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Review

Functional role of nitric oxide in regulation of ocular blood flow

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Abstract

Nitric oxide generated by three distinct enzyme systems appears to play a critical role in many diverse physiological processes. Using both conventional and immunohistochemical techniques, nitric oxide synthases have been identified throughout the body, including all regions of the eye. A large number of in vitro and in vivo preparations have been utilized showing nitric oxide to have an important role in regulation of regional ocular blood flow. Nitric oxide-mediated control of basal ocular blood flow is demonstrated by vasoconstriction seen in experiments where vascular endothelial cells are removed, or when nitric oxide synthase is inhibited. The endogenous source of nitric oxide in the eye appears to be both endothelial and neural. In addition, administration of drugs that can 'donate' nitric oxide produces vasodilation of the eye vasculature. Local vasodilation in response to illumination of the retina is controlled by generation and release of nitric oxide, whereas most other physiological adjustments of ocular blood flow (i.e., autoregulation and responses to altered blood gas levels) seem to be relatively independent of nitric oxide mechanisms. Nitric oxide is implicated in a variety of ocular pathophysiological states including uveitis, retinal ischemic disease, diabetes and glaucoma.

Keywords: Nitric oxide (NO); Eye; Ocular blood flow; Nitric oxide (NO) synthase; Vascular physiology; Ocular pathophysiology

1. Introduction

In 1980, Furchgott and Zawadzki made the seminal observation that in vitro relaxations of arterial smooth muscle produced by acetylcholine required an endogenous relaxing factor (EDRF) released from endothelial cells (Furchgott and Zawadzki, 1980). Over the ensuing years, it has been shown that EDRF is nitric oxide (NO), a gas that is produced from enzymatic breakdown of L-arginine. Nitric oxide is now implicated as an ubiquitous biological messenger acting to mediate a multiplicity of physiological responses throughout the body, usually related to increased intracellular cyclic GMP concentrations (Ignarro et al., 1987; Palmer et al., 1987; Murad et al., 1990). Numerous reviews concerning the general chemistry and pharmacology of this nitric oxide system are available and are summarized below (for specifics, see Moncada et al., 1991, 1997; Moncada and Higgs, 1993; Moncada, 1997).

Identification of nitric oxide, a primary relaxing factor released from endothelial cells, and the subsequent explosion of research effort in this area was facilitated by several disparate lines of research, including recognition that NADPH diaphorase is a marker for localization of enzymes producing nitric oxide, development of specific antibodies to nitric oxide synthase isoforms and characterization of the biosynthetic pathway, whereby these enzymes produce nitric oxide from L-arginine. Discoveries that certain analogs of L-arginine can inhibit nitric oxide synthesis and that some chemicals can act as an exogenous source for nitric oxide have provided additional powerful tools for determination of potential functional roles for this substance.

Nitric oxide is synthesized by three distinct isoforms of nitric oxide synthase (NOS) which are independently genetically encoded. One of these (referred to as NOS-II or iNOS), is usually induced only in response to allergic or inflammatory challenges and produces large amounts of potentially cytotoxic nitric oxide in inflammatory cells. The two other types of NOS isoforms are normally active in many cells and thus are called constitutive. These generate relatively small quantities of nitric oxide when activated by intracellular calcium. Of the constitutive enzymes, the first was originally discovered in brain and neural tissues and is referred to as NOS-I or nNOS. Neuronal (nNOS) is the main isoform present in the CNS where it is believed to act as a neurotransmitter or neuro-

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modulator. The second constitutive enzyme was the last to be discovered and was found to be largely localized to endothelial cells, thus the terminology, NOS-III or eNOS. Endothelial (eNOS) not only plays a major role in control of vascular tone, but also affects adhesion of leukocytes and platelets to blood vessel walls.

Many L-arginine analogs are available for use as competitive inhibitors of nitric oxide synthases (Nathan and Xie, 1994; Griffith and Gross, 1996). Among the nonselective inhibitors, the most widely used are N^G-monomethyl-L-arginine (L-NMA), N^G-nitro-L-arginine (L-NA), and N^G-nitro-L-arginine methyl ester (L-NAME). The latter is a favorite for in vivo experiments due to its good aqueous solubility and ease of systemic administration. One additional, commonly used nitric oxide synthesis inhibitor, 7-nitroindazole, has apparent selectivity for inhibition of nNOS over eNOS in vivo (Moore et al., 1993). Other approaches for inhibition of nitric oxide action are to treat tissues with hemoglobin (which binds to nitric oxide) or with a guanylate cyclase inhibitor such as methylene blue (Martin et al., 1985).

A final important development was recognition that certain chemicals can exogenously supply nitric oxide for use in agonist studies (Feelisch, 1998). These drugs, now referred to as NO donors, include the clinically useful nitrovasodilators (i.e., sodium nitroprusside, glyceryl trinitrate and various organic nitrites) as well as other agents used as research tools, including sydonimines (i.e., 3-morpholinosydnonimine (SIN-1)) and *S*-nitrosothiols (i.e., *S*-nitroso-*N*-acetylpenicillemine).

