Neurogenic Vasoconstriction as Affected by Cholinergic and Nitroxidergic Nerves in Dog Ciliary and Ophthalmic Arteries

Megumi Toda, Tomio Okamura, Kazuhide Ayajiki, and Noboru Toda

Purpose. To determine the involvement of noradrenergic and other vasoconstrictor nerves in the contraction of ocular arteries and the modification by cholinergic and nitroxidergic nerves of vasoconstrictor nerve function.

Methods. Changes in isometric tension were recorded in helical strips of the canine posterior ciliary and external ophthalmic arteries denuded of the endothelium, which were stimulated by transmurally applied electrical pulses (5 Hz). Vasoconstrictor mediators were analyzed by pharmacological antagonists, such as prazosin, αβ-methylene ATP, a P2X purinoceptor antagonist, and BIBP3226, a neuropeptide Y receptor antagonist.

Results. Transmural electrical stimulation produced contractions that were potentiated by Nω-nitro-l-arginine (L-NA), a nitric oxide (NO) synthase inhibitor. The contraction was partially inhibited by prazosin and abolished by combined treatment with αβ-methylene ATP but was not influenced by BIBP3226. Stimulation-induced contraction was attenuated by physostigmine and potentiated by atropine. Contractions induced by exogenous ATP were reversed to relaxations by αβ-methylene ATP. In the strips treated with L-NA, prazosin, and αβ-methylene ATP, the addition of L-arginine elicited relaxations by nerve stimulation. The ATP-induced relaxation was attenuated by aminophylline, whereas neurogenic relaxation was unaffected.

Conclusions. Ciliary and ophthalmic arterial contractions by nerve stimulation are mediated by norepinephrine and ATP, which stimulate α1-adrenoceptor and P2X purinoceptor, respectively. ATP from the nerve is unlikely involved in vasodilatation. Acetylcholine derived from the nerve impairs the neurogenic contraction, possibly by interfering with the release of vasoconstrictor transmitters, and neurogenic NO also inhibits the contraction postjunctionally by physiological antagonism. (Invest Ophthalmol Vis Sci. 1999;40:1753–1760)

A

utonomic innervation in the ocular vasculature plays important roles in the regulation of vascular tone and blood flow, and disturbances of the neurogenic control may lead to ophthalmic dysfunction (e.g., hypoperfusion of the retina and impairment of aqueous humor circulation, possibly responsible for normal-tension glaucoma).1,2 Classic knowledge of sympathetic and parasympathetic innervations in ocular blood vessels consists mainly in noradrenergic neurogenic vasoconstriction and cholinergic neurogenic vasodilatation. However, recent advances in the research of vascular innervation in the eye3 and the other organs and tissues, including the mesentery, skeletal muscle, heart, brain, and for example, indicate that the other neurotransmitters4 are also involved in the neurogenic vascular control. ATP and neuropeptide Y are postulated to be vasoconstrictors from postganglionic sympathetic nerves,5,6, and nitric oxide (NO), vasoactive intestinal polypeptide, and ATP would be vasodilator neurotransmitters.7–10 Acetylcholine released from cholinergic nerves acts as a vasodilator only when it enables the stimulation of muscarinic receptors in the endothelium, from which the relaxing factor (endothelium-derived relaxing factor, EDRF11) is liberated, or in the adrenergic nerve terminals, responsible for the inhibition of transmitter release.12 We have reported that vasodilatation induced by perivascular nerve stimulation in isolated retinal, ciliary, and ophthalmic arteries from dogs, pigs, and monkeys13–16 is mediated by NO synthesized from L-arginine and released from nerve terminals. However, little is known concerning the mechanisms underlying neurogenic vasoconstriction and the functional interactions between vasoconstrictor and vasodilator nerves in ocular arteries.

Aims of the present study were to determine the involvement of noradrenergic and other vasoconstrictor nerves in the contraction of isolated canine ciliary and external ophthalmic arteries, to compare the responsiveness to nerve stimulation in these arteries, to elucidate modifications by cholinergic and nitroxidergic nerves of vasoconstrictor nerve function, and to investigate whether a substance or substances other than NO, such as ATP, are involved in the neurogenic vasodilatation.

