Evidence for nitroxidergic innervation in monkey ophthalmic arteries in vivo and in vitro

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Ayajiki, Kazuhide, Toshiki Tanaka, Tomio Okamura, and Noboru Toda. Evidence for nitroxidergic innervation in monkey ophthalmic arteries in vivo and in vitro. Am J Physiol Heart Circ Physiol 279: H2006–H2012, 2000.—In anesthetized monkeys, electrical stimulation (ES) of the pterygopalatine or geniculate ganglion dilated the ipsilateral ophthalmic artery (OA). The induced vasodilatation was unaffected by phentolamine but potentiated by atropine. Intravenous N-nitro-L-arginine (L-NNA) abolished the response, which was restored by L-arginine. Hexamethonium-abolished vasodilator responses induced solely by geniculate ganglionic stimulation. The L-NNA constricted OA; L-arginine reversed the effect. Destruction of the pterygopalatine ganglion constricted the ipsilateral artery. Helical strips of OA isolated under deep anesthesia from monkeys, denuded of endothelium, responded to transmural ES with relaxations, which were abolished by tetrodotoxin and L-NNA but were potentiated by atropine. It is concluded that neurogenic vasodilatation of monkey OA is mediated by nerve-derived nitric oxide (NO), and the nerve is originated from the ipsilateral pterygopalatine ganglion that is innervated by cholinergic neurons from the brain stem via the geniculate ganglion. The OA appears to be dilated by mediation of NO continuously liberated from nerves that receive tonic discharges from the vasomotor center. Acetylcholine liberated from postganglionic cholinergic nerves would impair the release of neurogenic NO.

Nitric oxide; pterygopalatine ganglion; denervation of vasodilator nerve

Ocular circulation plays an important role in maintaining the functions of neural, retinal, and other ocular tissues. The tissues responsible for controlling ocular pressure seem to be particularly sensitive to circulatory disturbance (8). Vascular tone in the eye is regulated by autonomic neural and humoral factors, as that in other organs and tissues. Sympathetic nerves are involved in the ocular vasoconstriction and increased vascular resistance, mainly by mediation of neurogenic norepinephrine and also by neuropeptide Y (17) or ATP (33). However, the roles of parasympathetic nerves in dilating vasculature in the eye were not determined until recently.

The discovery of vasodilator innervation in dog and monkey cerebral arteries, in which nitric oxide (NO) acts as a neurotransmitter (28, 29), prompted us to reinvestigate autonomic innervation in ocular arteries. Functional and histological evidence of NO-mediated vasodilator nerves (nitroxidergic; see Ref. 31) have been reported in retinal, posterior ciliary, and ophthalmic arteries from monkeys (35, 36), dogs (25–27, 33), pigs (34), cattle (37), cats (10), and humans (13). In an earlier study (25), we have demonstrated that denervation of the pterygopalatine ganglion by injections of ethanol abolishes the perivascular neurons containing NO synthase in the ipsilateral middle cerebral arteries and the vasodilator response to electrical nerve stimulation of the isolated canine arteries. This denervation also abolishes the nicotine-induced relaxation of central retinal arteries (25), which is mediated by nerve-derived NO (30). Therefore, it is hypothesized that nitroxidergic neurons innervating the retinal artery are delivered from the parasympathetic, pterygopalatine ganglion. However, it awaits the direct evidence for the origin of vasodilator innervation in ocular arteries in vivo.

The present study was aimed to elucidate whether electrical stimulation of the pterygopalatine and geniculate ganglia dilates the ophthalmic artery of ipsilateral side in anesthetized monkeys and whether damage of the pterygopalatine ganglion constricts the artery for the determination of tonic innervation of this nerve. In addition, it was investigated whether the vasodilator responses to nerve stimulation by electrical pulses of the arteries in vivo and of those isolated from monkeys used for the in vivo study were mediated by NO.