With the above background in mind, this review addresses the functional involvement of constitutive nitric oxide in the eye with particular emphasis on ocular vascular mechanisms. It does not address ongoing controversies regarding the potential role of nitric oxide in retinal neurotransmission or in retinal neurotoxicity and cell death (for recent reviews, see Goldstein et al., 1996; Roth, 1997; Bonne et al., 1998). Specific topics covered include anatomical localization of nitric oxide synthases in the eye, functional effects seen using both in vitro and in vivo preparations and potential involvement of nitric oxide in ocular pathophysiology. Additional reviews in some of these areas have been published (Haefliger et al., 1994a; Brown and Jampol, 1996; Becquet et al., 1997; Buckley et al., 1997; Marin and Rodriguez-Martinez, 1997; Haefliger and Flammer, 1998).

2. Localization of nitric oxide synthase in the eye

Technical advances facilitating detailed anatomical localization of enzymes responsible for synthesis of nitric oxide include recognition that nitric oxide synthase is the agent mediating a previously known NADPH diaphorase histochemical reaction which stained a subpopulation of central nervous system (CNS) neurons (Dawson et al., 1991; Hope et al., 1991). Additional advancements in our understanding of location were fostered by development of specific antibodies that can be labeled and complexed with the enzyme in situ (Bredt et al., 1990) as well as by biochemical techniques to measure nitric oxide synthase activity (Geyer et al., 1997). For an overview of the development and impact of the histochemical methodologies, see review by Vincent and Hope (1992). It is now clear that nitric oxide synthases are ubiquitously found throughout the body and in all compartments of the eye. Indeed, ocular tissues were among the first in which presence of these nitric oxide producing enzymes were clearly identified (Yamamoto et al., 1993a,b).

2.1. NADPH diaphorase histochemistry

Prior to linkage of the NADPH diaphorase histochemical reaction with nitric oxide synthase, Sandell (1985) reported enzyme staining of retinal amocrine cells in the rat, cat, rabbit and several primates, including humans. Subsequent studies confirmed this observation in rats (Yamamoto et al., 1993b; Darius et al., 1995; Perez et al., 1995; Roufail et al., 1995), rabbits (Koistinaho et al., 1993), hamsters (Lau et al., 1994) and humans (Roufail et al., 1995). Staining has also been reported for nonamocrine retinal cells in rats (Goureau et al., 1993; Huxlin, 1995; Huxlin and Bennett, 1995), rabbits (Osborne et al., 1993), and birds (Fischer and Stell, 1999). It is of interest that synthesis of a constitutive form of nitric oxide synthase has been demonstrated in bovine retinal rod outer segments (Venturini et al., 1991) and that human retina can express mRNA for both inducible and constitutive isoforms (Park et al., 1994).

Although the initial report in rats showed an absence of NADPH diaphorase reaction in the cornea or trabecular meshwork (Yamamoto et al., 1993b), later studies demonstrated weak to moderate staining in the cornea of rabbits (Osborne et al., 1993) and primates, including humans (Chen et al., 1998). Similarly, the ciliary muscle and outflow channels also appear to contain nitric oxide synthase (Osborne et al., 1993; Nathanson and McKee, 1995a,b), as does the rabbit conjunctiva (Osborne et al., 1993), episcleral blood vessels in several species (Funk et al., 1994) and nerves to the arterial circle of the iris (Tamm et al., 1995). Some scleral and choroidal nonvascular cells also stain for NADPH diaphorase (Poukens et al., 1998).

A number of studies have led to the conclusion that the vascular endothelium of the retina and choroid contain nitric oxide synthase. Initial experiments showing NADPH diaphorase staining in rat choroidal blood vessels have been confirmed by others and expanded to other species including rabbits, pigs and cats (Flugel et al., 1994), birds (Bergua et al., 1996; Cuthbertson et al., 1997; Fischer and Stell, 1999) and primates (Flugel et al., 1994; Chen et al.,

1998). Reactivity has also been shown in human retinal vascular endothelial cells (Roufail et al., 1995).

In isolated ocular blood vessels, a positive NADPH diaphorase reaction is seen in dog ophthalmic and retinal arteries (Toda et al., 1995a,b) and posterior ciliary arteries of both pigs and monkeys (Toda et al., 1997, 1998b). The most likely source for these choroidal nitric oxide-containing nerves, appears to be the pterygopalatine ganglion (Toda et al., 1993; Yamamoto et al., 1993a) with the possible exception in birds where the ciliary ganglion may be involved (Sun et al., 1994; Bergua et al., 1996). In general, nitric oxide-containing neurons are most frequently found in parasympathetic ganglia where they also frequently contain vasoactive intestinal peptide (Alm et al., 1995).

2.2. Immunohistochemistry

Antibodies raised to individual isoforms of nitric oxide synthase have been used to differentially localize these enzymes in ocular tissues. Immunoreactivity for type I (neuronal) nitric oxide synthase is seen in retinal amocrine cells and photoreceptor cells from a variety of species (Yamamoto et al., 1993b; Koch et al., 1994; Liepe et al., 1994; Perez et al., 1995; Lopez-Costa et al., 1997; Oh et al., 1998; Fischer and Stell, 1999).

