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Sympathetic vasodilation in the rat anterior choroid mediated by β_1-adrenoceptors

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Abstract

Electrical stimulation of the preganglionic superior cervical nerve produced a frequency-dependent vasoconstrictor response in the anterior choroidal blood vessels of the eye of anesthetized rats. Systemic administration of phentolamine (5 mg kg^{-1}) reversed the vasoconstriction to a vasodilator response. This sympathetic-evoked vasodilation was not antagonized by inhibition of nitric oxide synthase with N^G-nitro-L-arginine methyl ester (L-NAME) (20 mg kg^{-1}) or by inhibition of cyclo-oxygenase with indomethacin (20 mg kg^{-1}). Intravenous administration of propranolol (1 mg kg^{-1}), as well as selective β_1-adrenoceptor antagonists atenolol (3 mg kg^{-1}), timolol (0.3 mg kg^{-1}), and betaxolol (0.1 mg kg^{-1}), totally abolished the sympathetic nerve evoked ocular vasodilation. In contrast, the selective β_2-adrenoceptor antagonist, ICI-118,551 ((±)-1-[2,3-(Dihydro-7-methyl-1H-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol) (0.3 mg kg^{-1}, i.v.), was without effect. These results support the conclusion that the residual sympathetic ocular vasodilation observed in the rat anterior choroid after α-adrenoceptor blockade is mediated exclusively by neurogenic release of norepinephrine acting on vascular β_1-adrenoceptors.

Keywords: Choroidal blood flow; (Rat); Laser-Doppler flowmetry; Sympathetic vasodilation; β-Adrenoceptor activation; β-Adrenoceptor antagonist; L-NAME (N^G-nitro-L-arginine methyl ester); Indomethacin

1. Introduction

Although adrenergic vasodilation in most blood vessels is believed to be mediated primarily by β_2-adrenoceptors (Bilski et al., 1983), there is accumulating evidence to support a physiological role for neurogenic vasodilation mediated by β_1-adrenoceptor mechanisms (Ngai et al., 1966; Belfrage, 1978; Armstead et al., 1984; Najafipour and Ferrell, 1993a).

In a recent study, we used laser-Doppler flowmetry to measure blood flow from the anterior surface of the rat eye (choroidal circulation) with the goal of identifying the type(s) of α-adrenoceptors involved in neurally evoked sympathetic vasoconstriction (Kawarai and Koss, 1998). During the course of these experiments, it was observed that α-adrenoceptor blockade not only prevented the neurally elicited vasoconstrictor response, but unmasked a sympathetic nerve-evoked ocular vasodilation. The present study was undertaken in an attempt to determine the mechanism responsible for this sympathetic vasodilator response by using inhibitors of nitric oxide and prostaglandin formation as well as β_1- and β_2-adrenoceptor antagonists. Taken together, our results suggest that, in the rat anterior choroid, neural release of norepinephrine from ocular sympathetic nerves initially results in vasoconstriction by preferentially activating α_1-adrenoceptors. Following α-adrenoceptor blockade, sympathetic nerve stimulation produces a residual vasodilation via β_1-adrenoceptor stimulation.

2. Materials and methods

2.1. General

Male Sprague–Dawley rats weighing 280–400 g were anaesthetized with sodium pentobarbital (60 mg kg^{-1}, i.p.), with supplementary pentobarbital (3–5 mg kg^{-1}, i.v.)

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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 laser-Doppler flowmeter (Omega flow FLO-C1, Omegawave, Japan) fitted with a 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 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 that 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 angles to the eye surface several mm posterior to the limbus with care taken not to record blood flow from the large external limbal blood vessels. The time constant was set at 1 s. To avoid influences of blood pressure on the laser-Doppler signal, electrical stimulation was performed under conditions of stable blood pressure and when the laser-Doppler recordings showed a stable baseline.

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. Because laser-Doppler flowmetry yields only relative flow values, the data were normalized to percentage of the basal blood flow values just before electrical stimulation of the sympathetic nerve. The level of zero blood flow 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 establishing the frequency–response relationship, the rats were treated with phentolamine (5 mg kg^{-1}, i.v.) in order to reduce adrenergic-mediated vasoconstriction.

