Weakness of Sympathetic Neural Control of Human Pial Arteries Compared With Superficial Temporal Arteries Reflects Low Innervation Density and Poor Sympathetic Responsiveness

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Background and Purpose—The primary goal of these studies was to understand and investigate the capacity of perivascular nerves to influence the tone of human pial arteries and to compare them with other human cephalic arteries, the superficial temporal and middle meningeal.

Methods—Responses to electrical activation of intramural nerves and related features of fresh segments of human cephalic arteries—the pial (PA; 478±34 μm ID), middle meningeal (MMA; 540±41 μm ID), and superficial temporal (STA; 639±49 μm ID)—obtained from patients aged 15 to 82 years during surgical procedures were studied on a resistance artery myograph.

Results—The PA segment responses to electrical nerve activation and to norepinephrine (NE; 10^{-5} mol/L) were 1% and 21% of tissue maximum, respectively, compared with 6% and 34% for the MMA and 14% and 90% for the STA. Tissue maximum was defined as the force increase to 127 mmol/L KCl plus arginine vasopressin (1 μm). All arteries dilated well to acetylcholine. Possible explanations for the PA marginal neurogenic responses were assessed. NE ED_{50} was 5.4±2.2×10^{-7} mol/L and did not vary with age or diameter. NE responsiveness did not increase in vessels with spontaneous or raised potassium-induced tone. Relaxation to isoproterenol was variable and propranolol did not increase the neurogenic response. Neither N^{G}-monomethyl-L-arginine, N^{G}-nitro-L-arginine methyl ester, endothelium removal, nor indomethacin consistently influenced the contractions to NE or neurogenic reactivity. The weak PA neurogenic response is in keeping with its poor innervation. As determined by catecholamine histofluorescence, innervation in the PA is sparse, with density increasing in the order PA, MMA, and STA. The incidence of nerve structures in the PA adventitio-medial junction was only 3% of those in the STA, and these were situated more than 3 μm from the closest smooth muscle cell.

Conclusions—We conclude that the weak neurogenic response of adult human pial artery reflects its poor innervation and responsiveness to NE, implying that these features are not important in the regulation of its diameter. (Stroke. 1998;29:212-221.)

Key Words: adrenergic responses ■ pial artery ■ sympathetic innervation ■ temporal arteries

Much of the mammalian vasculature receives a sympathetic innervation that contributes to the control of its caliber at rest and to the changes that occur in response to stress. However, the role of this innervation in regulation of the cerebral blood flow in humans is unclear. Although the presence of adrenergic perivascular nerves has been described in the cerebral arteries of a number of laboratory animals including humans, for some time there has been much debate about the level of functional control of this innervation (see, for a few pertinent references, references 3 to 6). This is particularly the case in humans since in vivo studies are limited to animals. In vitro preparations of larger arteries from many species are responsive to electrical perivascular nerve activation, but remarkable species variation in the magnitude and the direction of the response has been reported. Both the presence and absence of neurogenic responses of the larger human cerebral arteries have been observed. In the only study to date of human pial arteries, no response attributable to the innervation was observed. This is, perhaps, not surprising since neurogenic control in the rabbit decreases with decreasing diameter. However, in this latter study of human pial arteries no attempt was made to exclude functional factors that

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might mask or minimize a potential response or to understand its basis. In addition, since neurogenic contraction varies, at least in the rabbit, with diameter\(^2\) and since in humans there are age-related changes in cerebral vascular function,\(^{14-16}\) these were not excluded by these authors as explanations for the paucity of the responses sought.

In view of the remarkable species variation, an assessment of the extent of neurogenic control of these arteries in humans cannot be established by extrapolation from other species. The majority of prior studies on the human have been carried out on autopsy material at various times after death. In this paper on human PA we report on our assessment of the level of neurogenic control of fresh vessels (180 to 700 \(\mu\)m ID) obtained from patients 15 to 82 years old, average 41.5 years, obtained during neurosurgical procedures. Artery segments were studied in a resistance artery myograph, and nerves were excited by EFS. Several approaches were used to establish appropriate voltage parameters for the pial segments, and their effectiveness was demonstrated in a parallel study of human STA and MMA, some segments of which came from the same patients. To make this more inclusive of the human cephalic circulation, MMA segments were also included. In the initial experimental series the PA segments, in contrast to those of the STA, were essentially unresponsive to nerve activation. Responses from MMA were intermediate. For this reason a number of possible processes that might compromise or mask a contractile neurogenic response were assessed: weak responsiveness to NE mediated by \(\beta\)-adrenoceptors, concurrent \(\beta\)-adrenoceptor occupation, absence of basal tone, concurrent stimulation of dilator nerves, release of nitric oxide (NO)–endothelium-derived relaxing factor (EDRF), dilator prostanoids, and other endothelial factors. Our results showed that these did not provide an explanation for the absence of responsiveness. The size of the NE responsiveness did not change significantly with age over the range of 15 to 82 years or with diameter. Fluorescence histochemistry of the sympathetic catecholamine perivascular innervation showed a plexus of low density in the PA and quantitative electron microscopy, a low density of perivascular nerve bundles widely separated from the outer layers of the smooth muscle cells. These features were in contrast to those seen in the STA and seemed to provide an explanation for the absence of the nerve effect.