Immunohistochemistry techniques have also been utilized to demonstrate nitric oxide synthase positive nerve cells in ciliary muscle, which might indicate a role in accommodative mechanisms (Tamm et al., 1995), and in rabbit corneal endo- and epithelium where involvement in control of corneal thickness is suggested (Yanagiya et al., 1997). In human tissues, endothelial nitric oxide synthase is found in ciliary muscle and aqueous outflow pathways; the neuronal isoform is also seen in nerves of these structures (Nathanson and McKee, 1995a). All three isoforms are present in the human optic nerve head; however, only the neuronal isoform is seen in normal eyes (Neufeld et al., 1997).

With regard to vascular innervation, nitric oxide synthase immunoreactive autonomic nerve fibers innervate retinal blood vessels in rats (Bredt et al., 1990; Roufail et al., 1995) as well as both retinal blood vessels and pericytes in humans (Chakravarthy et al., 1995). A major function for nitric oxide may be to relax choroidal blood vessels, as heavy immunostaining is seen in the choroidal vasculature of rats, rabbits, guinea pigs and humans (Yamamoto et al., 1993b; Flugel et al., 1994; Flugel-Koch et al., 1994; Hashitani et al., 1998). Consistent with the results with NADPH diaphorase staining, neuronal nitric oxide synthase is also identified in parasympathetic ganglia (Alm et al., 1995). With regard to the eye, neuronal nitric oxide synthase is found in cell bodies of the pterygopalatine ganglia of pigeons (Cuthbertson et al., 1997) and in nerves innervating the dog and monkey retinal arterioles (Toda et al., 1994, 1996).

3. In vitro experiments

Support for an important role for nitric oxide in regulating vascular tone in ocular blood vessels comes from a variety of in vitro experiments. Many of these studies demonstrate increased smooth muscle tension or contraction after exposure to inhibitors of nitric oxide synthase. Other corroborative experiments entail effects of nitric oxide synthase inhibition on pharmacologically elicited vasodilation and observations of effects of drugs acting to release nitric oxide directly (i.e., nitric oxide donors).

In review of the literature cited below, it should be kept in mind that two distinct vascular systems perfuse the anterior and posterior aspects of the eye. Blood is supplied to the retina mainly by the retinal and short posterior ciliary arteries; the anterior segment receives blood primarily from the long posterior ciliary and anterior ciliary arteries (Alm, 1992). There are anatomical differences in response patterns between vessels of the same species (Vanhoutte and Miller, 1985) as well as potential variability between species. Thus, one might not readily be able to extrapolate responses seen in one artery of one species to that of another vessel or of another type of preparation.

Although nitric oxide is clearly involved in ocular blood vessel vasodilation, it is not the only mediator released as considerable relaxation potential persists in the absence of nitric oxide. Other locally released vasodilator candidates include, among others, members of the prostaglandin family and the still elusive endothelial-derived hyperpolarizing factor (Vanhoutte and Miller, 1985; Brown and Jampol, 1996).

3.1. Tonic release of nitric oxide

A number of studies now support the general conclusion that basal production and release of nitric oxide provides a degree of tonic vasodilator tone, even in totally isolated ocular arteries or their segments. Initially, Benedito et al. (1991b) reported that inhibition of guanylate cyclase with methylene blue results in tonic contraction in isolated bovine retinal arteries. At about the same time, other investigators observed that inhibition of nitric oxide production also leads to tonic contractions of isolated porcine (Yao et al., 1991; Haefliger et al., 1993) and human (Haefliger et al., 1992) ophthalmic artery segments.

Additional studies, using isolated porcine or bovine ciliary arteries, showed increased tension when the bathing media contained an inhibitor of nitric oxide (Haefliger et al., 1993; Su et al., 1994; Wiencke et al., 1994). In contrast, Buckley et al. (1998) observed no increased tone of the bovine anterior ciliary artery (supplying blood to the ciliary body and anterior segment) although other anterior segment arteries (i.e., rat iris arterioles) do contract when exposed to L-NAME (Hill and Gould, 1995).

3.2. Pharmacological stimulation of nitric oxide release

3.2.1. Removal of endothelium

In their classic study, Furchgott and Zawadzki (1980) demonstrated the presence of EDRF by the observation that the vasodilator action of acetylcholine on isolated peripheral blood vessels is lost when the endothelium is removed. Similar studies subsequently have been undertaken using a variety of ocular blood vessels.

Removal of the vascular endothelium prevents bradykinin relaxation of the isolated porcine ophthalmic and ciliary arteries (Yao et al., 1991; Zhu et al., 1997), as well as vascular relaxation produced by substance P in the canine retinal, ophthalmic and ciliary arteries (Kitamura et al., 1993; Wang et al., 1993a; Toda et al., 1995b, 1998a; Okamura et al., 1997). Both vasoactive intestinal peptide (Bakken et al., 1995) and histamine (Benedito et al., 1991a; Wang et al., 1993b) also relax isolated ocular arteries by an endothelium-dependent mechanism. In addition, dipyridamole (Meyer et al., 1996) and vasopressin (Okamura et al., 1997; Toda et al., 1998a) require an intact endothelium to produce relaxation of isolated ciliary arteries. Contractions of porcine ciliary arteries in response to serotonin are potentiated after endothelium removal (Yao et al., 1991), a result similar to that seen after inhibition of nitric oxide synthase in the porcine ophthalmic arteries (Haefliger et al., 1993).

