Experimental Eye Research 87 (2008) 251–256

Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Relaxing effect and mechanism of tafluprost on isolated rabbit ciliary arteries

Yaru Dong [a,b], Hiroshi Watabe [a], Guanfang Su [b], Hitoshi Ishikawa [c], Noriko Sato [a], Takeshi Yoshitomi [a,*]

[a] Department of Ophthalmology, Akita University School of Medicine, 1-1-1 Hondo, Akita, 010-8543, Japan
[b] Department of Ophthalmology, the Second Hospital of Jilin University, Jilin, China
[c] Department of Ophthalmology, Kitasato University, Kanagawa, Japan

ARTICLE INFO

Article history:
Received 16 January 2008
Accepted in revised form 8 June 2008
Available online 17 June 2008

Keywords:
Ca^{2+}
glaucoma
tafluprost
vasodilator

ABSTRACT

Our objective was to determine if tafluprost, a newly synthesized antiglaucoma drug, can relax precontracted rabbit ciliary arteries, and if so, to elucidate the underlying mechanism. We used isometric tension recordings of smooth muscle contractions and fluorescence photometry to monitor the change of intracellular free calcium concentration ($[Ca^{2+}]_i$) in isolated rabbit ciliary artery segments. Tafluprost induced a concentration-dependent relaxation in rabbit ciliary arteries precontracted with a high-K solution. The amplitude of relaxation induced by tafluprost was unchanged by 100 μM L-NAME, 10 μM indomethacin, denudation of vascular endothelium, 30 μM thapsigargin, or 100 μM ouabain. In Ca^{2+}-free solution, 30 μM tafluprost did not decrease the amplitude of contraction induced by 1 μM histamine or the amplitude of the $[Ca^{2+}]_i$ increase induced by 10 μM histamine. The mechanism of tafluprost-induced relaxation was different from diltiazem, a voltage-dependent Ca^{2+} channel blocker. However, in thapsigargin-pretreated preparations incubated in Ca^{2+}-free solution, tafluprost attenuated the capacitative increase of $[Ca^{2+}]_i$ upon Ca^{2+} reintroduction to the extracellular medium. Thus, we conclude that tafluprost relaxed isolated rabbit ciliary artery segments precontracted with a high-K solution. The relaxing mechanism was not dependent on endothelial-derived factors, and not affected by the intracellular Ca^{2+} cycles or the Ca^{2+} extrusion component of the extracellular Ca^{2+} cycles. Relaxation of rabbit ciliary artery smooth muscle by tafluprost may be due, at least in part, to inhibition of capacitative Ca^{2+} entry from the extracellular space.

1. Introduction

Tafluprost, a prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-type agonist, is a newly developed antiglaucoma drug now in preregistration in the USA, Europe, and Japan. In preclinical studies it exhibited potent intraocular pressure (IOP) reducing effects in ocular normotensive mice (Ota et al., 2005), monkeys (Nakajima et al., 2003), and ocular hypertensive monkeys (Takagi et al., 2004). Tafluprost is formulated as a pro-drug ester that facilitates corneal penetration. The active carboxylic acid form, AFP-172, is delivered to the aqueous humor and is responsible for reducing IOP (Takagi et al., 2004).

It is generally accepted that increased IOP is a major risk factor in glaucoma. However, loss of visual fields sometimes occurs in glaucoma patients with well-controlled IOP. Recently, attention has been focused on the role of ocular blood flow in the pathogenesis of glaucoma, especially in patients with normal tension glaucoma (Flammer et al., 2002). Therefore, a better understanding of the effect of antiglaucoma drugs on ocular circulation is important for optimizing their clinical use. Various antiglaucoma agents have effects on ocular circulation in addition to lowering IOP. We reported that betaxolol (Hayashi-Morimoto et al., 1999, Dong et al., 2006) and nipradilol (Yoshitomi et al., 2002), β-adrenergic antagonists used for treatment of glaucoma, relax rabbit ciliary artery with calcium antagonistic or nitric oxide (NO) producing activities, respectively. We also reported that unoprostone (Yoshitomi et al., 2004) and latanoprost (Ishikawa et al., 2002), both $PGF_{2\alpha}$ related compounds used for glaucoma therapy, relax rabbit ciliary artery. These drugs may also work by inhibition of calcium entry.

Interestingly, an in vivo experiment using laser speckle flowmetry in rabbit indicated that topical administrations of tafluprost stably increase the optic nerve head (ONH) blood flow (Akaishi et al., 2007). To clarify the underlying mechanism of effect of tafluprost on ocular blood flow, we investigated the effect of tafluprost upon smooth muscle contractions in isolated rabbit ciliary artery.

* Corresponding author. Tel.: +81 884 6164; fax; +81 836 2621.
E-mail address: yoshitom@med.akita-u.ac.jp (T. Yoshitomi).

0014-4835/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.exer.2008.06.005

2. Methods and materials

2.1. Preparations

All animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and with approval by the Animal Experiment Committee of Akita University. Male albino rabbits weighing 2–3 kg were killed with an overdose of intravenous pentobarbital sodium (Abbott, North Chicago, IL, USA). The eyes were immediately enucleated, ensuring that a maximum length of optic nerve was removed, and then placed in oxygenated Krebs solution of the following composition (mM): NaCl 94.8, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25.0, glucose 11.7 and bubbled with 95% O_2 and 5% CO_2. With the aid of a dissecting microscope, the ciliary artery and surrounding connective tissue were carefully isolated from the optic nerve.

