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Sympathetic vasoconstriction in the rat anterior choroid is mediated by α_1-adrenoceptors

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Abstract

These experiments were undertaken in an attempt to use laser-Doppler flowmetry to measure anterior choroidal blood flow in the anesthetized rat and to study the mechanism by which sympathetic nerve stimulation might produce vasoconstriction in this vascular bed. Electrical stimulation of the preganglionic cervical sympathetic nerve produced reproducible, frequency-related ocular vasoconstrictor responses with maximal vasoconstriction seen at about 32 Hz. Ocular vasoconstrictor responses were blocked by intravenous treatment with the nonselective α-adrenoceptor antagonists, phentolamine (5 mg kg^{-1}) and phenoxybenzamine (2 mg kg^{-1}), as well as with the selective α_1-adrenoceptor antagonist, prazosin (0.3 mg kg^{-1}). In contrast, the selective α_2-adrenoceptor blocker, rauwolscine (0.5 mg kg^{-1}), only potentiated the vasoconstriction. Neither intravenous atropine (1 mg kg^{-1}) nor propranolol (1 mg kg^{-1}) altered the magnitude of neurally evoked vasoconstriction. These results demonstrate the usefulness of laser-Doppler flowmetry in studies of the rat anterior choroidal circulation and suggest that adrenergic neurogenic vasoconstriction in the anterior segment of the rat eye is mediated almost exclusively by α_1-adrenoceptor mechanisms.

Keywords: Anterior choroidal blood flow, rat; Laser-Doppler flowmetry; α-Adrenoceptor subtype; Sympathetic nerve stimulation; Phentolamine; Phenoxybenzamine; Prazosin; Rauwolscine

1. Introduction

Although many previous investigations have demonstrated that sympathetic nerve stimulation causes vasoconstriction in the anterior ocular circulation (Adler et al., 1924; Greaves and Perkins, 1952; Bill, 1962; Alm and Bill, 1973; Alm, 1977), only a few in vivo studies have addressed the question of the specific subtypes of adrenergic receptors that may be involved (Mittag and Tormay, 1985; Okubo et al., 1990; Koss and Gherezghiher, 1993; Koss, 1994). This, in part, is due to technical difficulties in making repeated measurements of regional ocular blood flows so that frequency– and dose–response relationships can be established.

It is now generally accepted that postjunctional α_1- and α_2-adrenoceptors coexist in the systemic vasculature and that both receptor subtypes mediate vasoconstriction (Docherty et al., 1979; Drew and Whiting, 1979). It was originally hypothesized that α_1-adrenoceptors are preferentially innervated and that α_2-adrenoceptors are only 'extrasynaptic', responding mainly to circulating catecholamines (Langer et al., 1981; Timmermans and Van Zwieten, 1981). More recently, however, neuronally mediated activation of both α_1- and α_2-adrenoceptors has been reported in many, if not most, in vivo systems (see Koss et al., 1991).

The present experiments were undertaken in an attempt to utilize laser-Doppler flowmetry to measure blood flow from the rat anterior choroid on a continuous basis. The overall aim was to determine the extent to which the anterior ocular vasculature is under sympathetic neuronal control and to define the specific subtype(s) of α-adrenoceptor that may be involved in vasoconstriction in the anterior choroid of this common experimental animal. Our results suggest that neural release of norepinephrine from

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ocular sympathetic nerves produces vasoconstriction in the rat anterior choroid by preferentially activating α_1-adrenoceptors with little, if any, α_2-adrenoceptor involvement.

2. Materials and methods

2.1. General

All experimental animals were treated in a manner consistent with the ARVO statement for the Use of Animals in Ophthalmic and Vision Research. Male Sprague–Dawley rats weighing 300–400 g were anaesthetized with sodium pentobarbital (60 mg kg^{-1}, i.p.), with supplementary pentobarbital (3–5 mg kg^{-1}, i.v.) given as necessary to maintain stable anesthesia. Animals were paralyzed with gallamine triethiodide (5–10 mg kg^{-1}, i.v.). The trachea was intubated for ventilation with a Harvard respirator using room air at a frequency of 60 strokes min^{-1} and tidal volume of 1 ml 100 g^{-1}. A femoral artery and vein were cannulated for recording of systemic arterial blood pressure (Spectramed P23XL) and for the intravenous administration of drugs. Heart rate was derived from the femoral arterial pulse and processed using a cardiotachometer (Sanei 1321, Sanei, Japan). Body temperature was maintained at approximately 37°C with a thermostable chemical heating pad (Deltaphase Isothermal Pad; Braintree Scientific, Braintree, MA, USA). A Sanei model 365 polygraph was used to record all physiological parameters.

