Prostaglandin $F_2\alpha$, but Not Latanoprost, Increases the Ca^{2+} Sensitivity of the Pig Iris Sphincter Muscle

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PURPOSE. To determine the mechanisms underlying prostaglandin (PG) $F_2\alpha$-, carbachol (CCh)-, or latanoprost (a $PGF_2\alpha$ analogue)-induced contraction of the pig iris sphincter muscle.

METHODS. Effects of these agents on myofilament Ca^{2+} sensitivity were evaluated and compared with the use of receptor-coupled permeabilized preparations by α-toxin. The effects of $PGF_2\alpha$ and CCh on the phosphorylation of myosin light chain (MLC) were also analyzed.

RESULTS. In the intact strips, all three of these agents induced contractions. In permeabilized strips, $PGF_2\alpha$ and CCh, but not latanoprost, caused an additional tension development at a fixed intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and also shifted the $[Ca^{2+}]_i$-tension curve to the left, thus indicating that $PGF_2\alpha$ and CCh, but not latanoprost, induced increases in Ca^{2+} sensitivity (Ca^{2+} sensitization). This Ca^{2+} sensitization could have been inhibited by Y27632, a rho kinase inhibitor, but not by GF109203X, a protein kinase C (PKC) inhibitor or by PD98059, a mitogen-activated protein (MAP) kinase inhibitor. $PGF_2\alpha$ increased the level of MLC phosphorylation at a constant $[Ca^{2+}]_i$.

CONCLUSIONS. $PGF_2\alpha$, but not latanoprost, induced Ca^{2+} sensitization of the pig iris sphincter muscle in an MLC phosphorylation–dependent manner through the rho–rho kinase pathway. The effect of latanoprost on the Ca^{2+} sensitization mechanism was different from that of $PGF_2\alpha$ and was thought to play a beneficial role in glaucoma treatment. (*Invest Ophthalmol Vis Sci.* 2006;47:4865–4871) DOI:10.1167/iovs.05-1518

Prostaglandins (PGs) are derived from arachidonic acid and display a wide range of biologic functions.[1–4] Although there are enormous species differences regarding their physiological effects, in human ocular tissue, $PGF_2\alpha$, PGE_2,[5] and thromboxane B_2[5,6] have been shown to be involved in the pathogenesis of the neurogenic inflammation of the eye, which is characterized by noncholinergic long-lasting miosis, anterior uveal hyperemia, and increased intraocular pressure (IOP) because of a breakdown of the blood–aqueous barrier.[7] A mechanistically similar phenomenon called surgically induced miosis sometimes occurs during surgical procedures of the eye, in spite of the use of the sympathomimetic and anticholinergic agents.[8] The trauma of ocular surgery may stimulate prostaglandin synthesis to induce papillary constriction, which thus makes the operation more difficult and increases the risk for surgical trauma. Although nonsteroidal anti-inflammatory drugs have been used to block the production of prostaglandins by inhibiting cyclo-oxygenase in addition to making use of sympathomimetic and anticholinergic agents to prevent surgically induced miosis, the mechanisms underlying such $PGF_2\alpha$–induced contraction of the iris sphincter muscle are still poorly understood.

Although $PGF_2\alpha$ at high doses had been thought to increase IOP, a $PGF_2\alpha$ analogue, latanoprost (13,14-dihydro-17-phenyl-18,19,20-trinor- $PGF_2\alpha$-isopropyl ester), has been widely used for the treatment of glaucoma as an ocular hypotensive agent. Thus, it has been suggested that latanoprost might increase the uveoscleral outflow of aqueous humor. However, the mechanism for this is still not well understood. Although Poyer et al.[9] and Yousufzai et al.[10] have suggested the $PGF_2\alpha$-mediated increase in aqueous humor outflow is accompanied by relaxation of the ciliary muscle, Yoshitomi et al.[11] reported that neither $PGF_2\alpha$ nor latanoprost had a relaxant effect on this muscle. Weinreb et al.,[12] however, reported that these agents could induce a reduction of the extracellular matrix within the ciliary muscle by increasing the release of matrix metalloproteinases. In addition, $PGF_2\alpha$ is well known to induce miosis in pigs, bovines, and rabbits, but this effect is not as apparent as in humans.[13,14] Even if $PGF_2\alpha$-induced miosis is not a major mechanism for its antiglaucoma effect, it is still important to understand the effect of $PGF_2\alpha$ and its analogue, latanoprost, on the contractility of the iris sphincter muscle. Therefore, we considered that it would be of great interest to know the mechanism for the $PGF_2\alpha$- and latanoprost-induced contraction of the iris sphincter muscle.

