Presynaptic Muscarinic Facilitation of Parasympathetic Neurotransmission after Sympathectomy in the Rat Choroid

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ABSTRACT

The effect of sympathectomy on parasympathetic regulation of ocular perfusion was investigated. Uveal blood flow through the vortex veins was measured by laser Doppler flowmetry during electrical stimulation of the superior salivatory nucleus, which activates ocular parasympathetic nerves, in adult rats with intact innervation and 2 days or 6 weeks after excision of the ipsilateral superior cervical ganglion. In all groups, parasympathetic stimulation produced comparable increases in flux, which were abolished by the selective neuronal nitric-oxide synthetase inhibitor, 1-(2-trifluoromethylphenyl) imidazole. Atropine had no effect in control and acutely sympathectomized rats but abolished the flux increase in four of six chronically sympathectomized animals, and 1-(2-trifluoromethylphenyl) imidazole eliminated the residual response. The muscarinic receptor agonist bethanechol did not affect basal flow in control or sympathectomized rats. However, bethanechol enhanced parasympathetically mediated vasodilation, but only in rats studied at 6 weeks after sympathectomy, a finding consistent with the appearance of muscarinic prejunctional facilitation of nitrergic transmission. In chronically sympathectomized rats, the M2 and M4 receptor antagonists methoctramine and tropicamide did not affect choroidal flow during parasympathetic activation. However, pirenzepine increased flux, implying the presence of M3 inhibitory autoreceptors on these nerves. Parasympathetically mediated increased flux was partially blocked by the M3 antagonist 4-diphenylacetoxy-N-methylpiperdine, and the remaining vasodilation was blocked by atropine. We conclude that parasympathetic prejunctional facilitatory M3 and probably M4 receptors adopt a crucial role after chronic sympathectomy in maintaining nitrergic vasodilatory ocular neurotransmission in the face of down-regulated nitric oxide transmitter mechanisms.

Neural regulation of end organ function is dependent not only on impulse activity of central origin but also on interactions with adjacent peripheral nerve terminals. Transmitter release is known to be modulated by substances released from coprojecting axonal populations within the target organ. For example, acetylecholine from parasympathetic nerves can diminish norepinephrine release from sympathetic nerves within the heart (Vanhoutte and Levy, 1980), bladder (de Groat et al., 1999), and tarsal smooth muscle of the orbit (Beauregard and Smith, 1994). Similarly, sympathetic neurotransmitters can prejunctionally inhibit parasympathetically mediated vasodilation in the nasal mucosa (Lacroix et al., 1994). Therefore, transmitter release from heterologous nerves coprojecting to a common target represents an important short-term mechanism for modifying neural control of target activity. Less is known about the role of heterologous nerves in long-term regulation of neurotransmission. It is known that innervation density is affected by ablation of coprojecting axons. Thus, after sympathectomy numbers of both sensory axons and parasympathetic terminals increase in some targets (Terenghi et al., 1986; Smith and Marzban, 1998), and in at least one instance, this leads to altered neurotransmission. Hence, after long-term sympathectomy of the periorbital tarsal smooth muscle, parasympathetic nerves that normally inhibit sympathetic neurotransmission but do not directly affect muscle tone become excitatory to the smooth muscle cells (Smith and Beauregard, 1993; Križsan-Agbas et al., 1998). Therefore, long-term disruption of sympathetic nerves can dramatically influence properties of parasympathetic neurotransmission in this nonvascular smooth muscle target.

It is unclear whether sympathetic denervation elicits similar changes in targets other than the tarsal muscle, and whether transmitters other than acetylecholine are affected. To address these questions, we examined the effects of long-term sympathectomy on parasympathetic neuroeffector regulation of vascular smooth muscle function in the eye.