2.2. Assessment of ocular blood flow changes

Anterior segment choroidal blood flow was measured with a laser-Doppler flowmeter (Omega flow FLO-C1, Omegawave, Japan) fitted with an NX fiber optic probe (custom made, 1 mm diameter). This is the standard needle probe (type N) which has been modified (NX) to have a 45° angle at the tip for easier placement on the eye. The laser-Doppler flowmetry technique involves exposure of a small surface area to coherent light which is reflected from both stationary tissue and blood cells with the moving blood cells producing a Doppler frequency shift which creates Doppler beat-frequencies at a photodetector. The computer-processed Doppler beat-frequencies are proportional to the total blood flow within the volume of tissue measured and are dependent upon the relative concentration of blood cells and average blood cell velocity. We speculate that the maximal depth of laser light penetration in our preparations is about 0.5 mm.

The laser probe was placed at right angels to the eye surface several millimeters posterior to the limbus with care taken not to record blood flow from the large external limbal blood vessels (Morrison et al., 1995). The time constant was set at 1 s. To avoid influences of blood pressure on the laser-Doppler signal, all electrical stimulations were performed under conditions of stable blood pressure and when the laser-Doppler recordings showed a stable baseline.

Because laser-Doppler flowmetry yields only relative flow values, the data were normalized to percentage of the basal flow values. Thus, the change of blood flow (percentage decrease) was calculated according to the following formula: $(A - B)/A \times 100$, where A represents the blood flow just before electrical stimulation of the sympathetic nerve, and B represents the lowest blood flow level reached in response to stimulation.

Although laser-Doppler flowmetry has many advantages over other techniques, there are also limitations and drawbacks including sensitivity to movement, signal reduction by tissue pigment and restriction of the measurement area. The lack of calibration in units of flow and uncertainty concerning the volume of tissue in which blood flow is measured are additional limitations. As this technique gives only relative flow values, the data are usually normalized to percentage of the basal flow levels. This is justified in studies of vasoconstrictor mechanisms, as the level of zero blood flow is a known quantity that was determined in each experiment after sacrifice with pentobarbital.

2.3. Sympathetic nerve stimulation

One cervical sympathetic nerve was carefully separated and cut at the mid-cervical level. For electrical stimulation, bipolar silver stimulating electrodes were placed under the distal portion of this preganglionic cervical sympathetic nerve and covered with liquid paraffin. Stimuli were generated by an electronic stimulator and isolation unit (SS-1894S, Nihon Kohden Japan). Square wave (4 V) trains of 10 s duration were presented. The pulse width was 1 ms with the frequency varied between 1 and 32 Hz. Responses were allowed to recover fully before the next highest frequency of stimulation was tested. Pupillary dilation was used as a marker for effective sympathetic nerve stimulation. After a control period for response stabilization, antagonists were administered intravenously with at least 10–15 min allowed to reach steady state.

2.4. Drugs and statistical analyses

The following drugs were used: (±)-propranolol hydrochloride, atropine sulfate, gallamine triethiodide (Sigma, St. Louis, MO, USA); prazosin hydrochloride, rauwolscine hydrochloride, and phenoxybenzamine hydrochloride (Research Biochemical International, Natick, MA, USA); phentolamine mesylate (Regitine) (Ciba-Geigy, Japan). All drug solutions were prepared in physiological saline with the exception of prazosin [2.5% glucose (w/v):2.5% glyc-

erol(v/v)] and phenoxybenzamine (50% propylene glycol). Drug dosages refer to the respective salts.

Data are reported as means ± S.E.M. Changes of blood pressure, heart rate and ocular blood flow (mV), before and after antagonist administration were analyzed using Student's *t*-test for paired comparisons with $P < 0.5$ testing for significance.

3. Results

3.1. Anterior choroidal blood flow and responses to sympathetic nerve stimulation

Anterior choroidal blood flow was measured using laser-Doppler flowmetry before, during and after electrical stimulation of the efferent preganglionic cervical sympathetic nerve. Systemic arterial blood pressure and heart rate were also monitored. Prior to nerve stimulation, the ipsilateral sympathetic nerve was sectioned proximal to the site of stimulation and the effect of denervation was quantified. There was no significant change of ocular blood flow following sympathetic nerve section, which suggests that there is minimal vasoconstrictor tone in these pentobarbital-anesthetized preparations. When measured 3–5 min after section of the preganglionic nerve, mean anterior choroidal blood flow was 96 ± 4% of controls ($n = 11$).

