Regional regulation of choroidal blood flow by autonomic innervation in the rat

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Regional regulation of choroidal blood flow by autonomic innervation in the rat. Am J Physiol Regulatory Integrative Comp Physiol 279: R202–R209, 2000.—Regional influences of parasympathetic and sympathetic innervation on choroidal blood flow were investigated in anesthetized rats. Parasympathetic pterygopalatine neurons were activated by electrically stimulating the superior salivatory nucleus, whereas sympathetic neurons were activated by cervical sympathetic trunk stimulation and uveal blood flow was measured by laser Doppler flowmetry. Parasympathetic stimulation increased flux in the anterior choroid and nasal vortex veins but not in the posterior choroid. Vasodilation was blocked completely by the neuronal nitric oxide synthase inhibitor L-NAME but was unaffected by atropine. Sympathetic stimulation decreased flux in all regions, and this was blocked by prazosin. Parasympathetic stimulation did not affect vasoconstrictor responses to sympathetic stimulation in the posterior choroid but attenuated the decrease in blood flow through the anterior choroid and vortex veins via a nitrergic mechanism. We conclude that sympathetic a-adrenergic vasoconstriction occurs throughout the choroid, whereas parasympathetic nitrergic vasodilation plays a selective role in modulating blood flow in anterior tissues of the eye.

Parasympathetic nervous system; sympathetic nervous system; laser Doppler flowmetry; nitric oxide

Ocular blood flow must be closely regulated to maintain normal retinal function (8). The choroidal blood vessels, which lie between the outer retina and sclera, provide much of the retinal perfusion in humans and other mammals. The choroid contains dense sympathetic innervation originating in the ipsilateral superior cervical ganglion (24). Stimulation of the cervical sympathetic trunk (CST), which provides preganglionic innervation to the superior cervical ganglion, diminishes choroidal blood flow predominantly by \( \alpha_1 \)-adrenergic receptor activation (21). The choroid also receives parasympathetic innervation from the ipsilateral pterygopalatine ganglion (30), which facial nerve stimulation studies suggest is vasodilatory (35). Therefore, the choroid contains sympathetic vasoconstrictor and parasympathetic vasodilator nerves whose activity and interactions apparently determine the level of choroidal perfusion.

Although autonomic innervation may be important in regulating choroidal blood flow, it is not known whether sympathetic and parasympathetic nerves determine total choroidal perfusion through interactions on common vascular beds or whether different types of innervation predominate in different regions. Questions also remain regarding the extent to which parasympathetic nerves mediate the facial nerve vasodilatory response and the identity of vasodilatory neurotransmitters. In the present study, we used laser Doppler flowmetry, which offers a relatively high degree of spatial resolution, to determine whether responses to autonomic nerve stimulation vary within different regions of the choroid. We have combined this methodology with discrete stimulation of parasympathetic and sympathetic innervation and selective pharmacological blockade to assess the regional regulation of choroidal blood flow by autonomic innervation in the rat.

MATERIALS AND METHODS

Studies were conducted on 25 adult female Sprague-Dawley rats (Harlan) aged 60–80 days and weighing 180–240 g. Rats were anesthetized with urethane (1.25 g/kg ip), rectal temperature was maintained at 36°C, and a femoral artery and vein were cannulated for blood pressure recording (Statham; Costa Mesa, CA) and drug administration, respectively. The distal right facial nerve was exposed and cut to eliminate movement of the facial musculature. The CST was exposed through a ventral midline incision in the neck, and a cuff electrode (Roboz Microprobe; Rockville, MD) was placed around the intact nerve just caudal to the superior cervical ganglion for stimulation. The wires were externalized, the incision was closed with silk suture, and the rat was placed in a stereotaxic frame (Stoelting; Wood Dale, IL). Surgical procedures and all subsequent experimental manipulations were approved by the Institutional Animal Care and Use Committee of the University of Kansas Medical Center.