### Methods

Human PA segments were obtained during neurosurgical procedures from the pial circulation of normal regions of the cerebral cortex, MMA from the dura mater, and from the STA and its branches. This study was approved by the Ethics Committee of the University of Vermont. The patients selected were clinically free of cardiovascular and metabolic disease. Arterial segments were obtained (from patients aged: 15 to 82 years for PA, \(n=23\); 18 to 46 years for MMA, \(n=15\); 19 to 75 years for STA, \(n=15\)) and were either studied on the same day of removal or after storage for 24 hours at 4°C in Krebs’ physiological solution (PSS) (composition in mmol/L: NaCl 130, KCl 4.7, KH\(_2\)PO\(_4\) 1.18, MgSO\(_4\) 1.17, NaHCO\(_3\) 14.9, EDTA 0.026, and dextrose 11.0) and gassed continuously with deferoxamine (100 \(\mu\)mol/L), heparin (10 U/mL), penicillin (50 U/mL), and streptomycin (50 \(\mu\)g/ml). Similar functional results were obtained with the two groups of human tissues. Preservation for 24 hours under these conditions has been shown previously not to affect nerve, muscle, or endothelial function of animal arteries.\(^7\) The human tissues were transferred to the laboratory and prepared for in vitro study. Only segments free of adherent blood were studied.

### Functional Studies

PA, MMA, and STA were cut into two to four rings 3- to 4-mm long and suspended on wires in resistance artery myographs.\(^{18,19}\) They were suspended in Krebs’ PSS maintained at \(pH=7.4\pm0.1, 37^\circ C\), and gassed continuously with 95% O\(_2\)/5% CO\(_2\). The rings were connected to force transducers (model FT03, Grass Instruments) to record changes in isometric force. Internal diameter was measured using a video camera (Colorado video micrometer). The myograph mounting wires were slowly separated until a barely significant change in the force record was observed. Wire separation was taken to be half the unstretched circumference. Platinum wire electrodes (0.3 mm diameter, 3 mm long) placed on either side of the suspended segments were used for transmural EFS using a Grass stimulator. The internal diameters of PA, MMA, and STA used in the initial series of studies were 478±34, 540±41, and 639±49 \(\mu\)m, respectively. The STA segment diameters were significantly larger than those of the MMA or PA (\(P<.05\)). Diameters measured in this way correspond to unstretched diameter.

Each vascular segment was equilibrated for 90 minutes in PSS maintained at 37°C and gassed continuously with 95% O\(_2\)/5% CO\(_2\). The bathing solution was changed every 15 to 20 minutes. Because comparable anatomic artery segments from different patients varied in their internal diameter, wall thickness, and elasticity, the active length-tension relationship was determined for each segment before the experimental protocol. The rings were first stretched to an internal diameter known from experience to be less than the optimum and then were exposed to 30 mmol/L KCl. A step increase in passive wall tension of approximately 10% was then achieved by stretch and when equilibrium was reached, rings were exposed to KCl. The preload when the contractile response to KCl was within 20% of the prior response was considered to provide optimum length. The rings were then allowed to equilibrate for a further 30 minutes. The arterial segments were exposed to NE (10 \(\mu\)mol/L), and at the contractile plateau ACh (10 \(\mu\)mol/L) was added to assess the function of the endothelium. A vessel was included in the experimental series if the relaxation induced by ACh was >50% of the pre-addition NE-induced tone. When similar experiments were conducted on more than one segment from the same patient, results were averaged.

### Nerve Stimulation

Neurogenic responses of PA, MMA, and STA segments to transmural EFS of their intramural nerves were investigated. Various frequencies were applied as biphasic, square pulses of 0.3–millisecond duration at supramaximal voltage through platinum wire electrodes placed on either side of the arterial segment and connected to a Grass stimulator. A pulse duration was adopted of 0.3 millisecond, which in our experience with blood vessels from various species provides optimum selectivity of stimulation of neurons but not smooth muscle cells.\(^1\) Two approaches were used to determine stimulation voltage for selective activation of perivascular nerves, breakthrough and subtraction (see Fig 2). Only one method was used in a particular ring.
Breakthrough Method
A voltage was used that was less than that required to cause an increase in wall force in the presence of TTX (3 x 10^{-6} mol/L). TTX is considered to block neuronal conduction effectively and selectively. Thirty minutes after the addition of TTX, EFS was applied at 10-minute intervals at increasing voltages until a force response was recorded. This voltage reduced by one volt was used after TTX washout for the EFS.

Subtraction Method
At 10-minute intervals EFS was applied for 2 minutes at 5, 7.5, 10, 12.5, 15, and 20 V. In some experiments, pulses of 0.6 and 0.3 milliseconds' duration were used. TTX (3 x 10^{-6} mol/L) was added to the tissue bath before parameters of contraction before TTX addition were repeated. Differences in the force responses at the same stimulation parameters are considered to represent responses to activation of the perivascular innervation. In both methods, trains of biphasic square wave pulses of 0.3 milliseconds' duration were delivered to the electrodes at 8 and 16 Hz until the response reached equilibrium (0.5 to 2 minutes).