Materials and Methods

Preparation

All experimental procedures that used animals conformed to the ARVO Resolution on the Use of Animals in Ophthalmic and
Vision Research. The institutional review board at our university approved the use of animal blood vessels in this study.

Beagles of either sex, weighing 9 to 13 kg, were anesthetized with intravenous injections of sodium thiopental (30 mg/kg) and were killed by bleeding from the carotid arteries. Eyeballs attached with the optic nerves and extraocular tissues were removed from the orbital cavities. Posterior ciliary and external ophthalmic arteries were isolated and cut into helical strips of approximately 20 mm long. The endothelium was removed by gently rubbing the intimal surface with a cotton ball, and the endothelial denudation was verified by a suppression of the relaxation caused by 10^{-6} M acetylcholine. The specimens were fixed vertically between hooks in a muscle bath (20-ml capacity) containing the modified Ringer-Locke solution maintained at 37°C ± 0.5°C and aerated with a mixture of 95% O2 and 5% CO2. The hook anchoring the upper end of each experiment, papaverine (10^{-2} M) was added to the bathing media, during which time the medium was replaced three times every 10 to 15 minutes.

**Tension Recording**

Isometric contractions and relaxations were recorded on an ink-writing oscillograph. The contraction induced by 30 mM K+ was first obtained, and the arterial strips were repeatedly washed with the fresh media and equilibrated. Only one specimen per dog per individual type of experiment was used. The arteries were partially contracted with prostaglandin (PG) F2a (5 × 10^{-7} to 3 × 10^{-6} M); the contraction ranged between 25% and 40% of the contraction induced by 30 mM K+. 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 absolute values or relative values to the relaxation caused by 10^{-4} M papaverine and the contraction caused by 30 mM K+, respectively. Most of the arteries were placed between platinum electrodes to stimulate nerve terminals transmurally by the application of electrical square pulses of 0.2-msec duration at a frequency of 5 Hz for 40 seconds, which produced submaximal and reproducible responses. The concentration-response curves of norepinephrine (5 × 10^{-8} to 2 × 10^{-6} M) were obtained by cumulatively applying the amine to the bathing media. After responses to agonists or electrical stimulation were stabilized, the strips were treated for 20 to 30 minutes with blocking agents, and then responses to the agonists or electrical stimulation were obtained.

**Histochemical Study**

Tissue blocks containing the posterior ciliary arteries were fixed for 3 hours in ice-cold phosphate-buffered saline (PBS; 0.2 M, pH 7.4) containing 2% paraformaldehyde and were kept in 15% sucrose at 4°C until the next stage. The ciliary artery was dissected out microscopically in ice-cold PBS (0.1 M). NADPH diaphorase staining of whole-mounts was performed by incubating the free-floating arteries with PBS (0.1 M, pH 8.0), containing NADPH (1 mM; Kohjin, Tokyo, Japan), nitro blue tetrazolium (2 mM; Sigma Chemical, St. Louis, MO), and 0.3% (vol/vol) Triton X-100 at 37°C under a dissecting microscope with ×8 magnification. The period of incubation was based on staining intensity. The reaction was terminated by washing the arteries in PBS (0.1 M). After several washouts with distilled water, the whole-mount arteries were air-dried on gelatin/ chrome-alum-coated-glass and covered with a coverslip, using xylene (Entellan; Merck, Darmstadt, Germany). Histochemical control experiments by exclusion of NADPH from the reaction mixture gave no positive staining.

