The effects of electrical field stimulation (EFS) of rabbit middle cerebral arteries were examined using wire-mounted arterial segments. EFS of segments maintained at rest tension caused a tetrodotoxin-sensitive sympathetic contraction. In agonist-contracted segments maintained at approximately 60% of tissue maximum force, EFS caused a relaxation in two thirds of the preparations. Maximum response (mean±SEM) was 33±3.5% of maximal relaxation. The EFS relaxation was tetrodotoxin-sensitive but was not blocked by either chronic surgical sympathectomy or exposure to guanethidine (5 µM). Electron microscopy of chromaffin-fixed arterial sections showed the presence of chromaffin-positive large and small vesicles. Within the same sheath of Schwann were also a smaller number of nerve profiles containing many small clear vesicles. Removal of the vascular endothelium or treatment with atropine (10 nM) eliminated the EFS relaxation in approximately 50% of the segments and reduced the response in another 35–40%; in the remainder, relaxation was unaffected. Combined data for endothelium removal and atropine treatment showed that each caused a significant (p<0.01) reduction in the EFS relaxation. Atropine also significantly reduced EFS relaxation in guanethidine-treated segments. There was no reduction in EFS relaxation after procedures that antagonized ATP- or substance P-mediated relaxations. These results indicate that EFS of precontracted rabbit middle cerebral artery causes a neurogenic nonadrenergic relaxation. The neuroeffector mechanism mediating this response has a predominantly cholinergic endothelium-dependent component as well as a noncholinergic endothelium-independent component. (Circulation Research 1992;70:1104–1112)

**KEY WORDS** • cerebral artery • neurotransmitter • cholinergic mechanisms • endothelium

The responses of isolated cerebral arteries to electrical field stimulation (EFS) of their perivascular nerves are highly species dependent. Rabbit arteries, for example, exhibit a contractile response,1 and in the cat, a decrease in vascular wall force is the predominant effect.2 Dog and sheep arteries display a combination of contraction and relaxation.3

Cerebral arteries receive several types of innervation that may be associated with neurotransgenic responses. In addition to adrenergic and cholinergic innervation,4 cerebrovascular nerves contain peptide transmitters such as neuropeptide Y,5 vasoactive intestinal peptide,6 substance P,7 and calcitonin gene–related peptide.8

Although the EFS-mediated relaxation of cerebral arteries has been investigated in many species, there are only a few reports that have implicated specific neuroeffector systems in this response.9 In the cat, for example, there is evidence that vasoactive intestinal peptide and calcitonin gene–related peptide are present in perivascular nerves and may be neurotransmitters for the EFS relaxation.10,11 The cholinergic innervation of cerebral arteries has been described for several species on the basis of choline acetyltransferase activity and [3H]choline uptake.12,13 EFS relaxations in these species, however, are atropine resistant,2,3 although part of the response in the cat may be mediated by acetylcholine (ACh).14

In the rabbit basilar artery, the EFS response is mainly contraction with only a small, inconsistent relaxation component.2 EFS of the rabbit middle cerebral artery also results in contraction,15 but we have found the EFS-mediated relaxation of this artery to be more substantial than that of the basilar artery. We report here that in the middle cerebral artery a nonadrenergic neurogenic EFS relaxation occurs in the majority of segments and that it is mediated in most instances by an endothelium-dependent cholinergic mechanism.