2.4. Experimental protocols

Thirty-nine rats were divided into eight experimental groups of four to six animals in each group. In one group, frequency–response ocular vasoconstrictor relationships were observed before and after saline, and subsequently, after nonselective β-adrenoceptor blockade with propranolol (1 mg kg^{-1}, i.v.). In the remaining experimental groups, antagonists were given after α-adrenoceptor blockade with phentolamine (5 mg kg^{-1}, i.v.). After a control period for vasodilator response stabilization in these phentolamine-treated rats, antagonists were administered intravenously with at least 10–15 min allowed to reach steady state. For quantification of responses, only one additional antagonist was given per animal. However, in a few cases, additional blockers were subsequently administered for illustrative purposes (see Figs. 2 and 3).

2.5. Drugs and statistical analyses

The following drugs were used: (±)-propranolol hydrochloride, indomethacin, atenolol, and gallamine triethiodide (Sigma, St. Louis, MO, USA); *S*(−)-timolol maleate and N^G-nitro-L-arginine methyl ester hydrochloride (L-NAME; Research Biochemical International, Natick, MA, USA); betaxolol hydrochloride and ICI-118,551 hydrochloride ((±)-1-[2,3-(Dihydro-7-methyl-1H-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol) (Tocris Cookson, UK); phentolamine mesylate (Ciba-Geigy, Japan). All drug solutions were prepared in physiological saline with the exception of indomethacin (0.035 M sodium carbonate). Drug dosages refer to the respective salts.

Data are reported as means ± S.E.M. Comparisons of two groups of data (i.e., changes of blood pressure, heart rate and ocular blood flow (mV), before and after antagonist administration) were made using Student's *t*-test for paired comparisons. Three or more groups of results were

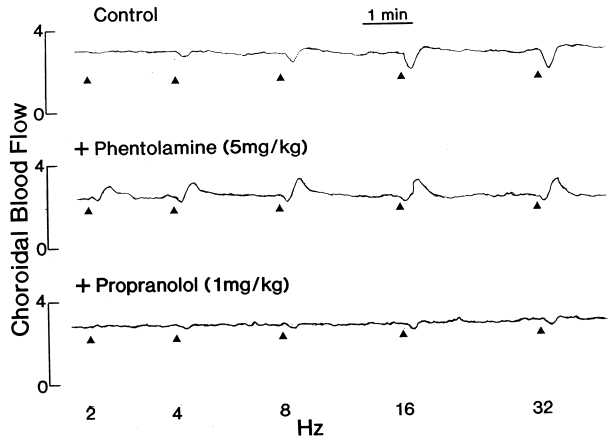

Fig. 1. Effects of increasing frequency (2–32 Hz) of electrical stimulation of the preganglionic cervical sympathetic nerve on anterior choroidal blood flow (ChBF) as measured using laser-Doppler flowmetry in a pentobarbital anesthetized rat (arbitrary units). Upper panel represents frequency-related vasoconstrictor responses measured from the anterior choroid under control conditions (4 V; 1 ms; 10 s trains). Middle panel represents responses repeated after intravenous administration of phentolamine (5 mg kg^{-1}); lower panel after subsequent intravenous administration of propranolol (1 mg kg^{-1}). Note that vasodilation unmasked after phentolamine is totally abolished by subsequent administration of propranolol.

analyzed using repeated measurement ANOVA and Tukey's post-hoc test. In all cases, values of $P < 0.05$ were regarded as statistically significant.

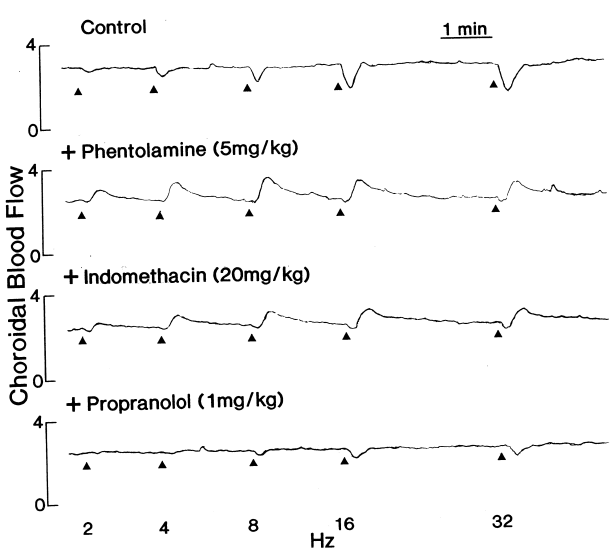

Fig. 2. Effects of increasing frequency (2–32 Hz) of electrical stimulation of the preganglionic cervical sympathetic nerve on anterior choroidal blood flow (ChBF) as measured using laser-Doppler flowmetry in a pentobarbital anesthetized rat (arbitrary units). Upper panel represents frequency-related vasoconstrictor responses measured from the anterior choroid under control conditions (4 V; 1 ms; 10 s trains). Second panel represents responses repeated after intravenous administration of phentolamine (5 mg kg^{-1}). Third and fourth panels represent responses after subsequent intravenous administration of indomethacin (20 mg kg^{-1}) followed by propranolol (1 mg kg^{-1}).