2.2. Isometric tension recording

Vascular segments (150–300 μm in diameter, 1–2 mm in length) cut from the distal section of the ciliary artery were immediately mounted in a chamber of a double Myograph System® (JP Trading, Denmark) with 10 ml Krebs solution. The temperature was raised to 37 °C in the chamber and maintained for the duration of the study. The vessels were stretched to an internal diameter, I_1, that was 0.9 of the diameter generated by 100 mmHg. This was the optimal diameter for active tension development (Halpern et al., 1978). After the vessels were equilibrated for 30 min, the experiments were performed. The Myograph System® directly determined vessel isometric tension and simultaneously transmitted the data to a computer that displayed the tension curves on a monitor. Detailed methods for isometric tension recordings by the Myograph System® have been described by Mulvany and Halpern (1976, 1977).

After the equilibration period, contractions evoked by a high-K solution were measured during a 20-min interval to establish preparation viability and stability. The high-K solution was prepared by replacing NaCl with isotonic, equimolar KCl to give a final K^+ concentration of 100.7 mM. Carbachol (1 μM), a cholinergic agonist that acts on receptors in the endothelium, was applied 20 min after contractions to induce relaxation of vascular smooth muscle (Keef and Bowen, 1989). This procedure established the susceptibility of each contracted ciliary artery to cholinergic relaxation before examining the effects of other agents. In certain experiments, the vascular segments with baseline tone were incubated with an inhibitor 30 min before the exposure to the test drug. In other experiments, we gently denuded the endothelium by rubbing the inside of the vascular ring with a scalp hair before the normalization.

The ability of tafluprost to relax isolated ciliary artery was determined in segments that were precontracted with the high-K solution. Generally, the high-K-induced contraction was maintained for 20 min, then tafluprost was applied every 10 min in a cumulative manner. Moreover, we also tested tafluprost suppression of contractions induced by 1 μM histamine in Ca^{2+}-free solutions. Calcium-free solutions were prepared by replacing $CaCl_2$ with isotonic, equimolar $MgCl_2$ and adding 1 mM EGTA, a chelating agent for Ca^{2+} in the presence of magnesium. The ability of diltiazem to induce relaxation of precontracted ciliary artery rings was also tested.

2.3. Fluorescence photometry

Vascular ring segments (150–300 μm in diameter, 3.5–4 mm in length) were cut longitudinally to form strips (500–1000 μm in width, 3.5–4 mm in length). To determine smooth muscle intracellular Ca^{2+} levels, the vascular strips were immediately incubated at room temperature in the dark for 3 h in a solution composed of 10 μM fura-2 AM (Wako Chemical, Osaka, Japan), 250 ppm cremophor EL (Sigma, Tokyo, Japan) in HEPES-buffered saline (HBS). The solution was aerated with 95% O_2 and 5% CO_2. Thereafter, the strips were rinsed several times with HBS to remove extracellular fura-2. Within 3 h after fura-2 loading, changes of intracellular free calcium concentration ($[Ca^{2+}]_i$) were measured using an Aquacosmos System (Hamamatsu Photonics K.K., Shizuoka, Japan) equipped with a Nikon epifluorescence microscope (TE2000-U; Nikon, Tokyo, Japan) and band-pass filters for wavelengths of 340 and 380 nm. After correction for the individual background fluorescence, the ratio of the fluorescence at both excitation wavelengths (F_{340}/F_{380}) was monitored simultaneously to determine the $[Ca^{2+}]_i$.

2.4. Drugs

The following drugs and chemicals were used: carbachol hydrochloride, histamine, ethylene glycol-bis (2-aminoethylether)-*N*,*N*,*N*,*N*-tetraacetic acid (EGTA), indomethacin, ouabain, thapsigargin (all from Sigma Chemical Co., St. Louis, MO, USA), N^G-nitro-L-arginine methylester (L-NAME), diltiazem (all from Wako Chemical, Osaka, Japan), tafluprost, and AFP-172 (Santen Pharm, Tokyo, Japan). Concentrations were expressed as final molar values in the organ chambers.

2.5. Statistical analysis

Unless indicated otherwise, the amplitudes of the contractions and of the $[Ca^{2+}]_i$ induced by the high-K solution were defined as 100%. Results were expressed as means ± SD with n representing the number of vessels studied. Statistical differences between values were determined by the unpaired two-tailed Student's t-test. Differences between the concentration–response curves were analyzed by two-way analysis of variance (ANOVA). $P < 0.05$ was considered statistically significant.

3. Results

For rabbit ciliary artery precontracted with high-K solution, both tafluprost (Fig. 1A) and AFP-172 (Fig. 1B) induced concentration-dependent relaxation. For tafluprost and AFP-172, the relaxation achieved at 30 μM was 70.6 ± 14.3% and 13.4 ± 1.6%, respectively ($n = 6$, Fig. 1C). Tafluprost, at concentrations up to 30 μM, did not affect the vascular tone of the resting state (data not shown).

L-NAME is a NO synthase inhibitor, and at 100 μM it abolishes relaxation of ciliary artery segments induced by 1 μM carbachol (data not shown). Therefore we determined its ability to inhibit tafluprost-induced relaxation. Vessel segments were pretreated with 100 μM L-NAME 30 min before application of the high-K solution. L-NAME did not change the concentration-dependent relaxation induced by tafluprost ($n = 6$, $P > 0.05$, Fig. 2A). The cyclooxygenase inhibitor indomethacin (10 μM) also did not modify the relaxation of tafluprost ($n = 6$, $P > 0.05$, Fig. 2A). Removal of vascular endothelium suppressed the relaxation induced by 1 μM carbachol (82.8 ± 3.9% vs 11.2 ± 11.2%; $P < 0.05$; Fig. 2 B,C). However relaxation induced by 30 μM tafluprost was not significantly affected ($P > 0.05$) by the presence (70.6 ± 14.3%; $n = 6$; Fig. 2B) or absence (91.4 ± 0.5%; $n = 4$; Fig. 2C) of the endothelium.