ABBREVIATIONS: SSN, superior salivatory nucleus; TRIM, 1-(2-trifluoromethylphenyl) imidazole; 4-DAMP, 4-diphenylacetoxy-N-methylpiperdine.
Blood flow to ocular tissues in the rat derives from the choroidal and ciliary vascular plexuses. The choroid consists of a network of arterial and venous vessels that lies just inside the posterior sclera and is responsible for nourishing the photoreceptor and neural cell layers of the retina (Foulds, 1990). Tissues of the anterior eye are perfused by limbal vessels, which extend into the ciliary processes and provide perfusion necessary for aqueous humor formation (Morrison et al., 1995). Regional differences exist in the functional representation of autonomic nerves to these plexuses, with sympathetic noradrenergic vasoconstrictive effects predominating within the posterior choroidal vessels, whereas both sympathetic vasoconstrictor and parasympathetic nitricergic vasodilator nerves regulate anterior blood flow (Koss and Gherzghiher, 1993; Steindle et al., 2000). Blood flow from throughout the uvea drains into the vortex veins present along the nasolateral and temporolateral aspects of the globe just posterior to the equator. In previous studies, we and others have shown that vortex venous blood flow can be accurately measured using laser Doppler flowmetry, and that recordings of flow through the nasolateral vortex veins reliably reflects the summation of sympathetic and parasympathetic effects on ocular tissues (Best et al., 1972; Bill, 1985; Steindle et al., 2000). Therefore, laser Doppler flowmetry of vortex venous flux represents a convenient means to assess autonomic regulation of blood flow throughout the eye. In this study, we determined the effects of short- and long-term sympathetic denervation on parasympathetically induced nitricergic ocular vasodilation by measuring changes in vortex venous blood flow during parasympathetic stimulation.

Materials and Methods

Superior Cervical Ganglionectomy. Female Sprague-Dawley rats (Harlan Sprague-Dawley, Inc., Indianapolis, IN) weighing from 180 to 200 g at 60 to 70 days of age were anesthetized by ketamine hydrochloride (27.5 mg/kg, i.p.; Sanofi Winthrop, New York, NY), xylazine hydrochloride (2.5 mg/kg, Rompun; Miles, Shawnee Mission, KS) and atropine sulfate (0.24 mg/kg; Vedco, St. Joseph, MO). A ventral midline incision was made in the neck and the right superior cervical ganglion was removed aseptically (Smith and Beauregard, 1993; Krizsan-Agbas et al., 1998). This produces complete and permanent loss of orbital sympathetic innervation in adult rats (Smith et al., 1940), and electrode placement was confirmed by histological examination of the SSN (Paxinos and Watson, 1986; Steinle et al., 1990) at coordinates (9.5 mm posterior, 9.5 mm ventral, 2.5 mm lateral to bregma) previously shown to elicit selective and complete activation of parasympathetic innervation to the rat orbit (Beauregard and Smith, 1994; Krizsan-Agbas et al., 1998; Steindle et al., 2000). This nucleus is the source of preganglionic innervation to the parasympathetic pterygopalatine ganglion (Spencer et al., 1990). Parasympathetic preganglionic axons originating in the SSN were stimulated electrically at 20 Hz, 0.5-ms pulse duration, 3 V for 40 to 60 s (Grass SD9 stimulator; Grass Instrument Co., Quincy, MA), which has been shown previously to elicit maximal activation of orbital parasympathetic innervation (Beauregard and Smith, 1994; Krizsan-Agbas et al., 1998). Parasympathetic activation of orbital structures during SSN electrical stimulation was confirmed by porphyrin discharge from the Harderian gland (Tashiro et al., 1940), and electrode placement was confirmed by histological examination at the end of the experiment, as described previously (Steindle et al., 2000).

Laser Doppler Flowmetry. Ocular blood flow through the vortex veins was measured using laser Doppler flowmetry (MP2 flow probe, floLAB; Moor Instruments, Devon, England). This method measures a Doppler shift in the laser light (flux), which is determined by erythrocyte number and velocity, and is proportional to the total blood flow within a given volume of tissue (Riva et al., 1994). This method has been shown to provide flux values that correlate linearly with blood flow in vessels of equal or greater size than that of the rat vortex vein (Kajiya et al., 1989). Flux values from the floLab were acquired and displayed as arbitrary units using Polyview software (Astro-Med; Grass Instrument Co.) on a Pentium computer. The pupil was dilated by a 0.01% epinephrine topical solution, a small region of the cornea was excised using micro-Vannas scissors, and the probe was positioned using a micromanipulator in the posterior nasolateral region of the globe corresponding to the vortex veins (Steindle et al., 2000). Mineral oil was applied to prevent drying of the eye. Rats were euthanized by an overdose of urethane (3 g/kg, i.v.) at the end of each experiment. Background flux was then measured and subtracted from all experimental determinations, and the recording site was inspected to confirm correct probe placement and the absence of intraocular bleeding. Because basal flux was increased in the chronically sympathetomized rats, responses to parasympathetic stimulation are presented as percentage change in flux.