It is generally accepted that smooth muscle contraction is primarily regulated by an increase in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and by subsequent phosphorylation of 20 kDa myosin light chain (MLC) by Ca^{2+}-calmodulin–dependent MLC kinase (MLCK).[15] It is also known that Ca^{2+} sensitivity of the contractile apparatus changes during stimulation by various agonists, indicating that secondary regulatory pathways are likely to play a role in the regulation of smooth muscle contraction.[16–19] However, the role of Ca^{2+} sensitivity in the contraction of the iris smooth muscle has not yet been elucidated.

In the present study, we investigated the mechanisms underlying $PGF_2\alpha$-, latanoprost-, and carbachol (CCh)-induced contraction of the pig iris sphincter muscle. In particular, we investigated whether these agents affect Ca^{2+} sensitivity on the contractile apparatus in the α-toxin–permeabilized pig iris sphincter muscle strips. We also investigated MLC phosphorylation in the α-toxin–permeabilized pig iris sphincter muscle

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during activation by $PGF_2\alpha$ at a fixed $[Ca^{2+}]_i$. We obtained evidence that latanoprost has a different effect on the Ca^{2+} sensitivity from $PGF_2\alpha$ and would thus play a beneficial role in glaucoma treatment.

Materials and Methods

Tissue Preparation

All animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Porcine eyes were enucleated at a local slaughterhouse after the animals had been killed. After the cornea was removed, iris sphincter muscle preparations were cut, under a binocular microscope, into strips measuring approximately 1.0 mm in width and 2.5 mm length. The strips were placed in normal physiological saline solution (PSS) consisting of the following: NaCl, 123 mM; KCl, 4.7 mM; $CaCl_2$, 1.25 mM; $MgCl_2$, 1.2 mM; H_2PO_4, 1.2 mM; $NaHCO_3$, 15.5 mM; D-glucose, 11.5 mM, 95% O_2, and 5% CO_2.

Tension Measurement

The strips were mounted between two tungsten wires, one fixed and the other attached to a force transducer (UL2; Minebea Co., Japan). Tension experiments were performed at room temperature. The developed tension was expressed as a percentage, assigning the 118 mM K^+-induced contraction to be 100%.

Permeabilization with α-Toxin

Iris sphincter muscle strips were permeabilized by α-toxin according to the methods described by Nishimura et al.[16,19] Tension measurements of permeabilized tissues were taken at room temperature. The developed tension was expressed as a percentage, and values in the relaxing solution ($[Ca^{2+}]_i < 10$ nM) and in the activating solution ($[Ca^{2+}]_i = 10$ μM; maximal tension) were assigned as 0% and 100%, respectively.

Immunoblot

Strips from the pig iris sphincter muscle and coronary artery were subjected to immunoblot analysis, as previously described.[20] Regarding the tissue samples, freshly dissected iris sphincter muscle and coronary artery strips were rapidly frozen and then were shattered by hammering. Ten micrograms total protein was separated with SDS-PAGE and then was transferred to the polyvinylidene difluoride membrane (Bio-Rad, Hercules, CA). Membranes were incubated with anti–CPI-17 polyclonal antibody[21] or anti–MYPT1 monoclonal antibody.[22] Antigen detection was performed with a chemiluminescent substrate (SuperSignal West Pico [Pierce, Rockford, IL] and ChemicDoc XRS-J [Bio-Rad]).