To investigate if Ca^{2+} released from intracellular Ca^{2+} stores was involved in the relaxant effect of tafluprost, we incubated the arterial segments in a Ca^{2+}-free solution. In the Ca^{2+}-free solution, histamine induces smooth muscle contractions that are mediated through release of intracellular Ca^{2+} stores (Ushio-Fukai et al.,

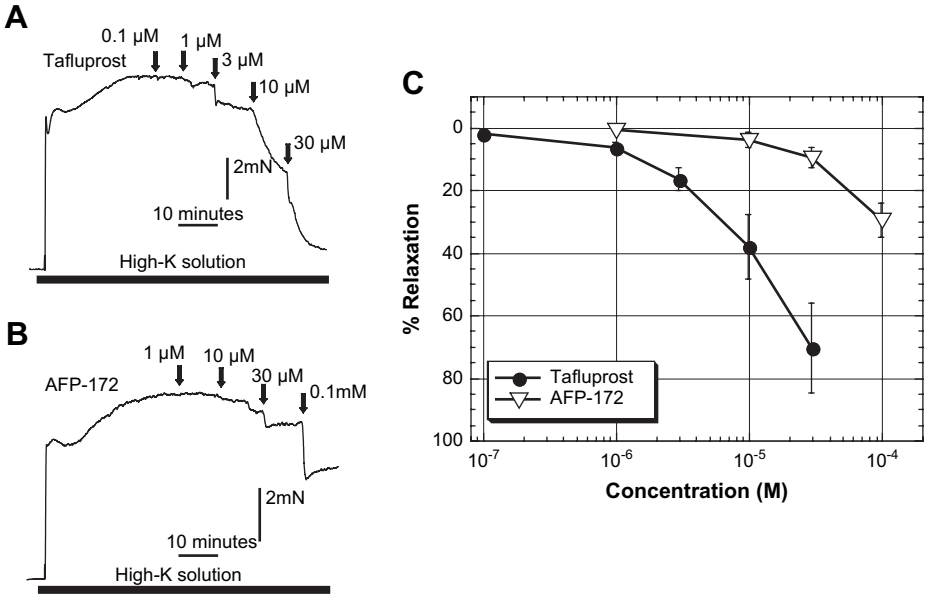

Fig. 1. Tafluprost- and AFP-172-induced relaxation of isolated rabbit ciliary artery segments. The arteries were precontracted by high-K solution. These representative concentration–response curves showed that (A) tafluprost and its metabolite (B) AFP-172 relaxed precontracted ciliary artery preparations. (C) The relaxation potency of tafluprost ($n = 6$) was greater than AFP-172 ($n = 6$, $P < 0.05$). Horizontal bars, time in minutes; vertical bars, isometric tension in milliNewtons (mN).

1993). We found that 1 μM histamine successfully caused contractions of the ciliary artery segments in the absence of extracellular Ca^{2+} (data not shown). Thus we determined if 30 μM tafluprost inhibited the contractions induced by 1 μM histamine in a Ca^{2+}-free solution (Fig. 3A). Contractions induced by histamine alone were 37.2 ± 9.8% ($n = 6$) of the high-K-induced contractions (Fig. 3B). In the presence of 30 μM tafluprost, the contractions were not significantly altered ($P > 0.05$; $n = 6$, Fig. 3B). By fluorescence photometry, endothelium-intact and -denuded vessels were studied to clarify whether the Ca^{2+} signals were derived from the smooth muscle or from the endothelium. Removal of the endothelium had no effect on the $[Ca^{2+}]_i$ change during these procedures (Fig. 4A,B). This indicates that the Ca^{2+} signals were derived from the smooth muscle. $[Ca^{2+}]_i$ was elevated by 10 μM histamine in Ca^{2+}-free solution to 26.8 ± 6.2% of the high-K value ($n = 8$, Fig. 4B). Incubation with 30 μM tafluprost did not decrease the amplitude of $[Ca^{2+}]_i$ (25.1 ± 5.0%, $n = 8$, $P > 0.05$).

Additionally, we determined if restoration of Ca^{2+} to the sarcoplasmic reticulum (SR) was involved in the relaxation mechanism of tafluprost by using thapsigargin, an inhibitor of the sarcoendoplasmic reticulum Ca^{2+}-ATPase. In preparations pretreated with 30 μM thapsigargin, 30 μM tafluprost induced a relaxation of 77.9 ± 9.2% ($n = 4$). These data were not different from controls of 70.6 ± 14.3% without thapsigargin ($n = 6$, $P > 0.05$).

