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Effect of Latanoprost, Prostaglandin $F_2\alpha$ and Nipradilol on Isolated Bovine Ciliary Muscle

Takeshi Yoshitomi*, Kazutsuna Yamaji*,
Hitoshi Ishikawa† and Yoshitaka Ohnishi*

*Department of Ophthalmology, Wakayama Medical University, Wakayama, Japan;
†Department of Ophthalmology, Kitasato University School of Medicine, Kanagawa, Japan

Purpose: Ciliary muscle tone is considered to be an important factor for control of uveoscleral outflow. In an attempt to clarify the functional roles of the ciliary muscle in uveoscleral outflow, the effects of latanoprost, prostaglandin (PG)$F_2\alpha$ or nipradilol, all of which are known to increase uveoscleral outflow, were investigated, using the bovine ciliary muscle.

Methods: We isolated longitudinal ciliary muscle from bovine eyes and investigated the effects of these three agents on the mechanical properties of this muscle using isometric tension recording methods.

Results: Latanoprost and $PGF_2\alpha$ evoked small but discrete contractions at a concentration of 0.1 μM even during the sustained contraction evoked by 10 mM acetylcholine (ACh). However, nipradilol did not evoke any response at concentrations up to 0.1 mM. None of these agents had an effect on the amplitude of the ciliary muscle twitch contraction evoked by electrical field stimulation.

Conclusions: Our findings indicate that these three agents have no relaxant effect on isolated bovine ciliary muscle even during the sustained contraction evoked by ACh. Further, these agents had no effect on the contraction evoked by field stimulation, which indicates that the drugs have no presynaptic effects. These results are inconsistent with the hypothesis that drugs that increase uveoscleral outflow relax the ciliary muscle with a consequent increase in uveoscleral outflow. Further investigation of the role of ciliary muscle contractility on uveoscleral outflow is warranted. **Jpn J Ophthalmol 2002;46:401–405**

Key Words: Ciliary muscle, latanoprost, nipradilol, prostaglandin F_2, uveoscleral outflow.

Introduction

Latanoprost is a selective prostaglandin (PG) FP receptor agonist that is widely used for the reduction of intraocular pressure (IOP) in the treatment of glaucoma.[1–4] Latanoprost reduces IOP primarily by increasing uveoscleral outflow without affecting aqueous humor flow rates or outflow facility.[5–7] However, the mechanisms by which uveoscleral outflow is increased by this agent are controversial. It has been reported that contraction of the ciliary muscle inhibits uveoscleral outflow,[8] probably by reducing the space between smooth muscle fiber bundles. Thus, the changes in intramuscular space in the ciliary muscle are important for uveoscleral outflow regulation. Morphological and tissue culture studies have revealed that PGs reduce the amount of extracellular matrix among the fiber bundles of monkey ciliary muscle[9,10] as well as among the fiber bundles of human[11,12] ciliary muscle, which may explain the mechanism of increased uveoscleral outflow. On the other hand, $PGF_2\alpha$ was reported to induce relaxation in monkey ciliary muscle,[13] which the authors argued increased uveoscleral outflow. However, others have reported that $PGF_2\alpha$ does not evoke relaxation in bovine[14] and porcine[15] ciliary muscle. There are no published data concerning the effect of latan-

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Correspondence and reprint requests to: Takeshi YOSHITOMI, MD, Department of Ophthalmology, Wakayama Medical University, 811-1 Kimiidera, Wakayama, Wakayama 641-0012, Japan

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oprost on isolated ciliary muscle. In an attempt to clarify the role of ciliary muscle contractility on uveoscleral outflow, we examined the effect of latanoprost and $PGF_2\alpha$ on isolated bovine ciliary muscle in vitro by conducting an isometric tension recording test. Nipradilol is a novel antiglaucoma agent, which has selective α_1 and β-adrenergic receptor antagonistic action and nitroglycerine-like activity.[16,17] Because this drug is also reported to reduce IOP by increasing uveoscleral outflow,[17] we also examined the effect of this drug on isolated bovine ciliary muscle.