METHODS

The Animal Care and Use Committee at Shiga University of Medical Science approved the use of monkeys in this study. In vivo study. Male and female Japanese monkeys (Macaca fuscata) weighing 6–10 kg were premedicated with intramuscular injections of 15 mg/kg ketamine and anesthetized intravenously with 20 mg/kg thiopental sodium. Stable anesthetic conditions were maintained by additional injections of thiopental as needed. The monkeys were intubated when needed but usually permitted to breathe spontaneously. PO2 and PCO2 were stable during the experiment. Arterial systolic and diastolic pressures were monitored with a pressure transducer (NEC San-ei, Tokyo, Japan) via a catheter in-
inserted into the left femoral artery. The monkeys’ body temperature was maintained at 37°C on a heated operating table. To make the right pterygopalatine ganglion visible, the zygomatic arch and underlying muscles were excised during microsurgery. Special care was taken to minimize bleeding when we reached deep into the fossa pterygopalatina. To reach the geniculate ganglion, a postauricular incision was made and the external auditory canal was cut and anteriorly retracted. After the temporal bone was removed with a cutting burr, the facial nerve was cut at the stylomastoid foramen and exposed along its course from the foramen to the geniculate ganglion. With the aid of a surgical microscope, a fine bipolar concentric stimulating electrode was inserted into the pterygopalatine or geniculate ganglion, and the electrode was then fixed by dental cement. The ganglion was stimulated by electrical pulses (1 ms duration and frequencies of 2, 5, and 10 Hz to the geniculate and 10 Hz to the pterygopalatine ganglion with 10 V intensity for 15 s; 5 s after the start of stimulation, the contrast medium for angiography was injected. Transfemoral internal carotid angiography was performed with a digital subtraction angiography system (DFA-3–30, Hitachi Medical, Tokyo, Japan) at the same magnification throughout the experiment. The contrast medium iopamidol (Iopamiron, 2 ml, Schering, Germany) was injected by an autoinjector (Anionatom 6600, Liebel-Flarsheim) connected to the angiography system in each angiography. The data obtained were stored in a digital data recorder (Hitachi Medical). The ophthalmic artery diameter was measured by the use of image analyzer included in the angiography system at two selected points. The two values were averaged, and the results were expressed as a percentage of the control artery diameter obtained before the electrical stimulation or the application of test drugs. In the preliminary study, diameters were measured six times with 1-h intervals; the mean values of the six measurements were 0.795 ± 0.026, 0.792 ± 0.027, 0.792 ± 0.027, 0.795 ± 0.026, 0.801 ± 0.019, and 0.810 ± 0.020 mm, respectively (n = 5). In the control series, arterial diameters before, during, and 10 min after the ganglionic stimulation were measured by angiography. The effects of treatment for 20 min with atropine and then phentolamine on the response to pterygopalatine ganglionic stimulation were determined. Except where otherwise mentioned, the studies were performed on monkeys treated with atropine and phentolamine. Modifications by N^\text{G}\text{-nitro-L-arginine (L-NAME), L-arginine, and hexamethonium were compared in vasodilator responses to stimulation of the pterygopalatine and geniculate ganglia. In the experimental series, the arterial diameter was measured 30 and 60 min after intravenous L-NAME, 20 min after L-arginine, or 10 min after the damage of the ganglion by electric cautery.

In vitro study. The monkeys from the in vivo study were placed under deep thiopental-induced anesthesia and were then euthanized by bleeding from carotid arteries. The eyes were removed and the eyeballs attached with optic nerves and extraocular tissues were then removed and stored overnight at 4°C. The ophthalmic arteries (0.5−0.7 mm outside diameter) were isolated and cut into helical strips ~15 mm in length, from which the endothelium was removed by gently rubbing the intimal surface with a cotton ball. The specimens were vertically fixed between hooks in a muscle bath (20 ml capacity) containing the modified Ringer-Locke solution, which was maintained at 37 ± 0.3°C and aerated with a mixture of 95% O₂-5% CO₂. The hook anchoring the upper end of the strips was connected to the lever of a force-displacement transducer (Nihon-Kohden Kogyo, Tokyo, Japan). The strips were placed between stimulating electrodes, and electrical pulses of 0.2 ms at a frequency of 5 Hz for 40 s were transmurally applied to stimulate perivascular nerves. Under these stimulus conditions, submaximal and reproducible relaxant responses were induced in monkey retinal arteries (33). The resting tension was adjusted to 1.0 g, which was optimal for inducing the maximal contraction. The composition of the bathing solution was as follows (in mM): 120 NaCl, 5.5 KCl, 2.2 CaCl₂, 1.0 MgCl₂, 25.0 NaHCO₃, and 5.6 dextrose. The pH of the solution was 7.38−7.44.

