of Density of Sympathetic Varicosities and Their Closeness to Smooth Muscle Cells in Rabbit Middle Cerebral and Ear Arteries and Their Branches

John T. Dodge, Rosemary D. Bevan, John A. Bevan

Abstract The density and nerve varicosity–smooth muscle cell separation of rabbit cerebral and ear arterial beds were compared. The rabbit middle cerebral artery and three of its successive branches and a comparable-sized ear artery and two branches were perfusion-fixed for electron microscopy and analyzed by quantitative morphometric procedures. The purpose was to determine if there are structural correlates to previously observed differences in the sympathetic control of these two vascular systems. The in vitro contractile response of isolated artery segments to electrical field stimulation of their intramural nerves is considerably less in cerebral arteries compared with the similar-sized ear arteries. Furthermore, in the cerebral but not the ear circulation, there is progressive diminution of the neurogenic response with successive branching. Although the total varicosity densities of the major ear and brain arteries studied are similar, and this parameter stays fairly constant with successive branching of the ear, it falls off considerably in the cerebral vessels. There is a significant difference in densities between the two vascular beds when "bare" varicosities located <1 μm from the medial smooth muscle are compared. The second-order branch of the ear artery has an average of 18 bare varicosities per 500-μm circumference, and the corresponding cerebral vessel has only 2.8 bare varicosities per 500-μm circumference. The mean bare varicosity–smooth muscle cell separation (mean±SEM) is significantly (P<0.05) less in the ear (1.18±0.06 μm) than in the cerebral arteries (4.95±0.23 μm). This is true of all vessels studied. Fifty-nine percent of the bare varicosities in the ear arteries are <1 μm from the smooth muscle cells, and 1.2% are more distant than 5 μm. These values for cerebral vessels are 9.5% and 37%, respectively. In the ear vessels, 25% of the bare varicosities make close neuromuscular contact (within 500 nm of the smooth muscle), whereas only 3% do so in cerebral vessels; in cerebral compared with ear vessels, the percentage becomes significantly less with branching. These structural features of brain vessels, taken together with the lower sensitivity to and the diminished capacity to respond to norepinephrine, probably account for their weak neurogenic control. The results indicate that the cerebral circulation of the rabbit receives a sympathetic innervation that is relatively ineffective in altering cerebrovascular tone. (Circ Res. 1994;75:916-925.)

Key Words • morphometry • sympathetic varicosities • arteries • ultrastructure

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fewer close contacts, and in the more proximal branches studied are less numerous than those in the ear bed. These structural features added to the differences found in functional characteristics—lower sensitivity and a smaller capacity to respond to NE—are consistent with the poverty of the sympathetic control of brain vessels previously studied in vitro,10 and with observations of the extent of sympathetic regulation observed in vivo.6-8

### Materials and Methods

From four adult New Zealand White rabbits weighing 2 to 2.3 kg, three arterial systems from the ear and four from the brain were studied. The arteries examined were the main side branch of the central ear artery, a proximal first-order branch (E1), a second-order (E2) and third-order (E3) branch, the middle cerebral artery (MCA), and its first-order (MC1), second-order (MC2), and third-order (MC3) branches.

#### Electron Microscopy

Four rabbits were anesthetized with Innovar (0.1 mL/kg IM), followed 5 minutes later with Rompun (xylazine, 10 mg/kg IM) and then ketamine (40 mg/kg IM) and prepared for vascular perfusion. Both carotid arteries for each animal were exposed and cannulated. Before perfusion, the animals were injected with heparin (1000 U/kg IV) and then perfused first with aerated physiological saline with 2% dextrose and sodium nitrate (10^{-3} mol/L) at 37°C at a pressure of 80 mm Hg, which was maintained by a pressure transducer. After the saline wash, perfusion was continued with fixative: 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 mol/L phosphate buffer, pH 7.4. The arteries were dissected out and placed in fresh fixative for an additional 1 to 2 hours. They were then washed overnight in buffer wash and postfixed in 2% osmium tetroxide in phosphate buffer for 1 hour at room temperature. Tissues were dehydrated in graded alcohols and embedded in Durcupan ACM (Fluka). Transverse sections were cut on a Sorvall MT 5000 Ultramicrotome at 1.3-μm thickness and stained with an epoxy tissue stain for examination of structural features and measurements of diameter and medial wall thickness by light microscopy. Ultrathin sections of the appropriate-sized artery were cut with a diamond knife and stained with uranyl acetate and lead citrate. The sections were examined in a JEOL 100 CXII electron microscope at 60 kV.

