Relaxation of Trabecular Meshwork and Ciliary Muscle by Release of Nitric Oxide

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Purpose. Recent evidence suggests that nitric oxide (NO) is a major messenger molecule regulating smooth muscle contractility. A role for NO in aqueous humor dynamics, and thus regulation of intraocular pressure, has been postulated. Recently, we described contractile properties of isolated bovine trabecular meshwork and ciliary muscle strips. To assess whether vasodilators contribute to the regulation of trabecular meshwork and ciliary muscle contractility, we measured the effect of various substances known to induce vasodilation by increasing intracellular cGMP production.

Methods. Measurements of isometric tension were performed on isolated bovine ciliary muscle and trabecular meshwork strips using a custom-built electromagnetic force-length transducer. The effects of a membrane-permeable cGMP and an inhibitor of nitric oxide formation (L-nitroarginine = L-NAG) were investigated. Organic nitrate (isosorbide dinitrate = ISDN, isosorbide-5-mononitrate = 5-ISMN) and non-nitrate (sodium nitroprusside = SNP, S-nitroso-N-acetyl penicillamine = SNAP) vasodilators were tested.

Results. Isolated strips were precontracted by carbachol $10^{-6}$ mol/l for 30 minutes (100% carbachol maximal contraction). 8-bromo-cGMP $10^{-4}$ mol/l evoked a relaxation to 86.7% ± 1.4% (n = 8) in ciliary muscle and 58.6% ± 5.4% (n = 7) in trabecular meshwork. Inhibition of NO-synthase by L-NAG increased the carbachol-induced contraction. The organic nitrovasodilators ISDN and 5-ISMN produced significant relaxations. The non-nitrates SNP and SNAP were the most potent relaxants. SNP $10^{-4}$ mol/l relaxed the isolated ciliary muscle to 55.5% ± 3.5% and the trabecular meshwork to 38.6% ± 3.6%. ISDN and SNP were also tested on isolated strips without carbachol-induced precontraction. Both vasodilators had significant relaxing activity under these conditions.

Conclusion. The data indicate that an increase of intracellular cGMP by application of cGMP and organic nitrate or non-nitrate vasodilators induces relaxation of the bovine trabecular meshwork and ciliary muscle. Thus, nitric oxide is a cotransmitter of smooth muscle relaxation in the chamber angle and may be involved in the regulation of aqueous humor dynamics.


Recently, it was shown that nitric oxide (NO) is a major messenger molecule involved in regulation of immune function and vasodilation and serves as an important neurotransmitter in the central and peripheral nervous system.1-3 NO activates soluble guanylate cyclase and thus increases the intracellular level of the second messenger cyclic GMP. NO is synthesized from L-arginine by the nitric oxide synthase (NOS). NOS and NADPH diaphorase, which generally stain cells with a similar distribution, have been localized in various parts of the eye,4-7 including retina, choroid, peripheral nerve fibers of blood vessels, and epithelial cells of the retinal pigment epithelium, ciliary process, iris, and conjunctiva. The observation that NOS-specific activity could be detected in ciliary process and trabecular meshwork8 is of special interest for the interpretation of the effect of vasodilators on intraocular pressure.

It has been established recently that trabecular meshwork cells contain contractile proteins, such as actin filaments, smooth muscle myosin, and smooth muscle-specific $\alpha$-isoactinin.9-16 The presence of smooth...
Muscle-specific actin- and myosin-containing cells suggests a direct effect of trabecular meshwork on outflow regulation in addition to the effect of the ciliary muscle. Indeed, the excitability of cultured bovine and human trabecular meshwork cells and the contractility of isolated meshwork strips from bovine eyes have been demonstrated. Furthermore, muscarinic, α- and β-adrenergic, and endothelin receptors have been demonstrated in both ciliary muscle and trabecular meshwork. The present study investigates the effect of various nitrovasodilators on contractile properties of the isolated bovine trabecular meshwork and ciliary muscle.

MATERIALS AND METHODS

Bovine eyes were obtained from a local abattoir and placed on ice after enucleation. Ciliary muscle and trabecular meshwork strips were dissected as described previously. In brief, after excision of the iris, only ciliary muscle and trabecular meshwork tissue remained on the cornea-scleral segment. Meridional ciliary muscle strips were excised at a right angle to the circular ciliary body. Trabecular meshwork strips were prepared in a circular direction. The tissue strips were approximately 0.5 mm wide and 1 to 3 mm long. Stretching of the tissue was carefully avoided, and tissues were rinsed with cold HEPES-buffered saline.

The effects of agents on ciliary muscle and trabecular meshwork tension were measured isometrically with a force-length-transducer system as described. In short, isolated strips were mounted on fine steel needles connected to the described equipment. It was possible to measure forces from 0.5 μN to 2000 μN with minimal length changes of the tissue strips (<10 μm). The data were recorded on floppy disk and plotted on the screen by a microcomputer. After attachment of the strips to the needles, the tissues were allowed to rest under control conditions for approximately 1 hour. Only strips showing a stable tone were used for further measurements. All isometric force measurements are given as relative values in comparison with the maximal carbachol (10^-6 mol/l) response. Thus, all tissues were precontracted with carbachol, and relaxation responses were expressed as percentages of the maximal effect elicited by carbachol for each tissue. In some experiments, the potency of maximal relaxation was also tested on resting tension not stimulated by carbachol.