Results with some other agonists on isolated ocular blood vessels are less straightforward. For example, calcitonin gene-related peptide produces a vasodilation that is either blocked (Bakken et al., 1995) or potentiated (Zschauer et al., 1992) by endothelial denudation of porcine or rabbit ophthalmic arteries. Some investigators found acetylcholine to relax bovine retinal (Benedito et al., 1991b) and porcine ciliary and ophthalmic arteries (Su et al., 1994; Bakken et al., 1995) by an endothelial-mediated mechanism. Others observed a lack of endothelial dependence for acetylcholine-induced relaxation of canine ophthalmic or retinal arteries (Wang et al., 1993a; Toda et al., 1995a). More recently, it was shown that endothelial cells are not required for cholinergic responses in monkey ciliary arteries (Toda et al., 1998b), nor does denudation prevent nicotine-induced vasodilation of dog retinal arteries (Toda et al., 1994). Denuded bovine and porcine ciliary

Table 1
Blockade of vasodilation by inhibitors of nitric oxide in experiments using isolated ocular blood vessels. Vasodilator responses produced by agonist administration or by electrical field stimulation

Vasodilator	Artery	Species	Antagonist	References
Acetylcholine	Ophthalmic	Human	L-NA	Haefliger et al. (1992)
		Dog	L-NA	Wang et al. (1993a)
	Retinal	Cow	MB	Benedito et al. (1991b)
	Ciliary	Pig	L-NMA	Yao et al. (1991)
		Monkey	L-NMA	Toda et al. (1998b)
Bradykinin	Ophthalmic	Pig	L-NMA	Haefliger et al. (1993)
		Human	L-NA	Haefliger et al. (1992)
	Ciliary	Pig	L-NMA	Yao et al. (1991); Haefliger et al. (1993)
			L-NAME	Zhu et al. (1997)
Substance P	Retinal	Dog	L-NA	Kitamura et al. (1993)
			MB	Kitamura et al. (1993)
Vasopressin	Ciliary	Dog	L-NA	Okamura et al. (1997)
			L-NA	Toda et al. (1998a)
Histamine	Ophthalmic	Cow	MB	Benedito et al. (1991a)
	Retinal	Human	L-NA	Haefliger et al. (1992)
Prostaglandin D_2	Choroidal	Pig	L-NA	Abran et al. (1997)
Dipyridamole	Ciliary	Pig	L-NAME	Meyer et al. (1996)
Nicotine	Ophthalmic	Dog	L-NA	Toda et al. (1993); Toda et al. (1995b)
	Retinal	Dog	L-NA	Toda et al. (1994)
		Monkey	L-NA	Toda et al. (1996)
	Ciliary	Pig	L-NA	Toda et al. (1997)
			MB	Toda et al. (1997)
Electrical field	Ophthalmic	Dog	L-NA	Toda et al. (1995b)
stimulation	Retinal	Dog	L-NA	Toda et al. (1994)
		Monkey	L-NA	Toda et al. (1996)
	Ciliary	Pig	L-NAME	Su et al. (1994)
			L-NA	Toda et al. (1997)
			MB	Toda et al. (1997)
		Human	L-NA	Nyborg and Nielsen (1994)
		Cow	L-NA	Wiencke et al. (1994)
			MB	Wiencke et al. (1994)
	Iris	Rat	L-NAME	Hill and Gould (1995)

MB: methylene blue. L-NA: N^G-nitro-L-arginine. L-NMA: N^G-monomethyl-L-arginine. L-NAME: N^G-nitro-L-arginine methyl ester.

arteries still respond with relaxation when stimulated electrically (Su et al., 1994; Wiencke et al., 1994), suggesting that if the relaxation is mediated by nitric oxide, it must be released from nerves and not the endothelium (see Section 3.2.2).

3.2.2. Inhibition of nitric oxide synthase

Administration of nonselective inhibitors of nitric oxide synthase, as well as inhibitors of formation of guanylate cyclase, inhibit agonist-induced relaxation of ocular blood vessels in a variety of species (Table 1). Exposure to nicotine or electrical field stimulation seems to release nitric oxide from nerves innervating ophthalmic, retinal and ciliary arteries. These effects are prevented with nitric oxide synthase inhibitors or with inhibitors of guanylate cyclase such as methylene blue (Table 1).

3.3. Nitric oxide donors

In further support for a vasodilator role for nitric oxide on ocular blood vessels, a number of studies clearly show arterial vasodilation in response to drugs which 'donate' nitric oxide. For example, sodium nitroprusside (as well as other nitric oxide donors) have been shown to relax bovine retinal and ciliary arteries (Benedito et al., 1991b; Delaey and Van de Voorde, 1998) and retinal pericytes in culture (Haefliger et al., 1994b, 1997). Nitric oxide donors also dilate isolated porcine ophthalmic, ciliary and retinal arteries (Yao et al., 1991; Haefliger et al., 1993; Meyer et al., 1993a), as well as ophthalmic and retinal arteries of dogs (Kitamura et al., 1993; Toda et al., 1993, 1994, 1997; Okamura et al., 1996).