Materials and Methods

Bovine eyeballs were obtained from a local slaughterhouse and were kept in an ice-cold Krebs solution. Under the microscope, the ciliary body was carefully dissected from the scleral spur, lens and vitreous body; then muscle specimens (1.0–1.5-mm wide and 4–5-mm long) were prepared. Meridional sections of ciliary muscle that included the entire anterior-posterior extent of the muscle were used in this experiment. Because preliminary experiments showed no difference in the response of circular and meridional sections of ciliary muscle to various agents, and meridional muscle bundles are more important for regulating uveoscleral outflow,[18] we examined the meridional sections of this muscle. Detailed methods for an isometric tension recording experiment have been reported previously.[19] To measure the development of isometric tension, ciliary muscle specimens were mounted in a 1.2-mL organ bath through which the test solution, at a temperature of 35°C, flowed continuously (0.3 mL/s). The muscle specimen was placed vertically in the organ bath with a silk thread tied to each end. The thread at the top of the specimen was fastened to a mechano-transducer (RCA5734; Nihon-Koden, Tokyo) and the thread at the bottom end was attached to a hook at the bottom of the bath (Figure 1). The resting tension was 100–200 mg. To investigate the neural effects on the motility of the muscle tissues, electrical field stimuli were applied through a pair of electrodes consisting of platinum plates, separated by 5 mm, and placed so that a current pulse would pass transversely across the tissue. Single and repetitive stimulations at 50 Hz were applied, with a current pulse of 1 millisecond in duration and 5–10 V in strength.

Modified Krebs solutions in the following ionic concentrations were used (mM): Na^+ 137.4, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.5, Cl^- 134.0, HCO_3^- 15.5, $H_2PO_4^-$ 1.2, and glucose 11.5. The solutions were aerated with 95% O_2 and 5% CO_2, and the pH was adjusted to 7.2–7.3.

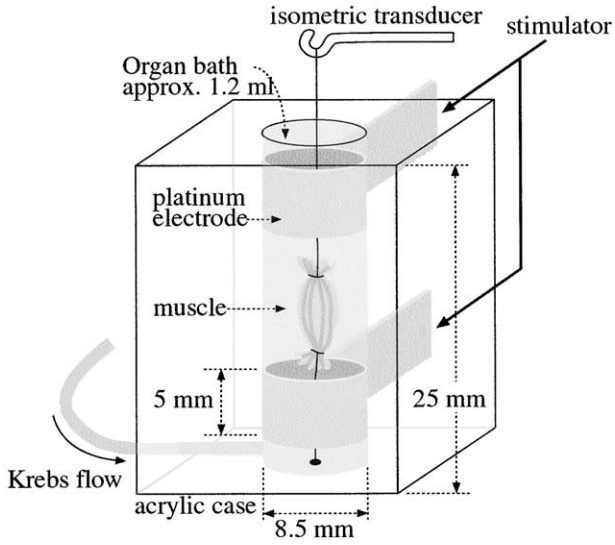

Figure 1. Schematic drawing of the isometric tension recording method. Krebs solution with or without various drugs flows continuously over isolated bovine ciliary muscle specimens. For full details, see text.

The following drugs were used: tetrodotoxin (Sankyo, Tokyo), atropine sulphate, prostaglandin $F_2\alpha$ (Sigma, St. Louis, MO, USA), acetylcholine (ACh; Tokyo Kasei), latanoprost (13,14-dihydro-17-phenyl-18,19,20-trinor-prostaglandin $F_2\alpha$-isopropyl ester; Xalatan®; Pharmacia Upjohn, Uppsala, Sweden) and nipradilol (Kowa, Tokyo).

Results

When specimens of ciliary muscle were mounted in the organ bath, the tissue gradually relaxed to a steady tension after 0.5–1-hour superfusion with Krebs solution. Spontaneous contraction did not occur at any time during the experiment. Application of ACh (10 mM) evoked reproducible contraction, which consisted of a phasic and a tonic component (Figure 2a). The amplitude of phasic and tonic contractions evoked by 10 mM ACh were 88.6 ± 34.4 mg (n = 12) and 73.1 ± 5.3mg (n = 12), respectively. Electrical field stimulation (1 millisecond in duration, 10 V in strength, 50 Hz and 50 train) evoked twitch contraction in the ciliary muscle (Figure 2b). This contraction was blocked by tetrodotoxin (0.1 μM) or atropine (1 μM), suggesting that the responses were of cholinergic nerve origin (data not shown). The amplitude of contraction evoked by field stimulation was 86.6 ± 12.0 mg (n = 6).