Isometric mechanical responses were measured on an ink-wrting oscillograph (Nihon-Kohde Kogyo). The contractile response to 30 mM K⁺ was first obtained, and the arterial strips were repeatedly washed with fresh media and equilibrated. The strips were partially contracted with prostaglandin F₂₀ (0.5 to 3 × 10⁻⁶ M). Nicotine (10⁻⁴ M) and NO (acetylated NaNO₂ solution) in one or two concentrations (10⁻⁷ and 10⁻⁹ M) were applied successively. Transmural electrical stimulation was applied every 10 min. At the end of each series of experiments, papaverine (10⁻⁶ M) was added to attain the maximal relaxation, which was taken as 100% for the relaxation induced by agonists or nerve stimulation.

Statistics and drugs used. The results shown in the text and figures are expressed as means ± SE. Statistical analyses were made using the Student’s paired and unpaired t-tests and the Tukey’s test after one-way analysis of variance. Drugs used were L-NAME, NG-nitro-L-arginine (L-NAME) (Peptide Institute, Minoh, Japan), L-arginine, nicotinamide, hexamethionium bromide (Nacalai Tesque, Kyoto, Japan), acetylcholine chloride (Daichi Pharmaceutical, Tokyo, Japan), atropine sulfate (Tanabe, Osaka, Japan), physostigmine (eserine) sulfate (Sigma, St. Louis, MO), phentolamine mesylate (Novartis Japan, Tsukuba, Japan), prazosin hydrochloride (Wako, Osaka, Japan), prostaglandin F₂₀ (Pharmacy-Upjohn, Tokyo, Japan), tetrodotoxin (Sankyo, Tokyo, Japan) and papaverine hydrochloride (Dainippon, Osaka, Japan). 1H[1,2,4]oxadiazolol[4,3-c]quinolxalin-1-one (ODQ) was a generous gift from Prof. S. Moncada (University College London, London, UK). Responses to NO were obtained by adding NaNO₂ solution, adjusted at pH 2 (6), just before the application, and the concentrations of NaNO₂ in the bathing media were expressed as those of NO.

RESULTS

In vivo study. In anesthetized monkeys, electrical stimulation of the right pterygopalatine ganglion at a frequency of 10 Hz for 15 s dilated the ipsilateral ophthalmic artery, as shown in Fig. 1. The diameter of the artery and the response to electrical stimulation were not influenced by treatment with phentolamine (1 mg/kg iv, n = 4). Atropine (1 mg/kg iv) did not alter the arterial diameter under control conditions (0.804 ± 0.044 vs. 0.814 ± 0.042 mm, n = 5) but potentiated the response to nerve stimulation from 11.0 ± 1.8 to 13.6 ± 2.2% (24.6 ± 5.2% increase, n = 5, P < 0.01, paired t-test). Atropine did not affect mean blood pressure (82.1 ± 4.3 vs. 82.9 ± 4.6 mmHg, n = 5, P > 0.05, paired t-test) and heart rate (152.6 ± 17.8 vs. 164 ± 21.7 beats/min, n = 5). Additional treatment with phentolamine (1 mg/kg iv) failed to alter the arterial diameter under basal conditions (0.789 ± 0.030 vs. 0.801 ± 0.023 mm, n = 5) and failed to alter the responses to nerve stimulation (16.4 ± 1.0 vs. 15.0 ± 1.2%, n = 5, P > 0.05). Phentolamine significantly decreased blood pressure from 81.3 ± 5.5 to 68.4 ± 3.9
mmHg \((n = 5, P < 0.05, \text{paired } t\text{-test})\) and increased heart rate from 159.3 ± 27.2 to 181 ± 28.2 beats/min \((n = 5, P < 0.05, \text{paired } t\text{-test})\). The remainder of this study was undertaken in the monkeys treated with atropine and phentolamine, unless otherwise mentioned.