To test for dilator neurogenic responses, after the voltage for EFS had been determined by either the breakthrough or the subtraction method in the absence of tone, EFS was applied in the presence of tone, either spontaneous or induced by PGF2α, 10^{-6} mol/L. EFS was often repeated in the presence of phenolamine (10^{-6} mol/L).

Frequency-response curves were determined from trains of stimuli delivered at 1, 2, 4, and 8 Hz in random sequence and finally at 16 Hz until an equilibrium response was derived. Responses to 8 Hz were elicited at the beginning and end of the series to assess time- and stimulation-dependent changes. These were found to be minimal.

Cumulative NE concentration-response curves of PA, MMA, and STA were obtained by adding increasing concentrations of NE (0.01 to 30 μmol/L) to the bath in half-log-unit steps. Each addition was made only after the response to the previous concentration had reached equilibrium. The effective concentration (EC50, mol/L) of NE that causes 50% of maximum contraction (Emax) was determined.

Relaxation to NE (10 μmol/L) was expressed as % pre-addition tone. A variety of other procedures or conditions were used. The extent of maximum relaxation that occurred on exposure to papaverine (10^{-5} to 3 x 10^{-6} mol/L) was considered basal tone. In experiments designed to test the influence of raised tone on NE responses to PA this was achieved by increasing the KCl concentration in the PSS. To assess possible β-adrenoceptor influences, after tone had been increased with PGF2α (10^{-6} mol/L), PA were exposed to isoproterenol (3 x 10^{-6} to 10^{-5} mol/L). Propranolol (10^{-6} mol/L) was used to show possible β-adrenoceptor involvement during EFS. Tissues were exposed to L-NNA (10^{-4} mol/L) or L-NAME (3 x 10^{-5} mol/L) for 30 minutes to inactivate NO synthesis. The endothelium was removed or inactivated by gentle rubbing with a hair. The effectiveness of the procedure was assessed by loss of dilation to ACh (10^{-6} mol/L). Exposure to indomethacin (10^{-6} mol/L) for 30 minutes was used to inhibit the possible synthesis of dilator prostanoids.

At the end of each experiment, the arterial segments were maximally contracted with 127 mmol/L KCl-Krebs' solution plus arginine vasopressin (1 μmol/L). This is defined as Emax.

Structural Studies
Catecholamine Nerve Histofluorescence
Segments adjacent to those used for the functional studies were processed for viewing of catecholaminergic perivascular nerves using the glyoxylic acid method20 plus pontamine sky blue (0.5% wt/vol) to mask the nonspecific background fluorescence21 and were viewed with a Zeiss epifluorescence microscope.

Electron Microscopy
Segments of arteries 1 to 2 mm in length were immersion-fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 mol/L phosphate buffer, pH 7.4, for 4 hours at room temperature or overnight at 4°C. After a phosphate buffer rinse, they were postfixed in 2% osmium tetroxide in phosphate buffer for 1 hour at room temperature. The segments were then dehydrated in graded alcohols and embedded in Durcupan ACM (Fluka). Ultrathin transverse sections were cut on a RMC Ultramicrotome (MT-7) with a diamond knife and stained with uranyl acetate and lead citrate. The sections were viewed in a JEOL 100 CXII electron microscope at 60 kV.

For the quantitation of nerves, the entire tunica adventitia of each artery was examined for the presence of nerve bundles. Nerve bundles are defined as contiguous groups of nerve axons ensheathed in Schwann cell processes. A bundle may be seen to contain nerve axons (nonvaricose) and varicosities with synaptic vesicles and mitochondria. Only entire bundles rather than individual nerve axons or varicosities were counted in this study. Low-magnification micrographs were taken to measure the perimeter of the adventicio-medial border. Higher magnification micrographs were made of each nerve bundle and were used to measure the distances separating the nerve bundle from the nearest smooth muscle cell; montages were taken for nerve bundles at greater distances, and to determine nerve densities, the number of nerve bundles per unit length of adventicio-medial border. Measurements of all micrographs were made using the Sigma-Scan measurement system with a Numonics Digitizing Tablet and linked to a computer.

Drugs Used
The following drugs were used: ACh hydrochloride, NE bitartrate, indomethacin (Sigma), 1, 2, 6, 7 isoproterenol HCl (Sigma), L-NAME (Sigma), L-NNA (Sigma), D6-propranolol HCl (Sigma), TTX (Sigma), and arginine vasopressin (Bachem). Drugs were dissolved in Krebs' solution, prepared freshly every day, and kept on ice.

Data Analysis
Contractile responses were expressed either as a percentage of the maximal tension produced by 127 mmol/L KCl-Krebs' and arginine vasopressin (1 μmol/L) or as active wall tension divided by the length of the individual arterial segment and the internal diameter (mg/cm per millimeter). The latter parameter was used in place of wall thickness because of the difficulty in making accurately the latter measurement on a tissue that would subsequently be used for in vitro study. Some of the segments available to us were short. It was assumed that wall thickness-to-lumen ratio remains constant over the small range of vessel diameter used in this study. The unstretched wall thickness-to-lumen ratio in the PA was approximately 6 (unpublished data, R.D. Bevan and J.A. Bevan). Values were expressed in relation to internal diameter to minimize the effect of changes in medial muscle mass associated with changes in diameter. Concentration-response curves based on a Hill relationship were fitted to individual concentration-response data by a computer program (least-square method).

