Functional Role of Nerve-Derived Nitric Oxide in Isolated Dog Ophthalmic Arteries

Noboru Toda, Yoshihiko Kitamura, and Tomio Okamura

Purpose. To determine if nitric oxide (NO) is involved in the relaxant response to nerve stimulation by nicotine and electrical pulses in dog external ophthalmic arteries (EOA) and internal ophthalmic arteries (IOA).

Methods. Changes in isometric tension were recorded in helical strips of the arteries, and the presence of perivascular nerve containing NADPH diaphorase was histochemically demonstrated.

Results. Nicotine (10^{-4} M, EOA and IOA) and transmural electrical stimulation (5 Hz, EOA) produced a slight or no contraction followed by a moderate relaxation in the strips contracted with prostaglandin F2a. The contraction was abolished by a-adrenoceptor antagonists. The relaxation was abolished and the contraction was potentiated by NG-nitro-L-arginine (L-NA), a nitric oxide (NO) synthase inhibitor; the relaxation was reversed by L-arginine. Contractile response in L-NA-treated EOA was greater than in the IOA, and the relaxation was less in nontreated EOA. NO-induced relaxation and norepinephrine-induced contraction were not influenced by L-NA. There were plenty of nerve fibers visualized by NADPH diaphorase staining method in the adventitia of EOA and IOA, indicating the presence of NO synthase-containing nerves.

Conclusions. The neurogenic relaxation appears to be mediated by NO released from the vasodilator nerve in EOA and IOA. There is a reciprocal innervation in vasodilator nitroergic and vasoconstrictor noradrenergic nerves; functionally, the latter is more predominant in EOA than in IOA. Invest Ophthalmol Vis Sci. 1995; 36:563-570.

Circulating blood in the eye and neighboring tissues in the dog is supplied by internal (IOA) and external ophthalmic arteries (EOA) that originate from the internal and external carotid arteries, respectively.1 The ophthalmic arteries are located extracranially; however, many branches of the internal carotid artery distribute to intracranial tissues. Although ophthalmic or retinal arteries have been considered to share functional characteristics with intracranial arteries, the supportive evidence is actually lacking. Our earlier articles indicate that acetylcholine produces only a slight relaxation that is endothelium independent.2-4 Substance P elicits an endothelium-dependent relaxation in isolated ophthalmic and cerebral arteries, whereas distinct actions of histamine and mechanisms of its action are observed in these arteries.2,7

We have reported evidence supporting the hypothesis that nitric oxide (NO) acts as a neurotransmitter of perivascular nerve innervating the dog cerebral artery that is responsible for vasodilatation10-11; the nerve is called “nitroergic.”12 Characteristic features of cerebral vasculature consist in a paucity of adrenergic neural and a-adrenoceptor functions, and an evident nitroergic nerve function.12 Little information is available concerning adrenergic innervation in ophthalmic arteries, whereas functional importance in nonadrenergic, noncholinergic nerves innervating the ophthalmic arterial wall has not been determined, except for an abstract16 reported during submission of our article to this journal. To know the neurogenic control of blood flow in ocular tissues, it is important to determine functional roles of autonomic innervation in ophthalmic vasculature in vitro in which the detailed analysis of neural function can be performed.
The present study was undertaken to elucidate the response to nerve stimulation by nicotine or electrical pulses in isolated dog ophthalmic arteries, to analyze the mechanism of neurally induced responses, and to compare the functional role of autonomic nerves in IOA and EOA. The responses to nerve stimulation in these arteries were compared with those in dog cerebral arteries previously demonstrated.17

METHODS

Preparations of Isolated Ophthalmic Arteries

All experimental procedures that used animals conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Thirty-eight mongrel dogs of either sex, weighing 7 to 13 kg, were anesthetized with intraperitoneal injections of sodium pentobarbital (30 mg/kg) and were killed by bleeding from the carotid arteries. The eyeballs attached with optic nerves and extraocular tissues were rapidly removed from the orbital cavities; EOA (0.4 to 0.7 mm outside diameter) and IOA (0.3 to 0.6 mm) were isolated. Both arteries were cut into helical strips approximately 10 mm long. The specimens were vertically fixed between hooks in a muscle bath containing the modified Ringer-Locke solution maintained at 37°C ± 0.3°C and aerated with a mixture of 95% O2 and 5% CO2. The hook anchoring the upper end of the strip was connected to the lever of a force-displacement transducer (Nihonkohden Kogyo, Tokyo, Japan). Resting tensions were adjusted to 0.7 g for the EOA and 0.5 g for the IOA, which are optimal for the EOA of the posterior ciliary artery previously demonstrated.17

