

Effects of Isopropyl Unoprostone on Rabbit Ciliary Artery

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Purpose: Isopropyl unoprostone (unoprostone), a prostaglandin F_{2α} (PG F_{2α})-related compound, is widely used for treatment of glaucoma in Japan and is reported to have effects on ocular circulation. To investigate the action of this drug, we have studied the effect of unoprostone on the isolated rabbit ciliary artery.

Methods: Under microscopic observation, ciliary arteries were prepared from rabbit eyes and mounted in a myograph system. The effects of unoprostone on the isolated rabbit ciliary artery were investigated in vitro using isometric tension recordings.

Results: Exogenously applied PG F_{2α} but not unoprostone evoked contraction in the rabbit ciliary artery. After precontraction with excess-[K]_o solution, unoprostone evoked dose-dependent relaxation. The relaxation was not blocked by 10 μM/L N^G-nitro-L-arginine methylester (L-NAME), 1 μM/L 8-37 calcitonin gene-related peptide (8-37 CGRP) or 10 μM/L indomethacin. Moreover, unoprostone could induce relaxation even in preparations without endothelium. The relaxation induced by diltiazem was greater in muscle precontracted in excess-[K]_o solution than that precontracted by 10 μM/L histamine. On the other hand, unoprostone induced a similar amplitude of relaxation in muscles precontracted by either drug.

Conclusions: These results indicate that unoprostone acts directly to relax rabbit ciliary artery. The relaxation was not dependent on the endothelium and was not caused by intrinsic prostoglandins CGRP, or nitric oxide. Moreover, the relaxation was different from that caused by a Ca²⁺ antagonist. The mechanism for this relaxation is not yet determined. **Jpn J Ophthalmol** 2000;44:214–220 © 2000 Japanese Ophthalmological Society

Key Words: Ciliary artery, isopropyl unoprostone, ocular blood flow, relaxation, vascular smooth muscle.

Introduction

Although it is generally accepted that increased intraocular pressure is a major risk factor in glaucoma, vascular factors are also considered to be important in the pathogenesis of optic nerve damage and visual field loss,¹ especially in patients with normal tension glaucoma.^{2,3} Unoprostone, a prostaglandin (PG) F_{2α}-related compound, has been widely used for the treatment of glaucoma in Japan.⁴ The drug is thought to reduce intraocular pressure by increasing conventional or uveoscleral outflow.^{5–7} This drug is reported to have some effect on ocular blood

flow, which has been studied in vivo by many investigators.^{8–11} Nishi et al⁸ reported that topical application of unoprostone increased end-diastolic velocity of both the central retinal artery and the short posterior ciliary artery in normal volunteers using color Doppler ultrasound imaging. Topical application of unoprostone is also reported to increase choroidal blood flow in rabbits, as determined by the hydrogen clearance method.^{9,10} Topical application of unoprostone increased normalized blur (NB; indicator of retinal blood flow rate) in humans using the laser speckle microcirculation analyzer.¹¹ These reports support the idea that unoprostone can increase ocular blood flow. However, the underlying mechanisms of this effect are not yet clear. In an attempt to clarify the mechanisms involved in the effectiveness of unoprostone on the ocular circulation, we have in-

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vestigated the effects of this drug on ciliary arterial smooth muscle in vitro using isometric tension recording methods.

Materials and Methods

All experiments were performed according to the ARVO Resolution on the Use of Animals in Research. Male albino rabbits weighing 2–3 kg were sacrificed with an overdose of intravenous pentobarbital sodium (Abbott, North Chicago, IL, USA). The eyes were immediately enucleated with a maximum length of optic nerve and placed in oxygenated Krebs solution of the following composition (mM): NaCl 94.8, KCl 4.7, MgSO₄ 1.2, CaCl₂ 2.5, KH₂PO₄ 1.2, NaHCO₃ 25.0, and glucose 11.7.