**Statistics and Drugs**

The results shown in the text and figures are expressed as mean ± SEM. Statistical analyses were made using the Student’s paired and unpaired t-tests for two groups and the Tukey’s test after one-way ANOVA for more than three groups. Drugs used were L-arginine, hexamethonium bromide (Nacalai Tesque, Kyoto, Japan), atropine sulfate (Tanabe Seiyaku, Osaka, Japan), a,β-methylene ATP, physostigmine sulfate, amiphyllyline, suramin sodium salt (Sigma), timolol maleate (Banyu, Tokyo, Japan), tetrodotoxin (Sankyo, Tokyo, Japan), prazosin hydrochloride (Wako Pure Chemical Industries, Osaka, Japan), PG F2a (Pharmacia Upjohn, Tokyo, Japan), N^6-nitro-L-arginine (L-NA), N^6-nitro-o-arginine (O-NA), neuropeptide Y (Peptide Institute, Osaka, Japan), acetylcholine chloride (Daichi, Tokyo, Japan), BBIPI3226 [RGF-4(diphenylacetyl)-N-(4-hydroxyphenyl)methyl-L-argininamide] (Peninsula Laboratory, Belmont, CA); and papaverine hydrochloride (Dainippon Pharmaceutical, Osaka, Japan). ODQ, 1H[1,2,4]oxadiazolo-[4,3-a]quinoxalin-1-one, was kindly provided by Salvador Moncada.

**RESULTS**

**Experiments with Ciliary Arteries**

**Vasoconstrictor Nerve.** In canine ciliary artery strips denuded of the endothelium and partially contracted with PG F2a, transmural electrical stimulation at 5 Hz produced a contraction that was abolished by tetrodotoxin (3 × 10^{-6} M). The contraction was moderately attenuated by prazosin (10^{-6} M) and was reversed to a relaxation by combined treatment with a,β-methylene ATP (10^{-6} M), A2A purinoceptor antagonist,17 which was abolished by tetrodotoxin (Fig. 1) or L-NA (10^{-6} M). Similar results were also obtained with an additional two strips. Therefore, the mechanism of contraction induced by electrical stimulation was analyzed in the strips treated with L-NA (10^{-5} M).

Treatment with L-NA potentiated the stimulation-induced contraction from 122 ± 28 mg (n = 11, 25.4% ± 7.7% relative to contraction caused by 30 mM K+) to 162 ± 36 mg (n = 11, 39.1% ± 7.6% increase, P < 0.01, paired t-test). D-NA (10^{-5} M) did not produce the potentiation (136 ± 35 versus 134 ± 31 mg, n = 4). The response in L-NA-treated strips was not influenced by yohimbine (10^{-7} M, n = 3) but was attenuated dose-dependently by 10^{-7} and 10^{-6} M prazosin. Raising the concentration of prazosin to 10^{-6} M did not produce additional inhibition (Fig. 2). Treatment with a,β-methylene ATP (10^{-6} M) abolished the response. No relaxation was induced by these treatments in the strips soaked in L-NA-containing media.
In L-NA-treated strips, the neurogenic contraction was significantly inhibited by physostigmine (10⁻⁵ M; n = 6, 35.4 ± 7.0% reduction; P < 0.01, paired t-test) from 132 ± 28 mg. Treatment with atropine (10⁻⁷ M) antagonized the inhibition (n = 4) or potentiated the contraction in the remaining two (5.4% and 11.1%), compared with the level before the physostigmine application.

** Vasodilator Nerve. ** In the strips treated with prazosin (10⁻⁵ M) and α,β-methylene ATP (10⁻⁶ M) and contracted with PG F₂α (10⁻⁶ M), transmural electrical stimulation induced a relaxation that was not significantly influenced by aminophylline (2 × 10⁻⁵ M, a P₁ purinoceptor antagonist); mean values before and after the treatment were 27.8% ± 4.8% and 26.0% ± 3.7% (95.2% ± 3.6% of control, n = 5), respectively. In these preparations, the response was abolished by L-NA (10⁻⁶ M) and restored by L-arginine (3 × 10⁻⁴ M). The relaxation by electrical stimulation in the strips treated with aminophylline was also abolished by ODQ (10⁻⁶ M; n = 5), a soluble guanylate cyclase inhibitor. Treatment with physostigmine (10⁻⁷ M) and atropine (10⁻⁷ M) did not affect the relaxation (n = 4).