Pharmacological Studies. Pharmacological agents were dissolved in distilled water and administered through a femoral venous cannula. Neurortransmitters mediating the effects of parasympathetic stimulation in control and sympathetomized rats were evaluated by administering the nonselective muscarinic antagonist atropine methyl nitrate (0.5 mg/kg i.v.; Sigma) and the selective neuronal nitric-oxide synthetase inhibitor 1-(2-trifluoromethylphenyl) imidazole (TRIM; Research Biochemicals International, Natick, MA) in graded doses (40–60 mg/kg total i.v. dose administered in 8- or 16-mg/kg increments alone or with atropine until maximal blockade was achieved). The dosages of antagonists used in this study have been shown to be effective in blocking ocular parasympathetic neurotransmission (Beauregard and Smith, 1994; Steinle et al., 2000). To further elucidate the role of muscarinic receptors, the nonselective agonist bethanechol (Research Biochemicals International) was administered at a dose (0.1 mg/kg i.v.) that has been shown to be maximally effective in modulating ocular parasympathetic neurotransmission (Beauregard and Smith, 1994; Krizsan-Agbas et al., 1998). Muscarinic receptor subtypes involved in parasympathetic neurotransmission after sympathetomy were assessed by sequential administration of the following selective receptor antagonists: tropicamide (0.2 mg/kg; Research Biochemicals International), a selective M1 receptor antagonist; pirenzepine dihydrochloride (0.2 mg/kg; Research Biochemicals International), a selective M1 receptor antagonist; 4-diphenylacetoxy-N-methylpiperidine (4-DAMP) methiodide (0.2 mg/kg; Research Biochemicals International), a selective M2 receptor antagonist; and methoctramine tetrahydrochloride (0.2 mg/kg; Research Biochemicals International), a selective M3 receptor antagonist. The dosages for these agents have been shown to be selective for the intended receptors and supramaxi-
nal for blocking responses mediated by these receptors (Blanquet and Gonella, 1992; Blanquet et al., 1994).

**Statistics.** Responses to stimulation and drugs were compared statistically by one-way ANOVA, two-way ANOVA, or one-way repeated measures ANOVA, with post hoc analysis using Student Newman-Keuls. All values are presented as mean ± S.E., with \( P < .05 \) taken as statistically significant.

## Results

### Effect of Acute and Chronic Sympathectomy on Ocular Blood Flow.

SSN stimulation in control rats elicited a 1.0- to 1.5-fold increase in vortex venous blood flux. Atropine methyl nitrate had no significant effect on choroidal blood flow in either unstimulated or stimulated conditions. TRIM did not alter vortex venous flux in unstimulated conditions but blocked the increase during stimulation (\( P < .001, n = 6 \), Fig. 1). Neither drug produced a significant change in mean arterial pressure (Steinle et al., 2000).

SSN stimulation in rats 2 days after superior cervical ganglionectomy increased flux to an extent similar to that of arterial pressure (Steinle et al., 2000). Fig. 1). Neither drug produced a significant change in mean arterial pressure (Steinle et al., 2000).

At 6-weeks postsympathectomy, vortex venous basal flux was increased relative to control and acutely sympathectomized preparations (control = 61 ± 24 arbitrary units; 2-day sympathectomy = 53 ± 5 arbitrary units; 6-week sympathectomy = 351 ± 42 arbitrary units). SSN stimulation produced an increase in flux, which, when expressed as a percentage of the basal flow, was comparable to that of both control and acutely sympathectomized animals (n = 12). However, atropine methyl nitrate (0.5 mg/kg, n = 6) significantly attenuated the increased flux during SSN stimulation (\( P < .030 \) versus control and 2-day sympathectomy, not significantly different from prestimulation basal flux, Fig. 1). The increased flux was essentially blocked in four of six rats, whereas two animals showed residual increases of 25 and 50%. Administration of TRIM (40–60 mg/kg) to these preparations abolished the flux increases during SSN stimulation. In another group of chronically sympathectomized rats (n = 6), TRIM administration without prior atropine treatment blocked the increase in flux during SSN stimulation (\( P < .003 \) versus control and 2-day sympathectomy, not significantly different from baseline, atropine alone, or atropine + TRIM, Fig. 1).