Measurement of MLC Phosphorylation

After incubation in 180 nM Ca^{2+} solution for 5 minutes (0 second), iris sphincter muscle strips were exposed to 10 μM $PGF_2\alpha$ (10, 30, 60, 120 seconds) or to 10 μM CCh (10, 20, 30, 60 seconds). At the indicated times, the phosphorylation of MLC was determined using urea–glycerol gel electrophoresis.[23,24]

Drugs and Solutions

The composition of the normal physiological saline solution (PSS) and the permeabilized preparations have been described. PSS (118 mM K^+) was made by an equimolar substitution of KCl for NaCl. The following were obtained from commercial manufacturers: $PGF_2\alpha$, carbachol, α-toxin, U46619, phorbol 12,13 dibutyrate (PDBu), and thapsigargin (Sigma Chemical, St. Louis, MO); latanoprost and PD98059 (2′-amino-3′-methoxyflavone; Cayman Chemical, Ann Arbor, MI); guanosine 5′-triphosphate (GTP), Y27632 ((R)-(+)-trans-N-(4-pyridyl)-4-(1-aminoethyl)-cyclohexanecalboxamide dihydrochloride, monohydrate), and GF109203X (2-[1-(3-dimethylaminopropyl)-1H-indol-3-yl]-3-(1H-indol-3-yl)-maleimide (Calbiochem, La Jolla, CA).

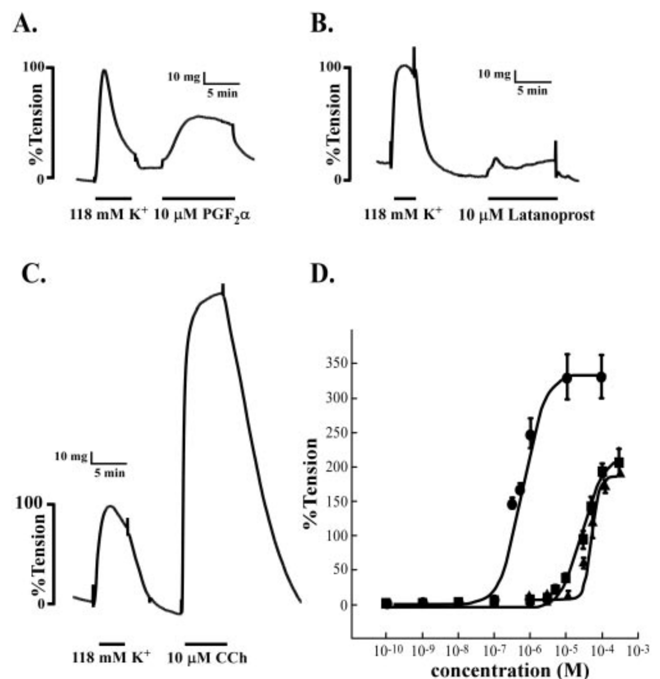

Figure 1. Effect of $PGF_2\alpha$, latanoprost, and CCh on the tension development of the intact pig iris sphincter muscle. (**A–C**) Representative traces of the contraction induced by 10 μM $PGF_2\alpha$ (**A**), latanoprost (**B**), and CCh (**C**). (**D**) Summary of the effect of the cumulative application of $PGF_2\alpha$ (■), latanoprost, (▲), and CCh (●) obtained from six to eight independent experiments. Vertical bars represent SEM. Contractions induced by each drug were plotted by assigning the 118 mM K^+-induced contraction a value of 100%.

Statistical Analysis

All data were expressed as the mean ± SEM along with the number of observations (*n*). One strip obtained from one animal was used for each experiment; therefore, the number of experiments (*n* value) also indicated the number of animals. Student *t* test was used to determine any statistical differences between the two mean values. $P < 0.05$ was considered significant. The four-parameter logistic model was used to fit the sigmoidal curve to the concentration response of each drug.[24] All data were collected using a computerized data acquisition system (MacLab [Analogue Digital Instruments, Australia]; Macintosh [Apple Computer, Cupertino, CA]).