Parasympathetic and sympathetic stimulation. A scalp incision was made over the sagittal suture, and a craniotomy was performed. A semi-microbipolar concentric electrode (100 µm contact diameter, Rhodes Medical Instruments; Woodland Hills, CA) was positioned stereotaxically within...
the superior salivatory nucleus [SSN, 9.5 mm posterior, 9.5 mm ventral, 2.5 mm lateral to bregma (27)], as described previously (2, 22, 32). This nucleus provides the preganglionic innervation to the parasympathetic pterygopalatine ganglion (34). Parasympathetic innervation was activated by electrical stimulation of the SSN at 12–20 Hz, 3 V for 40–60 s (Grass SD9 stimulator; Quincy, MA), which we have shown previously to elicit maximal activation of orbital parasympathetic innervation (2); this was confirmed for blood flow in preliminary experiments. Electrode placement within the preganglionic parasympathetic nucleus was confirmed by porphyrin discharge from the harderian gland during stimulation (36) and by electrolytic lesioning at the end of the experiment followed by examination of cresyl violet-stained frozen sections to verify electrode position within the SSN as described previously (2).

Ocular sympathetic innervation was stimulated using the cuff electrode placed around the preganglionic CST. Stimulations were performed at 12–20 Hz, 30 V for 40–60 s, which is supramaximal for sympathetic activation (2, 33), as confirmed for blood flow changes in preliminary investigations in the present study.

Blood flow measurements. Blood flow was measured using laser Doppler flowmetry (floLAB model with MP3 probe, Moor Instruments; Devon, UK). This technique is based on the concept that laser light reflected from a moving object, such as a red blood cell, undergoes a Doppler frequency shift that is determined by the relative concentration of blood cells and their average velocity (28). Flux, which is directly proportional to blood flow (28), was recorded (time constant of 0.5 s) on a computer using Polyview software (Astro-Med, Grass Instruments). Previous studies using this technique have shown linear flow measurements for vessels with diameters similar to those from which measurements were obtained in the present study (17).

To assess blood flow in the anterior choroid, a flow probe (1 mm tip diameter) was positioned extraocularly ~3 mm distal to the limbus, as described by others (18). To obtain recordings from the posterior choroid and the vortex veins, the pupil was dilated by topical application of a 0.01% solution of epinephrine, the cornea was incised, the fiberoptic probe was inserted using a micromanipulator through the anterior and posterior surface of the cornea. In a separate group of rats, CST stimulation caused a transient decrease in systemic arterial blood pressure and may reflect spontaneous uveal vasomotor activity reported by others (29). During SSN stimulation, peak flux increased oneto twofold (Figs. 1 and 2, \( P = 0.015 \)), reaching a maximum within 20 s that was maintained until stimulation was stopped after 40–60 s. Flux values returned to baseline levels in ~20–40 s. Although the oscillation frequency did not change during stimulation, amplitude increased substantially (Fig. 1). SSN stimulation did not alter systemic blood pressure (Table 1).

Atropine did not influence basal flux or systemic blood pressure (Table 1), and the increase in flux during SSN stimulation was not affected (Fig. 2). The selective neuronal NOS inhibitor TRIM caused a transient decrease in systemic arterial blood pressure and choroidal flux, both returning to baseline within 3 min (Table 1). TRIM blocked the response to SSN stimulation (\( P = 0.004 \) vs. stimulation before TRIM; not significant vs. prestimulation, Fig. 2).

In a separate group of rats, CST stimulation caused vortex venous flux to decrease significantly without obvious effects on amplitude or frequency of oscillations. CST stimulation decreased vortex venous flux by

RESULTS

Vortex veins. Basal blood flux recorded from the nasal vortex veins showed 0.2- to 0.3-Hz oscillations of low amplitude (Fig. 1), which were not synchronous with respiration and may reflect spontaneous uveal vasomotor activity reported by others (29). During SSN stimulation, peak flux increased one- to twofold (Figs. 1 and 2, \( P = 0.015 \)), reaching a maximum within 20 s that was maintained until stimulation was stopped after 40–60 s. Flux values returned to baseline levels in ~20–40 s. Although the oscillation frequency did not change during stimulation, amplitude increased substantially (Fig. 1). SSN stimulation did not alter systemic blood pressure (Table 1).

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60% (Fig. 3, P = 0.002). CST stimulation did not affect systemic arterial blood pressure (Table 1). The α-adrenoceptor antagonist prazosin significantly attenuated the CST stimulation-induced decrease (Fig. 3, P < 0.001 vs. stimulation before prazosin), and flux during stimulation was not significantly different from prestimulation basal flux. However, prazosin induced a marked decrease in systemic arterial pressure (Table 1) with an accompanying decrease in basal flux.