** Postjunctional Purinoceptors and Adrenoceptors. ** In PG F₂α–contracted strips, ATP (10⁻⁶ M) caused a phasic contraction, which was reversed to a relaxation by treatment with α,β-methylene ATP. The relaxation was significantly attenuated by aminophylline (2 × 10⁻⁵ M; Fig. 3) but was unaffected by suramin (3 × 10⁻⁴ M), a nonselective P₃X and P₂V-purinoceptor antagonist. Mean values of ATP (10⁻⁶ M)–induced relaxation before and after suramin were 39.0% ± 4.0% and 44.7% ± 5.3% (n = 6), respectively. Contractions to norepinephrine (2 × 10⁻⁸ to 2 × 10⁻⁶ M) under resting conditions were markedly inhibited by treatment with prazosin (10⁻⁷ and 10⁻⁶ M Fig. 4). L-NA (10⁻⁷ M) did not significantly alter the response to norepinephrine; mean values with 5 × 10⁻⁷ and 2 × 10⁻⁶ M norepinephrine were 180 ± 42 and 420 ± 62 mg (n = 6), respectively, in control media, and those were 176 ± 47 (91.0% ± 8.4% of control) and 419 ± 66 mg (99.3% ± 3.6% of control), respectively, after treatment with L-NA. ATP (10⁻⁶ M)–induced contractions were also unaffected by L-NA (64 ± 19 versus 60 ± 15 mg, n = 5).

** Experiments with External Ophthalmic Arteries **

** Vasoconstrictor and Vasodilator Nerves. ** In PG F₂α–contracted, endothelium-denuded ophthalmic arterial strips, transmural electrical stimulation (5 Hz) elicited a contraction (Fig. 5), which was potentiated by L-NA (10⁻⁵ M), from 163 ± 31 mg (19.7% ± 5.8% of 30 mM K⁺-induced contraction, n = 7) to 312 ± 56 mg (128% ± 30.4% increase, n = 7, P < 0.01, paired t-test). Percentage increase in the response was significantly greater than that in ciliary arteries (P < 0.01, unpaired t-test). Prazosin (10⁻⁶ M) approximately halved the stimulation-induced contraction, and α,β-methylene ATP (10⁻⁶ M) abolished the response (Figs. 5 and 6, left). After the addition of L-arginine (10⁻³ M), electrical stimulation produced a significant relaxation (Figs. 5 and 6, right), which was abolished by tetrodotoxin (3 × 10⁻⁷ M). Atropine (10⁻⁷ M) potentiated the response in prazosin-treated strips (11.2% ± 3.2% increase, n = 4, paired t-test).

** Effects of Acetylcholine and Neuropeptide Y. ** In L-NA–treated strips, the neurogenic contraction was inhibited by physostigmine (10⁻⁷ M) and potentiated by atropine (10⁻⁷ M; Fig. 7). These antagonists did not alter the contraction induced by norepinephrine (n = 5) and ATP (n = 3). Treatment with BBP3226, a neuropeptide Y Y₁-receptor antagonist, at 10⁻⁸ M did not alter the contractile response to nerve stimulation under L-NA treatment; mean values before and after the antagonist were 252 ± 74 and 237 ± 91 mg (85.8% ± 7.0% of control, P > 0.05, n = 5). BBP3226 in the same concentration abolished the contraction induced by exogenous neuropeptide Y at 10⁻⁹ M (n = 4) and markedly suppressed the contraction at 3 × 10⁻⁹ M (79.8% ± 9.2% of control [162 ± 58 mg], P < 0.001, n = 6, paired t-test).

** Histochemical Study **

Figure 8 shows networks of nerve fibers and bundles containing NADPH diaphorase in the whole-mount preparation of a ciliary artery. Similar findings were also observed in an additional two preparations from separate dogs.