#### Quantitative Morphometry

One transverse ultrathin section of a 2-mm segment from each of the three ear arteries from two rabbits and from the four cerebral arteries from another pair of rabbits was selected for quantitative morphometry. Depending on the diameter of the artery, four to six ultrathin sections were examined from each arterial segment. Each section was separated by a depth of at least 15 μm. Only grid openings that showed the adventitial-medial border and the complete depth of the adventitia were examined. Openings where grid bars obscured portions of the adventitia were not used. Low-magnification electron micrographs were taken of the adventitial-medial border. The entire tunica adventitia was scanned for nerve bundles, axons, and varicosities. Higher magnification micrographs were made of each nerve bundle, and when necessary, montages were taken for the distances between the nerve and the nearest smooth muscle cell of the tunica media. By use of the Sigma-Scan measurement system with a digitizing tablet linked to an AT PC6300 computer, the higher magnification micrographs were used (1) to measure the distances separating the axons and nerve varicosities from the nearest smooth muscle cell of the medial wall, (2) to measure the cross-sectional area of each nerve bundle, axon, and varicosity, (3) to determine nerve densities by counting the total number of nerve bundles and nerve terminals in relation to the length of the tunica media-adventitial border, (4) to determine the frequency of neuromuscular close contacts or junctions, and (5) to note the presence of fibroblast processes between the nerve and outer muscle layer and measure the distance from the nerve to the nearest fibroblast. A distinction was made between nerve varicosities that are either ensheathed (“covered”) or partially covered or completely devoid (“bare”) of Schwann cell processes (Table 1).

#### Identification of Catecholamine Neurons

The chromaffin reaction procedure modified by Gibbins was used to identify catecholamine-containing vesicles. Two rabbits were anesthetized with sodium pentobarbital (50 mg/kg IV), injected with heparin (1000 U/kg IV), and exanguinated. Segments of the E1 and MCA from each animal

### Table 1. Arterial Dimensions and Structural Features

<table>
<thead>
<tr>
<th></th>
<th>E1</th>
<th>E2</th>
<th>E3</th>
<th>MCA</th>
<th>MC1</th>
<th>MC2</th>
<th>MC3</th>
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<tbody>
<tr>
<td>Internal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>diameter, μm</td>
<td>400±38</td>
<td>235±21</td>
<td>130±16</td>
<td>380±29</td>
<td>200±25</td>
<td>120±19</td>
<td>105±17</td>
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<tr>
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<tr>
<td>Medial wall</td>
<td>14±2.8</td>
<td>6.3±1.1</td>
<td>3.3±0.8</td>
<td>10.5±2.3</td>
<td>5.7±0.8</td>
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<tr>
<td>varicosities, %</td>
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<td>56</td>
<td>47</td>
<td>66</td>
<td>65</td>
<td>45</td>
<td>46</td>
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<tr>
<td>Area of nerve</td>
<td>2.085±0.19</td>
<td>2.036±0.26</td>
<td>1.965±0.13</td>
<td>4.466±0.55*</td>
<td>4.123±0.61</td>
<td>2.798±0.41</td>
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<td>complex, μm²</td>
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<td>(n=4)</td>
<td>(n=4)</td>
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<td>Area of</td>
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<td>0.358±0.03*</td>
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<td>varicosities, μm²</td>
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<td>(n=4)</td>
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<td>process, %</td>
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<td>66</td>
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<td>45</td>
<td>79</td>
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</table>