The ciliary artery with connective tissue was carefully dissected free from the optic nerve. Vascular ring segments (150–300 μm in diameter, 1–2 mm length) were cut from the distal section of the ciliary artery and mounted in a double myograph system (JP Trading, Denmark)¹² under microscopic observation. The myograph system allowed direct determination of vessel isometric tension while the internal circumference was controlled. The blood vessels were equilibrated for 30 minutes in oxygenated Krebs solution with 5% CO₂ and 95% O₂ and maintained at 38°C. For active tension development, the blood vessels were stretched to their optimal lumen diameter, I₀, corresponding to 0.9 × I₁₀₀, where I₁₀₀ is the diameter the vessels would have in situ if subjected to a passive transmural pressure of 13.3 kPa (100 mm Hg).¹³

After the mounting and equilibration period, the amplitudes of three successive contractions evoked by excess-[K]_o solutions were measured at 10-minute intervals to establish preparation viability and stability. Excess-[K]_o solutions were prepared by replacing equimolar NaCl with KCl. During a contraction evoked by excess-[K]_o solutions, 10 μM/L carbachol was shown to induce relaxation in all preparations indicating that the preparations had an intact endothelium.

The endothelium was removed by gently rubbing the inside of the vessel with a human scalp hair inserted through the lumen. Successful denudation of the vessel was confirmed by the lack of the relaxant effect of carbachol in vessels precontracted by excess-[K]_o solutions.

Chemicals

The following drugs were used: carbachol hydrochloride, histamine (Sigma Chemical, St. Louis, MO,

USA), isopropyl 20-ethyl-9 α, 11 α-dihydroxy-15-ketocis-Δ⁵-prostanate (unoprostone; Ueno Seiyaku, Osaka), N^G-nitro-L-arginine methylester (L-NAME), diltiazem (Wako Pure Chemical, Osaka), 8-37 calcitonin gene-related peptide (8-37 CGRP; Peptide Institute, Osaka)

Results

Mechanical Responses to PG F_{2α}

As unoprostone is a PG F_{2α}-related compound, we investigated the effect of PG F_{2α} itself on rabbit ciliary artery. PG F_{2α} evoked contraction beginning at a concentration of 1 μM/L. As shown in Figure 1a, 10 μM/L PG F_{2α} evoked an initial twitch contraction followed by a slow contraction in rabbit ciliary artery. The contraction was also observed when the muscle was precontracted by excess-[K]_o solutions. Relaxation was not observed in any preparation tested (4 cases).

Mechanical Responses to Unoprostone

Unoprostone had no effect on the mechanical properties of rabbit ciliary artery up to a concentration of 10 μM/L (Figure 1b). However, after precontraction by excess-[K]_o solution, application of unoprostone elicited relaxation in a dose-dependent manner, and the minimum concentration of this drug required to generate relaxation was 1 μM/L (Figure 1b). Figure 2a shows dose-response relationships of unoprostone on rabbit ciliary artery precontracted with excess-[K]_o solution, where the amplitude of contraction evoked by excess-[K]_o solution was defined as 100%.

To further investigate the mechanisms on the relaxation induced by unoprostone, effects of a nitric oxide synthase (NOS) inhibitor (L-NAME), indomethacin, and the CGRP antagonist, 8-37 CGRP, were studied. Figure 2a shows the dose-response relationships of unoprostone, in the presence of 10 μM/L indomethacin, 10 μM/L L-NAME, or 1 μM/L 8-37 CGRP. None of these drugs had an inhibitory effect on the dose-response curves, which indicates that intrinsic NO, PG, or CGRP are not responsible for the unoprostone-induced relaxation.