** DISCUSSION **

Canine-isolated posterior ciliary and external ophthalmic arteries contracted in response to electrical nerve stimulation, and the response was potentiated by L-NA, a NO synthase inhibitor, but not influenced by the D-enantiomer. L-NA did not increase the contractile response to exogenous...
norepinephrine and ATP (present study) or the release of 
$^3$H-overflow by adrenergic nerve stimulation from super-
fused canine temporal arteries previously exposed to $^3$H-
norepinephrine.\textsuperscript{22} Relaxations induced by transmural elec-
trical stimulation of canine retinal and ophthalmic arteries
are reportedly mediated by NO released from perivascular
nerves,\textsuperscript{13,14} and the reciprocal innervation of adrenergic
vasoconstrictor and nitroxidergic vasodilator nerves is pos-
tulated.\textsuperscript{23} Dense networks of neurons containing NADPH
diaphorase, reported to be identical to NO synthase in
neural tissues,\textsuperscript{24} were histochemically determined in dog
ciliary arteries. These findings led us to conclude that the
potentiation by L-NA of the stimulation-induced contraction
is due to an elimination of counteracting action of NO rather
than a potentiation by L-NA of the response to norepineph-
rine and ATP or an increase in the release of the amine from
the nerve. Potentiation by L-NA of the neurogenic contraction
was significantly greater in the sympathetic trunk than in
the ciliary artery (128\% versus 39.1\%), suggesting that the
NO-mediated vasodilatation more effectively blunts the con-

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig2.png}
\caption{Modifications by prazosin (PZ, $10^{-7}$ to $10^{-5}$ M) and $\alpha$, $\beta$-methylene ($\alpha$, $\beta$mATP, $10^{-6}$ M) of the contractile response to trans-
mural electrical stimulation at 5 Hz of ciliary arterial strips treated with
L-NA ($10^{-5}$ M). The arteries were partially contracted with PG $F_2\alpha$.
Numbers in parentheses indicate the number of strips from separate
dogs. Significantly different from control (C), $^aP < 0.01$; significantly
different from the value with $10^{-7}$ M PZ, $^bP < 0.01$, $^cP < 0.05$;
significantly different from the value with $\alpha$, $\beta$mATP, $^dP < 0.01$
(Tukey’s test). Percentages shown in the columns are relative values to
control. Significantly different from control, $^eP < 0.01$; significantly
different from the value with $10^{-7}$ M prazosin, $^fP < 0.01$ (paired
$t$-test). Vertical bars represent SEM.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig3.png}
\caption{Modifications by $\alpha$, $\beta$-methylene ATP ($\alpha$, $\beta$mATP, $10^{-6}$ M) and $\alpha$, $\beta$-methylene ATP + aminophylline (AP; $2 \times 10^{-5}$ M) of the
contractile response to ATP ($10^{-6}$ M) of ciliary arterial strips con-
tracted with PG $F_2\alpha$. Contractions relative to those induced by 30 mM
K$^+$ were taken as 100\% contraction, and relaxations relative to those
by $10^{-4}$ M papaverine were taken as 100\% relaxation. Significantly
different from the value with $\alpha$, $\beta$mATP, $^aP < 0.05$ (unpaired
$t$-test), $^bP < 0.001$ (paired $t$-test). $^c$“n” denotes the number of strips from
separate dogs. Vertical bars represent SEM. C, control.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig4.png}
\caption{Concentration-response curves of norepinephrine before
and after treatment with prazosin (PZ, $10^{-7}$ and $10^{-6}$ M) in ciliary
artery strips. Contractions induced by $2 \times 10^{-6}$ M norepinephrine in
control media were taken as 100\%. Significantly different from corre-
sponding values in the control media, $^aP < 0.01$ (unpaired
$t$-test). $^b$“n” denotes the number of strips from separate dogs. Vertical bars repre-
sent SEM.}
\end{figure}
traction to electrical nerve stimulation in the ophthalmic arteries.