Ouabain is a Na^+-K^+-ATPase inhibitor that also prevents Ca^{2+} extrusion from cytosol to extracellular space. Based on an experiment of Weiss et al. (1993), we used 100 μM ouabain in present study. The relaxation of high-K precontracted rabbit ciliary arteries

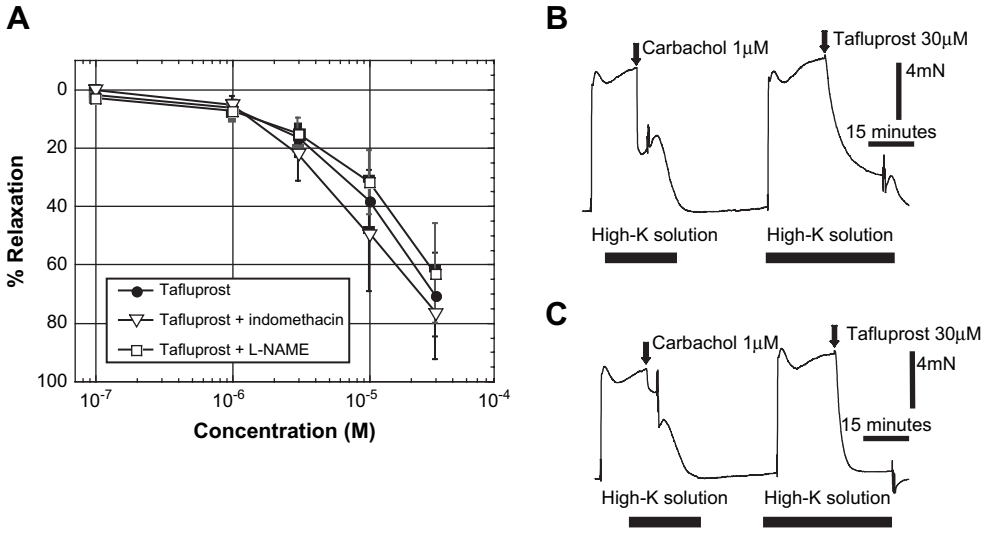

Fig. 2. Influence of L-NAME, indomethacin, or removal of the vascular endothelium on tafluprost-induced relaxation. (A) The relaxation curve of tafluprost alone ($n = 6$) was not significantly ($P > 0.05$) different from that in the presence of 100 μM L-NAME ($n = 6$) or 10 μM indomethacin ($n = 6$). (B) Carbachol- and tafluprost-induced relaxation in contracted artery segments with the endothelium intact. (C) For vessels in which the endothelium was denuded, the relaxation in response to 1 μM carbachol was suppressed. However, denudation did not inhibit the relaxation induced by 30 μM tafluprost. Horizontal bars, time in minutes; vertical bars, isometric tension in milliNewtons (mN).

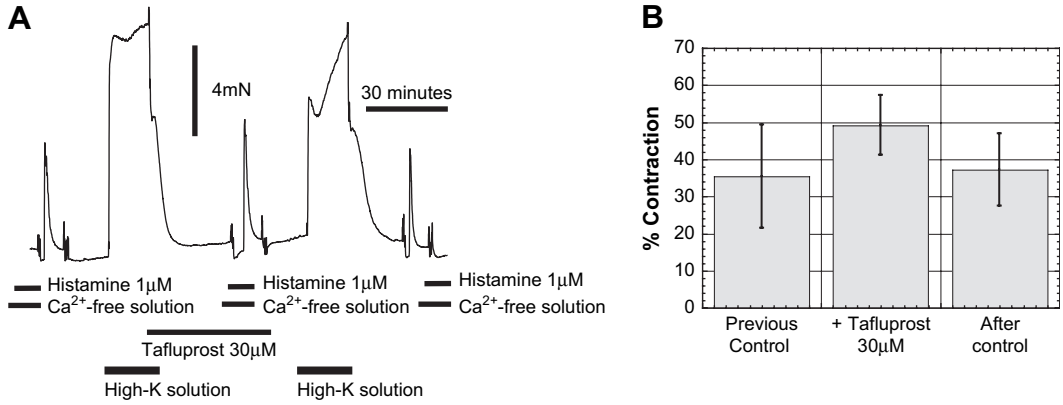

Fig. 3. Effect of tafluprost on histamine-induced ciliary artery contraction in Ca^{2+}-free solution. (A) In this representative experiment, the control contraction induced by histamine alone in Ca^{2+}-free solution was approximately 40% of the high-K solution contraction. Incubation with 30 μM tafluprost did not decrease the histamine-induced contraction. The contraction after the removal of tafluprost was identical to the first control contraction. (B) There was no statistical difference in the response to histamine with or without tafluprost ($n = 6$, $P > 0.05$). Horizontal bars, time in minutes; vertical bars, isometric tension in milliNewtons (mN).

induced by 30 μM tafluprost alone (70.6 ± 14.3%, $n = 6$) was not significantly different in the presence of 100 μM ouabain (80.0 ± 9.6%, $n = 4$). Thus, the effect of tafluprost was not dependent upon Ca^{2+} transport out of the cell.

To determine if the mechanism of tafluprost-induced relaxation was by blocking voltage-dependent Ca^{2+} channels, we compared the relaxation by 10 μM tafluprost with that of 1 μM diltiazem in isolated rabbit ciliary arteries (Fig. 5). These concentrations were chosen because they produced approximately 50% relaxation in the high-K precontracted arteries. Tafluprost induced relaxation of 48.0 ± 17.3% in high-K precontracted arteries, and 45.8 ± 29.1% in 10 μM histamine precontracted preparations ($P > 0.05$, Fig. 5A). While 1 μM diltiazem induced similar relaxation of the high-K precontracted arteries (49.8 ± 6.7%), it induced only 30.7 ± 7.1% relaxation in the histamine precontracted preparations ($P < 0.05$, Fig. 5B).