Tension Recording

Isometric contractions and relaxations were displayed on an ink-writing oscillograph. The contractile response to 30 mM K+ was first obtained, and the arterial strips were repeatedly washed with fresh media and equilibrated. Only one strip per dog per individual type of experiment was used. The concentration–response curves for nicotine (5 × 10^-6 to 5 × 10^-4 M) were obtained by applying a single concentration in each series to avoid tachyphylaxis. The EOA and IOA strips were partially contracted with prostaglandin (PG) F2a (10^-7 to 3 × 10^-6 M); the contraction ranged between 22% and 30% of the contraction induced by 30 mM K+. Except for the determination of concentration–response curve, 10^-4 M of nicotine was used to analyze the mechanism underlying the responses. At the end of each experiment, papaverine (10^-4 M) was added to obtain the maximal relaxation. Relaxations and contractions induced by test drugs were presented as relative values to the relaxation caused by 10^-4 M papaverine and the contraction caused by 30 mM K+, respectively. Some of the EOA strips were placed between platinum electrodes to stimulate nerve terminals transmurally by the application of electrical square pulses of 0.2-msec duration at 5 Hz for 40 seconds.19 The strips had been exposed for 20 to 30 minutes to blocking agents before the responses to agonists or electrical stimulation were obtained. Responses to nicotine and NO were compared in the EOA and IOA obtained from the same dogs. In some experiments, responses to nicotine were compared in the IOA strips with and without the endothelium obtained from the same dogs. The endothelium was removed by gently rubbing the intimal surface with a cotton ball. The endothelial function was determined from the relaxant response to substance P (10^-8 M).2

Histology

Isolated EOA and IOA were fixed in 0.1 M phosphate-buffered saline (PBS), containing 0.3% glutaraldehyde and 4% paraformaldehyde and then postfixed in 1% osmium tetroxide (Sigma Chemical, St. Louis, MO), and 0.3% Triton X-100 at 37°C. The period of incubation (30 to 60 minutes) was determined by staining intensity. The reaction was terminated by washing the sections in 0.1 M PBS. The slide-mounted tissue sections were stained with the NADPH-diazophase staining method, incubating the sections with 0.1 M PBS at pH 8.0, containing 1 mM NADPH (Kohjin, Tokyo, Japan), 2 mM nitro blue tetrazolium (Sigma Chemical, St. Louis, MO), and 0.3% Triton X-100 at 37°C. The period of incubation (30 to 60 minutes) was determined by staining intensity. The reaction was terminated by washing the sections in 0.1 M PBS. Counterstain with eosin was followed. The sections were air dried and coverslipped with Entellan (Merck, Darmstadt, Germany). A histochemical control experiment, in which NADPH was excluded from the reaction mixture, gave no positive staining.

Statistics and Drugs

The results shown in the text and figures were expressed as mean values ± standard error. Statistical analyses were made using the Student’s paired and unpaired t-tests for two groups and the Tukey’s method after one-way analysis of variance for more than three groups. Drugs used were nicotine, L- and D-arginine, yohimbine hydrochloride (Nacalai Tesque, Kyoto, Japan), atropine sulfate (Tanabe Seiyaku, Osaka, Japan), hexamethonium bromide (Yama-
nouchi, Tokyo, Japan), indomethacin (Sigma Chemical, St. Louis, MO), timolol hydrochloride (Banyu, Tokyo, Japan), N\(^{-}\)nitro-L-arginine (L-NA), N\(^{-}\)nitro-D-arginine (D-NA), substance P (Peptide Institute, Minoh, Japan), prazosin hydrochloride (Pfizer, Tokyo, Japan), dl-norepinephrine hydrochloride, tetrodotoxin (Sankyo, Tokyo, Japan), nitroglycerin (Nihon-Kayaku, Tokyo, Japan), prostaglandin (PG) \(\text{F}_2\alpha\) (Toray, Tokyo, Japan), and papaverine hydrochloride (Dainippon, Osaka, Japan). Responses to NO were obtained by adding the NaNO\(_2\) solution adjusted at pH 2.9.