Results

Effect of $PGF_2\alpha$, CCh, and Latanoprost on the Tension Development of the Intact Iris Sphincter Muscle in Normal PSS

The application of 10 μM $PGF_2\alpha$ induced a sustained increase in tension in normal PSS (32.6% ± 6.2%; $n = 8$; Fig. 1A). On the other hand, 10 μM latanoprost induced only a small contraction (9.60% ± 3.6%; $n = 8$; Fig. 1B). The addition of 10 μM CCh induced the development of tension significantly greater than that induced by $PGF_2\alpha$ or latanoprost (257% ± 22%; $n = 8$; $P < 0.01$). Figure 1D summarizes the data obtained by the cumulative application of various concentrations of $PGF_2\alpha$, latanoprost, and CCh (10 pM~30 μM). EC_{50} values were 33.7 ± 2.36 μM for $PGF_2\alpha$ ($n = 6$), 53.1 ± 2.3 μM for latanoprost ($n = 6$), and 429 ± 78 nM for CCh ($n = 6$). The tension induced by the cumulative application of each drug tended to be greater than

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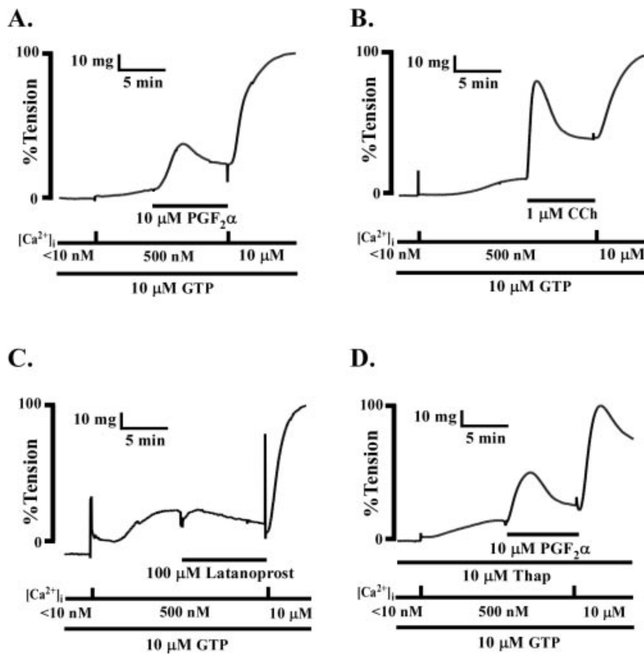

FIGURE 2. Effects of PGF$_2\alpha$ (**A**), CCh (**B**), and latanoprost (**C**) on the Ca^{2+}-induced contractions of the α-toxin–permeabilized iris sphincter muscle strips. (**D**) Effect of 10 μM thapsigargin, an inhibitor of SR ATPase (Ca^{2+} pump), on the PGF$_2\alpha$-induced contraction. The developed tension was expressed as a percentage, assigning the values in the relaxing solution ($[Ca^{2+}]_i < 10$ nM) and in the activating solution ($[Ca^{2+}]_i = 10$ μM; maximal tension) to be 0% and 100%, respectively. Ten micromolar GTP was added during all protocols.

that obtained by the single application of the same concentration.

Effect of PGF$_2\alpha$, CCh, and Latanoprost on the Tension Development of the α-Toxin–Permeabilized Iris Sphincter Muscle

Figures 2A, 2B, and 2C show the representative recordings of the effect of 10 μM PGF$_2\alpha$, 10 μM latanoprost, and 1 μM CCh, respectively, on the tension development induced by 500 nM Ca^{2+} and 10 μM GTP in the α-toxin–permeabilized pig iris sphincter muscle. The application of PGF$_2\alpha$ or CCh during the steady state contraction evoked by the mixture of 500 nM Ca^{2+} and 10 μM GTP induced an additional tension development at a constant $[Ca^{2+}]_i$. In contrast, the application of latanoprost did not induce any tension development (–2.88 ± 2.1%; $n = 4$) at the concentration of 100 μM, which induced a comparable contraction to PGF$_2\alpha$ in the intact strips (Fig. 2C). In Figures 2A and 2B, the additional tension development was composed of two phases, the initial transient phase and the sustained phase. To rule out the possibility that the intracellular Ca^{2+} release from the sarcoplasmic reticulum (SR) might have contributed to this biphasic nature, we treated the strips with 10 μM thapsigargin, an inhibitor of SR ATPase (Ca^{2+} pump), for 10 minutes before and during the protocol. As shown in Figure 2D, thapsigargin had no effect on the tension development induced by PGF$_2\alpha$ at a constant $[Ca^{2+}]_i$, thus indicating that the intracellular Ca^{2+} release may not be responsible for the biphasic tension development induced by PGF$_2\alpha$. Thapsigargin had no effect in either case of CCh stimulation (data not shown). Both PGF$_2\alpha$ and CCh induced significant increases in tension at a fixed $[Ca^{2+}]_i$ during the initial transient and the sustained phases. However, the CCh-induced initial transient phase (77.0% ± 5.7%; $n = 8$) was significantly ($P < 0.05$) greater than the PGF$_2\alpha$-induced phase (39.8% ± 2.9%; $n = 14$), whereas no significant difference was found in the sustained phase (25.3% ± 2.8%; $n = 14$ [PGF$_2\alpha$] and 24.8% ± 4.5%; $n = 8$ [CCh]). Thapsigargin had no significant effect on the tension development induced by PGF$_2\alpha$ or CCh in either phase (PGF$_2\alpha$: peak, 36.2% ± 8.4%; sustained, 22.9% ± 3.5% [$n = 4$]; CCh: peak, 71.6% ± 8.3%; sustained, 20.8% ± 4.1% [$n = 4$]). In addition, PGE$_2$, U46619 (a thromboxane A$_2$ analogue), and thromboxane B$_2$ also induced further tension development at a constant $[Ca^{2+}]_i$ (traces not shown).