**Materials and Methods**

New Zealand White rabbits were injected with heparin (1,000 units/kg i.v.) and sodium pentobarbital (30 mg/kg i.v.) and killed by exsanguination. The brain was removed and placed in ice-cold physiological salt solution, and the middle cerebral artery was dissected free from the brain surface. The physiological salt solution was composed of (mM) NaCl 130.0, KCl 4.7, KH₂PO₄...
1.18, MgSO₄ 1.17, NaHCO₃ 14.9, EDTA 0.026, CaCl₂ 1.6, and dextrose 11 and was aerated with 95% O₂-5% CO₂. Segments of the proximal end of the middle cerebral artery approximately 1.2 mm in length were mounted on 34-μm tungsten wires and placed in small vessel myographs maintained at 37°C. Tissues were allowed to equilibrate for 1 hour, during which time the buffer was changed every 20 minutes. Preliminary active length–force determinations using histamine established 100 mg as optimal rest tension. Tissue maximal responses were determined by the addition of histamine (100 μM) and KCl (89 mM). Vascular endothelium was removed by gentle rubbing with wire or by treatment with the α-toxin of Staphylococcus aureus (0.3 μg/ml) for 1 minute. The completeness of endothelial removal was assessed by loss of response to ACh (1 μM) and confirmed by scanning electron microscopy (see below). Endothelium-denuded vessels were routinely tested with sodium nitroprusside (10 μM) to ensure their relaxation capacity; only those arteries that responded to the directly acting vasodilator were included in this study. Experiments with these vessels were conducted without guanethidine to test EFS contractile responses after endothelium removal. Antagonists were incubated with guanethidine-treated arterial segments for 20 minutes before testing, and guanethidine was incubated for 1 hour. Tissues were field-stimulated with pulses from a stimulator (model S48, Grass Instrument Co., Quincy, Mass.) using platinum electrodes that were mounted in the myograph parallel to the long axis of the segment and separated from it by 0.5–1.0 mm. EFS responses were determined using biphasic square pulses at voltages just below (1 V) breakthrough, at a frequency of 16 Hz and a pulse width of 0.3 msec. Breakthrough voltage was defined as the lowest voltage producing contraction in the presence of tetrodotoxin (3×10⁻⁷ M). For relaxation experiments, tissues were routinely precontracted with histamine; experiments were also carried out using KCl (33 mM), L-norepinephrine (10 μM), or serotonin (10 μM).

**Surgical Denervation**

Surgery was performed under sterile conditions on 28-day-old rabbits anesthetized with a mixture of ketamine hydrochloride (40 mg/kg) and xylazine hydrochloride (10 mg/kg i.p.). Through a medial incision made in the neck, either the right or left superior cervical ganglion as well as the cervical sympathetic trunk was removed. The incision was closed with sutures, and the rabbit was returned to the animal care facility for 3–4 weeks before the day of the experiment. Completeness of denervation was confirmed by absence of contractile response to EFS and loss of perivascular catecholamine histofluorescence.

**Histofluorescence**

Segments of artery were incubated for 1 hour at room temperature in glyoxylic acid (2%) in phosphate buffer (0.1 M) at pH 7.0. After being rinsed in physiological salt solution, the vessels were mounted on glass slides, dried at room temperature, and then placed in an oven at 100°C for 4 minutes. Tissues were covered with mineral oil and cover slips and viewed using fluorescence microscopy.

**Electron Microscopy**

The chromaffin reaction method of Gibbins was used to visualize catecholamine-containing vesicles. Segments were immersion-fixed in glutaraldehyde (2.5%) and paraformaldehyde (1.5%) in sodium chromate/potassium dichromate (0.1 M), pH 7.2, at 0–4°C for 15 minutes. They were stored overnight in sodium chromate/potassium dichromate (0.2 M), pH 6.0, at 4°C. Postfixation was in OsO₄ (2%) in sodium chromate/potassium dichromate (0.1 M), pH 7.2, at 0–4°C for 1 hour. Tissue samples were then dehydrated in graded alcohols and embedded in EMbed 812 (Electron Microscopic Sciences, Ft. Washington, Pa.). Ultrathin sections were stained with uranyl acetate and lead citrate and examined with an electron microscope (model 100 CX, JEOL U.S.A., Inc., Peabody, Mass.). Some tissues were treated with 5-hydroxydopamine before fixation. Samples were incubated in 5-hydroxydopamine (1 mg/ml in physiological salt solution) for 30 minutes at room temperature and then fixed in glutaraldehyde (2.5%) and paraformaldehyde (2%) in sodium phosphate (0.1 M), pH 7.4, for 2 hours, washed overnight in phosphate buffer (0.1 M) with sucrose (7%), and postfixed with OsO₄ (1%) in phosphate buffer (0.1 M) for 1 hour. The segments were then dehydrated, embedded, sectioned, and stained.