RESULTS

Effect of Nicotine

The addition of nicotine produced a transient contraction followed by a relaxation or only a relaxation in the strips of EOA and IOA partially contracted with PGF\(_{2\alpha}\), whereas no response was induced under resting conditions. The contraction induced was abolished by combined treatment with prazosin (10\(^{-6}\) M) and yohimbine (10\(^{-6}\) M). Concentration–relaxation curves of nicotine in these arterial strips thus treated are shown in Figure 1. There was no significant difference in the responses of EOA and IOA. The maximal relaxation was attained at 10\(^{-4}\) M. The response at this concentration was consistent and reproducible; therefore, a concentration of 10\(^{-4}\) M was used to analyze the mechanism of action in the remainder of this study.

Nicotine (10\(^{-4}\) M) elicited a contraction followed by a relaxation in 6 out of 14 EOA strips from separate dogs and only a relaxation in the remaining 8 strips contracted with PGF\(_{2\alpha}\). Treatment with 10\(^{-5}\) M L-NA markedly potentiated the contraction, and abolished the relaxation (Fig. 2, left). All the treated strips (\(n = 14\)) responded to nicotine with contractions. Typical tracings of the response are illustrated in Figure 3. The addition of L-arginine (10\(^{-5}\) M) antagonized the effects of L-NA. In only 3 out of 14 IOA strips did nicotine produce slight contraction followed by relaxation, and in the remaining strips only relaxation was obtained. The contraction was increased, and the relaxation was almost abolished by treatment with L-NA (Fig. 2 right and Fig. 3). The contraction was seen in 10 out of 14 treated strips. The addition of L-arginine suppressed the contraction that had been potentiated by L-NA and restored the relaxation to a level seen under control conditions. In contrast, L-NA did not alter the relaxant response to NO (3 x 10\(^{-7}\) M) (Fig. 3); mean values of the response in control media and those containing L-NA and L-arginine in EOA were 62.0 \(\pm\) 3.9, 65.8 \(\pm\) 4.3, and 58.8 \(\pm\) 3.5\% (\(n = 9\)), respectively, and those in IOA were 57.8 \(\pm\) 3.3, 61.8 \(\pm\) 4.3, and 59.0 \(\pm\) 3.4\% (\(n = 9\)), respectively. Constrictions by nicotine in EOA and IOA in control media (8.4\% \(\pm\) 4.1\% and 2.4\% \(\pm\) 1.6\%, respectively, \(N = 14\)) and those containing L-NA and L-arginine (5.3\% \(\pm\) 3.8\% and 1.2\% \(\pm\) 0.8\%, \(n = 14\)) did not significantly differ, whereas the difference in contractions of EOA and IOA treated with L-NA (53.3\% \(\pm\) 3.2\% and 19.3\% \(\pm\) 6.4\%, \(n = 14\)) was statistically significant (\(P < 0.001\)). The relaxation of EOA in control media (54.1\% \(\pm\) 4.4\%, \(n = 14\)) was significantly less (\(P < 0.05\)) than that of IOA (65.1\% \(\pm\) 1.6\%, \(n = 14\)).

The nicotine-induced relaxation was not altered by 10\(^{-6}\) M indomethacin in 13 EOA and 13 IOA strips. The responses to nicotine were abolished by 10\(^{-5}\) M hexamethonium in all the treated EOA and IOA strips.

To determine the participation of endothelial and adrenergic neural functions in potentiating or inhibiting the nicotine-induced relaxation, the experiment was carried out in five IOA strips with the intact and denuded endothelium obtained from the same dogs treated with prazosin (10\(^{-6}\) M) and yohimbine (10\(^{-6}\) M).
FIGURE 2. Modification by L-NA (10^{-5} M) and L-arginine (L-arg., 10^{-3} M) of the responses to nicotine in EOA and IOA strips contracted with PGF_2α. Nicotine produced biphasic responses, a slight contraction followed by a relaxation, in some preparations as indicated in the text. Therefore, the contraction is indicated as plus, and the relaxation is indicated as minus. Contractions induced by 30 mM K^+ were taken as 100% contraction, and relaxations by 10^{-4} M papaverine were taken as 100% relaxation. Significantly different from control (C) and the value with L-NA and L-arginine, *P < 0.01 (Tukey’s method). EOA = external ophthalmic arteries; IOA = internal ophthalmic arteries.