3. Results

3.1. Choroidal vasoconstrictor responses to sympathetic nerve stimulation before and after phentolamine or propranolol

Stimulation of the preganglionic cervical sympathetic nerve produced frequency-dependent anterior choroidal vasoconstrictor responses ranging from $12 \pm 1\%$ reduction of blood flow at 2 Hz, to maximal vasoconstriction of $51 \pm 2\%$ seen at 32 Hz ($n = 34$). After intravenous administration of phentolamine (5 mg kg^{-1}), the uniform vasoconstrictor responses were converted to biphasic responses comprised of vasoconstrictor followed by vasodilator components (Figs. 1–3). Basal mean systemic arterial blood pressure decreased from 114 ± 2 to 78 ± 2 mm Hg after phentolamine administration ($P < 0.01$; $n = 34$). In these same animals, basal anterior choroidal blood flow also decreased following phentolamine blockade from 33 ± 1 to 30 ± 1 (arbitrary units; $P < 0.01$).

At 8 Hz, the initial control vasoconstriction in all 34 preparations was $32 \pm 1\%$ of basal blood flow levels. After

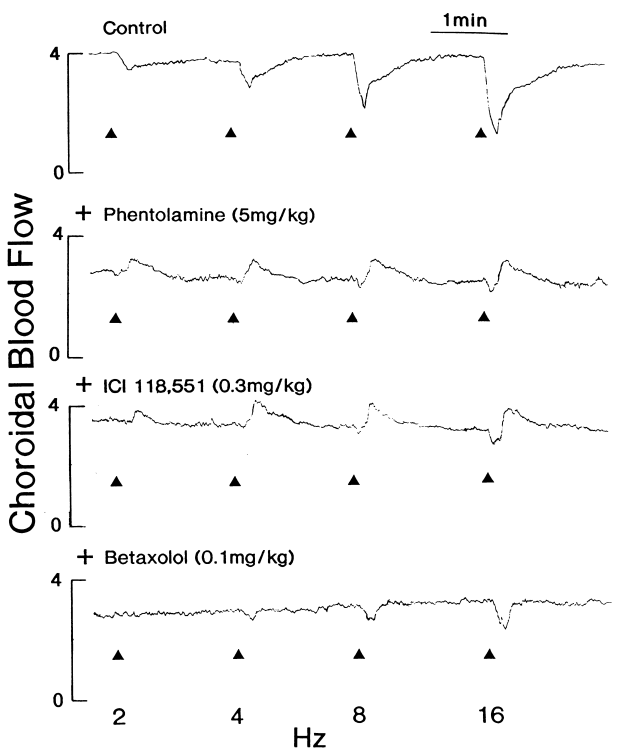

Fig. 3. Effects of increasing frequency (2–16 Hz) of electrical stimulation of the preganglionic cervical sympathetic nerve on anterior choroidal blood flow (ChBF) as measured using laser-Doppler flowmetry in a pentobarbital anesthetized rat (arbitrary units). Upper panel represents frequency-related vasoconstrictor responses measured from the anterior choroid under control conditions (4 V; 1 ms; 10 s trains). Second panel represents responses repeated after intravenous administration of phentolamine (5 mg kg^{-1}). Third and fourth panels represent responses after subsequent intravenous administration of ICI-118,551 (0.3 mg kg^{-1}) followed by betaxolol (0.1 mg kg^{-1}).