Segments were taken for scanning electron microscopy at the end of functional in vitro experiments and were pinned open, fixed in glutaraldehyde (2.5%) and paraformaldehyde (2%) in phosphate buffer (0.1 M), and postfixed in OsO₄ (2%). They were critical-point−dried and sputter-coated with gold/palladium, and the luminal surface was scanned with a Stereoscan 100 (Cambridge Scientific Computing, Cambridge, Mass.).

**Statistics**

Comparisons of tissue responses before and after treatments were made with the paired t test.

**Drugs**

The following drugs were used: (+)norepinephrine hydrochloride, serotonin creatinine sulfate, atropine sulfate, histamine dihydrochloride, 8-phenylthopelyline, ATP, adenosine, tetrodotoxin, and neuropeptide Y (all from Sigma Chemical Co., St. Louis, Mo.); substance P (BACHEM Bioscience Inc., Philadelphia, Pa.); guanethidine sulfate (CIBA-GEIGY Corp., Summit, N.J.); ketamine hydrochloride (Parke-Davis, Morris Plains, N.J.); and xylazine hydrochloride (Haver-Lockhart, Shawnee, Kan.).

**Results**

**Morphology**

Electron microscopic examination of sections of rabbit middle cerebral artery revealed that neuronal elements were present in the adventitia and that they contained at least two types of nerve profiles (Figure 1). In sections of chromaffin-fixed arteries, the majority of neural varicosities contained profiles having both small and large dense-cored vesicles. The small vesicles were chromaffin positive and had diameters (mean±SEM) of
FIGURE 1. Electron micrograph of chromaffin-fixed section of rabbit middle cerebral artery. Section contains at least two nerve profiles within the same sheath of Schwann: one having chromaffin-positive small granular vesicles (small arrow) as well as large granular vesicles (large arrow), and the other containing small clear vesicles (double arrow). Diameters of the vesicles are as follows: small granular vesicles, 36 nM; large granular vesicles, 64 nM; small clear vesicles, 34 nM. Calibration bar, 0.5 \( \mu \text{m} \).

36±0.7 nm (\( n=23 \)); many of the large vesicles (diameter, 64±1.9 nm; \( n=20 \)) were also chromaffin positive. Similar nerve profiles were seen in tissues that had been treated with 5-hydroxydopamine, an electron-dense false transmitter. Also present and within the same sheath of Schwann were profiles containing many small clear vesicles whose diameter was 34±0.9 nm (\( n=20 \)). Profiles containing clear vesicles represented approximately 13% of the profiles observed.

EFS Relaxation Response

EFS of segments of rabbit middle cerebral artery without active wall tone produced a tetrodotoxin-sensitive contraction (see Figures 2 and 3). In segments precontracted with histamine (0.5–5 \( \mu \text{M} \)), EFS caused a tetrodotoxin-sensitive relaxation in approximately two thirds of the segments tested (\( n=46 \)) (see Figure 4 for example). Segments were precontracted to approximately 60% of maximum tissue contraction (mean±SEM, 59.8±3.2%; range, 32–90%). The mean maximal relaxation response was 33±3% of maximum relaxation (range, 5–82%; \( n=30 \)). There was considerable variability in the response, which ranged from small transient relaxations (Figure 2) to responses of considerably longer duration (Figure 5). The presence of EFS relaxation was dependent on the agent used to precontract the vessel: tissues were routinely contracted with histamine, and EFS relaxations of comparable levels were observed in segments contracted with norepinephrine (\( n=10 \)) and serotonin (\( n=3 \)) but were not present in those contracted with KCl (\( n=9 \)).