Agonist potencies were calculated on the basis of data from individual vessels and are expressed as pD2 (−logEC50, where EC50 is the concentration of the agonist needed to produce 50% of the maximal response).

Statistical Analysis
Data are given as mean±SEM. In all experiments, n refers to the number of patients from whom the blood vessels were obtained. Statistical differences between the mean values were determined by ANOVA followed by a Scheffe’s F test. A value of P<.05 was accepted as significant for differences between groups. The relationship between parameters was evaluated by linear regression analysis of the data using a commercially available statistical analysis software (Statview).

Results
The primary goal of these studies was to understand and investigate the capacity of perivascular nerves to influence the tone of human pial arteries and to compare them with other human cephalic arteries, the superficial temporal and middle meningeal. Because the initial series of experiments showed...
only a marginal response of PA to nerve stimulation and a poor response to NE, subsequent experiments were carried out in an attempt to account for this. For this reason, results are presented in two sections—first, a comparison of the PA, MMA, and STA, and their general characteristics of response to nerve activation and NE and second, an analysis of the basis of the poor responsiveness of the PA.

Comparison of the Responsiveness of the Human PA, MMA, and STA

**Maximum Contractile Responsiveness**

The maximum change in wall force (E_{max}) produced by 127 mmol/L KCl-Krebs’ solution combined with arginine vasopressin (1 μmol/L) was 2.76±0.57 mg/μm per millimeter (n=15) in the STA, 0.90±0.14 mg/μm per millimeter (n=15) in the MMA, and 0.92±0.07 mg/μm per millimeter (n=23) in the PA. (See “Methods” for basis of expressing wall force.) The STA force development was significantly greater than in the PA and MMA.

**Responses to Exogenous NE**

NE (0.01 to 30 μmol/L) produced sustained concentration-dependent increases in wall tension in the three types of human arteries tested. Higher concentrations either did not cause additional force increase or resulted in relaxation. NE was equipotent in the different groups of arteries, with ED_{50} values of 5.4±2.2×10^{-7} mol/L (n=21), 1.1±0.057×10^{-6} mol/L (n=14), and 1.7±0.29×10^{-6} mol/L (n=10) in the PA, MMA, and STA, respectively. However, the maximum force developed in response to NE (30 μmol/L) was greater in STA (1.85±0.49 mg/μm per millimeter, n=10, P<.05) compared with MMA (0.29±0.06 mg/μm per millimeter, n=14), and PA (0.18±0.03 mg/μm per millimeter, n=21) (Fig 1a). The active tension produced by NE in the three types of segments, expressed as a percentage of their own E_{max} (Fig 1b), was 89.8±3.5% in STA (n=10), 34.0±5.3% in the MMA (n=14) (P<.05 versus STA), and 20.8±3.1% in the PA (n=21) (P<.05 versus STA).

**Responses to EFS**

Voltages used to selectively activate intramural nerves were determined using two techniques: breakthrough and subtraction (Fig 2, for details see “Methods”). Fig 3 shows the frequency-contractile response–curves (1 to 16 Hz) from human isolated STA, MMA, and PA to EFS applied as square pulses of 0.3 milliseconds’ duration and at supramaximal voltage using both supramaximal and subtraction techniques. In the STA, the contractile neurogenic responses obtained were frequency dependent, exhibiting a threshold change at 4 Hz and achieving 14.1±1.9% (n=21) of E_{max} at 16 Hz. In contrast, in the MMA EFS-induced contractions were absent at 4 Hz and significantly lower than those of the STA at 8 and 16 Hz. At the latter frequency it was only 5.7±1.4% of E_{max} (n=5 responding tissues of 14). In the PA, EFS contractile responses rarely occurred, reaching 0.96±0.67% of E_{max} at 16 Hz (n=9 responding tissues of 21). Unequivocal nerve-induced contractile responses were invariably elicited from the STA segments, some of which were obtained from the same patients whose PA segments were unresponsive.
Assessment of the Factors that Might Influence the Human PA Responses to EFS

Arteries from an additional 49 patients were used to assess possible reasons why the PA neurogenic response was absent or small (see below). A total of 116 segments were involved. Frequently more than one of the factors was tested in a particular segment.

Contraction to NE

The sizes of the maximum response of the PA to NE up to $3 \times 10^{-5}$ mol/L were variable, ranging from the barely detectable to 44% of tissue maximum (mean, 16.6±3.1). The NE ED$_{50}$ for these was 1.22±0.29×$10^{-6}$ mol/L (n=12). This was not significantly different from the value determined in the prior series.

Patient Age and Artery Diameter

Neither the magnitude of the maximum contraction to NE, as a percentage of E$_{\text{max}}$, nor the NE ED$_{50}$ correlated with the patient’s age ($r=.063$; $P=.076$ and $r=.054$; $P=.84$) nor with arterial internal diameter ($r=.071$; $P=.71$ and $r=.41$; $P=.1$, respectively).