E1 indicates the first-order main side branch of central ear artery; E2, second-order branch; E3, third-order branch; MCA, middle cerebral artery; MC1, first-order branch; MC2, second-order branch; MC3, third-order branch; n, number of arterial segments; %, percentage of the total. Values are mean±SEM. *P<.05 vs corresponding value for E1.
were immersion-fixed in 2.5% glutaraldehyde and 1.5% paraformaldehyde in 0.1 mol/L sodium chromate/potassium dichromate, pH 7.2, at 4°C for 15 minutes. The segments were stored overnight in 0.2 mol/L sodium chromate/potassium dichromate, pH 6.0 and then postfixed in 2% osmium tetroxide in 0.1 mol/L chromate/dichromate, pH 7.2, at 4°C for 1 hour. The tissues were dehydrated in alcohols and embedded in araldite. Transverse ultrathin sections were stained only in lead citrate and examined in a JEOL 100 CXII electron microscope.

Statistics

Results are reported as mean±SEM. Tests of significance were made by Student's t test for paired data. A value of P<.05 was considered statistically significant.

Results

Light Microscopy

Internal diameters, medial wall thickness, and layers of smooth muscle cells were determined by light microscopy in the perfusion-fixed and plastic-embedded stained sections of arterial segments (Fig 1). The internal diameters ranged from 400 μm (internal diameter) in E1 to 130 μm in E3 and from 380 μm in MCA to 105 μm in MC3 (Table 1).

When compared with ear arteries of similar diameter, the cerebral vessels generally have a thinner media, fewer layers of smooth muscle cells (Table 1), and (for the second- and third-order branches) a much thinner adventitia containing only sparse amounts of collagen (data not shown).

Electron Microscopy

A nerve complex was defined as a contiguous group of nerve profiles and Schwann cell processes. Varicosities were identified as larger nerve profiles with accumulations of synaptic vesicles, a few mitochondria, and microtubules. Schwann cell cytoplasm with small vesicles was distinguished from varicose and nonvaricose areas of axons by the presence of nerve axon microtubules. In the present study, the results we report for varicosities will pertain only to bare varicosities, those partially or completely devoid of Schwann cell cytoplasm (Fig 2).

Varicosity and Smooth Muscle Cell Separation

The average neuromuscular separation of varicosities is significantly (P<.05) less in the ear arteries (1.18±0.06 μm) than in the cerebral arteries (4.95±0.23 μm). When arteries of similar diameter were compared (Fig 3), the mean separation of varicosities from the closest smooth muscle cells for E1 (400 μm) was 1.43±0.11 μm, which was significantly less than that for the MCA (380 μm), where it was 5.03±0.41 μm. Differences between the two arterial systems were greater the more distal the branches being compared. E2 (internal diameter, 235 μm) showed the narrowest separation of all vessels examined, 0.82±0.06 μm. In the comparable-sized MC1 (internal
The neuromuscular separation is \( \approx 1.28 \) \( \mu \)m. A process of a fibroblast (fb) is interposed between the bare varicosity and the smooth muscle cell (SM) (perfusion fixation; bar=0.5 \( \mu \)m). B, Second-order branch of rabbit ear artery showing a close neuromuscular separation, \( \approx 0.46 \) \( \mu \)m. A varicosity (arrowhead) is separated from smooth muscle (SM) only by collagen fibers and basal lamina (bar=0.5 \( \mu \)m).

In the ear arteries, the mean separation for varicosities in E2 became significantly less than E1, from 1.43±0.11 to 0.82±0.06 \( \mu \)m. However, the separation for E3 became wider (1.23±0.12 \( \mu \)m), which was significantly different from the values for E1 and E2.

In the cerebral arteries, there was no significant difference in the neuromuscular separation between MCA (5.03±0.41 \( \mu \)m) and MC1 (5.17±0.49 \( \mu \)m). The difference was significant \( (P<0.05) \) when the smaller branches, MC2 (4.07±0.67 \( \mu \)m) and MC3 (3.05±0.44 \( \mu \)m), were compared with the major vessel (MCA).