Treatment of the middle cerebral artery with guanethidine (5 \( \mu \text{M} \)), which eliminates EFS contractions,\(^{13} \) caused a significant increase in EFS relaxation (Figure 6, Table 1). In addition to an increased maximum EFS relaxation, in many instances guanethidine

FIGURE 2. Effects of chronic surgical sympathectomy on electrical field stimulation–mediated responses of rabbit middle cerebral artery. Recordings show responses from one rabbit that had undergone unilateral removal of the superior cervical ganglion 21 days before the experiment. Responses on the left side of the figure show electrical field stimulation–mediated responses of vessels at rest tension, and those on the right are responses after contraction with serotonin (SHT; 0.1 \( \mu \text{M} \)). Electrical field stimulation was applied at the indicated voltages; its duration is indicated by bars. On the intact side, the response of the precontracted segment to electrical field stimulation was a transient relaxation followed by contraction. In comparison to the intact side, denervation eliminated the contractile response, but a prominent relaxation was displayed.
prolonged the duration of the relaxation. This effect, however, was difficult to quantify (Figure 4). Chronic superior cervical ganglionectomy also eliminated EFS contraction but not EFS relaxation (Figure 2). Exposure to the purinergic antagonist 8-phenyltheophylline (10 μM) at a concentration that antagonized the relaxations produced by adenosine (10 μM) or ATP (10 μM) did not antagonize the EFS relaxation (n=6).

In a group of 10 vessels, removal of the vascular endothelium caused a significant reduction in EFS relaxation (Table 1, Figure 3). Examination of the individual responses showed that the EFS relaxation was completely eliminated in five of the 10 preparations; in four of the remainder, the response was reduced; and in one, there was no effect. Completeness of endothelium removal was judged on the basis of elimination of ACh (1 μM)–mediated relaxation and the absence of endothelial cells on the vascular lumen as determined with scanning electron microscopy. Endothelium removal did not diminish the contractile response to histamine (1 μM) (Table 1).

Exposure to the muscarinic antagonist atropine (10 nM) had variable effects on the EFS relaxation (Figure 5): of 20 segments tested, atropine blocked the relaxation response in 10 (50%), reduced it in seven (35%), and had no effect in three (15%) (Figures 5A, 7, and 8B). Combined results for these segments showed the atropine effect to be significant (Figure 7, Table 1). This concentration of atropine blocked the response to a concentration of ACh (1 μM) that caused relaxations considerably greater than the EFS relaxation. Atropine (10 nM) did not affect the responses to the vasorelaxing agents substance P or adenosine. Significant antagonism of EFS relaxation by atropine (10 nM) was also seen in the presence of guanethidine (5 μM) (Table 1, Figures 5 and 7). The antagonism of EFS relaxation by atropine was reversible: EFS and ACh responses both returned after washout of atropine (data not shown). In one set of experiments, the effects of both endothelium removal and exposure to atropine on EFS relaxation were tested (n=3). The tissues responded in parallel to both treatments; i.e., the treatments eliminated the EFS relaxation in two preparations and caused similar reductions in response in the other.

The possibility that substance P might be involved in the EFS relaxation was tested by desensitizing the vascular smooth muscle cells to substance P. Tissues were exposed to high concentrations of substance P (0.1 μM) for a period of 5 minutes, after which time the tissue became refractory to its further addition. This procedure did not diminish either the atropine-sensitive or atropine-insensitive EFS relaxations (n=6) (Figure 5B).

Treatment with atropine (10 nM) also caused enhanced EFS-mediated contractions (Figure 8). To determine if this enhancement was due to the elimination of coincident relaxation or to other factors, experiments were carried out using segments that displayed either atropine-insensitive EFS relaxations or no EFS relaxation. In these segments, atropine significantly enhanced the EFS-mediated contraction (Table 1).

**Discussion**

The experiments described in this paper demonstrate that EFS causes relaxation of agonist-contracted segments of rabbit middle cerebral artery. This response is tetrodotoxin sensitive, indicating its neural basis. It is not blocked by either chronic sympathetic denervation or the adrenergic neuronal-blocking agent guanethidine, demonstrating its nonsympathetic and nonadrenergic nature. The EFS relaxation was not present in all segments, and approximately one third of the preparations did not display a relaxation. The absence of relaxation was seen in some vessels even after elimina-
tion of the EFS contraction component with guanethidine. The mean relaxation response of untreated vessels for pulse trains of 1 minute at 16 Hz was 33% of maximum relaxation; however, responses as great as 80% were observed. The voltages used in these experiments were determined for the contractile response so the possibility exists that they were suboptimal for relaxation.