Using fluorescence photometry to measure the capacitative Ca^{2+} entry associated with tafluprost-induced relaxation, the preparations were treated with 1 μM thapsigargin (Potocnik and Hill, 2001) throughout the measured interval. Diltiazem (1 μM) was also applied throughout the experiment to eliminate the possible effects of the voltage-dependent Ca^{2+} channels. After superfusion of Ca^{2+}-free solution, the $[Ca^{2+}]_i$ was raised by Ca^{2+} reintroduction into the Krebs solution (Fig. 6A). The amplitude of $[Ca^{2+}]_i$ induced by this first Ca^{2+} reintroduction was defined as 100%. A second round of Ca^{2+} depletion and restoration produced a second $[Ca^{2+}]_i$

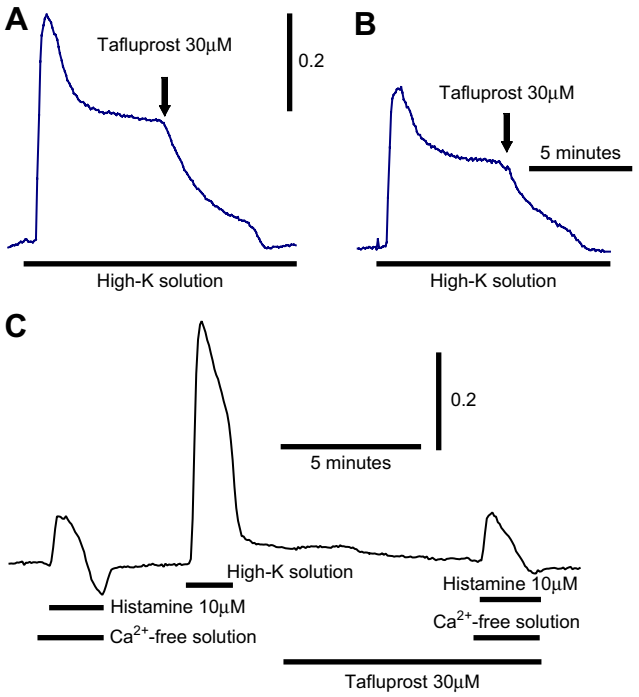

Fig. 4. Effect of high-K solution and 30 μM tafluprost on $[Ca^{2+}]$ in intact (A) and denuded (B) vessels. (C) Fluorescence photometry of histamine-induced Ca^{2+} release from sarcoplasmic reticulum (SR). In Ca^{2+}-free solution, 10 μM histamine induced a transient increase of $[Ca^{2+}]_i$ released from the SR. Incubation with 30 μM tafluprost did not alter the response ($n = 8$, $P > 0.05$). Horizontal bars, time in minutes; vertical bars, ratio of F_{340}/F_{380} which was monitored to determined the $[Ca^{2+}]_i$ in the Aquacosmos System.

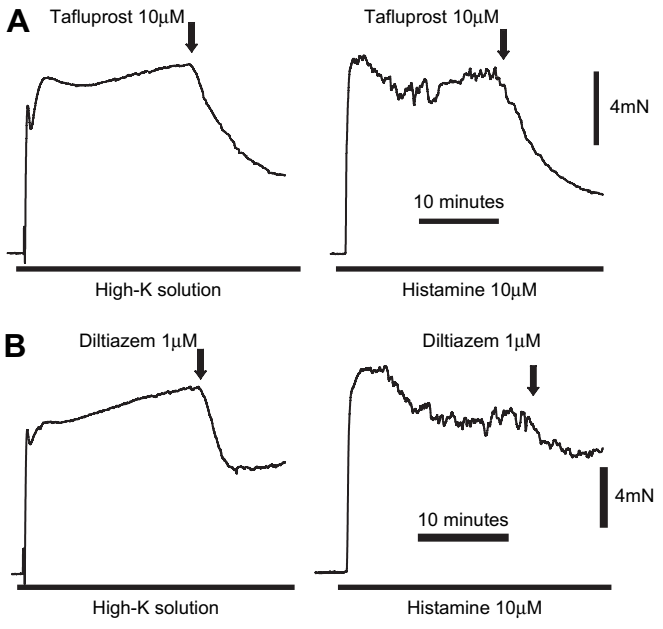

Fig. 5. Effect of tafluprost and diltiazem on high-K and histamine precontracted rabbit ciliary arteries. (A) The 10 μM tafluprost-induced relaxation of high-K precontracted arteries was equal to the relaxation of 10 μM histamine-induced contractions ($P > 0.05$). (B) In contrast, the 1 μM diltiazem-induced relaxation of the high-K precontracted arteries was stronger than the relaxation of the histamine-induced contraction ($P < 0.05$). In these experiments, the contraction induced by high-K or 10 μM histamine were defined separately as 100%. Horizontal bars, time in min; vertical bars, isometric tension in milliNewtons (mN).