Effect of PGF$_2\alpha$ and CCh on the Relationship between $[Ca^{2+}]_i$ and Tension in the α-Toxin–Permeabilized Preparation

Figure 3 shows a representative plot of 12 independent experiments of 10 μM PGF$_2\alpha$ or 10 μM CCh on the tension development induced by the cumulative application of the increasing function of $[Ca^{2+}]_i$. The Ca^{2+}–tension relationship in the presence of 10 μM PGF$_2\alpha$ or 10 μM CCh shifted to the left. Under control conditions, EC$_{50}$ was 2.66 ± 0.04 μM, which was significantly ($P < 0.05$) greater than that in the presence of 10 μM PGF$_2\alpha$ (1.70 ± 0.09 μM) or 10 μM CCh (1.03 ± 0.04 μM) by the paired t test ($n = 12$).

Effects of the Selected Kinase Inhibitors on the Increase in Ca^{2+} Sensitivity Induced by PGF$_2\alpha$ or CCh in the α-Toxin–Permeabilized Preparation

As shown in Figures 4A and 4B, 1 μM Y27632 inhibited 10 μM PGF$_2\alpha$– and 1 μM CCh–induced tension by 22.5% ± 6.0% ($n = 4$) and 22.7% ± 4.9% ($n = 4$), respectively. However, as shown in Figures 4C to 4F, neither GF109203X (10 μM), a protein kinase C (PKC) inhibitor, nor PD98059 (10 μM), a mitogen-activated protein (MAP) kinase inhibitor, had an effect (PGF$_2\alpha$-sustained phase, 16.4% ± 4.1%; GF109203X, 17.6% ± 5.9% [$n = 4$]; PD98059, 16.2% ± 5.1% [$n = 4$]; CCh-sustained phase, 14.9% ± 6.0%; GF109203X, 14.2% ± 2.5% [$n = 4$]; PD98059, 12.6% ± 2.4% [$n = 4$]). We next examined the effect of Y27632 on the tension induced by 500 nM $[Ca^{2+}]_i$ with 10 μM GTP. We chose a concentration of $[Ca^{2+}]_i$, which produces a tension comparable to that induced by 300 nM Ca^{2+} plus 10 μM PGF$_2\alpha$. As shown in Figures 5A to 5D, 1 μM Y27632 significantly inhibited PGF$_2\alpha$- and Ca^{2+}-induced contractions by 23.3% ± 4.1% for PGF$_2\alpha$ and 36.2% ± 8.7% for 500 nM $[Ca^{2+}]_i$, respectively ($P < 0.01$).