The response to field stimulation reflects activation of opposing systems, both of which display considerable variability. In some cases, the relaxation response is obscured or overwhelmed by the contractile response, and only small transient relaxations are observed. However, on denervation, either through ganglionectomy or guanethidine, the full relaxation response is obtained. In the untreated state, both transient relaxations and relaxations of longer duration were equally represented. Although guanethidine increased EFS relaxation, there were still some vessels that did not respond to EFS. There are no apparent reasons for an absence of response; however, the variability observed in these tissues suggests a basis for the variation in neural control.

The data indicate that there is an endothelium-dependent cholinergic component to the EFS relaxation. Atropine and endothelium removal separately caused significant decreases in EFS relaxation. In roughly half of the preparations, the EFS response was eliminated by atropine or by endothelium removal. In another third, the response was reduced by both procedures, and only in a small number (=10%) was the relaxation totally resistant to both treatments. The effects of atropine were considered specific since a low concentration (10 nM) was used and since this concentration did not affect responses to other vasodilators. This concentration of atropine is 10-fold greater than either the IC50 of atropine antagonism of [3H]quinuclidinyl benzilate binding to the cholinergic receptors of rabbit ear artery or the IC50 of atropine antagonism of methacholine relaxation of the same artery. Atropine has been shown to have noncholinergic effects such as inhibition of α-adrenoceptor-mediated responses, but these are generally seen when micromolar concentrations are used.20 Endothelium removal proved to be satisfactory; denuded segments did not respond to concentrations of ACh (1 µM) that caused relaxations in excess of the EFS relaxation, and endothelial loss was confirmed in most cases with scanning electron microscopy over the entire intimal surface of the arterial segment. In addition, the contractile response to histamine was not diminished.

The perivascular innervation of cerebral arteries contains a number of substances that can cause vasorelaxation. These include ACh, ATP, vasoactive intestinal peptide, calcitonin gene–related peptide, and substance
TABLE 1. Effects of Pharmacological Treatments and Endothelium Removal on Electrical Field Stimulation and Agonist Responses

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Treatment</th>
<th>n</th>
<th>Control</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>EFS relaxation (%)</td>
<td>Guanethidine</td>
<td>10</td>
<td>13.6±5.2</td>
<td>24.9±5.8*</td>
</tr>
<tr>
<td></td>
<td>Endothelium</td>
<td>10</td>
<td>32.4±4.8</td>
<td>8.9±3.3†</td>
</tr>
<tr>
<td></td>
<td>removal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Atropine</td>
<td>20</td>
<td>42.8±3.4</td>
<td>10.9±3.0†</td>
</tr>
<tr>
<td></td>
<td>Atropine+guanethidine</td>
<td>9</td>
<td>38.4±4.6</td>
<td>14.0±5.4*</td>
</tr>
<tr>
<td>Histamine contraction (%)</td>
<td>Endothelium removal</td>
<td>7</td>
<td>74.7±6.2</td>
<td>70.7±4.3‡</td>
</tr>
<tr>
<td>EFS contraction (%)</td>
<td>Atropine</td>
<td>10</td>
<td>8.5±1.8</td>
<td>23.3±3.0†</td>
</tr>
</tbody>
</table>

Values are mean±SEM. n, Number of arterial segments; EFS, electrical field stimulation. Relaxation responses are expressed as percent of maximum relaxation, and contractions are expressed as percent of tissue maximum contraction. Concentrations are as follows: atropine, 10 nM; guanethidine, 5 μM; histamine, 1 μM.

*p<0.05, †p<0.01, ‡p=NS vs. control arterial segments.

Since our findings indicate a considerable endothelium-dependent component to the EFS relaxation, the participation of the endothelium-dependent transmitters ACh, substance P, or ATP seemed most likely. Resistance of the EFS response to substance P desensitization or ATP antagonism indicates that these putative transmitters probably do not participate in EFS relaxation in the rabbit middle cerebral artery. Since the response was predominantly atropine sensitive, our findings are highly suggestive that cholinergic nerves are involved in the EFS relaxation.