Role of Endothelium in

Unoprostone-Induced Relaxation

To investigate the role of the endothelium in unoprostone-induced relaxation, the effect of unoprostone was compared in preparations with intact en-

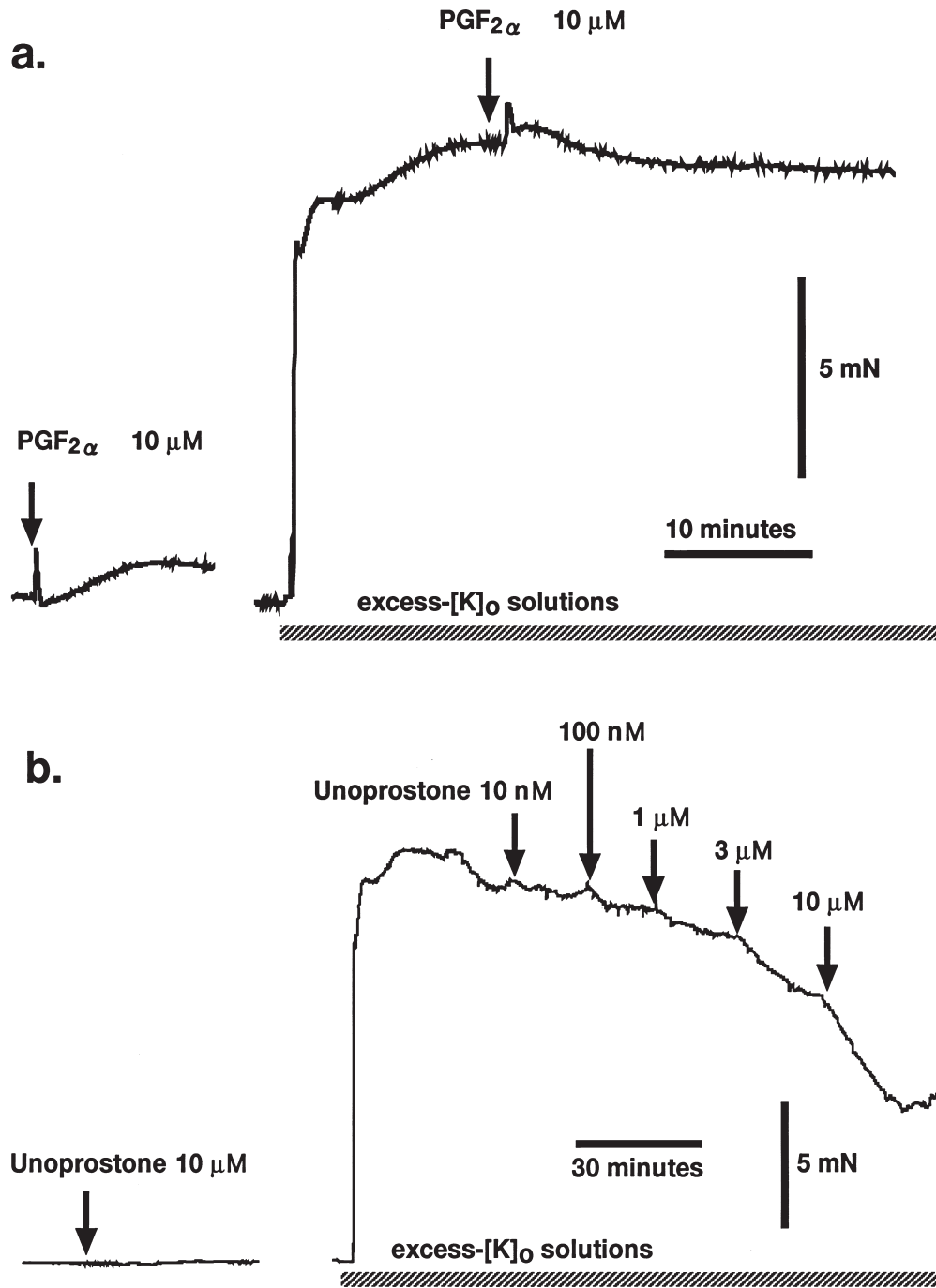


Figure 1. (a) Effects of $10 \mu\text{M/L}$ prostaglandin (PG) $\text{F}_{2\alpha}$ on rabbit ciliary artery (left). Effects of $10 \mu\text{M/L}$ PG $\text{F}_{2\alpha}$ on rabbit ciliary artery that was precontracted by excess- $[\text{K}]_o$ solution (right). Vertical scale shows active force in millinewtons (mN). **(b)** Effects of $10 \mu\text{M/L}$ unoprostone on rabbit ciliary artery (left). Effects of multiple applications of unoprostone on rabbit ciliary artery that was precontracted by excess- $[\text{K}]_o$ solution (right).