The bath medium of the perfusion chamber, which contained a modified Ringer’s solution (composition in mmol/l: Na+, 151; K+, 5; Ca^2+, 1.7; Mg^2+, 0.9; Cl^-, 131; SO_4^2-, 0.9; H_2PO_4^-, 1; HCO_3^-, 28; and glucose, 5; pH adjusted to 7.4), was maintained at 37°C and aerated with 5% CO_2/95% O_2.

Carbachol, 8-bromo-cGMP, L-nitroarginine (L-NAG), isosorbide dinitrate (ISDN), and sodium nitroprusside (SNP) were supplied by Sigma (Deisenhofen, Germany). Isosorbide-5-mononitrate (5-ISMN) was a gift from Boehringer (Mannheim, Germany). S-nitroso-N-acetyl penicillamine (SNAP) was synthesized and kindly supplied by Prof. Dr. E. Noack, Institut für Pharmakologie, Universität Düsseldorf, Germany. All substances except ISDN could be dissolved in the perfusion solution. ISDN was dissolved in DMSO. More than 95% of the solution in the perfusion chamber (volume, 4 ml) could be exchanged within less than 1 minute.

Data reported in this study are given as mean values ± SEM. Statistical significance was assessed by Student's t-test.
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student's t-test for paired observations (% changes versus carbachol-contracted tissues or versus tissues at rest). The unpaired Student's t-test was used for comparison between ciliary muscle and trabecular meshwork tissue. Significance was assumed when $P < 0.05$.

RESULTS

Effects of cGMP and L-nitroarginine

As has been shown before,\textsuperscript{19,20} carbachol $10^{-6}$ mol/l induced a maximal contraction in both ciliary muscle and trabecular meshwork strips. The forces at rest (baseline tension) were adjusted in the range of 100 to 300 μN. In carbachol-treated tissues, 8-bromo-cGMP $10^{-4}$ mol/l produced a relaxation in both tissues (Fig. 1). Figure 2 summarizes the data obtained with cGMP. In ciliary muscle strips, cGMP achieved a significant relaxation to 86.7% ± 1.4% of maximal carbachol effect at the $10^{-4}$ mol/l concentration ($n = 8$, $P < 0.001$). The relaxing effect of cGMP application was more pronounced in isolated trabecular meshwork strips. cGMP $10^{-5}$ mol/l relaxed to 83.9% ± 4.4% ($P < 0.05$) and $10^{-4}$ mol/l to 58.6% ± 5.4% ($P < 0.001$) as compared to carbachol (100%).

As inhibitor of nitric oxide formation, L-nitroarginine $10^{-4}$ mol/l was tested (Fig. 2). In precontracted tissues, L-NAG induced a further contraction to 108.5% ± 1% ($n = 7$) in ciliary muscle and 118.5% ± 5% ($n = 6$) in trabecular meshwork. In tissues not stimulated by carbachol, L-NAG was without significant effects (trabecular meshwork + 2.4% ± 1.3%, $n = 6$; ciliary muscle -0.43% ± 0.3%, $n = 8$).

Effect of Organic Nitrates

The organic nitrates ISDN and 5-ISMN were tested for their relaxation effects in precontracted tissues (Fig. 3). Both substances were effective in relaxing ciliary muscle and trabecular meshwork. With ISDN, maximal relaxation was obviously already obtained at a concentration of $10^{-5}$ mol/l.

Effects of Non-Nitrates

An original tracing illustrating the effect of sodium nitroprusside $10^{-5}$ mol/l is shown in Figure 4. In both ciliary muscle and trabecular meshwork strips, the non-nitrate reversibly relaxed the carbachol precontracted tissues. The data are summarized in Figure 5. SNP (dose-related) and SNAP potently relaxed the ciliary muscle and trabecular meshwork. In general, the relaxant effects were stronger in trabecular meshwork as compared to ciliary muscle ($P < 0.001$).
Baseline Relaxation
ISDN and SNP were also tested in strips that were allowed to rest under control conditions. The tissues were not stretched and not stimulated by carbachol. Both substances significantly relaxed resting ciliary muscle and trabecular meshwork; again, the relaxant effects were weaker in ciliary muscle than in trabecular meshwork (Fig. 6).

DISCUSSION
The present results indicate that the L-arginine–NO pathway is an important inhibitory system in the regulation of bovine trabecular meshwork and ciliary muscle tension. It could be demonstrated that increase of cGMP-induced relaxation; application of an L-arginine analog further contracted the precontracted tissues; NO-releasing nitrovasodilators have relaxant activity; and non-nitrate vasodilators, which are activators of the soluble guanylate cyclase, are also powerful relaxants of isolated bovine trabecular meshwork and ciliary muscle strips.