The cerebral circulations of several species, including rabbit, receive cholinergic innervation, as determined by choline acetyltransferase activity and [3H]choline uptake.6,12,13,24 The levels of choline acetyltransferase, [3H]choline uptake and release, and ACh content in the rabbit are comparable to those of the cat and dog6,12,13; however, the role of cholinergic nerves in this species has not been assessed. Our results indicate that the relaxation neuroeffector mechanism in the rabbit may be similar to that of the cat—a mechanism comprised of both atropine-sensitive and -insensitive components.14 Other studies of EFS relaxations in the cat, as well as those in the sheep and dog, however, have shown the relaxation mechanism to be atropine resistant,23,25 The reasons for the discrepancies are not clear; however, the influence of noncholinergic dilator systems may be more prominent in these preparations (see below).

The conclusion that the dilator innervation in the rabbit is cholinergic is also supported by the results of ultrastructural studies in which varicosities with small clear vesicles were found. The presence of small clear vesicles is often associated with cholinergic transmission but cannot be taken as conclusive evidence for this mechanism.20 Previous studies have described small clear vesicles in rat, rabbit, and cat cerebrovascular nerves and have shown that, after chemical or surgical sympathectomy, they remained intact, whereas the vesicles within adrenergic nerves degenerated.27-29 These findings are consistent with our results that showed that surgical sympathectomy or guanethidine did not affect the EFS-mediated relaxation but did eliminate contraction. The presence of cholinergic innervation is supported by in vivo studies in rabbits showing that intravenous physostigmine but not neostigmine causes atropine-sensitive increases in cerebral blood flow.30

ACh-mediated relaxations of arteries, including resistance vessels, are mostly endothelium dependent.31 However, there are exceptions such as the ACh-mediated relaxation of the cat auricular artery, which is endothelium independent.32 It has been postulated that neurogenic vasodilation must involve endothelium-independent mechanisms since it is unlikely that sufficient quantities of an endothelium-dependent transmitter would be able to diffuse from the site of neural release (the adventitial-medial border of the artery) to the endothelium.33 This would be especially true for a short-lived labile substance such as ACh. Impediments to the diffusion of ACh would include several layers of smooth muscle, connective tissue, and tissue choline esterases. Contrary to this reasoning, however, are studies of arteries, including cerebral arteries, showing that topical ACh is capable of effecting endothelium-dependent dilatation34,35 and that in perfused rabbit middle cerebral artery extraluminally applied ACh can cause relaxation (J. Garcia-Roldan and J.A. Bevan, unpublished observations). Cerebral arteries may be well suited for endothelium-dependent neural responses since they are relatively thin walled. The rabbit middle cerebral artery consists of two to four layers of smooth muscle cells, whereas a peripheral artery of comparable diameter, e.g., the ear artery, contains four to eight layers of muscle (J.A. Bevan and J. Dodge, unpublished observations).36 The possibility exists then that, in a thin-walled artery, neurally released ACh could traverse the vascular

![Figure 7](http://circres.ahajournals.org/)

**Figure 7.** Bar graph showing effects of atropine on electrical field stimulation–mediated relaxation of rabbit middle cerebral artery. Effects are expressed as a percentage (mean±SEM) of maximal relaxation. Atropine reduced electrical field stimulation–mediated relaxation either by itself or in the presence of guanethidine. For atropine, n=20; for guanethidine, n=9. *p<0.05 and **p<0.01.
smooth muscle and effect an endothelium-mediated response.