Influence of Basal Tone

Reactivity of blood vessels to contractile agonists has been reported to be enhanced when tone is increased. PA autoregulate and in vivo possess intrinsic tone. In 12 segments in this series from 5 patients, selected because they exhibited raised levels of spontaneous tone of 16.6±1.3% E$_{\text{max}}$, the additional contraction to NE ($3 \times 10^{-5}$ mol/L) was 20.3±3.7% E$_{\text{max}}$. When an equivalent level of tone was induced in segments without spontaneous tone by the addition of 17 mmol/L K$^+$, the NE contraction was 10.6±2.1% E$_{\text{max}}$. These levels of response are not significantly different from the contraction to the same dose of NE that occurred in the absence of tone.

Responses to EFS and Reactivity to NE and ACh

Contraction

Additional experiments using EFS at 8 and 16 Hz were carried out on arteries after equilibration in the absence of tone—fifteen using the subtraction and four the breakthrough method of determining stimulation voltage. Eight segments from 6 patients showed a small TTX-sensitive contraction at 16 Hz. A response was seen in one segment at 16 Hz, but was absent at 8 Hz. PA EFS responses were 2.74±0.55% E$_{\text{max}}$ (n=8) and 2.17±0.34% E$_{\text{max}}$ (n=11) at 8 and 16 Hz, respectively. The largest response of a PA was 5.6% E$_{\text{max}}$. The NE ED$_{50}$ and NE E$_{\text{max}}$ of the pial arteries that exhibited an EFS TTX-sensitive response were not significantly different from those that did not respond ($P=.74$ and $P=.63$, respectively). The maximum dilation to ACh of the segments that showed a significant TTX-sensitive contraction was not different from those that did not ($P=.91$).

Dilation

A dilator neurogenic response, if present, would be anticipated when tone is raised. EFS at 8 and/or 16 Hz of PA that was tonically contracted by PGF$_{\text{2a}}$ ($3 \times 10^{-6}$ mol/L) or that developed spontaneous tone was carried out in 10 segments. In most of these tissues some dilation, usually a small amount, was observed on EFS, which reversed on its cessation. However, an unequivocal TTX-sensitive relaxation was observed in only one segment. This was 45.5% of prestimulation tone, which was about 30% E$_{\text{max}}$. It was 13.3% after TTX. The reversible dilator response developed very slowly to prolonged EFS.

Dilation to ACh

The mean maximum dilation to ACh of the second series was 75.8±8.4%. In 6 vessels relaxation completely reversed pre-addition NE tone. The maximum ACh dilation of tissues that exhibited EFS-induced contraction was not different from that of those that did not respond ($P=.91$).

Factors that Might Mask the EFS Contractile Response

β-Adrenoceptors

Five PA were used to test the possibility that concomitant β-adrenoceptor activation by an adrenergic transmitter might mask neurogenic α-adrenoceptor–mediated contraction. Four of these arteries dilated 100%, and the fifth 46% to ACh ($3 \times 10^{-6}$ mol/L). In three arteries, isoproterenol up to $3 \times 10^{-6}$ or $10^{-5}$ mol/L caused no dilation. In one it completely reversed pre-addition tone of 24% E$_{\text{max}}$, and in the other it caused relaxation of 43% when the pre-addition tone level was 18% E$_{\text{max}}$. The addition of propranolol ($10^{-6}$ mol/L) did not reveal a TTX-sensitive contraction in tissues that in its absence had not responded to EFS, nor did it increase an existing neurogenic contraction at 16 Hz (n=5).

L-NNA/L-NAME

Smooth muscle contraction might be diminished by concomitant activation of the endothelium leading to release of NO/EDRF (for example see reference 24). The NE concentration-response relationship was determined after incubation for more than 30 minutes in either L-NNA ($10^{-4}$ mol/L) or L-NAME ($3 \times 10^{-4}$ mol/L) when the mean increase in baseline was 3.4±1.9% E$_{\text{max}}$. NE ED$_{50}$ and E$_{\text{max}}$ were not significantly altered by pretreatment with any of these agents ($P=.36$ and $P=.10$, respectively). The contractions to EFS (∼2.6% E$_{\text{max}}$ at 8 Hz) were decreased by L-NNA/L-NAME.
Hz) seen in 5 segments were unaltered by exposure to these drugs.

**Endothelial Factors**

There was no alteration in either the size (% E_max) or the sensitivity (ED_{50}) of the response to NE (P=.67 and P=.32, respectively) after endothelium inactivation.

**Indomethacin**

The possibility of the concomitant production and release of dilator prostanoids during EFS was assessed by exposure to indomethacin (10^{-5} mol/L). In 3 segments this treatment did not unmask a response to EFS where it was previously absent. Neither the NE E_max nor the ED_{50} was altered by this treatment (P=.34 and 0.26, respectively).

**Concurrent EFS Activation of Dilator Nerves**

EFS during increased tone, whether spontaneous or due to PGF_{2a}, failed to reveal a TTX-sensitive dilation either before or after exposure to phentolamine (10^{-6} mol/L).