In the L-NA–treated ciliary arterial strips, stimulation-induced contractions were reduced by prazosin in a dose-dependent manner, and the maximal inhibition was attained at $10^{-6}$ M. The prazosin-resistant contraction in the ciliary and ophthalmic arteries was abolished by $\alpha,\beta$-methylene ATP, a $P_{2X}$-purinoceptor antagonist. Contractions by exogenously applied norepinephrine were markedly inhibited by prazosin at $10^{-7}$ M, and those by exogenous ATP ($10^{-6}$ M) was reversed to relaxations by the $P_{2X}$-purinoceptor antagonist. Therefore, the neurogenic contraction seems to be associated with stimulation of $\alpha_1$-adrenoceptors by norepinephrine and also of $P_{2X}$-purinoceptors.

**FIGURE 5.** Modifications by prazosin (PZ; $10^{-6}$ M) and $\alpha,\beta$-methylene ATP ($\alpha,\beta$mATP, $10^{-6}$ M) of the contraction induced by transmural electrical stimulation (TES; 5 Hz) in external ophthalmic artery strips (left) and by l-arginine (L-Arg.; $10^{-5}$ M) of the response to the electrical stimulation in the strips treated with prazosin, $\alpha,\beta$-methylene ATP, and L-NA ($10^{-5}$ M; right). The arteries were contracted with PG F$_{2\alpha}$. In the left panel, the ordinate represents the absolute value of contraction; percentages shown in the columns indicate the relative value to control (C). In the right panel, the ordinate represents relaxations relative to those induced by $10^{-4}$ M papaverine. Significantly different from control, $^aP < 0.01$; significantly different from the value with prazosin, $^bP < 0.01$ (Tukey’s test). Significantly different from control, $^cP < 0.01$ (unpaired t-test), $^dP < 0.001$ (paired t-test). Numbers in parentheses indicate the number of strips from separate dogs. Vertical bars represent SEM.

**FIGURE 6.** Tracing of the response to transmural electrical stimulation (5 Hz) of an external ophthalmic artery strip before and after L-NA ($10^{-5}$ M), prazosin ($10^{-6}$ M), atropine ($10^{-7}$ M), $\alpha,\beta$-methylene ATP ($\alpha,\beta$mATP; $10^{-6}$ M), l-arginine ($10^{-5}$ M), and tetrodotoxin (TTX; $3 \times 10^{-7}$ M). The strip was contracted with PG F$_{2\alpha}$ ($2 \times 10^{-6}$ M). PA represents $10^{-4}$ M papaverine that produced the maximal relaxation. Upward arrows indicate the application of supplemental doses of PG F$_{2\alpha}$ to raise the arterial tone. Dots denote the application of electrical stimulation.

**FIGURE 7.** Modifications by physostigmine (ES; $10^{-7}$ M) and atropine (AT; $10^{-7}$ M) of the contraction induced by transmural electrical stimulation (TES; 5 Hz) of external ophthalmic artery strips treated with L-NA ($10^{-5}$ M) and contracted with PG F$_{2\alpha}$. Percentages shown in the columns indicate the relative value to control (C). Significantly different from control, $^*P < 0.001$: $^†P < 0.02$ (paired t-test). Numbers in parentheses indicate the number of strips from separate dogs. Vertical bars represent SEM.
purinoceptors by ATP, both of which are liberated from stimulated vasoconstrictor nerves. P2X-purinoceptor activation increases cytosolic Ca\(^{2+}\), possibly as a consequence of permeation of the ATP-regulated channel by Ca\(^{2+}\).\(^{25}\) Under the experimental conditions used, the ratios of noradrenergic and purinergic factors involved in the response are identical (3/2) in the ciliary and ophthalmic arteries. Histochemical study has demonstrated the presence of neuropeptide Y in the adrenergic nerve terminal,\(^{26}\) and this peptide actually contracted the arteries used in the present study. However, BIBP3226, a neuropeptide Y Y1-receptor antagonist, in a concentration sufficient to significantly reduce the neuropeptide Y–induced contraction (present study and in guinea-pig vena cava\(^{27}\)) was ineffective in the neurogenic contraction at 5 Hz. Stimulation of peptidergic nerves may be obtained by the use of a higher frequency of electrical pulses,\(^{4}\) but this is not the case in canine ciliary and external ophthalmic arteries, because contractions by electrical stimulation at 20 Hz were also unaltered by BIBP3223 at the same concentration (authors’ unpublished observation). These results indicate that the release of neuropeptide Y in effective concentrations may be excluded under the experimental conditions used.