Stimulation of the cervical preganglionic sympathetic nerve produced frequency-dependent ocular vasoconstrictor responses which were essentially linear between 4 and 16 Hz (Fig. 1). The maximal vasoconstriction seen at 32 Hz was approximately 50% reduction of blood flow.

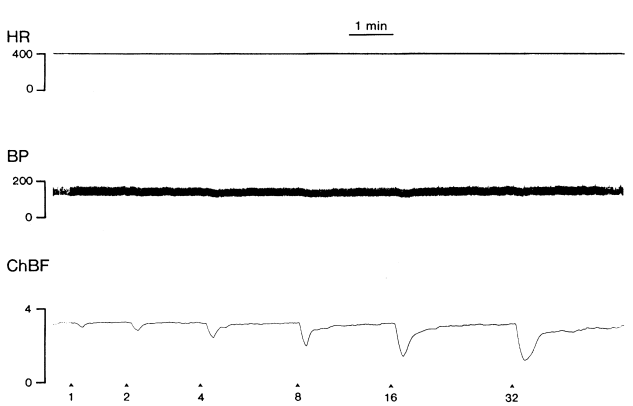

Fig. 1. Effects of increasing frequencies (1–32 Hz) of electrical stimulation of the preganglionic cervical sympathetic nerve on anterior choroidal blood flow (ChBF) as measured using laser-Doppler flowmetry in a pentobarbital-anesthetized rat (arbitrary units). Note the graded frequency-related vasoconstrictor responses measured from the anterior choroid. Neither systemic arterial blood pressure (BP) nor heart rate (HR) were altered by presentation of these trains of stimuli (4 V; 1 ms; 10 s trains).

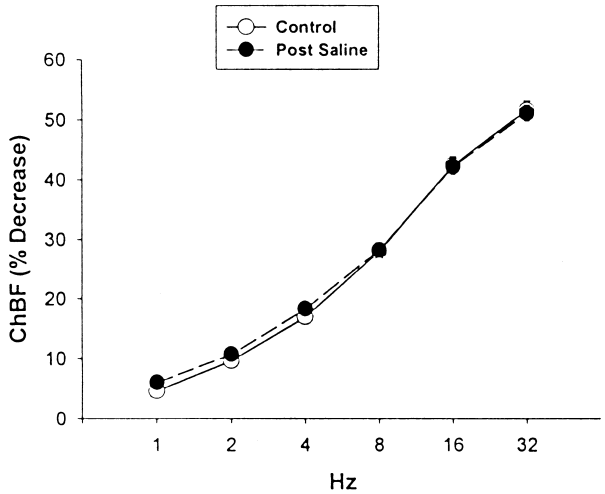

Fig. 2. Composite frequency–response relationships for ocular (anterior choroidal) vasoconstriction in response to electrical stimulation of the preganglionic cervical sympathetic nerve trunk (1–32 Hz) in 22 pentobarbital-anesthetized rats. Values represent percentage decrease from baseline in response to nerve stimulation. Solid lines with open circles represent control responses. Dashed lines with closed circles represent vasoconstrictor responses 15–20 min after intravenous administration of physiological saline. Note high degree of reproducibility between the two trials. Stimulation parameters: 4 V; 1 ms pulse width; 10 s trains of pulses. Values represent mean response ± S.E.M.

Fifteen to twenty minutes after intravenous administration of saline, the frequency-related vasoconstrictor responses were highly reproducible (Fig. 2).

3.2. Effect of autonomic blocking drugs

Vasoconstrictor frequency–response curves were generated before and after intravenous administration of autonomic antagonists. For nonselective α-adrenoceptor blockade, we chose phentolamine and phenoxybenzamine; for nonselective β-adrenoceptor and cholinergic muscarinic antagonism, we utilized propranolol and atropine, respectively. The data in Table 1 illustrate the effects of these autonomic receptor blockers on anterior choroidal vasoconstrictor responses produced by neuronal activation at 4, 8, and 16 Hz. These frequencies of stimulation were chosen as they best reflect the most linear segment of the frequency–response curves. As shown (Table 1), both of the nonselective α-adrenoceptor antagonists caused significant depression of neurally elicited anterior segment choroidal vasoconstriction at all frequencies on stimulation. Propranolol (1 mg kg^{-1}), was devoid of antagonistic effect on these responses. As expected, atropine (1 mg kg^{-1}) also had no effect and all neurally evoked responses were totally abolished by intravenous administration of 20 mg kg^{-1} of the ganglionic blocking drug, hexamethonium (data not shown). The respective cardiovascular responses to each individual antagonist are also illustrated (Table 1). Basal blood pressure responses in Table 1 represent values observed before and after each individual antagonist.