**Innervation Characteristics**

**Catecholamine Histofluorescence**

Fig 4 contains examples of the catecholaminergic histofluorescence of PA, MMA, and STA. Counter-staining techniques were adopted to reduce autofluorescence. In the STA, a rich, dense, and uniform plexus of catecholamine-containing nerve fibers could be observed. The density of catecholamine-containing neurons was less in the MMA. In the PA, nerve fibers were sometimes absent and when they occurred were predominantly longitudinally oriented rather than forming a plexus. Innervation was consistently poor compared with that in the MMA and STA. The majority of artery segments were from the distribution of the MCA. Pial segments from the anterior cerebral artery distribution, although sparsely innervated, appeared to have relatively more sympathetic neurons than those from the MCA distribution.

**Electron Microscopy**

Nerve bundle density or incidence relative to the circumferential length of the outer medial border (number/mm) of PA was assessed in 15 segments from 15 patients (see Table). Only 13 neuronal structures were identified in a total length of 12 mm of the adventitia-medial junction (Fig 5), an incidence of approximately 1/1000 μm. The mean closest distance was 3.8±0.46 μm. Nerve density was 26/1000 and 35/1000 μm in the MMA and STA, respectively, and the closest nerve muscle separations were on the order of 1 μm.

**Discussion**

This work brings together structural and functional measurements designed to assess the potential of the sympathetic nervous system to induce tone in fresh human STA, MMA, and PA. Arteries were obtained during neurosurgical procedures. The PA and MMA develop less maximum force than the STA, and the maximum capacity to respond to NE related to maximum force development increases in the order PA<MMA<STA. The sensitivity of the three vessels to NE is the same. The maximum nerve-induced responses of these three vessels were 1%, 6%, and 14%, respectively, of their maximum contractile capacity. Our study suggests that the basis of the marginal contractile response of the smooth muscle of adult human PA to EFS of their intramural nerves resides in their low density of adrenergic innervation combined with a limited capacity of the smooth muscle cells to respond to NE. In contrast, the capacity of the STA to contract to NE to an extent not different from tissue maximum, combined with a more dense innervation, suggests a significant control of this vessel by the sympathetic nervous system. The MMA is intermediate in its reactivity.
All three types of human arteries exhibited similar sensitivities to NE: that for the PA was $9 \times 10^{-7}$ mol/L. Duckworth et al.\(^\text{10}\) reported an NE ED\(_{50}\) of $7.9 \times 10^{-7}$ mol/L in the proximal human MCA, Shibata 9.3 $\times 10^{-8}$ mol/L,\(^\text{25}\) and Janson et al. an ED\(_{50}\) of $5.0 \pm 10^{-7}$ mol/L.\(^\text{26}\) Hardebo et al.\(^\text{27}\) concluded that the PA and STA were equally sensitive to NE, with a spread of values between $10^{-6}$ and $10^{-5}$ mol/L. These values are similar to the ED\(_{50}\) values reported for other human arteries.\(^\text{28,29}\) In the rabbit, the species in which variations in NE sensitivity and receptor affinity have been studied the most, the ED\(_{50}\) of most arteries is a reasonable approximation of receptor affinity.\(^\text{30}\) The NE dose-response curve of the human proximal middle arteries has two components: the first leveling at $3 \times 10^{-5}$ to $3 \times 10^{-4}$ mol/L. Higher doses are responsible for further contraction.\(^\text{10}\) This second phase is associated with the action of NE on a “nonspecific” adrenergic receptor not influenced by $\alpha$-adrenoceptor blockade, which has been called a $\gamma$-adrenoceptor or an extraceptor, one outside the confines of the synaptic cleft. The second component was not seen in the PA, which often relaxed to higher doses.

In the human, as in the monkey, cat, dog, and rabbit, NE causes contraction. However, in the cow and the pig, it elicits relaxation.\(^\text{31}\) Another variable is the size of the NE contraction relative to the maximum capacity to respond: that for the PA is 20%, for the MMA and STA are 34% and 90%, respectively. Experiments in the rabbit suggest that receptor number limits the response of a cerebral artery.\(^\text{32}\)

A semiquantitative assessment of the adrenergic innervation density was made by catecholamine histofluorescence plus pontamine sky blue counterstain.\(^\text{21}\) The brightness of the fluorescence is not of quantitative significance.\(^\text{33}\) This technique has validity only when gross comparisons are made as with the STA versus the PA. In the PA the catecholamine-containing nerve fibers were sparse, contrasting with the much denser uniform plexus of aminergic nerve fibers observed in the STA. There were intermediate levels in the MMA.