4. In vivo experiments

In an experiment bridging between in vitro and in vivo procedures, Meyer et al. (1993b) perfused isolated porcine eyes with a Krebs–Ringer solution, with and without the nitric oxide synthase inhibitor, L-NAME. L-NAME decreased perfusion through the eye by as much as 40% of control. This demonstrates basal release of nitric oxide even in the isolated eye, and independence from constituents of the blood or from neural influences. Nitric oxide also modulates basal ocular blood flow in most studies using intact animals. The majority of these studies use nitric oxide synthase antagonists combined with determinations of regional blood flow using microsphere techniques (Table 2). Use of other blood flow measurement techniques is documented in Section 4.1.

4.1. Basal ocular blood flow

As shown using microsphere techniques (Table 2), systemic inhibition of nitric oxide synthase reduces basal blood flow in the choroid, iris and ciliary body of most experimental animals studied, even in the presence of increased arterial blood pressure. The majority of studies report that retinal blood flow is not significantly altered (Table 2). This regional difference may be a reflection of the more powerful autoregulatory mechanisms found in retinal blood vessels combined with technical difficulties in measuring the relatively low retinal blood flow levels (Alm, 1992).

In contrast, others using direct visualization with fundus imaging techniques consistently find retinal arterioles to be constricted in response to systemic inhibition of nitric oxide synthase in many species including dogs (Kitamura et al., 1993; Toda et al., 1994), pigs (Donati et al., 1995, 1997; Gidday and Zhu, 1995) and cats (Harino et al., 1999).

Laser Doppler flowmetry has also been used to demonstrate reduced choroidal and retinal blood flow in response to inhibition of nitric oxide synthesis in cats (Buerk et al., 1996; Koss, 1996; Harino et al., 1999), in the pigeon choroid (Zagvazdin et al., 1996) and in the cat optic nerve head (Buerk et al., 1996). Nitric oxide has recently been shown to regulate basal ocular blood flow in rats with a variety of measurement techniques including radioactive microspheres (Granstam et al., 1998), [^{14}C]iodoantipyrine clearance (O'Brien et al., 1997; Kelly et al., 1998) and Laser Doppler flowmetry (Koss, 1998, 1999). In humans, systemic L-NA administration decreases basal choroidal blood flow as measured by pulsatile flow analysis (Schmetterer et al., 1997c).

4.2. Pharmacological activation of nitric oxide release

4.2.1. Agonist-induced release of nitric oxide

As shown previously with in vitro experiments (Table 1), a variety of agonists also produce ocular vasodilation by an apparent nitric oxide-mediated mechanism in intact preparations. Acetylcholine produces retinal and posterior choroidal vasodilator responses that are antagonized by inhibition of nitric oxide synthase (Gidday and Zhu, 1995, 1998; Mann et al., 1995; Granstam et al., 1998). Both vasodilator responses to substance P in retinal arteries of dogs (Kitamura et al., 1993) and bradykinin-induced increases in ophthalmic flow in perfused porcine eyes are prevented by inhibition of nitric oxide synthase (Meyer et al., 1993b). Using Laser Doppler flowmetry, others have shown endothelin-1 to produce an L-NAME sensitive vasodilation of the vasculature of the optic nerve head (Nishimura et al., 1996).

4.2.2. Nitric oxide donors

Similarly, donors of nitric oxide also produce vasodilation in vivo. For example, nitroglycerin dilates retinal arterioles in the intact dog eye (Kitamura et al., 1993) and sodium nitroprusside administration dilates choroidal and retinal blood vessels of the pig (Hardy et al., 1996a,b, 1998; Donati et al., 1998). In contrast, infusion of sodium nitroprusside does not significantly alter choroidal blood flow in rats (Granstam et al., 1998). In this latter case,

Table 2
In vivo microsphere experiments concerning effects of inhibitors of nitric oxide synthase on basal ocular blood flow

Preparation	Antagonist	Region	Effect	References
Dog	L-NAME	Retina	No effect	Deussen et al. (1993)
		Choroid	Decrease	
		Ciliary body	Decrease	
		Iris	Decrease	
Rabbit	L-NAME	Retina	Decrease	Seligsohn and Bill (1993)
		Choroid	Decrease	
		Ciliary body	Decrease	
		Iris	Decrease	
	L-NMA	Choroid	Decrease	Astin et al. (1994)
		Ciliary processes	Decrease	
		Iris	Decrease	
	L-NAME; L-NA	Retina	Decrease	Nilsson (1996)
		Choroid	Decrease	
		Ciliary body	Decrease	
		Iris	Decrease	
	L-NA (topical)	Retina	No effect	Chiou et al. (1995)
		Choroid	Decrease	
		Ciliary body	No effect	
Cat	L-NAME	Retina	Decrease	Granstam et al. (1993)
		Choroid	No effect	
		Ciliary body/Iris	Decrease	
	L-NAME	Retina	No effect	Ostwald et al. (1995)
		Choroid	No effect	
		Ciliary body	Decrease	
		Iris	Decrease	
	L-NAME	Retina	No effect	Ostwald et al. (1997)
		Choroid	Decrease	
	L-NAME	Retina	No effect	Kondo et al. (1997)
		ONH	Decrease	
Monkey	L-NAME	Retina	No effect	Kondo et al. (1995)
		Optic nerve	Decrease	
Pig	L-NAME	Retina	No effect	Hardy et al. (1996a)
	L-NMA	Retina	No effect	Hardy et al. (1996b)
		Choroid	Decrease	
	L-NAME	Whole eye	Decrease	Van Gelderen et al. (1993)
	L-NAME	Retina	No effect	Jacot et al. (1998)
		Choroid	Decrease	
		Uvea	Decrease	
Rat	L-NMA	Choroid	Decrease	Granstam et al. (1998)
		Uvea	Decrease	
	L-NMA	Retina	No effect	Tilton et al. (1999)
		Choroid	No effect	
		Uvea	No effect	