FIGURE 3. Typical tracings of the response to nicotine (10^{-4} M) and NO (3 × 10^{-7} M) of an EOA and IOA before and after treatment with L-NA (10^{-5} M) and L-arginine (L-arg., 10^{-3} M). The strips were partially contracted with 5 × 10^{-7} M PGF_2α. PA = 10^{-4} M papaverine that produced the maximal relaxation. EOA = external ophthalmic arteries; IOA = internal ophthalmic arteries.

FIGURE 4. Typical tracings of the response to nicotine (10^{-4} M) and NO (3 × 10^{-7} M) of an EOA and IOA before and after treatment with L-NA (10^{-5} M) and L-arginine (L-arg., 10^{-3} M). The strips were partially contracted with 5 × 10^{-7} M PGF_2α. PA = 10^{-4} M papaverine that produced the maximal relaxation. EOA = external ophthalmic arteries; IOA = internal ophthalmic arteries.

TABLE 1. Modification by L-NA and L-arginine of the Relaxant Response to Nicotine in IOA Strips

<table>
<thead>
<tr>
<th>Relaxation (%)</th>
<th>n</th>
<th>E-Intact</th>
<th>E-Removed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotine 10^{-4} M</td>
<td>5</td>
<td>63.2 ± 5.4</td>
<td>68.2 ± 2.8</td>
</tr>
<tr>
<td>L-NA</td>
<td>5</td>
<td>9.0 ± 2.5*</td>
<td>6.4 ± 2.1*</td>
</tr>
<tr>
<td>L-NA + L-arginine</td>
<td>5</td>
<td>60.6 ± 7.7</td>
<td>65.0 ± 6.2</td>
</tr>
<tr>
<td>Substance P 10^{-8} M</td>
<td>5</td>
<td>57.2 ± 5.2</td>
<td>7.6 ± 2.6†</td>
</tr>
</tbody>
</table>

The strips with intact and denuded endothelium (E) were treated with prazosin (10^{-6} M) and yohimbine (10^{-7} M). Concentrations of L-NA and L-arginine = 10^{-5} and 10^{-3} M, respectively. Significantly different from control values (no treatment) and those with L-NA + L-arginine, *P < 0.01 (Tukey’s method). Significantly different from the value in strips with intact endothelium, †P < 0.001 (unpaired test). n = Number of strips from different dogs. The strips with and without the endothelium were obtained from the same dogs.

Effect of Transmural Electrical Stimulation

In EOA strips, transmural electrical stimulation at 5 Hz produced a relaxation preceded by a slight contraction (n = 3) or by no contraction (n = 4). Electrical neural stimulation was verified by abolishment of the response by treatment with 3 × 10^{-7} M tetrodotoxin (n = 7). Typical responses are demonstrated in Figure 4. Treatment with 10^{-5} M L-NA potentiated the contraction and suppressed the relaxation. L-arginine (10^{-3} M) reversed the responses. Quantitative data are shown in Figure 5. D-NA (10^{-3} M) did not alter the response (n = 3), and D-arginine (10^{-3} M) did not antagonize the effect of L-NA (n = 3). Relaxations induced by electrical stimulation were not influenced by 10^{-5} M indomethacin (15.3% ± 2.5% versus 15.7% ± 2.4%, n = 10) nor by 10^{-5} M atropine (16.3% ± 3.1% versus 18.1% ± 3.3%, n = 7) and 10^{-7} M timolol (15.7% ± 4.2% versus 16.6% ± 3.2%, n = 3) (Fig. 4). Contractions seen in L-NA-treated strips were abolished by combined treatment with 10^{-6} M prazosin and 10^{-6} M yohimbine (n = 5).
**Effects of Nitroglycerin and Norepinephrine**

Nitroglycerin in concentrations ranging from 10^{-10} to 10^{-6} M produced a dose-related relaxation in EOA and IOA strips contracted with PGF2α (Fig. 6). At 10^{-8} M, the agent produced a significantly greater response in EOA than in IOA.

Norepinephrine (2 × 10^{-8} to 5 × 10^{-5} M) produced a concentration-dependent contraction in EOA and IOA. Mean values of the median effective concentrations in these arteries were [10.7 ± 1.8] × 10^{-7} M (n = 7) and [9.5 ± 1.4] × 10^{-7} M (n = 5), respectively (Fig. 7). The amine-induced contraction was not significantly influenced by treatment with 10^{-5} M L-NA.

**Histology**

Nerve fibers and bundles containing NADPH diaphorase are demonstrated in the adventitia of the EOA and IOA (Fig. 8). Similar results were also obtained in two more EOA and one more IOA.