Perivascular catecholaminergic nerves have been identified by specific histofluorescence in a number of species, for example, the cat and monkey pial arteries\(^\text{34,35}\) and rabbit\(^\text{12}\) and human\(^\text{10,36}\) middle cerebral arteries. The latter authors describe a fairly thick, densely packed, mesh-like system of varicose sympathetic fibers in the adventitia and outer media of the STA and PA. They did not distinguish between the two types of

### Table: Incidence of Nerve Bundles and Closest Nerve Muscle Separation in Adult Human STA, MMA, and PA

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<th>STA</th>
<th>Adult MMA</th>
<th>PA</th>
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<tbody>
<tr>
<td>Patients, n</td>
<td>3</td>
<td>3</td>
<td>15</td>
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<tr>
<td>Nerve bundles/artery</td>
<td>58±20</td>
<td>41±12</td>
<td>0.87±0.41*</td>
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<tr>
<td>Length of adventitio-medial border studied, $\mu$m</td>
<td>1629±282</td>
<td>1570±289</td>
<td>806±59</td>
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<tr>
<td>Incidence of nerve bundles/1000 $\mu$m of adventitio-medial borders of all arteries</td>
<td>35.5</td>
<td>26.1</td>
<td>1.07*</td>
</tr>
<tr>
<td>Closest nerve-muscle cell separation, $\mu$m</td>
<td>0.9</td>
<td>1.3</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Data are mean±SEM. *P<.05 vs STA and MMA.

![Figure 5](https://example.com/image5)  
**Figure 5.** A transmission electron micrograph of a PA from a 19-year-old woman showing a nerve bundle (NB) in the inner adventitia. The distance to the outer medial muscle is approximately 3.1 $\mu$m. SM indicates smooth muscle (); COL, collagen. (Bar=0.5 $\mu$m.)
arteries. They found a broad multiaxonal plexus, typical of preterminal axons in the outer adventitial layer. No bright focal collections indicative of terminal axons were seen.

These general conclusions are confirmed and extended by quantitative electron microscopy. In the STA the nerve bundle density was 35/1000 μm. In the PA, it was 1/1000 μm. Between these two extremes is the middle meningeal artery with 25 bundles/1000 μm. In the PA, the few nerve bundles seen were widely separated from the closest smooth muscle cells.

Two technical approaches to the determination of the stimulation voltage for the EFS of the perivascular nerves were used. Both used TTX. It is assumed that the TTX-sensitive component of the change in force with EFS reflects action potential–initiated transmitter release. The pulse duration adopted (0.3 milliseconds) provides optimum selectivity for nerve activation in the intact tissue.1 In some experiments a pulse duration of 0.6 milliseconds failed to influence the size of the response. A frequency of 8 Hz, and sometimes 16 Hz, is known to provide maximum or near maximum neurogenic responses. Tissues were exposed to NE before EFS in an attempt to replenish neuronal transmitter stores. In the break-through technique, the maximum voltage that just failed to activate smooth muscle cells was used. The main criticism of this method is that the voltage used may not activate all the perivascular nerves. With the subtraction method, the responses to pulses delivered at voltages set above the threshold for muscle activation are reduced by those responses seen after nerve inactivation with TTX. One criticism of this approach is that if there is concomitant muscle depolarization caused by nerve inactivation with TTX, vascular smooth muscle responsiveness to released neurotransmitter could be altered. The level of the membrane potential is an important determinant of smooth muscle reactivity. The comparable findings obtained by both approaches increases our confidence in the conclusions.

In no instance was an unequivocal nerve response greater than 5.6% of the maximum possible contraction of the human PA observed. Among those that contracted, it varied from threshold to 3%. No neurogenic response was seen in 60% of the artery segments studied. This is in agreement with the observations of Hardebo et al.12 Fresh human arteries were examined both in the study by Hardebo et al and in the current study. In postmortem human MCA,10 neurogenic responses were absent in 80% of tissues that were examined within 4 hours after death. Mean responses, when they occurred, were about 6%. In autopsy material Shibata et al14 recorded that 2 of the 4 segments of human MCA (diameters unspecified) from patients aged 63 to 68 years exhibited neurogenic responses that were between 25% and 30% of NE Emax. They used extreme parameters of stimulation: 80 V, 100 Hz, and duration 10 milliseconds. The contraction was TTX– and phentolamine-sensitive. Toda and Fujita15 were unable to demonstrate nerve responses in basilar, posterior cerebral, and intracranial carotid systems in vessels obtained after death. Sizable neurogenic responses, both constrictor and dilator, have been found by many laboratories in major cerebral arteries of a variety of animal species, for example, the rabbit,37 dog and sheep,38 cat,39 and monkey.40 To generalize, human proximal cerebral arteries appear to be modestly responsive to nerve activation. PA are virtually unresponsive. The STA was the most responsive to EFS and the MMA was intermediate. The STA is a cutaneous artery. The cutaneous vessel whose adrenergic control has been analyzed in some detail40 is the rabbit central ear artery. Neurogenic responses of the main trunk and the first- and second-order branches of that artery are 73%, 60%, and 35%, respectively, of maximum. This artery, however, probably has the highest density of innervation of any artery studied.