**Distribution of Varicosity–Smooth Muscle Cell Separation**

The distribution of the neuromuscular separation for varicosities, expressed as a percentage of the total, showed that in the ear arteries the varicosities were more closely grouped within the inner adventitial layer of the artery, whereas in cerebral arteries they were spread throughout the adventitia. Ear arteries had 59% of their total varicosities within 1 \( \mu \)m of the nearest smooth muscle cell. For the cerebral arteries, this value was only 9.5%. Only 1.2% of the varicosities in the ear artery system were \( >5 \) \( \mu \)m from the medial wall, whereas 37% of the varicosities were separated by \( >5 \) \( \mu \)m in the cerebral arteries.

In the ear arteries, the narrowest distribution of varicosities was in E2, with 71% within 1 \( \mu \)m of the medial wall; E1 had 48%; and E3 had 64% (Fig 4).

In the cerebral arteries, the MCA, MC1, and MC2 showed a similar distribution of varicosities within 1 \( \mu \)m: 9% in MCA, 11% in MC1, and 7.5% in MC2. In MC3, no varicosities within 1 \( \mu \)m were observed.

**Neuromuscular Close Contacts**

A neuromuscular close contact is defined as a nerve terminal that is devoid or bare of Schwann cell processes and filled with synaptic vesicles and that approaches a medial smooth muscle cell within 500 \( \mu \)m. In the ear vessels, 25% of the bare varicosities met these criteria, whereas for cerebral vessels only 3% qualified. E2 had the highest percentage of close-contact varicosities (44.5%). In the comparable-sized MC1, the number was 1.6%.

In the cerebral arteries, the percentage of close contacts became less with branching. The MCA had 3.7% varicosities within 500 \( \mu \)m of the medial smooth muscle. There was only 1.6% in MC1, and there were none in the smaller successive branches. This pattern was different in the ear arteries. E2 had more than twice the percentage of varicosities <500 \( \mu \)m (44.5%) than E1 (18%) and three times more than E3 (13%).

**Density of Innervation**

Nerve densities are expressed as the number of varicosities per 500 \( \mu \)m of the medial-adventitial border.
In the present study, 5860 \( \mu \)m of the border was surveyed for the ear arteries, and 4230 \( \mu \)m was surveyed for the cerebral vessels. Table 2 compares for each animal the total numbers of bare varicosities, the length of the adventitial-medial border sampled for each artery, the densities based on 500-\( \mu \)m border length, the varicosities that are within 1 \( \mu \)m of the smooth muscle cell, and the neuromuscular close contacts.

There was little difference in the density of innervation for comparable-sized arteries in the two vascular beds when all varicosities throughout the adventitia were considered. For example, E2 had an average of 25.3 varicosities per 500 \( \mu \)m, and the similar-sized MC1 had an average of 24.7 varicosities per 500 \( \mu \)m. In the cerebral and ear vascular beds, as the size (internal diameter) of the vessel decreased, the density of inner-

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**Figure 4.** Bar graphs showing the distribution of varicosity-smooth muscle cell separation for all "bare" varicosities in ear arteries (A) and cerebral arteries (B). The proportion of varicosities between 0 and 10 \( \mu \)m and \( \geq 10 \mu \)m to the nearest smooth muscle cell (expressed as a percentage of the total for each artery) is presented. SMC indicates smooth muscle cell; E1, E2, and E3, the first-, second-, and third-order branches, respectively, of the central ear artery; MCA, middle cerebral artery; and MC1, MC2, and MC3, the first-, second-, and third-order branches, respectively, of the MCA. E2 has the highest percentage of varicosities within 1 \( \mu \)m of the muscle cell at 71%; the highest for the cerebral arteries (MC1) is 11%. The cerebral arteries have a separation \( > 5 \mu \)m for 37% of their varicosities; the ear arteries, only 1.2%.
vigation was reduced. The MCA had an average of 31.5 varicosities per 500 \( \mu m \), and the smallest branches (MC2 and MC3) had only 7.6 and 7.3 varicosities per 500 \( \mu m \), respectively. In the ear, the E1 and E2 had similar densities of 28.0 and 25.3 varicosities per 500 \( \mu m \), respectively, whereas the smallest branch (E3) had 13.2 varicosities per 500 \( \mu m \), about half the number.