dothelium and in preparations from which endothelium was mechanically removed. Carbachol induced relaxation of the ciliary artery muscle with intact endothelium, and it had no effect in the preparation

without the endothelium (Figures 2b, c). Unoprostone, on the other hand, provoked relaxation in preparations both with and without endothelium. These results indicate that the relaxation induced by

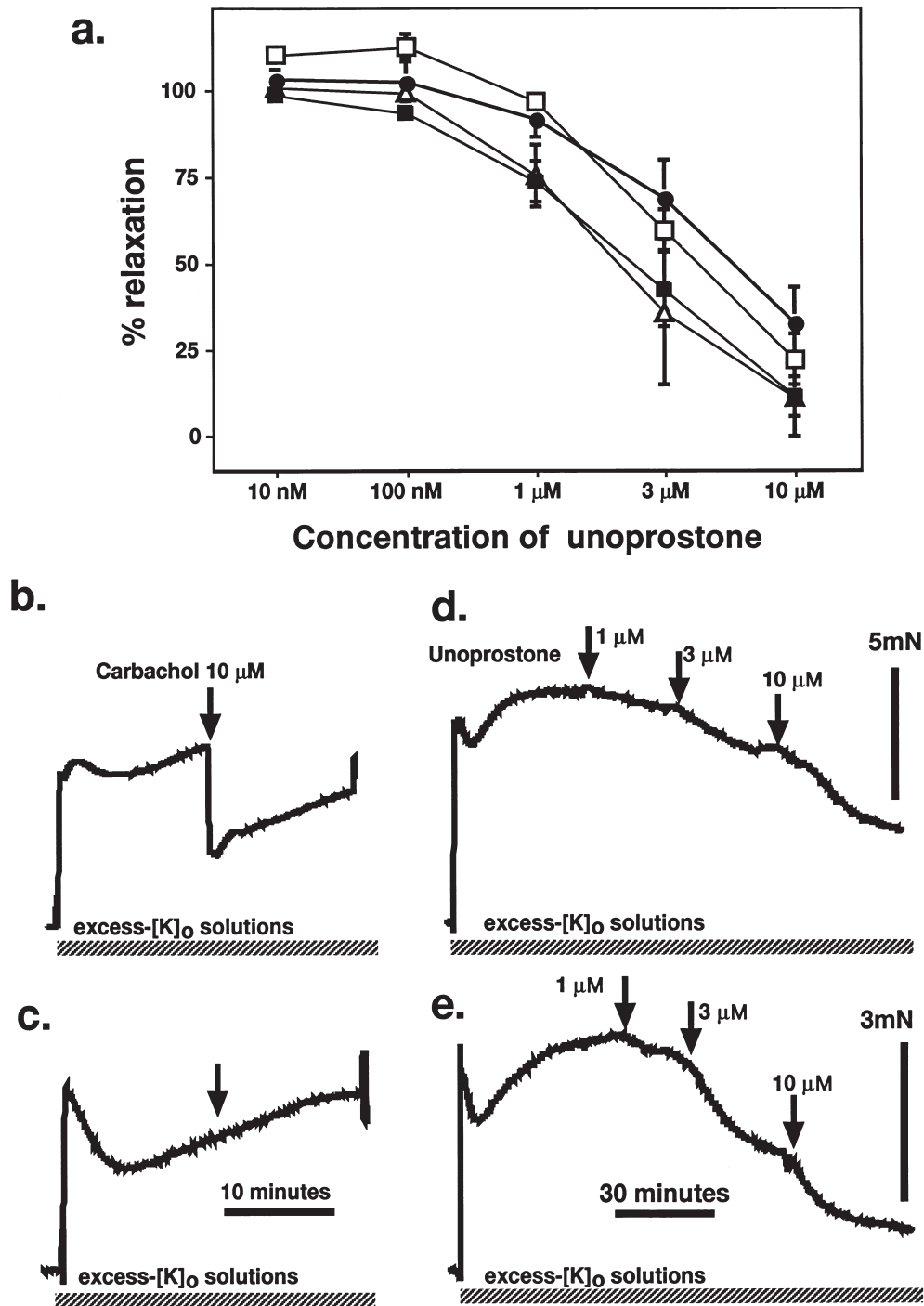


Figure 2. (a) Dose–response relationships of unoprostone (●) on rabbit ciliary artery precontracted with excess-[K]_o solution, where the relative amplitude of contraction evoked by excess-[K]_o solution was defined as 100%. Unoprostone relaxation was not affected by pretreatment with 10 μM/L indomethacin (□), 10 μM/L N^G-nitro-L-arginine methylester (■), and 1 μM/L 8-37 calcitonin gene-related peptide (Δ). Each point is mean and vertical bars indicate 2 × SD (n = 6 to 8). (b) Effects of carbachol (10 μM/L) on rabbit ciliary artery that was precontracted by excess-[K]_o solution (intact endothelium preparation). (c) Effect of carbachol (10 μM/L) in preparations from which endothelium was mechanically removed. (d) Effects of multiple application of unoprostone on rabbit ciliary artery that was precontracted by excess-[K]_o solution (intact endothelium preparation). (e) Effects of multiple application of unoprostone in preparations without endothelium.

unoprostone was not dependent on the vascular endothelium.

Comparison Between Diltiazem- and Unoprostone-Induced Relaxation

Figures 3(a-d) shows the effect of 10 $\mu\text{M/L}$ diltiazem, a Ca^{2+} channel antagonist, and 3 $\mu\text{M/L}$ unoprostone on rabbit ciliary artery that was precontracted with 10 $\mu\text{M/L}$ histamine or excess- $[\text{K}]_o$ solutions. The relaxation induced by diltiazem was greater in the muscle precontracted