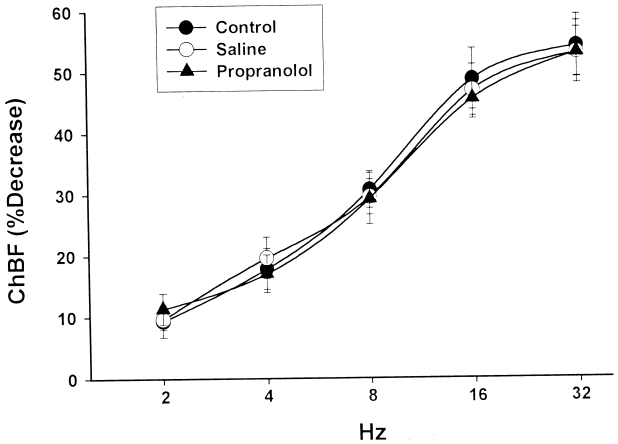

Fig. 4. Composite frequency–response curves representing electrically evoked anterior choroidal vasoconstriction produced by stimulation of the ipsilateral cervical sympathetic nerve of five pentobarbital anesthetized rats. Choroidal vasoconstriction (ChBF) expressed as percentage decrease from baseline levels. Solid circles represent control responses. Open circles represent responses after intravenous saline and triangles represent responses after subsequent administration of propranolol (1 mg kg^{-1}). Values represent means ± S.E.M.

α-adrenoceptor blockade with phentolamine (5 mg kg^{-1}), the vasoconstriction was reduced to 10 ± 1%. This was followed by a vasodilator response equal to 119 ± 2% of basal control blood flow levels (Figs. 1–3). All vasomotor responses to sympathetic nerve stimulation were statistically significant ($P < 0.01$).

In five animals, frequency-related sympathetic vasoconstrictor responses were elicited before and after saline and again after systemic administration of propranolol (1.0 mg kg^{-1}, i.v.). As shown in Fig. 4, the magnitude of vasoconstriction was virtually identical in all three trials.

3.2. Anterior choroidal neurogenic vasodilation before and after L-NAME and indomethacin

Systemic administration of the nitric oxide synthase inhibitor, L-NAME (20 mg kg^{-1}) failed to significantly alter the magnitude of duration of the residual vasodilation seen in animals pretreated with phentolamine in six anesthetized rats. Similarly, the cyclo-oxygenase inhibitor, indomethacin (20 mg kg^{-1}) also did not alter sympathetic neurogenic ocular vasodilation in five additional preparations (Fig. 2). Neither pretreatment altered basal anterior choroidal blood flow. A composite representation of these responses is shown in Table 1.

3.3. Neurogenic ocular vasodilation before and after β-adrenoceptor antagonism

In contrast with the lack of effect produced by L-NAME and indomethacin, the nonselective β-adrenoceptor antagonist, propranolol (1 mg kg^{-1}), consistently abolished the residual sympathetic vasodilation seen after α-adrenoceptor blockade (Figs. 1 and 2). Intravenous betaxolol (0.1 mg kg^{-1}; Fig. 3) together with other β_1-adrenoceptor antagonists (atenolol, 3 mg kg^{-1} and timolol, 0.3 mg kg^{-1}) also totally prevented the neurally evoked sympathetic vasodilator responses (Table 1).

It is of interest that systemic administration of the highly selective β_2-adrenoceptor antagonist (ICI-118,511, 0.3 mg kg^{-1}) was without effect, whereas, in these same animals, subsequent administration of betaxolol again totally prevented sympathetic neural vasodilation of the choroidal circulation (Table 1). Also shown in Table 1 are the cardiovascular responses to the above-mentioned treat-

Table 1
Effects of β-adrenoceptor antagonists, L-NAME and indomethacin on anterior choroidal blood flow (ChBF) vasodilator responses (8 Hz) expressed as percentage of basal blood flow level before stimulation, mean systemic arterial blood pressure (MSAP; mm Hg), and heart rate (HR; beats/min). Values represent means ± S.E.M. in animals pretreated with phentolamine (5 mg kg^{-1}). Vasodilator responses (8 Hz) taken before and 10–20 min after drug treatment listed.