The diameter of the ophthalmic artery angiographically measured was increased by electrical nerve stimulation by 16.6 ± 2.2% in eight monkeys compared with that before the nerve stimulation (0.850 ± 0.026 mm). Systemic blood pressure and heart rate were not affected. Hexamethonium (4 mg/kg iv) constricted the artery from 0.850 ± 0.05 to 0.801 ± 0.044 mm (6.2 ± 0.8% decrease, \(n = 5, P < 0.01\)) but did not significantly alter the vasodilator response to pterygopalatine ganglionic stimulation (13.4 ± 1.7 vs. 15.6 ± 3.0%, \(n = 5\)). The response (16.6 ± 2.2%, \(n = 8\)) was abolished by intravenous injections of L-NNA (5 mg/kg) when measured 30 and 60 min later and restored to 13.4 ± 2.0% by L-arginine (500 mg/kg iv) 20 min later. The ophthalmic artery constricted by the injection of L-NNA 30 and 60 min later, and the response was reversed by L-arginine. Typical responses are shown in Fig. 2. The L-NNA significantly increased mean blood pressure (from 59.0 ± 6.2 to 80.5 ± 5.5 mmHg, \(n = 8, P < 0.001, \text{paired } t\text{-test}\)) and decreased heart rate (from 164.6 ± 8.8 to 150.1 ± 10.6 beats/min, \(P < 0.05\)), and the addition of L-arginine restored the blood pressure (to 62.8 ± 6.2 mmHg, \(P < 0.001\) vs. the value with L-NNA) but did not influence heart rate (143.9 ± 12.3 beats/min, \(n = 8, P > 0.05\)). After the artery diameter was restored, the pterygopalatine ganglion was damaged by electrical cauterization, which induced ipsilateral arterial constriction averaging 9.0 ± 1.6% \((n = 5)\) 10 min later. Quantitative data on the effects of L-NNA, L-arginine, and denervation are summarized in Fig. 3. Destruction of the ganglion by cauterization did not affect the blood pressure (from 53.6 ± 5.0 to 53.7 ± 7.1 mmHg, \(n = 5, P > 0.05\)) and heart rate (from 129.2 ± 13.9 to 131.0 ± 14.3 beats/min, \(n = 5, P > 0.05\)).

Electrical stimulation of the geniculate ganglion at 2–10 Hz for 15 s produced frequency-dependent vasodilation. Mean values of the response obtained by 10 Hz stimulation of the pterygopalatine and geniculate ganglia did not differ (16.6 ± 2.2 vs. 19.7 ± 3.2%, \(n = 5\)). The dilator response almost abolished by treatment...
with L-Arg, treatment with L-NNA

treatment with PGF2

denuded of the endothelium and partially contracted

this study. In helical strips of the ophthalmic artery

attached to the optic nerve, was stored overnight for

iment was finished. The eyeballs of nonoperated side,

and were then euthanized by bleeding after the exper-

study were given additional injections of thiopental

nerve stimulation (Fig. 5). Typical responses as af-

Ordinate represents stimulation-induced increments in the arterial

Fig. 4. Frequency-vasodilatation relationship of the ophthalmic ar-

tery in response to geniculate ganglionic stimulation (2, 5, and 10 Hz) before and after L-NNA (5 mg/kg iv) and L-Arg (500 mg/kg iv) in

anesthetized monkeys (n = 4 for 2 and 5 Hz, n = 5 for 10 Hz). Ordinate represents stimulation-induced increments in the arterial

diameter relative to that before the stimulation. Significantly differ-

ent from control, *P < 0.01; significantly different from the value with L-Arg, *P < 0.01 (Tukey’s test). Vertical bars represent means ±