When nerve densities were determined for only those varicosities that were <1 \( \mu m \) and within 500 nm of the medial smooth muscle, differences between the two vascular beds and the successive branches were seen. E2 had the highest density at <1 \( \mu m \) of all vessels examined, with 18 varicosities per 500 \( \mu m \) and 11.3 varicosities per 500 \( \mu m \) within 500 nm, and the comparable-sized MC1 had only 2.8 varicosities per 500 \( \mu m \) and <1 varicosity per 500 \( \mu m \) within 500 nm of the smooth muscle (Table 2).

In the ear arteries as E1 branches to E2, the density within 1 \( \mu m \) increased from 13.4 to 18 varicosities per 500 \( \mu m \) and from 5.0 to 11.3 varicosities per 500 \( \mu m \) within 500 nm; however, for the smallest branch (E3), the densities were less for varicosities <1 \( \mu m \) (8.5 varicosities per 500 \( \mu m \)) and within 500 nm (1.8 varicosities per 500 \( \mu m \)).

The cerebral vessels had much lower densities for varicosities <1 \( \mu m \): 2.9 and 2.8 varicosities per 500 \( \mu m \) for MCA and MC1, respectively, and 1.2 and <1 varicosity per 500 \( \mu m \), respectively, for varicosities within 500 nm. For the smaller branches, MC2 was <1 varicosity per 500 \( \mu m \), and MC3 had no varicosities <1 \( \mu m \).

### Area of Nerve Complex and Varicosity

The mean cross-sectional area for a nerve complex in the main cerebral artery studied (MCA) was 4.46±0.55 \( \mu m^2 \), which was significantly greater than in E1, where it was 2.08±0.19 \( \mu m^2 \) (Table 1). The size of varicosities was also significantly greater in the MCA at 0.35±0.03 \( \mu m^3 \) compared with E1 at 0.21±0.02 \( \mu m^3 \) (P<.05). The differences became less as the vessel diameter decreased.

In the cerebral arteries, there was a progressive decrease in the area of varicosities with successive branches. For the ear vessels, only in the smallest branch (E3) was the varicosity size significantly different from the parent vessel (Table 1).

### Neuronal Vesicles

When the chromaffin reaction technique, which detects biogenic amines, was used, all varicosities observed in the ear arteries contained only small (40- to 50-nm-diameter) and some large (90- to 120-nm-diameter) dense-cored vesicles, presumably adrenergic. In the cerebral arteries, 86% of the varicosities showed mainly small and some large dense-cored vesicles (non-adrenergic), and 14% of the varicosities contained small (40- to 50-nm-diameter) clear vesicles.

### Interposed Fibroblasts

In the ear and cerebral artery systems, a fibroblast process was frequently found to be interposed between a varicosity and a smooth muscle cell in approximately half of the separations studied. There appeared to be a trend to a greater number of interpositions in the smaller arteries.

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**Table 2. Comparison of Total Numbers, Densities, and Close “Bare” Varicosities**

<table>
<thead>
<tr>
<th>Animal</th>
<th>E1</th>
<th>E2</th>
<th>E3</th>
<th>Animal</th>
<th>MCA</th>
<th>MC1</th>
<th>MC2</th>
<th>MC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Varicosities</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Length of adventitial-medial border, ( \mu m )</td>
<td>1</td>
<td>1143</td>
<td>753</td>
<td>1278</td>
<td>3</td>
<td>785</td>
<td>506</td>
<td>375</td>
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<tr>
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<td>887</td>
<td>954</td>
<td>4</td>
<td>929</td>
<td>749</td>
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<td>68</td>
<td>38</td>
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<td>3</td>
<td>46</td>
<td>22</td>
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<tr>
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<tr>
<td>Total number of bare varicosities</td>
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<td>21</td>
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<td>3</td>
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<tr>
<td>Density, number of bare varicosities/500-( \mu m ) length</td>
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<tr>
<td>Total number of bare varicosities</td>
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<td>13</td>
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<td>1</td>
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<td>0</td>
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<tr>
<td>Density, number of bare varicosities/500-( \mu m ) length</td>
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</table>