Treatment	*N*	ChBF vasodilation					
		Percentage basal flow		MSAP		HR	
		Before	After	Before	After	Before	After
Propranolol (1 mg kg^{-1})	4	24 ± 5	0[a]	88 ± 7	97 ± 8	363 ± 11	264 ± 10[a]
Atenolol (3 mg kg^{-1})	4	29 ± 7	0[a]	88 ± 8	94 ± 6	362 ± 10	265 ± 16[a]
Timolol (0.3 mg kg^{-1})	4	13 ± 3	0[a]	74 ± 6	86 ± 11	373 ± 16	290 ± 8[a]
Betaxolol (0.1 mg kg^{-1})	5	15 ± 3	0[a]	75 ± 3	77 ± 5	382 ± 14	288 ± 17[a]
ICI-118,551 (0.3 mg kg^{-1})	6	10 ± 2	12 ± 2	74 ± 7	90 ± 6	386 ± 17	377 ± 21
ICI-118,551 + Betaxolol (0.3 mg kg^{-1}) (0.1 mg kg^{-1})	4	17 ± 3	0[a]	77 ± 2	76 ± 6	394 ± 11	287 ± 21[a]
L-NAME (20 mg kg^{-1})	6	16 ± 3	18 ± 3	85 ± 9	146 ± 5[a]	372 ± 12	353 ± 13
Indomethacin (20 mg kg^{-1})	5	28 ± 5	22 ± 3	92 ± 2	91 ± 3	378 ± 13	366 ± 15

[a] $P < 0.01$.

ments. None of the β-adrenoceptor antagonists administered produced a significant effect on systemic arterial blood pressure or on anterior choroidal blood flow. As expected, all compounds with β_1-adrenoceptor antagonist properties lowered basal heart rate (Table 1).

4. Discussion

In the present study, stimulation of the sympathetic cervical nerve decreased anterior choroidal blood flow in the rat eye by activation of α-adrenoceptors. This is consistent with results of others using a variety of species (see Koss and Gherezghiher, 1993; Kawarai and Koss, 1998). After blockade of α-adrenoceptors with phentolamine, sympathetic nerve stimulation resulted in ocular vasodilation that was not prevented by indomethacin, L-NAME, or ICI-118,551. This suggests lack of involvement of prostaglandin-like products, nitric oxide or β_2-adrenoceptor mechanisms. In contrast, nonselective as well as β_1-adrenoceptor selective antagonists totally prevented the residual neurogenic sympathetic vasodilation.

There is a large body of evidence, using in vitro techniques, suggesting presence of β-adrenoceptor-mediated vasodilation in a variety of isolated peripheral blood vessels. For example, precontracted rat jugular veins and pulmonary arteries are relaxed with both β_1- and β_2-adrenoceptor agonists (Cohen and Wiley, 1978; O'Donnell and Wanstall, 1981). Precontracted monkey facial veins also are mainly relaxed by β_1-adrenoceptor mechanisms (Chiba and Tsukada, 1991). In dogs, adrenergic relaxation of coronary (O'Donnell and Wanstall, 1984; Toda and Okamura, 1990; Begonha et al., 1995) and hepatic arteries (Shiraishi et al., 1997) is mediated by activation of β_1-adrenoceptors. In addition, transmural electrical stimulation produces a neurogenic adrenergic vasodilation in rabbit facial veins (McPherson and Bevan, 1987) and monkey coronary arteries (Toda and Okamura, 1990) that is primarily β_1-adrenoceptor mediated.

Although presence of β-adrenoceptor binding sites are clearly demonstrable in ocular blood vessels of the choroidal circulation (Elena et al., 1987; Grajewski et al., 1991), there is minimal in vitro evidence of functional implications. Several investigators failed to find any involvement of β-adrenoceptor-mediated relaxation of the isolated long posterior ciliary artery taken from rabbits or pigs (Dalske, 1974; Su et al., 1994). Others did observe a modest β_2-adrenoceptor-mediated relaxation that was restricted to the intraocular segment of the bovine long posterior ciliary artery (Nyborg and Nielsen, 1995). In contrast, a dobutamine-induced vasodilation was seen in the isolated arterially perfused rabbit eye (Van Pinxteren and Van Alphen, 1985), thus demonstrating presence of β_1-adrenoceptor-mediated relaxation in the choroidal vasculature. One explanation for these disparate observations might be due to selective activation depending on vessel size (i.e., small ''downstream'' blood vessels may be differentially under β-adrenergic control).

As with the present study, a number of in vivo preparations also respond to sympathetic nerve stimulation with vasodilation after α-adrenoceptor blockade. Sympathetic vasodilation has been extensively characterized in the submandibular gland of a variety of animals, where both nitric oxide and β-adrenoceptor mechanisms appear to be responsible (Izumi and Karita, 1994; Anderson and Garrett, 1998) and in the skin where the precise neurochemical mediators remain unclear (Kawarai and Koss, 1992). A clearly β_1-adrenoceptor-mediated sympathetic vasodilation is seen in the vasculature of canine subcutaneous adipose tissue (Ngai et al., 1966; Belfrage, 1978), cat mesentery (Armstead et al., 1984) and rabbit knee joint (Najafipour and Ferrell, 1993a). It is of interest that the neurally evoked vasoconstriction in the rabbit knee joint is mediated mainly by α_2-adrenoceptors (Najafipour and Ferrell, 1993b), whereas in the present study, using the rat anterior choroid, vasoconstriction is exclusively due to α_1-adrenoceptor activation (Kawarai and Koss, 1998).