Table 1
Effects of autonomic blocking drugs on choroidal blood flow (ChBF) expressed as percentage of initial control vasoconstriction, mean systemic arterial blood pressure (MSAP; mmHg) and heart rate (HR; beats/min)

Treatment	*N*	ChBF percentage control vasoconstriction			MSAP		HR	
		4 Hz	8 Hz	16 Hz	Before	After	Before	After
Saline	22	113 ± 6	102 ± 3	100 ± 2	121 ± 8	114 ± 10	412 ± 11	405 ± 12
Phentolamine (5 mg kg^{-1})	6	68 ± 1[a]	55 ± 1[a]	49 ± 3[a]	122 ± 7	77 ± 3[a]	394 ± 13	373 ± 11
Phenoxybenzamine (2 mg kg^{-1})	7	38 ± 1[a]	41 ± 1[a]	41 ± 2[a]	120 ± 5	90 ± 6[a]	397 ± 7	393 ± 7
Propranolol (1 mg kg^{-1})	5	96 ± 3	92 ± 4	95 ± 3	107 ± 5	113 ± 5	428 ± 4	338 ± 5[a]
Atropine (1 mg kg^{-1})	5	103 ± 1	98 ± 2	96 ± 2	120 ± 5	118 ± 6	387 ± 5	379 ± 8
Prazosin (300 μg kg^{-1})	8	62 ± 1[a]	47 ± 2[a]	45 ± 1[a]	113 ± 7	74 ± 3[a]	417 ± 8	410 ± 9
Prazosin + Rauwolscine (300 μg kg^{-1}) (500 μg kg^{-1})	8	83 ± 2	52 ± 2[a]	45 ± 1[a]	77 ± 4	68 ± 6	404 ± 10	395 ± 9
Rauwolscine (500 μg kg^{-1})	9	157 ± 2[a]	163 ± 2[a]	123 ± 3[b]	103 ± 4	86 ± 3[a]	404 ± 9	408 ± 8
Rauwolscine + Prazosin (500 μg kg^{-1}) + (300 μg kg^{-1})	9	40 ± 2[a]	46 ± 2[a]	35 ± 2[a]	80 ± 4	66 ± 4[b]	409 ± 8	397 ± 9

[a] $P < 0.01$; [b] $P < 0.05$.
Values represent means ± S.E.M. Measurements taken before and 10–20 min after drug administration.

In order to more completely evaluate the relative contribution made by activation of specific α-adrenoceptor subtypes, we studied, in more detail, the effects of prazosin and rauwolscine on neurally evoked anterior choroidal vasoconstriction (given both alone and in combination). As shown (Fig. 3), prazosin (300 μg kg^{-1}) produced a shift of the frequency–response curve to the right that was more pronounced at higher frequencies of stimulation. This antagonism of choroidal vasoconstriction was not further affected by administration of 500 μg kg^{-1} of rauwolscine (Fig. 3).

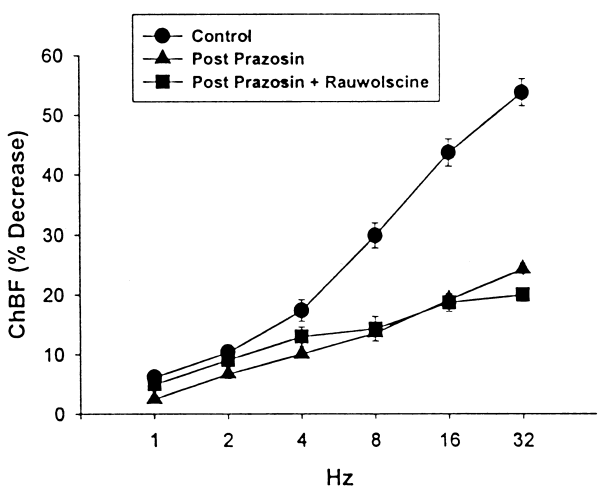

Fig. 3. Composite frequency–response curves representing electrically evoked anterior choroidal vasoconstriction produced by stimulation of the ipsilateral cervical sympathetic nerve of eight pentobarbital-anesthetized rats. Choroidal vasoconstriction (ChBF) expressed as percentage decrease from baseline levels. Circles represent control responses. Triangles represent responses after intravenous prazosin (300 μg kg^{-1}). Squares represent responses after subsequent administration of rauwolscine (500 μg kg^{-1}). Values represent means ± S.E.M.