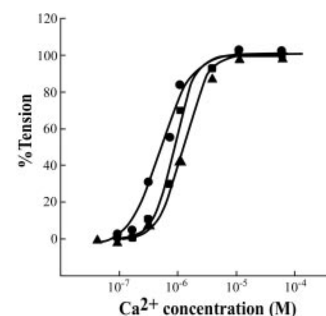

FIGURE 3. Effect of PGF$_2\alpha$ and CCh on the Ca^{2+} sensitivity of the myofilament. A representative plot obtained from three strips of one pig. Ca^{2+} tension–relationship curves were constructed by the cumulative application of the increasing function of Ca^{2+} solution in the absence (control, ▲) or presence of 10 μM PGF$_2\alpha$ (■) or 10 μM CCh (●). Ten micromolar GTP was added during all protocols.

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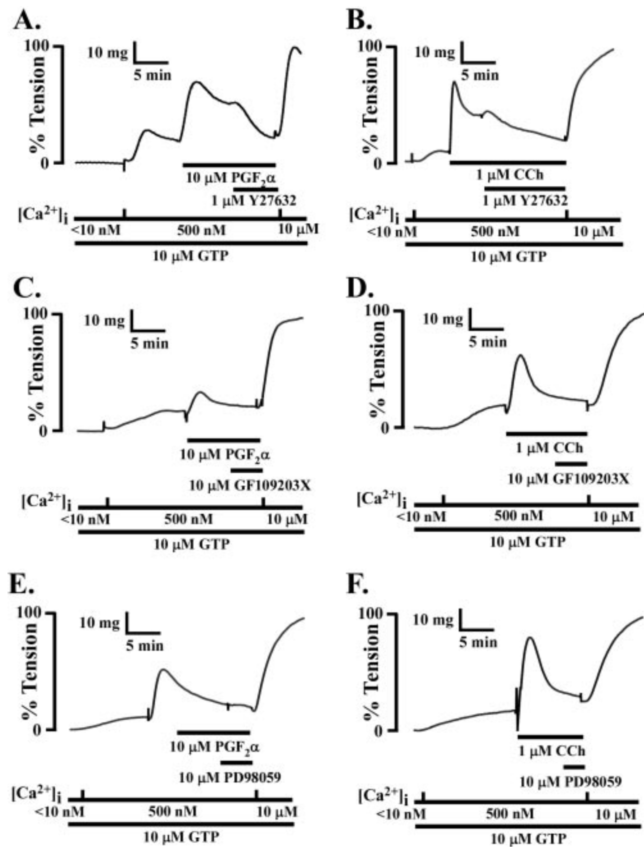

FIGURE 4. Effect of 1 μM Y27632 (**A**, **B**), a rho kinase inhibitor, 10 μM GF109203X (**C**, **D**), a PKC inhibitor, and 10 μM PD98059 (**E**, **F**), a MAP kinase inhibitor on the $PGF_2\alpha$- or CCh-induced contraction at a constant $[Ca^{2+}]_i$.