### Characterization of Muscarinic Transmission after Sympathectomy.

To determine the site at which muscarinic receptors act to increase ocular flux at 6 weeks postsympathectomy, the nonselective muscarinic agonist, bethanechol, was administered i.v. Bethanechol (0.1 mg/kg) before SSN stimulation produced a transient decrease in mean arterial pressure that quickly returned to baseline, but had no effect on vortex venous flux in either the control (n = 5) or the 6-week (n = 5) ganglionectomized animals. In the rats with intact sympathetic innervation, SSN stimulation 100 s after bethanechol administration increased ocular flux to a degree that was comparable to that observed in the absence of bethanechol. However, at 6 weeks postsympathectomy, bethanechol administration increased vortex venous flux during SSN stimulation by approximately 30% (\( P = .041 \), Fig. 2).

To determine which muscarinic receptor subtypes are responsible for the atropine-sensitive enhancement of parasympathetic neurotransmission at 6 weeks postsympathectomy, selective antagonists to M1 to M4 receptors were administered at doses of 0.2 mg/kg (n = 4). All antagonists produced a transient decrease in mean arterial pressure that returned to predrug levels within 100 s with no change in baseline flux. The M4 antagonist tropicamide did not affect the response to SSN stimulation (Fig. 3). Pirenzepine dihydrochloride, an M1 antagonist, increased flux by 40% during SSN stimulation (\( P = .007 \) versus SSN stimulation and after tropicamide, Fig. 3). 4-DAMP methiodide, an M2 antagonist, caused a 40% decrease in flux relative to that after pirenzepine (\( P = .003 \), Fig. 3). The M4 antagonist methoctramine tetrahydrochloride did not have any apparent effect on flux (Fig. 3). Atropine methyl nitrate significantly eliminated the remaining increase in vortex venous flux during SSN stimulation (\( P < .001 \), not significant versus prestimulation values, Fig. 3). In a separate group of rats with intact sympathetic innervation (n = 4), these selective muscarinic receptor antagonists had no effect on choroidal flux during basal conditions or during parasympathetic stimulation.

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**Fig. 1.** Changes in vortex venous flux during SSN stimulation (Control) alone and after the administration of atropine only (Atropine alone, 0.5 mg/kg), atropine followed by TRIM (Atropine + TRIM), and TRIM only (TRIM alone, 40–60 mg/kg) in control rats, and 2 days and 6 weeks after sympathectomy (SNX). Data are displayed as mean ± S.E. for six rats. *\( P < .05 \) versus prestimulation flux, **\( P < .005 \) versus Control, ***\( P < .005 \) versus control and Atropine alone; †, not significant versus baseline flux.
Discussion

Parasympathetic Nerves and Control of Choroidal Blood Flow. Parasympathetic innervation from the SSN and pterygopalatine ganglion plays a major role in regulating ocular blood flow. Stimulation of this pathway increases uveal blood flow substantially in rats, cats, and rabbits as demonstrated by both labeled microspheres and laser Doppler flowmetry (Bill, 1985; Nilsson et al., 1985; Steinle et al., 2000), providing evidence that parasympathetic nerves subserve a major vasodilatory role. There is general agreement that parasympathetic vasodilation is not cholinergic in nature, as atropine normally does not affect uveal blood flow (Bill, 1985; Nilsson et al., 1985), and there is strong evidence from studies using both nonselective and neuronally selective blockers of nitric-oxide synthetase that the vasodilatory transmitter is in fact nitric-oxide (Deussen et al., 1993; Jacot et al., 1998; Steinle et al., 2000). Observations from control preparations in this study confirm a strong nitrergically mediated vasodilation whereas cholinergic effects on ocular blood flow were absent.