E1 indicates the first-order main side branch of central ear artery; E2, second-order branch; E3, third-order branch; MCA, middle cerebral artery; MC1, first-order branch; MC2, second-order branch; MC3, third-order branch; and SMC, smooth muscle cell.
In E2, 70% of the varicosities were separated from the muscle wall by only basal lamina and connective tissue, often including elastin (Fig 5A), compared with only 37% in the similar-sized MC1.

Discussion

The neuromuscular morphology of arteries and their branches from two different vascular beds of the rabbit, the brain and the ear, have been compared. The successive segmental vessels examined from these two beds are comparable in their internal diameter. These vessels have been previously shown to respond differently to sympathetic nerve activity. The neurogenic response of the ear vessels is large, occurs after a short latency, and is little diminished with successive branching. Those of the brain have been found to be small; in the MCA they only occur after a long latency and diminish to almost negligible responses in subsequent branches. The varicosity densities in the larger branches of both arterial systems are similar, this similarity decreases for the smaller divisions of the cerebral vessels. In addition, the varicosities of the brain vessels are situated at a much greater distance from the smooth muscle cells than those in the ear bed. Close contacts (<500 nm) between nerve and muscle, which presumably are of considerable functional significance, are present in the ear vessels but are essentially absent in the cerebral vessels.

The perivascular sympathetic innervation in these two vascular beds in the rabbit is generally constrictor. The transmitter in the ear artery bed is exclusively NE, and in the rabbit cerebral system, both NE and neuropeptide Y are involved. Vascular smooth muscle cells respond to the local concentration of neurotransmitter in their vicinity achieved during nerve activity. This concentration is a function of the number of vesicles released per unit adventitial-medial junction and the distance of the site of release from the smooth muscle cells. Vesicles are presumably released from varicosities by exocytosis. The concentration of transmitter after release, assuming that diffusion occurs in three dimensions from a point source, falls off as a second power of the radius. In the cerebral arterial system, because the average distance the transmitter has to diffuse is much greater than in the ear, the concentration of these effector cells will be lower, increase more slowly, and for any given frequency achieve a lower equilibrium concentration than in the ear. The transmitter diffusion path would be hindered by structures that interpose themselves between the nerves and smooth muscle cells, such as fibroblasts. However, fibroblast densities are comparable for both arterial systems.

Differences in the distance between varicosities and smooth muscle cells are apparent between all comparable branches of the two arterial systems (Fig 3). In the ear artery, the mean varicosity–smooth muscle distance is only 0.3 that of the MCA; the difference is even greater in the next branch. The distribution profiles of the nerve-muscle separation for the two arterial beds are quite different. In the ear, the varicosity density falls off rapidly with increasing distance; in the cerebral artery system, however, the varicosity distribution is relatively uniform throughout the adventitial thickness. Varicosity density differences are particularly marked between the smaller branches; they are essentially absent in the brain vessels. Differences are also considerable in the close-contact category (see Table 2).

Innervation density varies widely in the vasculature and, in general, is assessed semiquantitatively. Catecholamine fluorescence has been used, although the brightness of the fluorescence is not of quantitative significance, and is of questionable validity except for fairly gross comparisons. Other measurements of some function of the adrenergic innervation such as NE content and [³H]NE uptake have been used. None of these methods are entirely satisfactory even when the density is expressed per unit surface area of the adventitial-medial junction. They do not take into account the number of varicosities that are likely to release transmitter, i.e., the number of varicosities that are deficient of Schwann cell sheaths over their surface. This distinction has been made in the present study. The bare area is the site presumably through which exocytosis occurs. Nor do other indexes take into account the distance of varicosities from the smooth muscle cells. The latter emphasizes a refractory prob-
lem, that of distinguishing between those neurons that pass distally to the smaller vessels and those that are responsible for the local release of transmitter and the development of neurogenic tone.