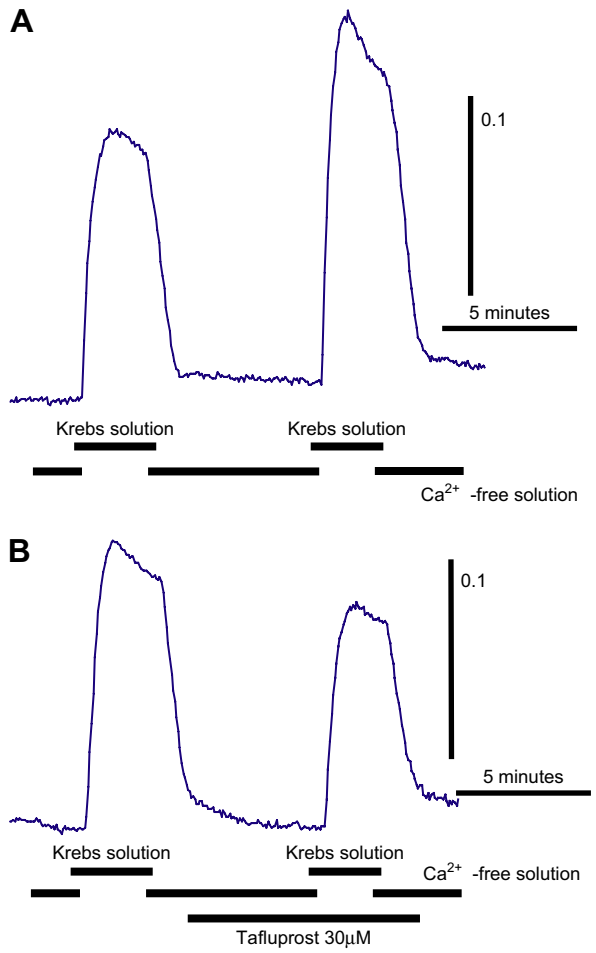

Fig. 6. Fluorescence photometry of tafluprost-reduced capacitative Ca^{2+} entry into ciliary arteries. The preparations were treated with 1 μM thapsigargin and 1 μM diltiazem throughout the measured intervals. (A) In control experiments, after superfusion of Ca^{2+}-free solution, the $[Ca^{2+}]_i$ was raised by Ca^{2+} reintroduction using the standard Krebs solution. The amplitude of $[Ca^{2+}]_i$ induced by first Ca^{2+} reintroduction was defined as 100%. The ratio of second over first of $[Ca^{2+}]_i$ amplitude was considered the control value ($n = 6$). (B) After superfusion of Ca^{2+}-free solution with 30 μM tafluprost, the $[Ca^{2+}]_i$ was again raised by Ca^{2+} reintroduction using the standard Krebs solution. The increase of $[Ca^{2+}]_i$ under these circumstances was significantly smaller than the control ($n = 8$, $P < 0.05$). Horizontal bars, time in min; vertical bars, ratio of F_{340}/F_{380} which was monitored to determined the $[Ca^{2+}]_i$ in the Aquacosmos System.

spike. The ratio of second $[Ca^{2+}]_i$ amplitude to the first was considered to be the control (138.5 ± 3.8%, $n = 8$). For arterial strips treated with 30 μM tafluprost during the second round of Ca^{2+}-free solution, upon extracellular Ca^{2+} restoration the ratio of the second to the first $[Ca^{2+}]_i$ amplitude was 75.9 ± 8.6% ($n = 12$), which was significantly less than the control ($P < 0.05$, Fig. 6B).

4. Discussion

In preclinical studies, tafluprost decreased IOP in mice (Ota et al., 2005), monkeys (Nakajima et al., 2003; Takagi et al., 2004), and increased the ONH blood flow in rabbits in vivo (Akaishi et al., 2007). The present study is the first report that tafluprost relaxes isolated rabbit ciliary arteries in vitro. These results suggest that tafluprost may increase ONH blood flow by two mechanisms: reduction of IOP and direct relaxation of ocular blood vessels.

Tafluprost, a prostanoid receptor agonist, relaxed precontracted preparations, and its potency was greater than AFP-172, one of its metabolites. It was reported that AFP-172 is the responsible form of tafluprost for reducing IOP by activating prostanoid receptor (Nakajima et al., 2003). Tafluprost has similarities to unoprostone, another antiglaucoma drug. Unoprostone is also a prostaglandin agonist that is metabolized while penetrating the cornea, and its metabolite is responsible for reducing IOP (Kashiwagi et al., 1999; Numaga et al., 2005). Our previous experiments indicated that unoprostone relaxed precontracted rabbit ciliary arteries, and its metabolite also had no effect on these preparations (Yoshitomi et al., 2004). In principle, both drugs could reach the ciliary artery via the loose connective tissue around the eyeball, without penetrating the cornea or sclera, and successfully increase ocular blood flow in vivo.

To reveal the underlying mechanism of the relaxation in response to tafluprost, we first determined if the endothelial-derived vasodilators such as NO (Hutchinson et al., 1987), prostacyclin (Vanhoutte, 1986), and endothelium-derived hyperpolarizing factor (EDHF) (Komori and Vanhoutte, 1990) were involved. However, the NO synthase inhibitor L-NAME (Palmer et al., 1988) and cyclooxygenase inhibitor indomethecin (Vanhoutte, 1986) had no effect on tafluprost-induced relaxation. These results suggest that endothelial-derived NO and prostacyclin are not involved in the relaxation mechanism of tafluprost. Latanoprost, another prostanoid agonist and antiglaucoma drug, increases the ONH blood flow in rabbits and monkeys (Ishii et al., 2001). The increase is abolished by pretreatment with indomethacin. The discrepancy between tafluprost and latanoprost may be due to the presence of different signaling pathways of vascular relaxation for the two drugs. To exclude the influence of other endothelial-derived vasodilators, such as EDHF, we denuded the endothelium of ciliary artery segments and found that the tafluprost-induced relaxation was unchanged. These results indicate that the relaxing mechanism of tafluprost is not dependent on endothelial-derived vasodilators.

Further, we investigated the influence of tafluprost on the Ca^{2+} cycle of vascular smooth muscles. Cytosolic Ca^{2+} homeostasis is regulated in part by an extracellular cycle that controls the entry and removal of Ca^{2+} between the cytosol and extracellular space. There is also an intracellular cycle that controls Ca^{2+} fluxes between the cytosol and intracellular stores in the sarcoplasmic reticulum (SR) (Katz, 1997).