After the observation that organic nitrates produce an increase in the intracellular concentration of cyclic GMP in smooth muscle cells,21 the cascade of events leading to relaxation has been established.1–3 It is now generally accepted that nitrovasodilators and NO activate intracellular soluble guanylate cyclase. The consequent increase in intracellular cyclic GMP leads to protein phosphorylation and, finally, to smooth muscle relaxation. Extracellularly applied cGMP in a membrane-permeable form also induces relaxation. In in vitro experiments, the concentrations used are usually in the range of $10^{-3}$ mol/l. In our experimental set-up with the isolated trabecular meshwork and ciliary muscle strips, we could demonstrate a relaxant effect of 8-bromo-cGMP at $10^{-4}$ mol/l in ciliary muscle and trabecular meshwork, and at $10^{-5}$ mol/l in trabecular meshwork. Thus, compared to other in...
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**FIGURE 6.** Relaxant effect of isosorbide dinitrate and sodium nitroprusside on resting strips that were allowed to rest under control conditions.

Vitro experiments, cGMP exhibited a strong potency to relax the carbachol-precontracted trabecular meshwork and ciliary muscle.

Several L-arginine analogs with different potencies have been described as inhibitors of NO release in smooth muscle cells and have been used as tools to investigate this pathway.1–3 We used L-nitroarginine, which is a potent competitive inhibitor of NO synthase. L-NAG induced a further constriction in precontracted ciliary muscle and trabecular meshwork strips, the effect on trabecular meshwork again being stronger. This indicates that in both precontracted tissues there is a continuous release of NO, and this release of NO might play an important role in regulating contractility of the trabecular meshwork. Removal of this relaxing activity is responsible for the contraction induced by L-NAG and could influence the outflow of aqueous humor. In quiescent (unstimulated) ciliary body and trabecular meshwork, L-NAG did not induce a significant contraction. This indicates that without precontraction, there is no or very little release of NO.

Organic nitrates such as ISDN and 5-ISMN require the interaction with a thiol such as cysteine and, consequently activate the soluble guanylate cyclase.1–3 The non-nitrate vasodilators (SNP and SNAP) are known to be direct activators of the cyclase.1–3 The powerful relaxing effect of the vasodilators demonstrates the presence of this enzyme in trabecular meshwork and ciliary muscle tissues. It is noteworthy that the vasodilators ISDN and SNP also relax the tissues not precontracted by carbachol, indicating that there is also a continuous release of NO at resting conditions.

At least two different NO synthases have been described,1–3 a constitutive and an inducible NO synthase. The major difference seems to be that the inducible synthase is independent of Ca²⁺/calmodulin and releases NO in nanomolar concentrations for a long period, whereas the constitutive form depends on Ca²⁺/calmodulin and releases much smaller amounts of NO over short periods. From our experiment, we cannot define which of the NO synthases is active in ciliary muscle and trabecular meshwork. In general, the inducible NO synthase produces larger amounts of NO in smooth muscle cells than does the constitutive enzyme. The long-lasting effect and the slow time course (within minutes) of induced relaxation or contraction in trabecular meshwork and ciliary muscle suggests that the inducible NO synthase was stimulated in the tissues we tested.

Nitrovasodilators have been used in clinical medicine for more than 100 years.1 The effect of nitrovasodilators on intraocular pressure of man and experimental animals has also been tested. However, variable effects have been reported when nitrovasodilators were applied locally or systemically. The systemic application of nitrates for ophthalmologic purposes is complicated by the well-described systemic hypotensive effect. The literature has been reviewed recently.22 Because of the presence of the L-arginine/NO system in various parts of the eye, further experiments on the effect of vasodilators on regulation of intraocular pressure seem to be necessary. It is of special importance that the NO system has been localized in ciliary body and trabecular meshwork.8 Relative to the NOS activity in retina–choroid in the bovine eye, an activity of 34% was measured in trabecular meshwork and of 23% in ciliary processes.

From our experiments on relaxation–contraction, it can be deduced that in trabecular meshwork and ciliary muscle of the bovine eye, cGMP is the final common effector molecule of the vasodilators activating the NO system. A continuous release of NO under resting and precontracted conditions seems to maintain a relaxing effect. The NO-mediated basal relaxation could functionally antagonize constrictor responses, as has been described for the regulation of retinal vascular muscle tone.25–27 The present concept of outflow regulation mechanisms considers the trabecular meshwork to be a more passive system with resistance against the secreted aqueous humor. We have recently presented evidence that bovine15 and human trabecular meshwork cells17 are excitable, smooth-muscle-like cells.19–20 The data presented on the function of nitric oxide support our hypothesis.
that the trabecular meshwork, in addition to the ciliary muscle, is a resistance system which at least in the bovine eye could actively regulate the outflow pathway and might be modulated by neural and hormonal contractile and relaxing influences. The relationship of the present model system to the primate eye has to be demonstrated.

**Key Words**
trabecular meshwork, ciliary muscle, isometric force measurement, nitric oxide, nitrosodilators

**References**