One hour after the muscle tone had reached a steady level, electrical field stimuli (10 V, 1 millisecond, 50 stimuli at 50 Hz) were applied every 1.5 min-

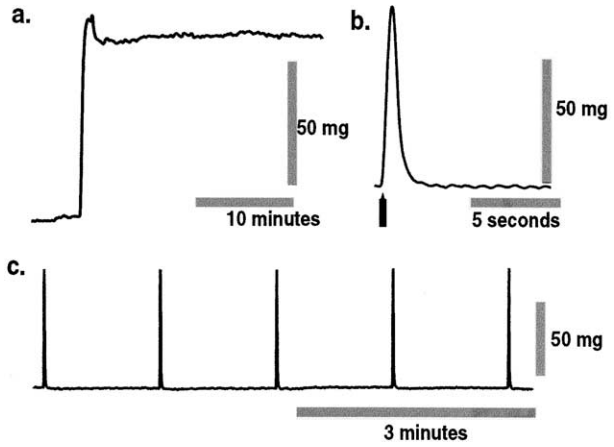

Figure 2. (**a**) Effects of 10 mM acetylcholine (ACh) on the ciliary muscle specimens. ACh induced phasic and tonic contraction. (**b**) Effect of electrical field stimulation on the ciliary muscle. The arrow shows the application of field stimulation (1 millisecond in duration, 10 V in strength, 50 Hz and 50 train). (**c**) Effect of electrical field stimulation applied every 1.5 minutes on the ciliary muscle.

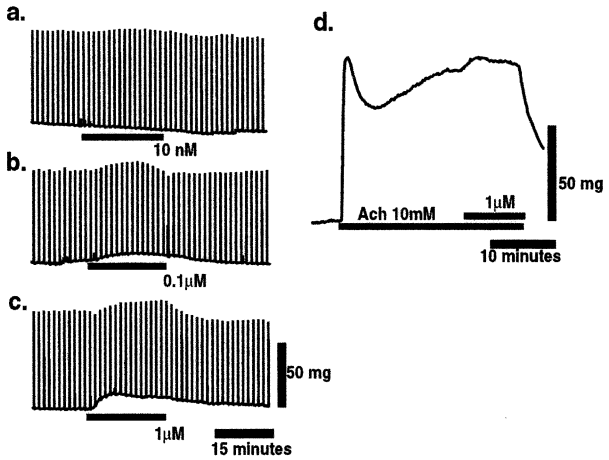

Figure 4. Effects of prostaglandin (PG)$F_{2\alpha}$ [(**a**) 10 nM; (**b**) 0.1 μM; (**c**) 1 μM] on the resting muscle tone or mechanical responses evoked by field stimulations, when the field stimulations were applied every 1.5 minutes. (d) Effects of $PGF_{2\alpha}$ (1 μM) during the sustained contraction evoked by 10 mM acetylcholine.

utes. Electrical field stimulation evoked twitch contraction of this muscle (Figure 2c). As shown in Figure 3a, latanoprost did not modify the resting tone of the ciliary muscle at a concentration of 10 nM. However, latanoprost evoked small but discrete contractions at concentrations of 0.1 and 1.0 μM. The amplitude of contraction evoked by 1 μM latanoprost was 5.8 ± 3.9 mg (n = 6). Latanoprost at 1 μM did not affect the amplitude of contraction induced by field stimulation (Figure 3c). The effect of latanoprost on the bovine ciliary muscle was also investigated during sustained contraction evoked by 10 mM ACh. Latanoprost evoked a small contraction at a concentration of 1 μM (Figure 3d) in 2 out of 6 specimens tested. Relaxation did not occur in any of the specimens tested (n = 6).