by excess- $[\text{K}]_o$ solutions than that by 10 $\mu\text{M/L}$ histamine. The amplitude of relaxation induced by diltiazem (10 $\mu\text{M/L}$) in histamine precontracted muscle was $49.3 \pm 17.7\%$ while with the excess- $[\text{K}]_o$ precontracted muscle, it was $8.7 \pm 4.6\%$ (Student's *t*-test, $P = .0032$, $n = 6$). On the other hand, unoprostone (3 $\mu\text{M/L}$) induced a similar amplitude of relaxation in muscles that were precontracted by either histamine ($69.2 \pm 7.4\%$) or excess- $[\text{K}]_o$ solutions ($74.4 \pm 19.5\%$). This difference was not significant (Student's *t*-test, $P = .576$, $n = 6$).

Figure 3e also shows the time course of relaxation evoked by unoprostone, carbachol, and diltiazem overlaid on the same time scale. Unoprostone evoked slower relaxation than carbachol or diltiazem.

Discussion

We have investigated the effects of unoprostone, which is used clinically in the treatment of glaucoma in Japan,⁴ on the mechanical properties of rabbit ciliary artery. Our results showed clearly for the first time that unoprostone relaxes rabbit ciliary artery in a dose-dependent manner. The results demonstrate that the drug has direct vascular relaxing effects on rabbit ciliary artery muscle, which seems to be consistent with previous data showing that topically applied unoprostone increased ocular blood flow in rabbits^{9,10} and humans.^{8,11} It is unlikely that the action is due to $\text{PG F}_{2\alpha}$, because $\text{PG F}_{2\alpha}$ itself did not evoke relaxation but only contracted this muscle. Moreover, exogenously applied indomethacin had no effect on the relaxation evoked by unoprostone. Thus, we conclude that even though unoprostone is a $\text{PG F}_{2\alpha}$ -related compound, its $\text{PG F}_{2\alpha}$ -like action or intrinsic PG is not related to this relaxation.