In the present study, this has been taken into account to some extent by those bare varicosities found within 1000 nm of the smooth muscle cell. We assume that more distant varicosities contribute relatively little to the neurogenic response. In the rabbit thoracic aorta, where average nerve separation is ~2.5 μm, the maximum neurogenic response is ~35% of the maximum. In this vessel, the NE ED<sub>50</sub> is 0.56 μmol/L, whereas in the rabbit middle cerebral artery, the NE ED<sub>50</sub> is 45 μmol/L. It would seem reasonable therefore to tentatively assume (somewhat empirically) that in the cerebral vessels, varicosities more distant than 1 μm are essentially ineffectual. This is consistent with calculations of transmitter diffusion.<sup>1</sup>

It is a reasonable possibility that the nerves closest to the smooth muscle cells would dominate the neurogenic response of the artery wall, especially if the smooth muscle cells form a true syncytium. In the rabbit ear artery, 25% of the bare varicosities are within 500 nm, but in the cerebral arteries, only 3%. In E2, the value is 44.5%; in MC2, 1.6% (see Fig 6 for other values).

Earlier studies of systemic vessels have described neuromuscular contacts where the varicosities are devoid of Schwann cell cytoplasm on the adluminal surface, the basal laminae of the smooth muscle and Schwann cell fuse to form a single layer, synaptic vesicles are packed toward the varicosity membrane, and the neuromuscular distance is ~100 nm.<sup>4,15</sup> More recent studies have quantified the frequency of such “junctions” in a variety of arteries of different diameter from various species.<sup>17,24</sup> Nerve terminals make synaptic junctions with the smooth muscle cells of the outer media of nearly all muscular arterial vessels with diameters between 20 μm and 1 mm in the rat, rabbit, and guinea pig.<sup>24</sup> In the basilar artery, these junctions were reported to be frequent in rats but absent in rabbits.<sup>17</sup> The finding in rabbits is consistent with our observations. In rabbit cerebral vessels, no bare varicosities occurred within 100 nm of the smooth muscle, a contrast with values up to 6% of the total number obtained from E2.

It is difficult to assess the role of the close contact in the vascular response to nerve activity. It is self-evident that a certain density would be necessary to initiate a significant contraction. In the middle cerebral artery, the latency of response to field stimulation for 50% of observations was 2.7 ± 3.5 seconds; in another third, 20.5 ± 3.9 seconds. The balance was larger still. In the rabbit ear artery, the latency was 0.95 ± 0.17 seconds. These figures imply that if close neuromuscular contacts dominate the response in the ear, they have relatively little influence in the MCA. In MC3, the response to nerve activation is negligible and may be due to nonadrenergic transmission.<sup>9</sup> This is consistent with the finding that threshold frequency for the MCA is >4 Hz, whereas that for the corresponding ear artery is <1 Hz. Thus, it is difficult to avoid the conclusion that whereas close contacts may be important in some vessels, such as the ear, they contribute little to the response of the MCA and its divisions. The absence of a close innervation, presumably a prerequisite for effective sympathetic control, is consistent with previous observations in rat small cerebral arteries, where there were only one or two layers of smooth muscle without nerves,<sup>14</sup> and in cat MCA, where the closest nerve terminal separations were 280 to 500 nm and, for the branches of the MCA, 900 to 1300 nm.<sup>26</sup>