SE. Basal absolute values for control at 2, 5, and 10 Hz are 0.828 ±

0.029 (n = 4), 0.828 ± 0.029 (n = 4), and 0.788 ± 0.045 mm (n = 5), respectively. Basal absolute values under treatment with L-NNA at

2, 5, and 10 Hz are 0.765 ± 0.032 (n = 4), 0.765 ± 0.032 (n = 4), and 0.725 ± 0.047 (n = 5) mm, respectively. Basal absolute values under treatment with L-NNA + L-Arg at 2, 5, and 10 Hz are 0.808 ± 0.043 (n = 4), 0.808 ± 0.043 (n = 4), and 0.766 ± 0.053 (n = 5) mm, respectively.

with L-NNA and restored by L-arginine (Fig. 4). The stimulation-induced vasodilatation was abolished by hexamethonium (1 mg/kg iv, n = 4).

In vitro study. The monkeys used for the in vivo study were given additional injections of thiopental and were then euthanized by bleeding after the exper-

iment was finished. The eyeballs of nonoperated side, attached to the optic nerve, was stored overnight for this study. In helical strips of the ophthalmic artery denuded of the endothelium and partially contracted with PGF2α, transmural electrical stimulation at 5 Hz produced reproducible relaxations, which were abol-

ished by tetrodotoxin (3 × 10⁻³ M). Treatment with L-NNA (10⁻⁶ M) abolished the relaxant response in five strips and reversed to a slight contraction in the remaining three, which was suppressed by treatment with prazosin (10⁻⁶ M). Additional treatment with L-arginine (3 × 10⁻⁴ M) restored the relaxation to nerve stimulation (Fig. 5). Typical responses as af-

ected by L-NNA, D-NNA, L-arginine, and tetrodotoxin

are illustrated in Fig. 6A. The neurogenic relaxation was abolished by treatment with ODQ, a soluble gua-

ylate cyclase inhibitor (10⁻⁶ M, n = 4). Effects of the inhibitors used are quantitatively compared in Fig. 5. D-NNA (10⁻⁶ M) did not affect the relaxation induced by nerve stimulation; mean values before and after the treatment were 21.4 ± 4.9 and 22.8 ± 4.1% (n = 5), respectively.

The stimulation (5 Hz)-induced relaxation was po-

tentiated by atropine (10⁻⁷ M); mean values of the response before and after the treatment were 22.7 ±

3.4 and 29.1 ± 6.6% increase, respectively. On the other hand, the neurogenic

Fig. 5. Quantitative data concerning the effect of L-NNA (10⁻⁶ M), L-Arg (3 × 10⁻⁴ M), and 1H[1,2,4]oxadiazolol[4,3-a]quinoxalin-1-one (ODQ, 10⁻⁶ M) on the relaxant response to 5 Hz transmural electrical stimulation (TES) of ophthalmic arterial strips denuded of the endothelium and partially contracted with PGF₂α. Ordinate represents the response to nerve stimulation relative to that induced by 10⁻⁴ M papaverine. Significantly different from control (C), *P < 0.01; significantly different from the value with L-Arg, *P < 0.01. n = 5 strips from separate monkeys. Vertical bars represent means ±

SE. Basal absolute values for control at 2, 5, and 10 Hz are 0.83 ±

0.03 (n = 4), 0.83 ± 0.03 (n = 4), and 0.79 ± 0.05 mm (n = 5), respectively.