Acute Sympathectomy. Observations from other systems indicate that sympathetic nerve degeneration can elicit rapid effects on parasympathetic nerves. In the tarsal smooth muscle of the eyelid with intact innervation, parasympathetic nerves act to inhibit sympathetic excitatory nerves prejunctionally, but do not exert detectable effects on the smooth muscle itself (Beauregard and Smith, 1994). However, within 2 days after superior cervical ganglionectomy, parasympathetic stimulation evokes smooth muscle contraction, apparently through enhanced acetylcholine release via excitatory prejunctional α receptors, which are activated by circulating adrenal medullary catecholamines (Krizsan-Agbas et al., 1998). In contrast, short-term sympathectomy in this study had no obvious effects on parasympathetic modulation of uveal blood flow despite a common origin from pterygopalatine ganglion neurons. In this regard, it is interesting to note that there appears to be a close functional relationship between sympathetic and parasympathetic nerves in the tarsal muscle (Beauregard and Smith, 1994), although we have been unable to demonstrate prejunc- tional interactions between sympathetic and parasympathetic nerves modulating uveal blood flow (Steinle et al., 2000). In any event, findings from these studies provide evidence that different populations of parasympathetic nerves do not respond uniformly to acute sympathectomy.

Fig. 2. Effect of bethanechol (Bethanechol, 0.1 mg/kg⁻¹) on vortex venous flux during SSN stimulation (Control) in animals with intact (Intact) innervation and 6 weeks after sympathectomy (6-week SNX). Data are expressed as mean ± S.E. for five rats. *P < .05 versus basal flux after 6-week sympathectomy.

Fig. 3. Effect of SSN stimulation on vortex venous flux under control conditions (Control) and after the administration of tropicamide (Tropicamide, M4 antagonist, 0.2 mg/kg), pirenzepine (Pirenzepine, M1 antagonist, 0.2 mg/kg), 4-DAMP (M3 antagonist, 0.2 mg/kg), methoctramine (Methoctramine, M2 antagonist, 0.2 mg/kg), and atropine (Atropine, nonselective antagonist, 0.5 mg/kg). Data are expressed as mean ± S.E. for four rats. *P = .001 versus Control and tropicamide, **P < .05 versus Control and tropicamide, ***P < .001 versus Control and all other drugs.
**Long-Term Sympathectomy.** In contrast to acute sympathectomy, chronic sympathetic denervation elicited significant changes in vortex venous flux and in parasympathetic neurotransmission properties. Flux was consistently elevated in the eyes of rats at 6 weeks after sympathectomy and, although it is not possible to directly infer altered blood flow simply on the basis of flux (Riva et al., 1994), microsphere measurements of blood flow and morphometric analysis of vessel numbers support the idea that uveal blood flow and vessel numbers are increased in chronically sympathectomized eyes (J. J. Steinle, J. D. Pierce, R. L. Clancy, and P. G. Smith, manuscript in preparation).

Despite augmented flow after chronic sympathectomy, the increase in vortex venous flux above baseline during parasympathetic stimulation was proportional to that observed in intact and acutely sympathectomized preparations, implying that a similar degree of parasympathetic vasodilatory influence is maintained. Furthermore, the neuronal nitric-oxide synthetase inhibitor, TRIM, completely blocked the vasodilation in response to parasympathetic nerve stimulation, indicating that the increased blood flow in the chronically sympathectomized rats is again mediated by nitrergic vasodilatory mechanisms. However, in contrast to control and acutely sympathectomized preparations, muscarinic receptor blockade by atropine now resulted in a marked reduction in the parasympathetically mediated response. Therefore, long-term sympathectomy results in the appearance of a muscarinic component of parasympathetic neurotransmission that is critical to maintaining ocular vasodilatory function.

Muscarinic receptors are present on vascular smooth muscle (Linville and Hamel, 1995) and endothelial (Tracey and Peach, 1992; Traish et al., 1994) cells, as well as on parasympathetic varicosities themselves (Miranda et al., 1994). It is therefore possible that acetylcholine from parasympathetic nerves plays a role in parasympathetically mediated vasodilation after chronic sympathectomy by acting postjunctionally to directly promote vasodilation via smooth muscle relaxation, or prejunctionally to enhance the formation and release of nitric-oxide. Evidence from this study suggests that a postjunctional site of action is unlikely. The muscarinic agonist bethanechol did not produce any significant change in ocular blood flow in either control or chronically sympathectomized preparations under basal conditions. This supports the idea that muscarinic receptors on vascular smooth muscle or endothelial cells do not play important roles in inducing ocular vasodilation after sympathectomy. However, when parasympathetic innervation was stimulated in the presence of the bethanechol, vasodilation was increased significantly, but only in chronically sympathectomized preparations. These findings are accordant with the notion that acetylcholine acts prejunctionally to enhance vasodilatory neurotransmitter release from parasympathetic nerves, and that enhanced release is necessary to maintain ocular vasodilation after long-term sympathectomy.