We investigated the effect of tafluprost on SR function. Histamine provokes contractions using Ca^{2+} that enters through voltage-dependent or receptor-operated Ca^{2+} channels, as well as from intracellular Ca^{2+} stores (Laporte and Laher, 1997). We used a Ca^{2+}-free solution, and then added histamine to induce contraction by SR-dependent Ca^{2+} release (Dong et al., 2007). Tafluprost did not decrease these histamine-induced transient contractions in the Ca^{2+}-free solution. These results were supported by fluorescence photometry that directly showed that tafluprost did not inhibit $[Ca^{2+}]_i$ elevation induced by histamine in Ca^{2+}-free solution. Thus, tafluprost does not inhibit Ca^{2+} release from the SR in this preparation. Further, we tested whether Ca^{2+} restoration to the SR was involved in the relaxation mechanism of tafluprost. Thapsigargin, an inhibitor of the sarco-endoplasmic reticulum Ca^{2+}-ATPase in mammalian cells, can deplete the intracellular Ca^{2+} stores (Treiman et al., 1998). However, incubation with thapsigargin did not affect tafluprost-induced relaxation in high-K precontracted preparations. This indicates that Ca^{2+} restoration of the SR did not contribute to tafluprost-induced relaxation.

Additionally, ouabain, which prevents Ca^{2+} efflux (Laporte and Laher, 1997), also did not affect the relaxation induced by tafluprost. Thus, tafluprost did not exert its relaxing effect by promoting Ca^{2+} extrusion.

Together, the above results indicate that tafluprost does not affect the intracellular Ca^{2+} cycle or the Ca^{2+} extrusion component of the extracellular Ca^{2+} cycle. Thus, we tested the possibility that tafluprost relaxes rabbit ciliary arteries by inhibiting Ca^{2+} entry from the extracellular space through voltage-dependent Ca^{2+}

channels. We compared the relaxation induced by tafluprost with diltiazem, a typical voltage-dependent Ca^{2+} channel blocker (Gunthorpe and Lummis, 1999). While the percent of relaxation induced by tafluprost was the same in both high-K-induced and histamine-induced contractions, the relaxing force of diltiazem in high-K-induced contractions was stronger than in histamine-induced contractions. Therefore, the relaxing mechanism of tafluprost was not by blocking voltage-dependent Ca^{2+} channel.

Among a variety of Ca^{2+} entry models, capacitative entrance occurs upon emptying of the intracellular Ca^{2+} stores (Putney, 1986, 1990; Putney and Bird, 1993, 1994). After we removed extracellular Ca^{2+}, the $[Ca^{2+}]_i$ increased upon reintroduction of extracellular Ca^{2+}. Tafluprost decreased the amplitude of $[Ca^{2+}]_i$ upon this reintroduction. This phenomenon suggests that tafluprost inhibits Ca^{2+} entry, at least in part by suppressing the capacitative pathway through the plasma membrane.

In summary, tafluprost induced concentration-dependent relaxation of isolated rabbit ciliary artery. The relaxation mechanism was not dependent on endothelial-derived factors. The effect was not mediated through the intracellular Ca^{2+} cycle or the Ca^{2+} extrusion component of the extracellular Ca^{2+} cycle. Our data suggest that the relaxant effect of tafluprost may be due, at least in part, to inhibition of capacitative Ca^{2+} entry from the extracellular space.

Acknowledgments

The authors thank Santen Pharm. Co. for providing tafluprost for these experiments. The authors thank Ms. Sanae Takaseki for her excellent technical assistance. Supported by Japan Society for the promotion of Science Grant-in-Aid for Scientific Research #12671718 and 12671719.

References

Akaishi, T., Kurashima, H., Odani, N., Ishida, N., Shimazaki, A., 2007. Comparison of the effect of tafluprost, latanoprost and travoprost on the optic nerve head blood flow in rabbits. Invest. Ophthalmol. Vis. Sci. 48 (E-abstract 2278).

Dong, Y., Ishikawa, H., Wu, Y., Shimizu, K., Goseki, T., Yoshitomi, T., 2006. Effect and mechanisms of betaxolol and timolol on vascular relaxation in isolated rabbit ciliary artery. Jpn. J. Ophthalmol. 50, 504–508.

Dong, Y., Ishikawa, H., Wu, Y., Yoshitomi, T., 2007. Vasodilatory mechanism of levobunolol on vascular smooth muscle cells. Exp. Eye Res. 84, 1039–1046.

Flammer, J., Orgul, S., Costa, V.P., Orzalesi, N., Krieglstein, G.K., Serra, L.M., Renard, J. P., Stefansson, E., 2002. The impact of ocular blood flow in glaucoma. Prog. Retin. Eye Res. 21, 359–393.

Gunthorpe, M.J., Lummis, S.C., 1999. Diltiazem causes open channel block of recombinant 5-HT3 receptors. J. Physiol. 519, 713–722.

Halpern, W., Mulvany, M.J., Warshaw, D.M., 1978. Mechanical properties of smooth muscle cells in the walls of arterial resistance vessels. J. Physiol. 275, 85–101.

Hayashi-Morimoto, R., Yoshitomi, T., Ishikawa, H., Hayashi, E., Sato, Y., 1999. Effects of beta antagonists on mechanical properties in rabbit ciliary artery. Graefes Arch. Clin. Exp. Ophthalmol. 237, 661–667.

Hutchinson, P.J., Palmer, R.M., Moncada, S., 1987. Comparative pharmacology of EDRF and nitric oxide on vascular strips. Eur. J. Pharmacol. 141, 445–451.