Fig. 6. Relaxant responses to transmural electrical stimulation (5 Hz) of an ophthalmic arterial strip denuded of the endothelium before and after treatment with L-NNA (10⁻⁶ M), L-Arg (3 × 10⁻⁴ M), ODQ (10⁻⁶ M), and tetrodotoxin (TTX, 3 × 10⁻³ M). The strip was partially contacted with PGF₂α. After the first series of experiment was over (A), the preparation was repeatedly rinsed and equili-

brated, and the second series (B) was performed. PA denotes 10⁻⁴ M papaverine that produced the maximal relaxation.
response was attenuated by $10^{-7}$ M eserine from 28.3 ± 4.2 to 18.3 ± 3.4% (36.4 ± 8.1% inhibition, $n = 9$, $P < 0.01$) and also by $10^{-6}$ M acetylcholine (47.0 ± 10.4% inhibition, $n = 3$, $P < 0.05$).

The addition of nicotine ($10^{-4}$ M) and NO (acidified NaNO$_2$ solution, $10^{-7}$ and $10^{-6}$ M) in the arterial strips contracted with PGF$_{2a}$ and treated with prazosin ($10^{-6}$ M) elicited transient relaxations (Fig. 7). The response to nicotine, but not NO, was abolished by $10^{-5}$ M hexamethonium ($n = 3$). Treatment with L-NNA ($10^{-6}$ M) abolished the nicotine-induced relaxation, and L-arginine ($10^{-3}$ M) restored the response (Figs. 7 and 8). The response to NO was unaffected. Relaxations by nicotine (29.6 ± 5.9%, $n = 4$) and NO at $10^{-7}$ and $10^{-6}$ M (22.5 ± 5.3 and 57.0 ± 6.9%, $n = 4$) were abolished by ODQ ($10^{-6}$ M).

**DISCUSSION**

The present study revealed that electrical stimulation of the pterygopalatine ganglion-induced vasodilation of the ipsilateral ophthalmic artery, which was abolished by intravenous injections of L-NNA and restored by L-arginine in anesthetized monkeys. Our previous study (38) has demonstrated the presence of abundant nerve cells, bundles, and fibers containing NO synthase immunoreactivity in monkey pterygopalatine ganglion. The ophthalmic artery isolated from monkeys, in which the vasodilator response had been elicited in vivo by the ganglionic stimulation, responded to transmural electrical stimulation with frequency-related relaxations that were suppressed by L-NNA but not by d-NNA and abolished by ODQ, a guanylate cyclase inhibitor (7). The L-NNA-induced inhibition was reversed by l-arginine. Relaxations induced by nicotine that chemically stimulates perivascular nerve terminals to liberate neurotransmitters (12, 20, 28) were also abolished by L-NNA and ODQ. Similar results with the electrical and chemical stimulation have also been obtained in canine, monkey, porcine, and human cerebral arteries (9, 21, 28, 29) and canine, monkey, and porcine retinal or ciliary arteries (25, 34, 35). In addition, release of NO from isolated, superfused canine cerebral arteries into superfusate in response to transmural electrical stimulation or nicotine (28) and increase in cGMP content in the tissue by nicotine (30) are reportedly abolished by treatment with NO synthase inhibitors and tetrodotoxin (for electrical stimulation) or hexamethonium (for nicotine). These findings support the hypothesis that NO plays a crucial role as a neurotransmitter in the vasodilator nerve innervating monkey ophthalmic arteries as well as cerebral arteries from various mammals (32). In ipsilateral retinal arteries isolated from dogs in which the unilateral pterygopalatine ganglion is degenerated, the relaxant response to nicotine is abolished, whereas the response is unaffected in the contralateral arteries, suggesting the nitroxidergic vasodilator innervation of ocular arteries from the ipsilateral pterygopalatine ganglion in dogs (25). The present study with anesthetized monkeys provided a direct evidence that the same ganglion projects the nitroxidergic nerve to ophthalmic arteries. Influence of anesthetics could not be ruled out in the present study in vivo that was performed only under persistent anesthesia.