Thus, these data support the presence of a cholinergic nerve mechanism mediating relaxation of cerebral arteries. Although the cholinergic innervation of cerebral arteries has been well described, only in one other tissue, the rat gastric mucosa, is there evidence of functional involvement of the endothelium. Whether or not these findings are unique to these particular vessels awaits further investigation; however, the participation of cholinergic nerves in the control of blood flow is supported by these results. Our findings indicate that there are atropine-sensitive and -insensitive components as well as endothelium-dependent and -independent components to the neurovasodilator response. These components could exist in four possible combinations and are presented in Table 2 along with possible mediators for each combination. Although there are experimental findings consistent with each combination, several of our observations suggest that the EFS relaxation comprises an atropine-sensitive endothelium-dependent component and an atropine-insensitive endothelium-independent component. First, there is little evidence that the other two combinations participate in the response. The endothelium-dependent atropine-resistant transmitters substance P and ATP are not involved, and there was no evidence of an ACh-mediated relaxation in endothelium-denuded segments. Second, the effects of atropine and endothelium removal paralleled each other in a group of experimental preparations exposed to both treatments. And third, the distribution of effects of atropine and endothelium removal are highly similar. If one of the other combinations from Table 2 were to be active, the individual distributions would not be similar. The similarity of distribution suggests that the treatments were affecting the same target, the cholinergic receptors on the vascular endothelium, without affecting the noncholinergic mechanism on the vascular smooth muscle.

The nerves and transmitters associated with the noncholinergic endothelium-independent component are unknown. There is evidence that the neurogenic relaxation of cat cerebral vessels is mediated by such endothelium-independent peptide transmitters as vasoactive intestinal peptide and calcitonin gene-related peptide. Although these transmitters could play a role in rabbit arterial responses, they are found in rabbit cerebral vessels, at levels lower than reported for other species.

Another concern pertinent to this investigation is that EFS-mediated vasorelaxation may result from nonneurogenic factors such as the "electrogenic" production of oxygen radicals or chlorine gas by the stimulating electrodes or vascular smooth muscle membrane hyperpolarization. Several aspects of our findings argue against the participation of these factors in our results: the response is prompt, it can be eliminated by endothelium removal, and it is tetrodotoxin sensitive. The EFS relaxations described in the above reports do not share these characteristics.

Our results also indicated that treatment with atropine enhanced EFS-mediated contractions. This enhancement may have been simply due to the elimination of coincident EFS relaxation; however, enhancement was seen in segments displaying no EFS relaxation as well as in those that were atropine resistant. In these situations the effects of atropine would be independent of the postjunctional mechanism and thus might result

**TABLE 2. Possible Combinations of Neural and Endothelial Components Involved in Electrical Field Stimulation–Mediated Relaxation, Including Examples of Possible Neurotransmitters**

<table>
<thead>
<tr>
<th>Component</th>
<th>Endothelium dependent</th>
<th>Endothelium independent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atropine sensitive</td>
<td>ACh-receptors on endothelium</td>
<td>ACh-receptors on vascular SM</td>
</tr>
<tr>
<td>Atropine insensitive</td>
<td>Substance P, ATP</td>
<td>VIP, CGRP</td>
</tr>
</tbody>
</table>

ACh, acetylcholine; SM, smooth muscle; VIP, vasoactive intestinal polypeptide; CGRP, calcitonin gene–related peptide.
from prejunctional actions. Atropine blockade of muscarinic prejunctional inhibitory sites on sympathetic nerves would lead to the enhanced release of constrictor transmitters. Our findings are consistent with the observation that exogenous ACh diminishes the neurally mediated release of ^3H^norepinephrine from prelabeled rabbit basilar artery.43

The response of the isolated rabbit middle cerebral artery to EFS comprises several different components. EFS causes the release of norepinephrine and neuropeptide Y from adrenergic nerves, and these transmitters occupy both postjunctional sites, causing contraction, and inhibitory prejunctional sites, resulting in decreased transmitter release.15,44 The present results demonstrate that EFS also causes a relaxation mediated by cholinergic and noncholinergic mechanisms, with the former appearing to be the predominant component. There is also evidence that the constrictor mechanism may be subject to prejunctional cholinergic inhibition. EFS of isolated arteries activates all neural systems and results in a complex and, not surprisingly, variable response. Through various methods, however, each of the components of the overall response can be described in the absence of other complicating mechanisms. When so handled, sizable responses, both contractile and relaxing, have been observed. These findings suggest that in vivo, when simultaneous activation of all perivascular neuroeffector systems does not occur as it does with EFS of isolated preparations, single neural pathways may significantly affect smooth muscle tone of the middle cerebral artery.

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Electrical field stimulation-mediated relaxation of rabbit middle cerebral artery. Evidence of a cholinergic endothelium-dependent component.

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