To assess interactions between parasympathetic and sympathetic innervation on vortex venous flux, another group of rats was prepared for SSN and CST costimulation. In these rats, either the SSN or CST was stimulated for 20 s, after which stimulation of the other site commenced in combination with the initial stimulus. Stimulations of both sites were maintained for a total 60 s, with measurements obtained 40 s after costimulation commenced. Maximal changes in vortex venous flux were essentially identical to those obtained in previous experiments in which one site or the other was stimulated alone (Fig. 4). Irrespective of the order of stimulation, activation of the second site attenuated the effect of the first. SSN stimulation reduced the CST-induced decrease in flux to $-11.5 \pm 2.5\%$ of prestimulation baseline, and CST stimulation attenuated the SSN-induced increase in flux to $-7.7 \pm 5\%$ of baseline. Flux during sympathetic and parasympathetic costimulation was not significantly different from prestimulation basal flux [Fig. 4, not significant by paired t-test].

Costimulation after TRIM administration produced a decrease in flux that was significantly greater than that obtained in the absence of TRIM ($P < 0.001$) and was not significantly different from that during CST stimulation alone (Fig. 4). Administration of atropine following TRIM did not elicit any further change (Fig. 4). Prazosin, when given after TRIM and atropine, prevented any significant change in flux (Fig. 4, $P < 0.001$ vs. CST stimulation prior to prazosin, not significantly different from prestimulation flux).

**Posterior choroid.** In another group of rats prepared for costimulation with the flow probe positioned to record from the posterior choroidal vasculature, activi-

### Table 1. Mean arterial blood pressures

<table>
<thead>
<tr>
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<th>Before</th>
<th>During</th>
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<tr>
<td>SSN stimulation</td>
<td>91 ± 6</td>
<td>93 ± 6</td>
</tr>
<tr>
<td>CST stimulation</td>
<td>90 ± 10</td>
<td>88 ± 10</td>
</tr>
<tr>
<td>Costimulation</td>
<td>82 ± 5</td>
<td>84 ± 6</td>
</tr>
<tr>
<td>Atropine</td>
<td>91 ± 6</td>
<td>82 ± 8</td>
</tr>
<tr>
<td>TRIM</td>
<td>86 ± 6</td>
<td>83 ± 7</td>
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<tr>
<td>Prazosin</td>
<td>75 ± 7</td>
<td>53 ± 1*</td>
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</table>

Values are means ± SE for 8 rats in all categories except superior salutatory nucleus (SSN) stimulation ($n = 10$) and prazosin ($n = 6$). Mean arterial blood pressure (mmHg) is measured before and during neuronal stimulation and in the presence of drugs in different groups of rats. SSN stimulation was conducted at 12–20 Hz, 3 V. Cervical sympathetic trunk (CST) stimulation was conducted at 12–20 Hz, 30 V. Drug dosages were 0.5 mg/kg atropine, 40–60 mg/kg 1-(2-trifluoromethylphenyl)imidazole (TRIM), and 1 mg/kg prazosin, and all measurements were obtained 5–10 min after intravenous administration. *$P < 0.001$ vs. before prazosin.
DISCUSSION

Autonomic regulation of choroidal perfusion. Stimulation of parasympathetic preganglionic neurons of the SSN elicits a pronounced increase in flux within the vortex veins. Because these vessels represent the primary route of choroidal blood efflux, this finding is consistent with increased overall blood flow throughout the choroid and associated tissues. Furthermore, because this was not accompanied by changes in systemic blood pressure, it is most consistent with a decrease in vascular resistance due to vasodilation. These findings are in agreement with previous studies showing increased choroidal blood flow and microsphere accumulation during facial nerve stimulation (35). Moreover, because increased flow was elicited by selectively stimulating preganglionic parasympathetic neurons, our findings provide strong evidence that vasodilation accompanying facial nerve stimulation is mediated by parasympathetic axons and not by other fiber populations [e.g., sensory, sympathetic (23), or somatic motor] that also travel in this nerve. We therefore conclude that a parasympathetic nerve pathway originating in the SSN and with postganglionic fibers traveling in the facial nerve is a potent modulator of uveal blood flow.