The ocular vasodilator response to sympathetic nerve stimulation seen after α-adrenoceptor blockade was abolished by administration of β-adrenoceptor antagonists. However, propranolol, given alone, had no appreciable effect on the control neurogenic vasoconstriction. A similar lack of effect of β-adrenoceptor antagonism is seen with neuronally evoked vasoconstriction in canine adipose tissue (Belfrage, 1978) and in the vasculature of the cat anterior choroid (Koss and Gherezghiher, 1993). Although a number of explanations are possible, such as a counterbalancing presynaptic β-adrenoceptor activation (Belfrage, 1978), we have no experimental data to clarify this paradox.

Sympathetic neurogenic vasodilation has been reported in cats even in absence of α-adrenoceptor blockade (Abe et al., 1995). However, in contrast to the present study, β-adrenoceptor blockade with propranolol failed to alter the evoked response, although both the vasoconstriction and vasodilation were antagonized by phentolamine (Abe et al., 1995). Others have demonstrated parasympathetic ocular vasodilation in the cat (Nakanome et al., 1995), rabbit (Nilsson, 1996), and pigeon (Zagvazdin et al., 1996) that, in the later two cases, is mediated by nitric oxide (Nilsson, 1996; Zagvazdin et al., 1996). Obviously, use of different species may contribute to some of the diverging experimental results.

As β-adrenergic antagonists are effective anti-glaucoma drugs, it is thought that the reduced intraocular pressure produced may be due to decreased ciliary body perfusion (Nyborg and Nielson, 1995). However, results regarding effects of β-adrenergic blockers on ocular blood flow are ambiguous. For discussion of divided results concerning β-adrenergic antagonists in human studies, see Yoshida et al. (1991), Harris and Martin (1997), and Kiel and Patel (1998).

Results concerning involvement of β-adrenergic mechanisms on ocular blood flow in animals are equally inconsistent. For example, one group of investigators found topical timolol to produce an initial reduction of choroidal blood flow in ocular hypertensive rabbits (Chiou and Chen, 1992) whereas others, also using microsphere techniques, saw no effect in response to topical administration of timolol (Jay et al., 1984; Green and Hatchett, 1987). Timolol has been claimed to induce constriction of arteries supplying blood to the ciliary body in rabbits (Van Buskirk et al., 1990) and systemic propranolol produces choroidal vasoconstriction, also in rabbits (Kiel and Lovell, 1996). These same investigators, however, found timolol to cause only a slight constriction and betaxolol to be ineffective when given topically to rabbits (Kiel and Patel, 1998). We saw no propranolol-induced alteration of choroidal blood flow when it was administered systemically to anesthetized cats (Koss, 1994; Koss and Gherezghiher, 1993) or rats (Kawarai and Koss, 1998). Finally, timolol reduces choroidal blood flow in isolated perfused bovine eyes (Millar et al., 1995), whereas others saw no effect of timolol on choroidal blood flow in arterially perfused rabbit eyes (Van Pinxteren and Van Alphen, 1985).

We have no explanation to offer for the above-mentioned discrepant findings. The present results, however, support both the existence of functional β-adrenoceptors in the rat anterior choroid (unmasked after α-adrenergic blockade), as well as the findings of no significant effect of β-adrenoceptor antagonism in nonblocked control eyes.

In summary, sympathetic nerve stimulation produces a frequency-related vasoconstriction of the anterior choroidal circulation of the rat eye. After α-adrenoceptor blockade, a residual vasodilation is unmasked. This ocular sympathetic nerve-mediated vasodilation is not mediated by products of cyclo-oxygenase or nitric oxide synthase, nor is it prevented by β_2-adrenoceptor antagonism. In contrast, both nonselective and β_1-adrenoceptor selective blockers totally abolished the vasodilator response, demonstrating that neurogenic sympathetic vasodilation in the eye is mediated exclusively by β_1-adrenoceptor mechanisms.

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