Unilateral denervation of the monkey pterygopalatine ganglion decreased the diameter of ipsilateral ophthalmic arteries. In anesthetized dogs and monkeys, intracisternal injections of L-NNA constrict the basilar artery, and the effect is reversed by L-arginine (14, 23). The vasoconstrictor action of L-NNA is significantly
attenuated by treatment with hexamethonium, suggesting that neurogenic NO continuously released under resting conditions is involved in the basilar arterial dilatation (14, 23). These findings strongly suggest that nitrooxidergic tonic discharges from the vasomotor center contribute to the maintenance of ocular and cerebral arterial dilatation and of decreased arterial resistance. In vivo studies on rat, mouse, goat, and pig pial arteries and arterioles or cerebral vascular resistance, the intravenous injection or topical application of NO synthase inhibitors produces vasoconstriction or decreases blood flow (1, 3–5, 18). Suppression of basal release of NO from the endothelium due to the NO synthesis inhibition is considered to be involved in the response. In the present study, L-NNA intravenously injected also produced vasoconstriction (about 16% of the diameter of preinjection control, Fig. 3) of the ophthalmic artery, and L-arginine reversed the action. Cauterization of the pterygopalatine ganglion constricted the artery by an average value of 9%. Therefore, under the experimental conditions used, it is postulated that vasodilatation is partly mediated by NO synthesized from L-arginine in piaevalveous nerve terminals innervating the ophthalmic artery, and the remaining vasodilatation is associated with NO liberated from extraneuronal tissues including the endothelium. One may argue the influence of autoregulation in the change of the arterial diameter, because intravenous administration of L-NNA elevates the blood pressure (present study, 15). However, phentolamine did not change the arterial diameter despite a significant fall of systemic blood pressure, suggesting that auto-regulation in ocular circulation is, if any, minimal under the experimental conditions used. Intracisternal injections of L-NNA in anesthetized monkeys constrict basilar arteries without any change in blood pressure, and the response was blunted under treatment with hexamethonium (14). Therefore, it is concluded that ophthalmic arterial constrictions induced by intravenous L-NNA are associated at least mainly with suppression of nitrooxidergic function, although the auto-regulatory influence cannot completely be excluded.

Stimulation of the geniculate ganglion also dilated the ipsilateral ophthalmic artery to a similar extent to that of the pterygopalatine ganglion. Responses to geniculate ganglionic stimulation, but not those to stimulation of the pterygopalatine ganglion, were abolished by hexamethonium, whereas those to stimulation of these ganglia were suppressed by L-NNA. These findings support the hypothesis that cholinergic preganglionic neurons from the brain stem via the geniculate ganglion and greater petrosal nerve innervate the pterygopalatine ganglion.

Atropine failed to alter the arterial tone under resting conditions but potentiated the response to vasodilator nerve stimulation. In isolated monkey, bovine, and porcine cerebral and ocular arteries (2, 22, 24, 34, 36), relaxations induced by electrical nerve stimulation are attenuated by treatment with acetylcholine and physostigmine but potentiated by atropine. We hypothesized that acetylcholine from cholinergic nerves acts on prejunctional muscarinic receptors, possibly the M2 subtype (24), and inhibits the synthesis and release of NO from vasodilator nerves. This is also true in the case of isolated monkey ophthalmic arteries. In addition, the present study revealed evidence supporting the hypothesis that the prejunctional inhibition by neurogenic acetylcholine is operative in monkey ophthalmic arteries in vivo. The reason why the potentiation by atropine of the ophthalmic arterial response was seen only when the nerve was stimulated may be because the liberation of acetylcholine from cholinergic nerves in vivo under resting conditions is not sufficient to induce prejunctional inhibition.

In conclusion, the monkey ophthalmic artery is innervated in vivo by vasodilator nerve in which NO acts as a neurotransmitter; the nerve is originated from the ipsilateral pterygopalatine ganglion that receives the projection of cholinergic nerves from the brain stem. Our recent study has demonstrated that NO per se, but not stable analogs of NO such as S-nitrosothiols (11), is involved in the neurotransmission in monkey cerebral arteries (16, 19). NO derived from the nerve would participate importantly in the arterial dilatation under resting conditions and also when nitrooxidergic nerves are activated by impulses from the vasomotor center. The nitrooxidergic nerve function appears to be negatively controlled by cholinergic nerve activation.

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