Although previous microsphere studies provide evidence of parasympathetically mediated choroidal vasodilation, regional influences were not examined. Unlike microsphere analyses, laser Doppler flowmetry can readily measure flow in relatively discrete volumes of tissue (~1.5 mm³) (1) and therefore is well suited for assessing regional differences in changes to perfusion and the posterior choroid (Fig. 6). However, flux values during costimulation were significantly greater in the anterior choroid than in the posterior choroid (P < 0.001, Fig. 6). Administration of TRIM before costimulation resulted in a decrease in flux that was comparable to that obtained from the vortex veins and was significantly different from flux changes in response to costimulation alone (P < 0.001). Atropine did not affect the response to costimulation. Prazosin effectively blocked the remaining change in flux (Fig. 6).
This technique provides clear evidence of major regional differences in parasympathetic modulation of choroidal flow. Thus, whereas the nasal vortex veins showed substantial flux increases during parasympathetic stimulation, all other sampled regions of posterior choroid (even as close as 1 mm to the vortex veins) showed little to no change in perfusion during stimulation. In contrast, the anterior choroid showed large increases in flux. We conclude that increased vortex venous flux during parasympathetic stimulation is primarily due to increased flow in anterior vasculature without detectable changes in posterior choroidal flow. These findings therefore may imply that different neural control mechanisms exist within different ocular regions.

These striking regional differences in parasympathetic regulation may reflect differences in origin and function of the choroidal vasculature. Anterior and posterior ocular structures derive their primary blood supplies from different sources. In the rat, the posterior choroid is perfused by the short posterior ciliary and central retinal arteries, whereas the anterior uvea is perfused by the long posterior and anterior ciliary arteries (6, 9). It is therefore probable that the arterioles supplied by the long posterior and anterior ciliary arteries are strongly influenced by pterygopalatine parasympathetic innervation, whereas those of the short ciliary and central retinal artery vessels are appreciably less affected. The absence of detectable parasympathetic influences on posterior choroidal flow is somewhat surprising in light of immunohistochemical studies showing NOS-immunoreactive nerves in the posterior choroid of the rat (39) and pigeon (4). Nonetheless, these fibers may simply be axons of passage en route to more anterior targets, or the posterior choroidal vascular smooth muscle may be relatively insensitive to released parasympathetic neurotransmitters.

Functionally, these findings may provide insight as to the role of parasympathetic innervation. The anterior tissue from which we recorded likely contains some anteriorly projecting blood vessels from posterior choroid but is probably dominated by branches from the major arterial circle and pars plana venules that drain the more anterior tissues (supplied by the long posterior and anterior ciliary arteries), particularly the ciliary body and processes (10). These structures are integrally involved in formation of aqueous humor. Because aqueous humor formation is determined in part by the extent of perfusion of the anterior uveal circulation, these findings may be significant.
structures, increased blood flow via a parasympathetic projection may provide a selective mechanism for modulating perfusion of these tissues. Indeed, it is well established that stimulation of the facial nerve increases intraocular pressure (11), as does stimulation of diencephalic regions (12) that project to the SSN [e.g., lateral hypothalamus (34)]. These findings therefore are accordant with the view that a parasympathetic pathway from the SSN via the pterygopalatine ganglion plays an important role in regulating anterior uveal perfusion.

**Neuronal NOS mediates parasympathetic choroidal vasodilation.** Previous studies have shown that facial nerve vasodilatation is atropine resistant and therefore not likely to be cholinergic (3), a finding confirmed in the present experiments. In light of the potent vasodilatory effects of NO in other systems (13), this neurotransmitter would be a logical candidate. Indeed, nonselective NOS inhibitors decrease basal choroidal blood flow in pigs, dogs, and rats (7, 15, 20) and attenuate the increased flow during facial nerve stimulation in the rabbit (26). However, because nonselective NOS inhibitors elevate basal systemic blood pressure, thereby increasing choroidal perfusion, it has been unclear whether other transmitters (e.g., vasoactive intestinal polypeptide) may also participate in parasympathetically mediated vasodilation (26). In the present study, the highly selective neuronal NOS inhibitor TRIM (14) did not affect systemic blood pressure or basal uveal flow, a finding similar to that reported for another neuronal NOS antagonist, 7-nitro indazole (7-NI) (20). However, TRIM completely abolished effects of parasympathetic stimulation on anterior choroidal and vortex venous blood flow, even at the relatively high stimulation frequencies used in these studies. Although this is the first study to document the involvement of NO in neurally evoked uveal vasodilatation of the rat, similar findings were obtained in the pigeon, where vasodilatation mediated by oculomotor nerve efferents was eliminated by 7-NI (40). However, the selectivity of this NOS inhibitor has been questioned recently (5). In contrast to previous studies using nonselective NOS inhibitors (19), TRIM in the present study did not affect basal flux at doses that completely blocked vasodilation during parasympathetic nerve stimulation. These findings support the idea that parasympathetic nitric mechanisms do not play an appreciable role in determining basal choroidal blood flow but are responsible for choroidal vasodilation that accompanies parasympathetic stimulation in the anesthetized rat.