**DISCUSSION**

The present study revealed that nicotine produced a transient contraction followed by a moderate relaxation or only a relaxation in EOA and IOA strips contracted with PGF2α. The responses were abolished by hexamethonium. The contraction caused by nicotine and transmural electrical stimulation was abolished by combined treatment with prazosin, a selective α1 adrenoceptor antagonist, and yohimbine, an α2 antagonist, suggesting the involvement of α1 and α2 receptors. The contractile response under treatment with L-NA was significantly greater in EOA than in IOA (53.3% versus 19.3%), whereas the maximal contractions induced by and the median effective concentration of nicotine in EOA were also effective in suppressing or reversing the relaxant response to transmural electrical stimulation in EOA and IOA. The NO-induced relaxation was not inhibited by the NO synthase inhibitor. The abolition of relaxation by nicotine is not due to increased contraction by L-NA because L-NA was effective to a similar extent in the control strips and in those treated with antagonists. Our accumulated data on dog cerebral arteries responding to nicotine and electrical stimulation with L-NA-sensitive relaxations indicate that NOx (nitroxy compounds) is released by the chemical or electrical nerve stimulation from superfused, endothelium-denuded arterial strips, and the cyclic GMP content is increased in the tissue. These findings are consistent with the hypothesis that EOA and IOA are innervated by vasodilator nerves that liberate NO upon excitation as a neurotransmitter. Nerve fibers and bundles containing NADPH diaphorase strongly suggest the presence of perivascular nerves containing NO synthase (present study).

Adrenergic vasoconstrictor innervation in EOA and IOA was suggested from pharmacologic evidence. Reasons for the combined use of prazosin and yohimbine in this study were that norepinephrine-induced contractions were inhibited by prazosin or yohimbine, and treatment with prazosin alone did not always abolish the nicotine-induced contraction (unpublished data, 1994). A nonselective α-receptor antagonist phentolamine in concentrations of 10^{-6} M or higher has been reported to inhibit the response to nicotine by the mechanism distinct from adrenoceptor blockade.

Treatment with L-NA potentiated the nicotine-induced contraction or reversed the relaxation to a contraction in all EOA used and in most of IOA (10 out of 14 strips). The contractile response under treatment with L-NA was significantly greater in EOA than in IOA (53.3% versus 19.3%), whereas the maximal contractions induced by and the median effective concentration of nicotine were also effective in suppressing or reversing the relaxant response to transmural electrical stimulation in EOA and IOA. The NO-induced relaxation was not inhibited by the NO synthase inhibitor. The abolition of relaxation by nicotine is not due to increased contraction by L-NA because L-NA was effective to a similar extent in the control strips and in those treated with antagonists. Our accumulated data on dog cerebral arteries responding to nicotine and electrical stimulation with L-NA-sensitive relaxations indicate that NOx (nitroxy compounds) is released by the chemical or electrical nerve stimulation from superfused, endothelium-denuded arterial strips, and the cyclic GMP content is increased in the tissue. These findings are consistent with the hypothesis that EOA and IOA are innervated by vasodilator nerves that liberate NO upon excitation as a neurotransmitter. Nerve fibers and bundles containing NADPH diaphorase strongly suggest the presence of perivascular nerves containing NO synthase (present study).

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centrations of exogenous norepinephrine did not significantly differ in these arteries, suggesting that adrenergic neural function in EOA predominates over the function in IOA. This potentiation does not seem to be associated with the increased responsiveness to norepinephrine because the response to exogenous norepinephrine did not differ in control and L-NA-treated EOA and IOA strips. Inability of L-NA to increase the amine release from adrenergic nerves is demonstrated in dog mesenteric and temporal arteries previously exposed to \(^{3}H\)-norepinephrine, in which \(^{3}H\)-overflow evoked by adrenergic nerve stimulation is measured.26,27 Therefore, the potentiation appears to be derived from a suppression by L-NA of the counteracting relaxant action of nicotine.