L-NA: N^G-nitro-L-arginine. L-NMA: N^G-monomethyl-L-arginine. L-NAME: N^G-nitro-L-arginine methyl ester. ONH: Optic nerve head.

ocular vasodilation may have been countered by an overall decrease in systemic blood pressure and consequently, reduction in the head of perfusion pressure.

4.2.3. Neuronal mechanisms

Studies using isolated blood vessels connote a strong neural component to nitric oxide vasodilation (see Section 4.2.2). Experiments with whole animals suggest that the origin of these nitric oxide-containing nerves is primarily from the pterygopalatine ganglia, via the facial nerve, in mammals (Nakanome et al., 1995; Nilsson, 1996) and through the ciliary ganglia in birds (Bergua et al., 1996; Zagvazdin et al., 1996). The nonselective nitric oxide synthase inhibitor, L-NAME, effectively blocks parasympathetic neural vasodilation in rabbits (Nilsson, 1996) and pigeons (Zagvazdin et al., 1996). Neurally evoked choroidal vasodilator responses in birds are also antagonized by the neuronal nitric oxide synthase inhibitor, 7-nitroindazole (Zagvazdin et al., 1996).

A question remains, however, as to whether nerves containing nitric oxide are tonically active. In two studies, 7-nitroindazole failed to depress basal ocular blood flow when administered to anesthetized pigeons or rats (Zagvazdin et al., 1996; Koss, 1998), although others report a depression in nonanesthetized rats (Kelly et al., 1998). The problem is further confused by the claim that 7-nitroindazole may not be as selective for neuronal nitric oxide as previously believed (Reiner and Zagvazdin, 1998).

The presence of sympathetic neural tone may also influence the extent of ocular vasoconstriction in response to drugs like L-NAME (Seligsohn and Bill, 1993).

4.3. Physiological adjustments in ocular blood flow

Autoregulatory mechanisms resulting in vasoconstrictor or vasodilator responses occur in regional ocular vasculature beds and are especially important in the retina where control of blood flow is most critical (Alm, 1992). There is little clear evidence for a significant role for nitric oxide in autoregulatory blood flow mechanisms. For example, hypercapnia leads to vasodilation in the retina that is independent of nitric oxide production (Donati et al., 1995; Gidday and Zhu, 1995), although there is some evidence for blunting of hypercapnic vasodilation in human choroid by L-NMA (Schmetterer et al., 1997b). Hypoxic vasodilation also appears to be refractory to inhibition of nitric oxide synthase (Gidday and Zhu, 1995; Bouzas et al., 1997; Schmetterer et al., 1997b), with the exception that removal of the endothelium alters the reactivity of isolated cat ophthalmociliary arteries to changing oxygen tensions (Alder et al., 1993). Interestingly, hyperoxia does not produce the expected choroidal vasoconstriction in newborn pigs, which is credited to compensatory production or release of nitric oxide and prostaglandins (Hardy et al., 1996a,b, 1997). Spontaneous oscillations of blood flow also occur, which do not appear to involve nitric oxide mechanisms (Buerk and Riva, 1998).

There are only a few studies of acute responses of ocular blood flow following ischemia or in response to decreasing ocular perfusion. In cats, post-occlusive hyperemia in the choroid and retina was not consistently altered by inhibition of nitric oxide synthase (Ostwald et al., 1995, 1997). In addition, the reperfusion pattern following ischemia secondary to asphyxia in newborn pigs was not further impaired by L-NMA (Gidday and Zhu, 1998). In rats, inhibition of nitric oxide synthesis with L-NAME greatly attenuated post-ischemic cerebral hyperemia, but had no significant effect on choroidal reperfusion (Koss, 1999). Finally, ocular autoregulation (vasodilation) in response to decreased perfusion pressure has been claimed to be independent of nitric oxide synthesis (Gidday and Zhu, 1995; Harino et al., 1999), although L-NAME may alter autoregulatory gain at low retinal perfusion pressures (Jacot et al., 1998).