**Interactions between parasympathetic and sympathetic innervation in the regulation of choroidal perfusion.** Together with previous studies, our findings show that sympathetic innervation exerts potent $\alpha_1$-adrenoceptor-mediated vasoconstriction in the anterior (18) and posterior choroid, whereas parasympathetic vasodilation influences primarily anterior uveal tissues. Because both sympathetic and parasympathetic nerves regulate anterior perfusion, blood flow in this region should reflect the summation of the interactions between these populations.

Recordings of flux from the vortex veins show that under normal conditions, flow during sympathetic and parasympathetic costimulation is similar to or slightly below prestimulation levels. Because the posterior choroid is markedly vasoconstricted during costimulation, vortex venous outflow may reflect a relative predominance of parasympathetic vasodilation in the anterior choroid, and indeed flux during costimulation is significantly greater in the anterior choroid than in the posterior choroid. Therefore, whereas sympathetic vasoconstriction is not detectably affected by parasympathetic activation in the posterior choroid, it appears to be negated by parasympathetic nerves in the anterior region.

Parasympathetic nerves can nullify sympathetic actions through two potential mechanisms. Target cell activity may reflect equal or preferential responsibility to parasympathetic transmitters or these neuronal populations can interact prejunctionally, with parasympathetic nerves inhibiting sympathetic transmitter release. This latter mechanism is particularly important in many target systems. Parasympathetic nerves inhibit sympathetic neurotransmitter release via prejunctional muscarinic receptors in a variety of organ systems, including the heart, vasculature, vas deferens, and periorbital smooth muscle (2, 25, 37, 38). If this mechanism is important in regulating choroidal perfusion, then parasympathetic stimulation during sympathetic activation in the neuronal NOS-inhibited preparation should still attenuate sympathetic vasoconstriction and this should be countered by the administration of atropine. However, our findings from anterior choroidal and vortex venous recording sites show that NOS inhibition completely blocks the parasympathetic effects and that atropine has no significant actions. Therefore, prejunctional muscarinic inhibition of sympathetic neurotransmission by parasympathetic nerves apparently is not an important mechanism in regulating choroidal blood flow.

It is unclear why this mechanism, which is widespread in the peripheral nervous system, is not operative in the choroid. One possibility is that sympathetic neurons that project to the choroid lack prejunctional muscarinic receptors. Although a high proportion (65–85%) of superior cervical ganglion neurons express high-affinity muscarinic binding sites (16), we cannot rule out the possibility that choroidal sympathetic nerves lack muscarinic receptors. Alternatively, even though both parasympathetic and sympathetic axons travel together within Schwann cells in some parts of the choroid (31), their terminals may be segregated, such that acetylcholine released by parasympathetic nerves is unable to activate prejunctional muscarinic receptors. In any event, it appears that autonomic projections to the choroid display a high degree of functional segregation as revealed by their selective regional distribution and absence of prejunctional muscarinic interactions.

**Perspectives**

This study provides evidence that regional differences exist in the autonomic regulation of blood flow in
the eye. Specifically, the posterior choroid is dominated by sympathetic vasoconstrictive input, whereas parasympathetic nitricergic vasodilatory nerves preferentially regulate flow in anterior tissues. Because many ocular disease states are associated with regional disturbances in ocular perfusion, it is interesting to speculate as to possible roles of autonomic innervation in the etiology of these diseases. Moreover, manipulation of either sympathetic or parasympathetic innervation may selectively impact different tissues, depending on their autonomic profile. Indeed, it may be predicted from these studies that vascular parasympathetic innervation plays an important role in regulating perfusion of the anterior tissues, including those associated with aqueous humor formation. It is particularly interesting therefore that NOS inhibitors are proving effective in clinical trials at combating elevated intraocular pressure associated with glaucoma. Whether other therapeutic strategies can also take advantage of regional selectivity of ocular autonomic effects remains to be determined.

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REFERENCES