Endothelium-derived relaxing factor (EDRF) is known to be important for vascular smooth muscle relaxation.¹⁴ Various agents are reported to generate relaxation through activation of EDRF, one of which is now recognized as nitric oxide (NO).^{15,16} Carbachol induced relaxation through activation of EDRF

in rabbit ciliary artery because endothelium denudation or application of L-NAME, an NO biosynthesis inhibitor,¹⁷ abolished the relaxation. Similar behavior has been reported with other agents, including acetylcholine, bradykinin, substance P, and serotonin.¹⁸⁻²¹ So, it is of interest to know if unoprostone evoked relaxation through EDRF. We observed that endothelium denudation or application of L-NAME did not affect the relaxation evoked by unoprostone. These results suggest that endothelium and NO play minor roles in the relaxation induced by unoprostone.

It has been reported that the relaxation evoked by field stimulation is partly mediated by CGRP released from sensory nerve terminals in bovine ciliary artery.²² To investigate the possible role of CGRP on unoprostone relaxation, we have investigated the effect of 8-37 CGRP, a CGRP antagonist, on this relaxation. As shown, 8-37 CGRP had no effect on unoprostone relaxation. This indicates that CGRP was not involved in the relaxation evoked by unoprostone.²³

We have reported that betaxolol and timolol, other important antiglaucoma agents, did relax rabbit ciliary artery muscle and their action was similar to those of Ca^{2+} antagonists.²⁴ We have investigated and compared the effect of diltiazem, a typical L-type Ca^{2+} channel antagonist, and unoprostone on the rabbit ciliary artery to determine if the relaxing action of these drugs are similar. Diltiazem relaxed this muscle more in excess- $[\text{K}]_o$ precontracted preparations than in histamine precontracted preparations. This is presumably because the contraction evoked by excess- $[\text{K}]_o$ solutions occurs mostly through activation of voltage-dependent Ca^{2+} channels; whereas, voltage-dependent Ca^{2+} channels play a minor role in the contraction evoked by histamine.²⁵ Unlike diltiazem, unoprostone evoked a similar amplitude of relaxation in both histamine and excess- $[\text{K}]_o$ solution precontracted muscle. Moreover, this time course of relaxation induced by diltiazem and unoprostone relaxation is quite different from that of diltiazem. Thus, it is unlikely that unoprostone relaxation is induced by blocking voltage-dependent Ca^{2+} channels.

Watanabe et al²⁶ reported the concentration of $^3\text{H-UF-021}$ (unoprostone) in various ocular tissues after topical application of this drug. They showed 153 ng/g wet tissue $^3\text{H-UF-021}$ in the choroid, 500 ng/g wet tissue in the posterior sclera and 12 ng/g wet tissue in the optic nerve of rabbit 30 minutes after single topical applications. From our results, these concentrations (3 nM to 1 $\mu\text{M/L}$) seem likely to evoke relaxation in the ciliary artery, and this may explain previous reports which indicate that unoprostone increased ocular blood flow.