4.4. Response to light

Flickering diffuse illumination presented to the cat retina stimulates retinal neuronal activity and subsequently, increased local ocular blood flow (Riva et al., 1991; Buerk et al., 1995). Nitric oxide is a primary candidate for mediating this light-evoked vasodilation as nitric oxide levels rise and fall at the retinal surface synchronously with either flickering or continuous light stimulation (Donati et al., 1995; Buerk et al., 1998; Neal et al., 1998). Long-term continuous light exposure also increases rat neuronal nitric oxide synthase levels as measured by the NADPH diaphorase technique (Lopez-Costa et al., 1995).

A pivotal role for nitric oxide in light-evoked hyperemia in the retina is further supported by experiments using nitric oxide synthase inhibitors. In one study, Buerk et al. (1996) show (using Laser Doppler flowmetry) that both light-evoked increases in nitric oxide levels and blood flow increases in the cat optic nerve head are blunted after enzyme inhibition. Similar results were found in cats using microsphere technology, where flickering light induced blood flow increases in the optic nerve and retina which were antagonized by administration of L-NAME (Kondo et al., 1997). However, a similar linkage between retinal light-induced hyperemia and nitric oxide could not be shown in monkeys (Kondo et al., 1995).

5. Pathophysiological implications

5.1. Uveitis

Injection of endotoxin (bacterial lipopolysaccharides), systemically or directly, into the eye produces inflammation of the ocular anterior chamber (Rosenbaum et al., 1980). This animal model for uveitis has been utilized in a number of studies attempting to assess the role of nitric oxide generation in the resultant hyperemia, breakdown of the blood-aqueous barrier and cellular infiltration.

In initial studies, the footpads of Lewis rats were injected with endotoxin to produce a delayed ocular inflammation which was antagonized by pretreatment with inhibitors of nitric oxide synthase that were either nonselective (Mandai et al., 1994; Parks et al., 1994; Goureau et al., 1995) or selective for the inducible isoform of the enzyme (Tilton et al., 1994). The hyperemia seen in an animal model of allergic conjunctivitis is also reduced by inhibition of nitric oxide synthesis (Meijer et al., 1995, 1996) as is endotoxin-induced corneal edema (Behar-Cohen et al., 1998).

A large increase of expression of the inducible enzyme is also seen 2–24 h after endotoxin treatment (Goureau et al., 1995; Jacquemin et al., 1996; Wang et al., 1996; McMenamin and Crewe, 1997) and in animal models of acute autoimmune uveoretinitis (Hoey et al., 1997; Rocha et al., 1997). Paradoxically, mice deficient in inducible nitric oxide synthase still remain fully susceptible to endotoxin-induced uveitis (Smith et al., 1998). In addition, direct intraocular administration of nitric oxide donors fail to produce inflammation in the rabbit eye (Behar-Cohen et al., 1996).

Subsequent studies have implicated both inducible and constitutive isoforms of nitric oxide synthase in endotoxin-induced uveitis, with the constitutive isoform playing a pivotal role in the early stages of the inflammatory response (Allen et al., 1996; Mandai et al., 1996). A potentially cytotoxic nitric oxide by-product, peroxynitrite,

is also produced in models of autoimmune uveitis (Wu et al., 1997).

Other recent investigations suggest that nitric oxide acts indirectly by releasing neuropeptides (i.e., substance P and calcitonin gene-related peptide) from sensory nerves which mediate part of the endotoxin-induced inflammatory response (Wang and Hakanson, 1995; Wang et al., 1996, 1997). Calcitonin gene-related peptide has been shown to produce a number of nitric oxide-mediated actions in rabbits, including the breakdown of blood-aqueous barrier (Andersson, 1992) as well as ocular inflammatory responses to infrared irradiation of the iris (Wang and Hakanson, 1995) and electroconvulsive treatment (Wang et al., 1997). In contrast, others have recently found this peptide to suppress activated macrophage production of nitric oxide in the aqueous humor (Taylor et al., 1998).

There is evidence for interaction between nitric oxide and prostaglandin-like substances in genesis of endotoxin-induced uveitis (Bellot et al., 1996, 1997). In rabbits, inhibitors of nitric oxide synthesis block surface hyperemia in response to topical prostaglandins (Astin et al., 1994), as well as prostaglandin-mediated break down of the blood-aqueous barrier (Hiraki et al., 1996). Laser irradiation of the rabbit iris also produces increased levels of prostaglandin E_2 in the aqueous that is associated with inflammation mediated by constitutive nitric oxide (Taniguchi et al., 1998).

5.2. Retinal ischemia

The role played by nitric oxide in response to ischemia-reperfusion is complex and, as has been shown in the cerebral circulation (Iadecola, 1997), likely involves both protective and cytotoxic mechanisms (for reviews, see Roth, 1997; Bonne et al., 1998). It is probable that nitric oxide protects the retina from early ischemic damage by maintaining blood flow. Inducible nitric oxide may, later, trigger neurotoxic mechanisms.

Results from experiments using delayed recovery of electroretinogram waves as an index of ischemic retinal damage have been inconsistent. On one hand, Ostwald et al. (1995, 1997) found L-NAME to have no effect on post-ischemic recovery of retinal electrical activity in cats, although the amplitude was depressed in the controls. In contrast, Veriac et al. (1993) found intravitreal L-NA to depress b-wave recovery in rabbits and Hangai et al. (1999a,b) found inhibition of endothelial nitric oxide synthesis to greatly depress b-wave recovery following ischemia-reperfusion in rats. They suggest that hypoperfusion of the retina accounts for the depressed retinal function seen after nitric oxide synthesis inhibition (Hangai et al., 1999a,b). Others also observed L-NA to depress, and L-arginine to improve retinal function in a nonvascular isolated rabbit retina preparation (Maynard et al., 1996).