Contractile responses to nerve stimulation were attenuated by physostigmine, an acetylcholinesterase inhibitor, in a dose that does not directly stimulate muscarinic receptors,\(^{28}\) and potentiated by atropine. However, treatment with these inhibitors did not alter the vasoconstrictor response to norepinephrine and ATP. Cholinergic innervation in ciliary body blood vessels has been demonstrated by cholinesterase histochemistry.\(^{29}\) Therefore, acetylcholine liberated from perivascular cholinergic nerves by electrical stimulation is expected to interfere with the release of vasoconstrictor neurotransmitters from adrenergic nerves by acting on muscarinic receptors in the nerve terminal. Similar prejunctional inhibition by exogenous acetylcholine has been widely recognized in other arteries from studies on mechanical responses and measurements of the norepinephrine release from adrenergic nerves.\(^{30–32}\)

Transmural electrical stimulation elicited a relaxation in the arteries treated with prazosin and \(\alpha,\beta\)-methylene ATP, which was abolished by L-NA and restored by \(\beta\)-arginine, as demonstrated in isolated ocular arteries from a variety of mammals.\(^{13–16,33,34}\) The response was also abolished by ODQ, an inhibitor of soluble guanylate cyclase, suggesting an involvement of cyclic guanosine monophosphate. The stimulation-induced relaxation was not inhibited by aminophylline, a P1 purinoceptor antagonist, in a concentration (2 \(\times\) \(10^{-5}\) M) sufficient to significantly depress the ATP-induced relaxation. Relaxations by P1-receptor stimulation are reportedly mediated by cAMP,\(^{35}\) contrary to NO (which relaxes arteries by a mediation of cyclic guanosine monophosphate).\(^{36}\) These findings support the idea that NO, but not ATP, liberated from nerve terminals is involved in the response. The data shown in Figure 6 indicate that in the L-NA– and prazosin-treated strip, relaxation is not evoked even when the P2X-receptor antagonist in a
concentration sufficient to reverse the ATP-induced contraction to a relaxation is used but is restored by the addition of L-arginine, a substrate of NO synthesis. The distinct effectiveness of nerve-derived ATP may be explained as follows: P2X receptors are present in smooth muscle cell membranes of sympathetic and extrasympathetic nerves, whereas P1 purinoceptors, responsible for relaxation, are located mainly in the extrasympathetic area; thus, endogenous ATP fails to induce significant relaxation, but exogenous ATP does. Suramin, a P2X and P2Y receptor antagonist, did not inhibit the relaxation to ATP, suggesting that P2Y purinoceptors do not contribute to the relaxation of the arteries used in the present study. Therefore, ATP might act solely as a vasoconstrictor neurotransmitter in the arteries tested.

Autonomic innervations and effects of neurotransmitters on canine ciliary and ophthalmalic arteries are summarized in Figure 9. The contractile response to perivascular nerve stimulation of the arteries appears to be mediated by norepinephrine and ATP liberated from adrenergic nerves. The response is impaired by acetylcholine released from cholinergic nerves that stimulates prejunctional muscarinic receptors. The M2 receptor subtype is reportedly involved in the prejunctional inhibition of adrenergic nerve function in dog saphenous vein and cat cerebral arteries. Although similar prejunctional inhibition by cholinergic nerve of nitroxidergic nerve has been reported in monkey cerebral arteries, this is not the case for canine ciliary and external ophthalmalic arteries. The neurogenic relaxation is expected to be mediated solely by NO and not by ATP.

References