Nicotine elicited a greater relaxation in IOA than in EOA (65.1% versus 54.1%) under control conditions but relaxed these arteries treated with \(\alpha\) blockers to a similar extent (67.7% versus 65.4% in Fig. 1). The relaxation caused by NO did not significantly differ in these arteries (57.8% versus 62.0%). These findings

![Figure 5](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933409/)

**Figure 5.** Modification by L-NA (10\(^{-5}\) M) and L-arginine (L-arg., 10\(^{-6}\) M) of the responses to transmural electrical stimulation at 5 Hz in EOA strips contracted with PGF\(_{2}\alpha\). Contractile responses (plus) followed by relaxations (minus) were presented as relative values to K\(^{+}\) (30 mM)-induced contraction and papaverine (10\(^{-4}\) M)-induced relaxation, respectively. Significantly different from control (C) and value with L-NA and L-arginine, *\(P < 0.01\) (Tukey's method). There were seven strips from separate dogs. Vertical bars = SE.

![Figure 6](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933409/)

**Figure 6.** Cumulative concentration–relaxation curves of nitroglycerin in EOA and IOA strips contracted with PGF\(_{2}\alpha\). Relaxations induced by 10\(^{-4}\) M papaverine were taken as 100%. Significantly different from the value in EOA, *\(P < 0.05\) (unpaired t-test). Numbers in parentheses indicate the number of strips from separate dogs. Vertical bars = SE.

EOA = external ophthalmic arteries; IOA = internal ophthalmic arteries.

![Figure 7](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933409/)

**Figure 7.** Concentration–contraction response curves of norepinephrine in EOA and IOA before and after treatment with 10\(^{-5}\) M L-NA. The ordinate represents the amine-induced contraction relative to that caused by 30 mM K\(^{+}\). Numbers in parentheses indicate the number of strips from separate dogs. Vertical bars represent SE. EOA = external ophthalmic arteries; IOA = internal ophthalmic arteries.
suggest that functioning of vasodilator nerve stimulated by the same concentration of nicotine is identical in IOA and EOA; however, adrenergic neurogenic vasconstriction attenuates the relaxation due to nerve-derived NO in EOA. Nitroglycerin liberates NO intra-cellularly in the presence of sulfhydryl compounds, which activates soluble guanylate cyclase and produces cyclic GMP for relaxation, as does NO liberated from vasodilator nerves. IOA responded to nitroglycerin with a relaxation to a lesser extent than EOA (57.7% versus 77.0% at $10^{-8}$ M). Although a greater response to this compound of the proximal coronary artery than the distal artery is suggested to result from the different availability of sulfhydryl compounds for NO production, whether such is true in the ophthalmic arteries could not be determined.

According to Seligsohn and Bill, L-NA methylester injected intravenously causes a reduction in uveal blood flow of anesthetized rabbits, which is more pronounced on the sympathetically intact side than the denervated side. Physiologic antagonism of vasoconstrictor norepinephrine and vasodilator NO is possible, although it was not determined that the NO involved is derived from the endothelium, nerves, or another source. Our previous study indicates a greater involvement of NO derived from the vasodilator nerve than from the endothelium in diluting basilar arteries in anesthetized dogs. Facial nerve stimulation increases uveal blood flow in anesthetized rabbits, and the effect is not abolished by cholinergic blockade.

Although the authors speculated the participation of vasoactive intestinal polypeptideergic nerve, this is not the case in isolated dog EOA and IOA because the neurogenic relaxation in our study was abolished solely by NO synthase inhibition, and the vasoactive intestinal polypeptide-induced relaxation was not influenced by L-NA (unpublished data, 1994). The non-cholinergic vasodilatation in the rabbit eye may have been elicited by NO derived from the nerve. This is a first demonstration of reciprocal innervation in EOA and IOA by nitroxynergic and noradrenergic nerves. As far as the concentrations of nicotine ($5 \times 10^{-6}$ to $5 \times 10^{-4}$ M) and the frequency of electrical stimulation (5 Hz) used are concerned, the vasodilator nerve is functionally more evident than the vasoconstrictor nerve, as seen in dog, monkey, and human cerebral arteries. In contrast, in other arteries—including mesenteric, temporal, and saphenous arteries (unpublished data, 1994)—although reciprocally innervated, vasoconstrictor nerve function is markedly greater, and the response to vasodilator nerve stimulation is observed only when the vasoconstriction is suppressed by $\alpha$ blockade. Vasodilator nerves that liberate NO as a neurotransmitter may play an important role in the regulation of blood supply through EOA and IOA to ocular and neighboring tissues as has been speculated for the brain.

**Key Words**
nitric oxide, nicotine, transmural electrical stimulation, ophthalmic artery, innervation

**References**

5. Onoue H, Nakamura N, Toda N. Endothelium-dependent and independent responses to vasodilators of