Further support for an initial protective role for nitric oxide is provided by studies showing that administration of either nitric oxide donors or L-arginine augments b-wave recovery following retinal ischemia in rats, again, presumably due to enhanced retinal blood flow (Veriac et al., 1993; Liu et al., 1995, 1997).

In contrast, when examined days after the ischemic challenge, structural retinal damage is ameliorated by treatment with nitric oxide synthesis inhibitors (Geyer et al., 1995; Hangai et al., 1996; Lam and Tso, 1996). Selective inhibitors for the inducible isoform are also protective (Geyer et al., 1995; Hangai et al., 1996) and appear to enhance b-wave recovery when measured 1–3 days after the ischemic insult (Hangai et al., 1996).

Intravitreal injection of *N*-methyl-D-aspartate (NMDA) produces a delayed retinal damage that is prevented by pretreatment with either an NMDA receptor antagonist (i.e., dizocilpine; MK-801) or with L-NAME (Morizane et al., 1997). A similar protection is also seen when retinal damage is produced by transient ischemia (Adachi et al., 1998). Finally, both intravitreal NMDA and vascular occlusion produce a L-NAME sensitive retinal toxicity in knockout mice that appears to depend on the presence of the neuronal, rather than the endothelial, isoform of nitric oxide synthase (Vorwerk et al., 1997).

5.3. Diabetes

Several studies implicate nitric oxide in the pathogenesis of diabetes; particularly concerning retinal vascular dysfunction and retinal cell damage. For example, several reports show elevated nitric oxide levels that may contribute to the vascular dysfunction seen in streptozotocin-induced diabetic rats (Tilton et al., 1993; Alder et al., 1997; Do Carmo et al., 1998). A similar alteration is seen in diabetic humans as they respond with significantly less reduction of choroidal blood flow when administered an inhibitor of nitric oxide synthase (Schmetterer et al., 1997a). Finally, a direct nitric oxide-mediated vasodilator action of insulin is seen in experimental animals (Su et al., 1996; Alder et al., 1997) as well as in humans (Schmetterer et al., 1997d).

5.4. Glaucoma

There is an accumulating body of evidence supporting a role for nitric oxide in control of intraocular pressure. Nonvascular cells in the aqueous humor resistance pathways have been shown to possess inherent contractile capabilities (Lepple-Wienhues et al., 1991) and to contain enriched nitric oxide synthase concentrations and activity (Nathanson and McKee, 1995a,b; Haufschild et al., 1996). Isolated strips of bovine ciliary muscle and trabecular meshwork contract in response to inhibition of nitric oxide synthase and relax when exposed to organic nitrovasodilators (Wiederholt et al., 1994). In a similar fashion, donors of nitric oxide produce dose-related relaxation of feline and bovine isolated ciliary muscles, also through a guanylate cyclase mechanism (Goh et al., 1995; Azuma et al., 1997; Kamikawatoko et al., 1998). It has also recently been shown that norepinephrine increases nitric oxide pro-

duction in isolated porcine ciliary processes via β-adrenoceptor activation (Liu et al., 1998).

Topical administration of nitroglycerine and other nitrovasodilators markedly decreases intraocular pressure in rabbits and monkeys by a mechanism involving increased facility of aqueous humor egress from the eye (Nathanson, 1988, 1992; Schuman et al., 1994). Although other investigators have confirmed these observations in rabbits (Behar-Cohen et al., 1996), the observed nitroglycerine ocular hypotension was not replicable in the monkey (Wang and Podos, 1995). In recent human studies, Hessemer and Schmidt (1997) found oral isosorbide dinitrate to produce only a slight reduction of intraocular pressure that could easily be a result of drug-induced reduction of systemic arterial blood pressure, whereas others (Grunwald et al., 1997), found chronic nitrate therapy to produce retinal venous dilation in glaucoma patients.

6. Conclusion

Capacity to generate nitric oxide is found on all regions of the eye where this simple molecule is believed to be involved in a vast array of physiological events including cellular toxicity, neuronal visual processing and control of ocular perfusion. The role played by nitric oxide in control of ocular blood flow appears to contribute to its pathophysiological impact where it likely contributes to the hyperemia and cellular infiltration in uveitis, the vascular dysfunction seen in diabetes and control of intraocular pressure mechanisms in glaucoma. As in the brain, nitric oxide seems to have both protective and cytotoxic actions in responses to ocular ischemia-reperfusion.

Questions needing clarification include further definition of the role of neuronal nitric oxide in control of ocular blood flow in vivo, as well as the degree to which this simple molecule contributes to overall normal physiological adjustments of ocular perfusion. Additional information concerning the extent to which nitric oxide is involved in the above-mentioned pathophysiological conditions, may assist in development of appropriate therapeutic interventions.

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