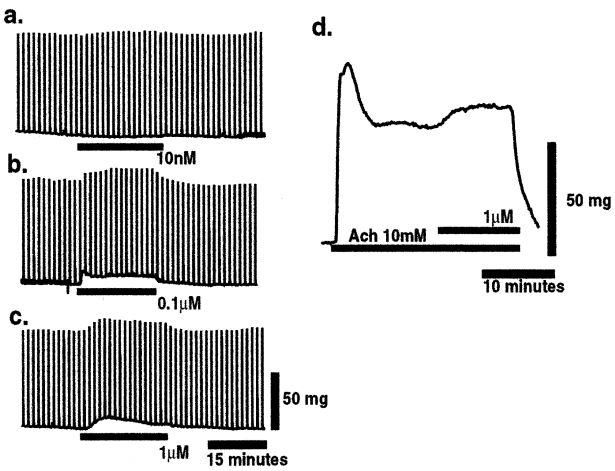

Figure 3. Effects of latanoprost [(**a**) 10 nM; (**b**) 0.1 μM; (**c**) 1 μM] on the resting muscle tone or mechanical responses evoked by field stimulations, when the field stimulations were applied every 1.5 minutes. (**d**) Effects of latanoprost (1 μM) during the sustained contraction evoked by 10 mM acetylcholine.

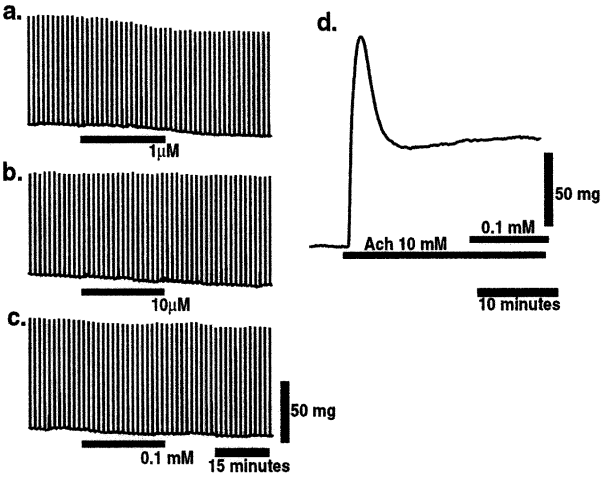

Figure 5. Effects of nipradilol [(**a**) 1 μM; (**b**) 10 μM; (**c**) 0.1 mM] on the resting muscle tone or mechanical responses evoked by field stimulations, when the field stimulations were applied every 1.5 minutes. (**d**) Effects of nipradilol (0.1 mM) during the sustained contraction evoked by 10 mM acetylcholine.

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As shown in Figures 4a–c, $PGF_2\alpha$ did not modify the resting tone of the ciliary muscle, at a concentration of 10 nM. However, $PGF_2\alpha$ evoked small but discrete contractions at concentrations of 0.1 and 1.0 μM (Figures 4b,c). The amplitude of contraction evoked by 1 μM $PGF_2\alpha$ was 22.3 ± 12.0 mg (n = 6). The amplitude of contraction induced by field stimulation was not affected by $PGF_2\alpha$ at concentrations up to 1 μM. $PGF_2\alpha$ (1 μM) also evoked contraction during the sustained contraction evoked by 10 mM ACh (Figure 4d) but produced no relaxation in any of the specimens tested (n = 6). Nipradilol did not have any effect either on the basal tone, the amplitude of contraction evoked by field stimulation or on the ACh-induced sustained contraction of the bovine ciliary muscle up to a concentration of 0.1 mM (Figures 5a–d).