Ishii, K., Tomidokoro, A., Nagahara, M., Tamaki, Y., Kanno, M., Fukaya, Y., Araie, M., 2001. Effects of topical latanoprost on optic nerve head circulation in rabbits, monkeys, and humans. Invest. Ophthalmol. Vis. Sci. 42, 2957–2963.

Ishikawa, H., Yoshitomi, T., Mashimo, K., Nakanishi, M., Shimizu, K., 2002. Pharmacological effects of latanoprost, prostaglandin E_2, and $F_{2\alpha}$ on isolated rabbit ciliary artery. Graefes Arch. Clin. Exp. Ophthalmol. 240, 120–125.

Kashiwagi, K., Iizuka, Y., Tsukahara, S., 1999. Metabolites of isopropyl unoprostone as potential ophthalmic solutions to reduce intraocular pressure in pigmented rabbits. Jpn. J. Pharmacol. 81, 56–62.

Katz, A.M., 1997. Molecular biology of calcium channels in the cardiovascular system. Am. J. Cardiol. 80, 171–221.

Keef, K.D., Bowen, S.M., 1989. Effect of ACh on electrical and mechanical activity in guinea pig coronary arteries. Am. J. Physiol. 257, H1096–H1103.

Komori, K., Vanhoutte, P.M., 1990. Endothelium-derived hyperpolarizing factor. Blood Vessels 27, 238–245.

Laporte, R., Laher, I., 1997. Sarcoplasmic reticulum–sarcolemma interactions and vascular smooth muscle tone. J. Vasc. Res. 34, 325–343.

Mulvany, M.J., Halpern, W., 1976. Mechanical properties of vascular smooth muscle cells in situ. Nature. 260, 617–619.

Mulvany, M.J., Halpern, W., 1977. Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. Circ. Res. 41, 19–26.

Nakajima, T., Matsugi, T., Goto, W., Kageyama, M., Mori, N., Matsumura, Y., Hara, H., 2003. New fluoroprostaglandin $F_{2\alpha}$derivatives with prostanoid FP-receptor agonistic activity as potent ocular-hypotensive agents. Biol. Pharm. Bull. 26, 1691–1695.

Numaga, J., Koseki, N., Kaburaki, T., Kawashima, H., Tomita, G., Araie, M., 2005. Intraocular metabolites of isopropyl unoprostone. Curr. Eye Res. 30, 909–913.

Ota, T., Murata, H., Sugimoto, E., Aihara, M., Araie, M., 2005. Prostaglandin analogues and mouse intraocular pressure: Effects of tafluprost, latanoprost, travoprost, and unoprostone considering 24-hour variation. Invest. Ophthalmol. Vis. Sci. 46, 2006–2011.

Palmer, R.M., Rees, D.D., Ashton, D.S., Moncada, S., 1988. L-Arginine is the physiological precursor for the formation of nitric oxide in endothelium-dependent relaxation. Biochem. Biophys. Res. Commun. 153, 1251–1256.

Potocnik, S.J., Hill, M.A., 2001. Pharmacological evidence for capacitative Ca(2+) entry in cannulated and pressurized skeletal muscle arterioles. Br. J. Pharmacol. 134, 247–256.

Putney Jr., J.W., 1986. A model for receptor-regulated calcium entry. Cell Calcium. 7, 1–12.

Putney Jr., J.W., 1990. Capacitative calcium entry revisited. Cell Calcium. 11, 611–624.

Putney Jr., J.W., Bird, G.S., 1993. The signal for capacitative calcium entry. Cell. 75, 199–201.

Putney Jr., J.W., Bird, G.S., 1994. Calcium mobilization by inositol phosphates and other intracellular messengers. Trends Endocrinol. Metab. 5, 256–260.

Takagi, Y., Nakajima, T., Shimazaki, A., Kageyama, M., Matsugi, T., Matsumura, Y., Gabelt, B.T., Kaufman, P.L., Hara, H., 2004. Pharmacological characteristics of AFP-168 (tafluprost), a new prostanoid FP receptor agonist, as an ocular hypotensive drug. Exp. Eye Res. 78, 767–776.

Treiman, M., Caspersen, C., Christensen, S.B., 1998. A tool coming of age: thapsigargin as an inhibitor of sarco-endoplasmic reticulum Ca^{2+}-ATPases. Trends Pharmacol. Sci. 19, 131–135.

Ushio-Fukai, M., Abe, S., Kobayashi, S., Nishimura, J., Kanaide, H., 1993. Effects of isoprenaline on cytosolic calcium concentrations and on tension in the porcine coronary artery. J. Physiol. 462, 679–696.

Vanhoutte, P.M., 1986. The endothelium and arterial reactivity. J. Mal. Vasc 11, 213–221.

Weiss, D.N., Podberesky, D.J., Heidrich, J., Blaustein, M.P., 1993. Nanomolar ouabain augments caffeine-evoked contractions in rat arteries. Am. J. Physiol. 265, C1443–C1448.

Yoshitomi, T., Yamaji, K., Ishikawa, H., Ohnishi, Y., 2002. Vasodilatory effects of nipradilol, an alpha- and beta-adrenergic blocker with nitric oxide releasing action, in rabbit ciliary artery. Exp. Eye Res. 75, 669–676.

Yoshitomi, T., Yamaji, K., Ishikawa, H., Ohnishi, Y., 2004. Vasodilatory mechanism of unoprostone isopropyl on isolated rabbit ciliary artery. Curr. Eye Res. 28, 167–174.