Fig. 4 is a composite representation of the effects of rauwolscine followed by administration of prazosin. In these experiments, rauwolscine produced facilitation of the choroidal vasoconstriction, rather than the inhibition seen with prazosin as shown in the composite illustration (Fig. 4) and in the table. Subsequent prazosin administration (300 μg kg^{-1}) produced dramatic attenuation of these responses (Fig. 4). Basal cardiovascular responses to these selective α-adrenoceptor antagonists are also shown (Table 1).

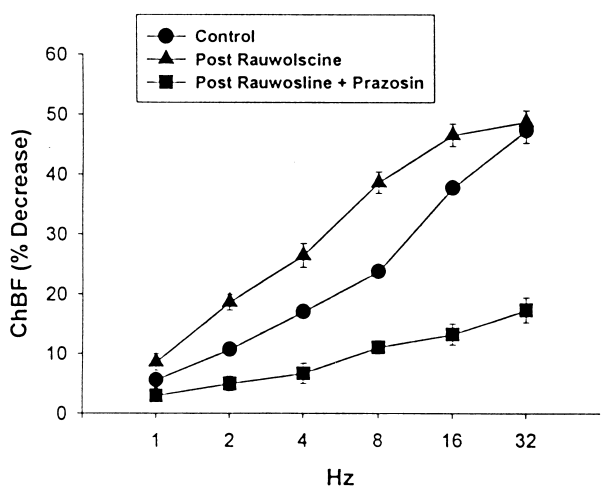

Fig. 4. Composite frequency–response curves representing electrically evoked anterior choroidal vasoconstriction produced by stimulation of the ipsilateral cervical sympathetic nerve of nine pentobarbital-anesthetized rats. Choroidal vasoconstriction (ChBF) expressed as percentage decrease from baseline levels. Circles represent control responses. Triangles represent responses after intravenous rauwolscine (500 μg kg^{-1}) and squares represent responses after subsequent administration of prazosin (300 μg kg^{-1}). Values represent means ± S.E.M. Note potentiation of vasoconstriction produced by rauwolscine (α_2-adrenoceptor blockade) and antagonism of responses by prazosin (α_1-adrenoceptor antagonism).

4. Discussion

Laser-Doppler flowmetry has become accepted as a suitable technique for measurement of small vessel blood flow in a variety of organs such as brain, nerve and skin which have been proven difficult to study by other methods (for overview, see Shepherd and Oberg, 1990). The major limitations of laser-Doppler flowmetry are the lack of calibration in units of flow, the restriction to a very small measurement site, and uncertainty of the precise tissue volume in which blood flow is measured. The main advantages are the noninvasive nature of the procedure and the ability to make continuous measurements, so that frequency–response and dose–response relationships can be established. We previously have utilized this technique to measure microcirculation in the skin of the cat paw (Koss, 1990; Koss et al., 1991) and from the surface of the long posterior ciliary artery in rabbits (Okubo et al., 1990). In more recent studies, we also have made blood flow measurements from the cat anterior choroidal blood vessels (Gherezghiher et al., 1991; Koss and Gherezghiher, 1993; Koss, 1994). The present experiments are an expansion of these earlier studies in that we have utilized laser-Doppler flowmetry to continuously measure blood flow from the surface of the anterior aspect of the rat eye.

Stimulation of sympathetic nerves decreases uveal blood flow in all species studied (Adler et al., 1924; Greaves and Perkins, 1952; Bill, 1962; Alm and Bill, 1973; Alm, 1977), while having little effect on retinal blood vessels (Alm, 1977, 1992). It has been proposed that the role of the sympathetics is to protect the eye from over perfusion (Alm, 1992). In the present study, we found that preganglionic nerve stimulation consistently decreased blood flow in the rat anterior choroid as measured by laser-Doppler flowmetry. In addition, the lack of vasodilation after sympathetic nerve section suggests a paucity of sympathetic tone in these pentobarbital-anesthetized animals. Taken together, the present results are in complete agreement with previous reports both from the literature (see above) and our prior studies using cats (Koss and Gherezghiher, 1988, 1993; Gherezghiher et al., 1991; Koss, 1994).