Some additional factors that might contribute to the poor sympathetic response were assessed. The possibility was considered that the poor neurogenic response might have resulted from the overnight storage. However, these conditions did not influence the neurogenic constriction of the rabbit ear artery7 or that of the human STA. The neurogenic responses of the PA that did occur were not restricted to first-day studies. The tissue samples did not come from a predominantly aged population. The NE response did not diminish with age between 15 and 75 years, a feature observed in some vascular preparations.15 A sizable nerve response was rarely found in any tissue. The sensitivity of the α-adrenoceptor to NE in the PA is not uniquely low. Furthermore, responses did not change when NE was added to a vessel with background tone. Finally, EFS would potentially activate all nerves in the artery wall, both constrictor and dilator. However, when tone occurred spontaneously or was induced with an agonist, EFS failed to reveal a TTX-sensitive dilation; nor was this observed in the main MCA under similar circumstances after α-adrenoceptor block with phentolamine.10 Nerve-induced dilation of PA has not been recorded in any animal species (see for example reference 12), although this is the dominant response in the proximal cerebral arteries of the cat12 and pig.41 A variety of dilator nerves have been visualized using different techniques in a number of species42–44; those containing ACh,42,43 neuropeptide Y, vasoactive intestinal peptide, substance P, calcitonin gene-related peptide,43,45 and NO.46 Evidence for a functional role for some of these dilator neurotransmitters in nonhuman arteries has been reviewed.47 Our electron microscopy survey does not identify specific types of neurons in the artery wall, and we can only conclude that innervation of any form is present only in minimal amounts.

There is little evidence for a significant β-adrenoceptor population in cerebral arteries.40,49 The inconsistent dilator effect of isoproterenol in the present studies suggests that β-adrenoceptors could not account for the consistent absence of a neurogenic contractile response. Furthermore, propranolol failed to reveal neurogenic dilation where this was previously absent or to potentiate a response when manifest. EDRF/NO influences basal and constrictor tone in the cerebral circulation.25 Exposure to L-NNA or L-NAME, and also endothelium removal, had no significant effect on the response of the PA to NE or EFS. Local endogenous production of dilator prostanoids can modulate vascular responsiveness.39 Treatment with indomethacin (10 μmol/L) did not change consistently reactivity of the PA to NE or EFS.

This study supports the conclusion that in the human PA the marginal neurogenic contractile response reflects innervation of low density separated widely from smooth muscle cells. Compounding this picture is the low capacity of the artery to respond to NE. By contrast, the STA has a sizable neurogenic
response, because it has a high nerve density; the nerve bundles are closer to the smooth muscle cells and respond well to NE. Since PA in toto comprise an important part of an effective autoregulating bed and since the role of the sympathetic nerves is questionable, pressure and flow are undoubtedly major regulatory mechanisms. The sympathetic nervous system may have roles other than those assessed in this study. It has been suggested that the innervation extends the range of autoregulation in vivo. Sympathetic innervation has been claimed to be protective against stroke and hypertension in animals, possibly reflecting a neurotrophic role, particularly during development.

Acknowledgment

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References


The functional significance of sympathetic innervation in regulating vascular tone is dependent on multiple factors, including density of innervation, synaptic cleft distance, receptor type, receptor population and characteristics, and coupling mechanisms. These factors are markedly variable among species and from region to region within the same species. In the cerebral circulation of animal models, dense sympathetic innervation is found in arteries at the base of the brain. The density of the innervation decreases gradually with a decrease in arterial diameter and disappears in the small pial arteries. Accordingly, the pial arteries are very weak or unresponsive upon stimulation of sympathetic nerves. On the other hand, pial veins from some species, such as the pig, receive denser sympathetic innervation and are more sensitive to norepinephrine (NE) than pial arteries.

In the study reported here, Bevan and colleagues demonstrated that freshly obtained human pial arteries, like those of experimental animals, receive sparse adrenergic/sympathetic innervation. These authors further demonstrated that, compared with human middle meningeal and superficial temporal arteries, human pial arteries were only marginally responsive to transmural nerve stimulation and exogenous NE. Bevan et al also examined the mechanisms responsible for weak reactivity to adrenergic/sympathetic nerve stimulation. Pial artery responses to transmural nerve stimulation were not affected by propranolol, inhibitors of nitric oxide synthase and cyclooxygenase, or endothelial denudation. Results from histochemical and ultrastructural studies demonstrated that pial adrenergic nerve density was sparse, and synaptic cleft distances were largely compared with middle meningeal and superficial temporal arteries. Results of this structural and functional correlation study suggest that the weak neurogenic response of adult human pial arteries reflects their poor innervation and responsiveness to NE, implying that these features are not important in the regulation of the arteries’ diameters. This study extends a variety of findings on sympathetic control of pial arterial tone made in experimental animals to the human cerebral circulation.

The conclusion given by the authors on the functional significance of pial artery sympathetic nerves is logical. These authors imply that NE released from the sympathetic nerves is ineffective in directly causing constriction of the smooth muscle. Recently, it has been shown that NE released from the adrenergic nerves can elicit release of nitric oxide from the neighboring nitric oxidergic nerves,2 suggesting that transmitter NE can indirectly affect the pial arterial tone. This possibility, however, cannot be concluded from results of the Bevan et al study in human pial arteries since the presence of nitric oxidergic or other autonomic innervation in these arteries was not evaluated. The exact functional significance of the pial artery sympathetic innervation in humans remains to be determined. Since isolated pial arteries were used in this study, the results of these findings may be more reasonable in suggesting that the perivascular sympathetic innervation in pial arteries is not important in direct regulation of its diameter.

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References
Weakness of Sympathetic Neural Control of Human Pial Compared With Superficial Temporal Arteries Reflects Low Innervation Density and Poor Sympathetic Responsiveness

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