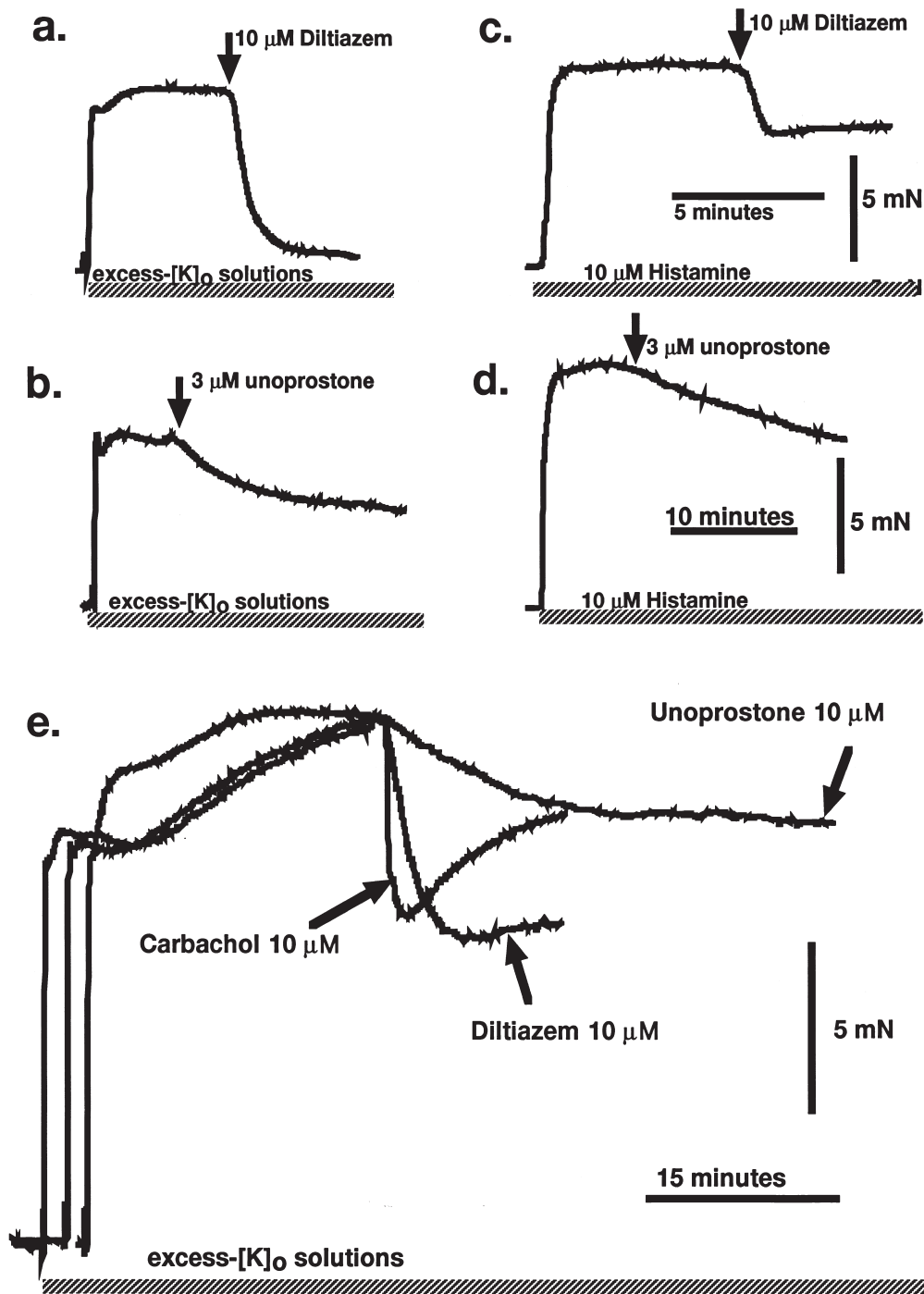


Figure 3. (a) Effect of 10 $\mu\text{M/L}$ diltiazem on rabbit ciliary artery that was precontracted with excess-[K]_o solution. (b) Effect of 3 $\mu\text{M/L}$ unoprostone on rabbit ciliary artery that was precontracted with excess-[K]_o solution. (c) Effect of 10 $\mu\text{M/L}$ diltiazem on rabbit ciliary artery that was precontracted with 10 $\mu\text{M/L}$ histamine. (d) Effect of 3 $\mu\text{M/L}$ unoprostone on rabbit ciliary artery that was precontracted with 10 $\mu\text{M/L}$ histamine. (e) Recordings of relaxation evoked by 10 $\mu\text{M/L}$ unoprostone, carbachol, and diltiazem overlaid on same time scale. mN: milliNewtons.

In conclusion, unoprostone relaxed rabbit ciliary artery muscle beginning at a concentration of 1 $\mu\text{M/L}$. The relaxation was not dependent on endothelium and was not caused by intrinsic PG, CGRP, or NO. The relaxation was also not due to Ca^{2+} antagonism. The mechanism of this relaxation is not yet clear.

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