It is the general consensus that eye blood vessels do not contain β-adrenoceptors (Chandra and Friedman, 1972; Dalske, 1974; Morgan et al., 1981; Alm, 1992) although one group has presented evidence for the existence of vasodilator β-adrenergic receptors in the pig (Malik et al., 1976). Using propranolol as a β-adrenoceptor antagonist, we found no evidence to support a vascular role for innervated vasodilatory β-adrenoceptors in the present experiments using rats or in prior studies of the anterior choroidal circulation of the cat (Koss and Gherezghiher, 1993; Koss, 1994).

Blockade of muscarinic receptors with atropine also did not alter neurally evoked anterior choroidal vasoconstriction. As the pupils were widely dilated by this dose of atropine, it is unlikely that sympathetic-evoked mydriasis plays any role in the observed ocular vasoconstrictor responses. Similarly, lowering of systemic blood pressure produced by α-adrenoceptor blockade also does not appear to contribute to the decreased anterior choroidal vasoconstriction as both prazosin and rauwolscine produced significant hypotension with only prazosin antagonizing the ocular responses. All of the α-adrenoceptor antagonists given either alone or in combination produced expected decreases of systemic arterial blood pressure. Basal choroidal blood flow was also depressed by α-adrenoceptor blockade ranging from $6 \pm 3\%$ with phenoxybenzamine to $26 \pm 8\%$ with combined treatment with rauwolscine followed by prazosin. However, only the decrease in the group treated with prazosin followed by rauwolscine was statistically significant (to $75 \pm 6\%$ of control; $P < 0.05$).

The present study is the first concerning adrenergic subtype characterization in response to neural activation of ocular blood vessels in the rat. In these experiments, it was clear that norepinephrine released from sympathetic nerve endings is a potent activator of α_1-adrenoceptors. This is similar to what we reported previously in cats (Koss and Gherezghiher, 1993). We found no evidence for α_2-adrenoceptor activation following sympathetic nerve stimulation. However, one must keep the possibility in mind that a small contribution of neurally activated postjunctional α_2-adrenoceptor vasoconstriction might be masked by concomitant potentiation of the vasoconstrictor effect following blockade of inhibitory presynaptic α_2-adrenoceptors on the sympathetic nerve endings (Starke et al., 1989). We believe that such a presynaptic effect is the most likely explanation for the potentiation of vasoconstriction seen after administration of rauwolscine. However, we cannot rule out other mechanisms. For example, it is possible that these ocular blood vessels also might possess α_2-adrenoceptors mediating vascular relaxation which might be selectively removed by α_2-adrenoceptor blockade with rauwolscine.

There is compelling evidence both for and against the presence of postjunctional α_2-adrenoceptors on ocular blood vessels. Autoradiography with ^3H-clonidine demonstrate α_2-adrenoceptor binding sites in the rabbit anterior segment (Elena et al., 1989). Radioligand binding studies suggest that the majority of α-adrenergic receptors of the iris-ciliary body are of the α_2-subtype (Mittag and Tormay, 1985). From a functional standpoint, the α_2-adrenoceptor stimulants, B-HT 920 and B-HT 933, produce a reduction in choroidal blood flow when given topically to conscious rabbits (Thorig and Bill, 1986) and anesthetized cats (Koss and Gherezghiher, 1994). Finally, epinephrine, given intra-arterially, produced vasoconstriction in the anterior choroidal vasculature of the cat that was selectively antagonized by α_2-adrenoceptor antagonists (Koss, 1994).

In contrast, only α_1-adrenoceptor agonists are effective in decreasing uveal flow in isolated perfused rabbit eyes (Van Pinxteren and Van Alphen, 1985). Similarly, Ohkubo and Chiba (1987a,b) found that only α_1-adrenoceptor stim-

ulants contract perfused canine and primate ophthalmic and ciliary arteries. These discrepant results are reminiscent of other vascular beds where α_2-adrenoceptors are difficult to demonstrate in vitro (Nielsen et al., 1989).

In summary, sympathetic nerve stimulation produces a frequency-dependent vasoconstriction in the anterior choroid of rats that appears to be mediated by norepinephrine acting on postjunctional α_1-adrenoceptors, with little, if any, involvement of postjunctional α_2-adrenoceptors or of β-adrenoceptors. It would appear that this vascular bed is a clear example of preferential α_1-adrenoceptor innervation and that the α_2-adrenoceptors are either prejunctional or 'extrasynaptic'.

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