Discussion

We have demonstrated in this study that both latanoprost and $PGF_2\alpha$ have small contractile effects (the effect is smaller for latanoprost than for $PGF_2\alpha$) on isolated bovine longitudinal ciliary muscle without having any relaxant effect even during the sustained contraction evoked by ACh. The data are consistent with previous data that $PGF_2\alpha$ evoked dose-dependent contraction of bovine ciliary muscles.[20] Others have reported that $PGF_2\alpha$ has no effect on the same tissues.[14,21] Moreover, nipradilol has no effect on ciliary muscle up to a concentration of 0.1 mM. Presynaptic effects of these agents are also important in addition to postsynaptic effects because the ciliary muscle receives mainly cholinergic innervation[19,22] and the effect on the nerve terminals may affect the contractility of this muscle in vivo. We have demonstrated the effect of latanoprost, $PGF_2\alpha$, and nipradilol on the amplitude of contraction evoked by field stimulation; this contraction is produced by cholinergic nerve activation. These agents have no effect on the contraction evoked by field stimulation, which indicates that the drugs have no presynaptic effect on bovine ciliary muscle tissue (see Table 1).

It is well established that $PGF_2\alpha$ and latanoprost reduce intraocular pressure by increasing uveoscleral outflow in various species,[6] including humans.[5,7] Nipradilol is also reported to increase fluorophotometrically determined uveoscleral outflow in the rabbit.[17] Ciliary muscle relaxation has been suggested as a possible mechanism of increase in uveoscleral outflow.[13] In light of that suggestion, these three agents, all known to increase uveoscleral outflow, should have had either a postsynaptic effect, which would relax this muscle, or a presynaptic effect, which would inhibit the contraction evoked by the cholinergic nerve. However, our present results clearly show that these three agents have no relaxant effect on ciliary muscle either pre- or postsynaptically. Moreover, latanoprost and $PGF_2\alpha$ at high concentrations have only small contractile effects on ciliary muscle. Other in vitro studies revealed that $PGF_2\alpha$ only at a concentration of 10 μM generated relaxation in cat ciliary muscle.[23] However, 10 nM $PGF_2\alpha$ was found to evoke relaxation in the monkey ciliary muscle.[13] High concentrations of $PGF_2\alpha$ increase ^{45}Ca efflux in cultured human ciliary muscle cells.[24] Yousufzai et al reported that exogenous $PGF_2\alpha$ had little effect on cyclic adenosine monophosphate accumulation or Ca^{2+} mobilization in bovine ciliary muscle.[25] However, $PGF_2\alpha$ induces other PG release, such as PGE_2, from the iris-ciliary body.[26] As PGE_2 is a potent activator of the adenylate cyclase system in ciliary muscle, this PG-induced PG release may explain in part how $PGF_2\alpha$ increases uveoscleral outflow.[26] However, there is no direct evidence that ciliary muscle relaxation per se induces increased uveoscleral outflow with a consequent IOP reduction because atropine, one of the most potent ciliary muscle relaxants[22] has no such effect. In addition, ciliary muscle relaxation should lead to a hyperopic shift in accommodation. However, topical application of unoprostone, another PG-related

Table 1. Summary of the Results

Agents	Effect on Basal Tone	Effect on Sustained Contraction Evoked by Acetylcholine	Effect on the Amplitude of Contraction Evoked by Field Stimulation
$PGF_2\alpha$ (1 μM)	Contraction (22.3 ± 12.0 mg)	Small contraction in 6/6 specimens	No effect
Latanoprost (1 μM)	Contraction (5.3 ± 3.9 mg)	Small contraction in 2/6 specimens	No effect
Nipradilol (up to 0.1 mM)	No effect	No effect	No effect

compound known to increase uveoscleral outflow, did not have any effect on the accommodative state in humans, although it significantly reduced IOP.[27]

There are no data on the investigation of the effect of agents other than $PGF_2\alpha$, which increase uveoscleral outflow, on the contractility of the ciliary muscle. We have investigated for the first time the effect of three agents, known to increase uveoscleral outflow, on the isolated bovine ciliary muscle. None of these agents had a relaxant effect on this muscle. The results are inconsistent with the hypothesis that drugs like latanoprost relax ciliary muscle with a consequent increase in uveoscleral outflow. Mechanisms other than ciliary muscle relaxation may be considered as explaining the latanoprost or nipradilol action in increasing uveoscleral outflow. However, it is reported that there are species differences in latanoprost action on intraocular pressure.[28] So, further investigation on the effect of latanoprost or nipradilol on isolated ciliary muscle in various species is necessary.

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