One possible explanation for this finding is that parasympathetic nitrergic transmission is down-regulated after sympathectomy, but prejunctional facilitatory muscarinic autoreceptors are acting to enhance transmitter release. Evidence to support this hypothesis derives from work in which we have shown that pterygopalatine ganglion neuronal immunoreactivity for neuronal nitric-oxide synthetase and NADPH-diaphorase enzymatic activity, as well as axonal nitric-oxide synthetase immunoreactivity in the cerebral vasculature, are substantially reduced after long-term sympathectomy (Warn et al., 1997). Although it is difficult to extrapolate directly between expression of transmitter-associated proteins and functional indices of neurotransmission, this study is consistent with a down-regulation of transmitter function. An alternative possibility is that, although parasympathetic sprouting does occur after sympathectomy (Smith and Marzban, 1998), innervation fails to expand to an extent that maintains normal nerve density of newly formed vasculature. Irrespective of the mechanism, parasympathetic nitrergic transmission appears to be incapable of maintaining optimal vasodilation in the absence of muscarinic facilitation in sympathectomized preparations.

Evidence from other studies supports the concept that acetylcholine can act prejunctionally to enhance the release of nitric oxide. In the guinea pig ileum, neuronal nitric oxide formation is increased under conditions in which acetylcholine release is increased (Wiklund et al., 1993). The present study favors the idea that acetylcholine can similarly enhance release of nitric oxide from parasympathetic vasodilatory nerves of the eye in the chronically sympathectomized preparation.

**Prejunctional Facilitation of Parasympathetic Vasodilation Is Mediated by the M<sub>1</sub> and M<sub>2</sub> Receptor.** There are five currently recognized muscarinic receptor subtypes, and four of these are amenable at this time to selective pharmacological blockade. Antagonism of the M<sub>2</sub> receptor by tropicamide or the M<sub>2</sub> receptor by methoctramine did not affect the vasodilatory response, thus excluding these receptors as candidates for cholinergic modulation of parasympathetic vasodilation after sympathectomy. The M<sub>1</sub> receptor antagonist pirenzepine, however, produced a significant increase in dilation. Although the M<sub>1</sub> receptor is excitatory at many sites, it does act as a prejunctional inhibitory autoreceptor in the guinea pig ileum (Lambrecht et al., 1999), and this study provides evidence that the M<sub>1</sub> receptor is a prejunctional inhibitory autoreceptor on parasympathetic
M5 excitatory prejunctional receptors are demonstrable. The ide synthetase, both M1 inhibitory and M3 and presumptive nitrergic transmission. However, after chronic sympathetic cholinergic muscarinic modulation of parasympathetic innervation, it is not possible to demonstrate prejunctional M5 receptors in the sympathetically innervated rat choroid. As M3 receptor blockade reduced vasodilation by only 30%, this receptor can account for only a portion of prejunctional muscarinic facilitation observed after sympathetic.

Because atropine was still effective in suppressing parasympathetic vasodilation after blocking M1, M2, M3, and M4 receptors, it is likely that the M3 receptor also facilitates parasympathetic nitrergic transmission. This receptor is abundant in neural tissue (Wang et al., 1994; Reever et al., 1997), and Chinese hamster ovary cells transfected to express high levels of M3 receptor protein show a 24-fold increase in neuronal nitric oxide release in the presence of acetylcholine (Wang et al., 1994). Therefore, these studies support the idea that prejunctional M3 receptors may be acting to enhance nitric oxide release in parasympathetic neurons in which nitric-oxide synthetase has been downregulated after long-term sympathetic denervation. These findings show that prejunctional receptor modulation of parasympathetic nitrergic transmission in the rat choroid is strongly influenced by the status of sympathetic innervation (Fig. 4). Under conditions with intact sympathetic innervation, it is not possible to demonstrate prejunctional cholinergic muscarinic modulation of parasympathetic nitrergic transmission. However, after chronic sympathetic denervation with concomitant down-regulation of nitric-oxide synthetase, both M1 inhibitory and M3 and presumptive M3 excitatory prejunctional receptors are demonstrable. The effects of the M3 and M5 receptors apparently predominate, leading to the facilitation of nitrergic transmission and maintenance of the parasympathetic vasodilatory response.

**References**


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