Two distinct profiles of nerve varicosities can be distinguished on the basis of vesicle size and type: dense-core (granular) and agranular vesicles. From a number of structural studies using various histochemical methods, a dual adrenergic and nonadrenergic innervation in cerebral arteries has been identified in rat,<sup>12,27,28</sup> cat,<sup>12,23,26</sup> and rabbit.<sup>29</sup> Our findings are consistent with this concept. The cerebral arteries (MCA) exhibited both small granular vesicle–containing varicosities (adrenergic) and small agranular vesicle–containing terminals (nonadrenergic). The percentage of granular to agranular for the MCA was 86%:14%. In E1, there was no evidence of a nonadrenergic component. In the rabbit basilar artery, which constricts on electrical stimulation of its intramural nerves, only a few agranular vesicle–containing varicosities were seen (2%),<sup>29</sup> although a small phentolamine-resistant component of vasoostriction has been observed.<sup>30</sup>

Differences in innervation distribution between adventitia and media would be expected to influence the neurogenic response. In the majority of blood vessels, sympathetic innervation is limited to the adventitial-medial junction, but in others it penetrates into the muscle layer and is associated with sizable responses. However, all nerves are in the adventitia in the vessels included in the present study.

Absence of neural regulation of tone does not exclude other effects attributable to vascular innervation. Adrenergic nerves are well known to influence vascular growth and maturity in systemic vessels,<sup>21</sup> and there is evidence for trophic influences in the cerebral circulation.<sup>32</sup> Sympathetic<sup>33</sup> and also parasympathetic<sup>34</sup> innervation has been claimed to offer protection against stroke-producing insults, presumably, at least in part, because of neurotrophic influences. Not all, however, agree.<sup>35</sup>

Detailed functional studies have been carried out on successive segments of both rabbit MCA and ear arteries. In the ear artery and its successive branches, NE effective dose (ED<sub>50</sub>) is of the order of 2 × 10<sup>-7</sup> mol/L;<sup>36</sup> in the MCA, it is 4.5 × 10<sup>-6</sup> mol/L; and in the MC1, it is 5.9 × 10<sup>-4</sup> mol/L. There is no significant consistent response of MC2 and MC3 to this catecholamine.<sup>10</sup> By contrast, NE in the ear artery and its branches caused a maximum increase in force not different from tissue maximum. In the MCA, the maximum NE effect mediated through the α-adrenergic receptor was 12%; in MC1, it was 5.2% of the force that the tissue was capable of achieving. NE failed to cause an α-adrenergic receptor–mediated contraction in the smaller branches. In the MCA, the constrictor effect of sympathetic nerve activity is associated with concurrent release of neuropeptide Y,<sup>9</sup> an observation consistent with the characteristics of the content of the vesicles. Presumably, because of the complex interaction of these two transmitters, α-adrenergic receptor blockers do not influence the response to field stimulation, nor does neuropeptide Y desensitization. It was prevented only by a combination of the two procedures. If there is a nonadrenergic component to sympathetic vasoconstric-
tion in the rabbit ear vessels, it is relatively small, and its basis is unknown. These functional studies, taken together with the anatomic measurements presented in the present study, suggest that differences between the MCA and ear arteries and their branches reflect a combination of multiple factors, each of which would be expected to contribute to the smaller responses of the brain vessels—lower nerve density, greater separation from the closest smooth muscle cells, and lower sensitivity to and reduced capacity to respond to \( \alpha \)-adrenergic receptor activation probably due to lower receptor number.4

It is interesting to speculate on the role of innervation in the regulation of cerebral vascular tone. The lack of evidence for a functionally effective innervation of small pial arteries (absence of response of human pial arteries has recently been documented) suggests that the diameter of the smaller cerebral vessels are regulated by nonneural mechanisms, which for those distant from metabolically active tissue are probably intraluminal pressure and flow. Changes of intravascular flow and pressure in the middle-sized and smaller pial arteries may occur not only as a result of changes in cardiac output and central arterial pressure but secondary to upstream neural influences on the larger inlet cranial arteries. These, although small in rabbits and humans, are reported to be sizable in other species. Such activity would result in downstream changes in pressure and flow, which in turn would influence diameter. This arrangement would allow the cerebral circulation to accommodate variation in regional brain blood flow while maintaining an overall constancy.

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**References**


Comparison of density of sympathetic varicosities and their closeness to smooth muscle cells in rabbit middle cerebral and ear arteries and their branches.

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