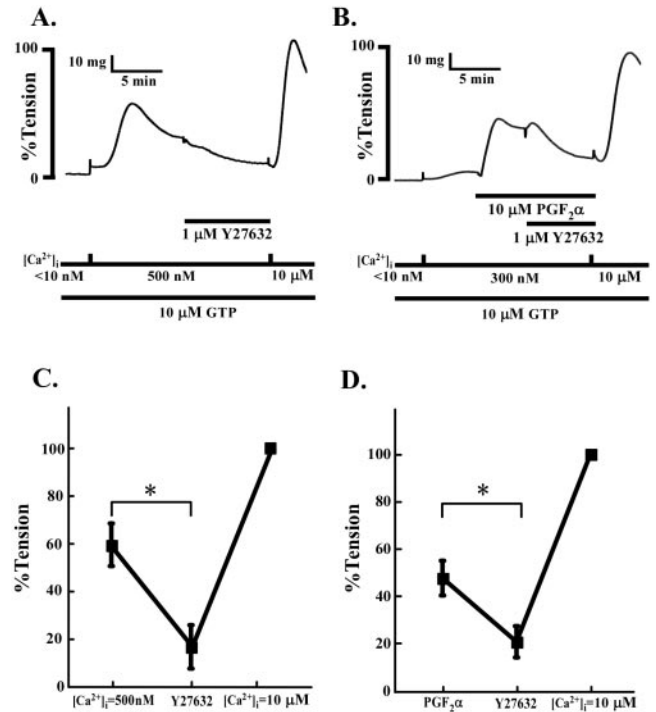

FIGURE 5. Effect of 1 μM Y27632 on the contractions induced by 500 nM Ca^{2+} alone (**A**) or 300 nM Ca^{2+} plus 10 μM $PGF_2\alpha$ (**B**) in the presence of 10 μM GTP. (**C**, **D**) Summary of the results obtained from four independent experiments (**C**, 500 nM Ca^{2+} alone; **D**, 300 nM Ca^{2+} plus 10 μM $PGF_2\alpha$). Tension levels induced by 500 nM Ca^{2+} alone or 300 nM Ca^{2+} plus $PGF_2\alpha$, those following the application of 1 μM Y27632, and maximal tension levels induced by 10 μM Ca^{2+} were plotted. $^*P < 0.01$.

Lack of CPI-17 Expression in the Pig Iris Sphincter Muscle

Given that a protein kinase C (PKC) inhibitor had no effect on the $PGF_2\alpha$- induced increase in the Ca^{2+} sensitivity of the pig iris sphincter muscle, we next examined the effect of an activator of PKC (PDBu). PDBu did not induce any further tension development in the intact and permeabilized pig iris sphincter muscle ($n = 4$; Fig. 6A). We thus investigated the expression of PKC-potentiated protein phosphatase-1 inhibitory protein (CPI-17) and an isoform of a large subunit of MLC phosphatase (MYPT1). As shown in Figure 6B, CPI-17 expression was under detectable levels in the pig iris sphincter muscle ($n = 4$), whereas MYPT-1 expression was observed to be similar to that of the coronary smooth muscle (114% ± 5.6% of coronary artery; $n = 4$).

Effect of $PGF_2\alpha$ and CCh on the Level of MLC Phosphorylation in the α-Toxin–Permeabilized Preparation

To determine whether the $PGF_2\alpha$- or CCh- induced increase in the Ca^{2+} sensitivity of pig iris sphincter muscle was accompanied by an increase in MLC phosphorylation, a protocol similar to that shown in Figure 2 was used and the level of MLC phosphorylation was measured at 180 nM $[Ca^{2+}]_i$. As shown in Figure 7, the tension development induced by $PGF_2\alpha$ and CCh at steady state ($[Ca^{2+}]_i$ = 180 nM) was accompanied by an increase in MLC phosphorylation.

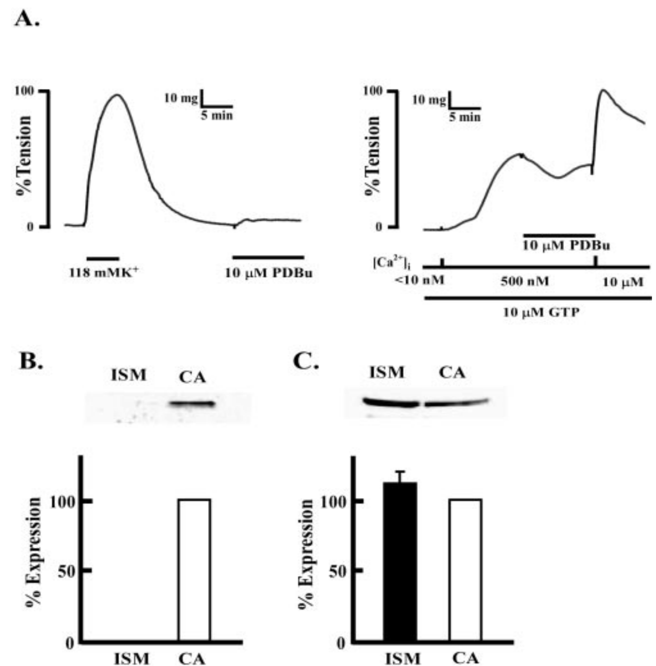

FIGURE 6. Effect of 10 μM PDBu on the intact iris sphincter muscle and the contraction induced by 500 nM Ca^{2+} in the presence of 10 μM GTP in the α-toxin–permeabilized strip (**A**). Expression of CPI-17 (**B**) and MYPT1 (**C**) in the pig iris sphincter muscle (ISM) compared with the pig coronary artery (CA).

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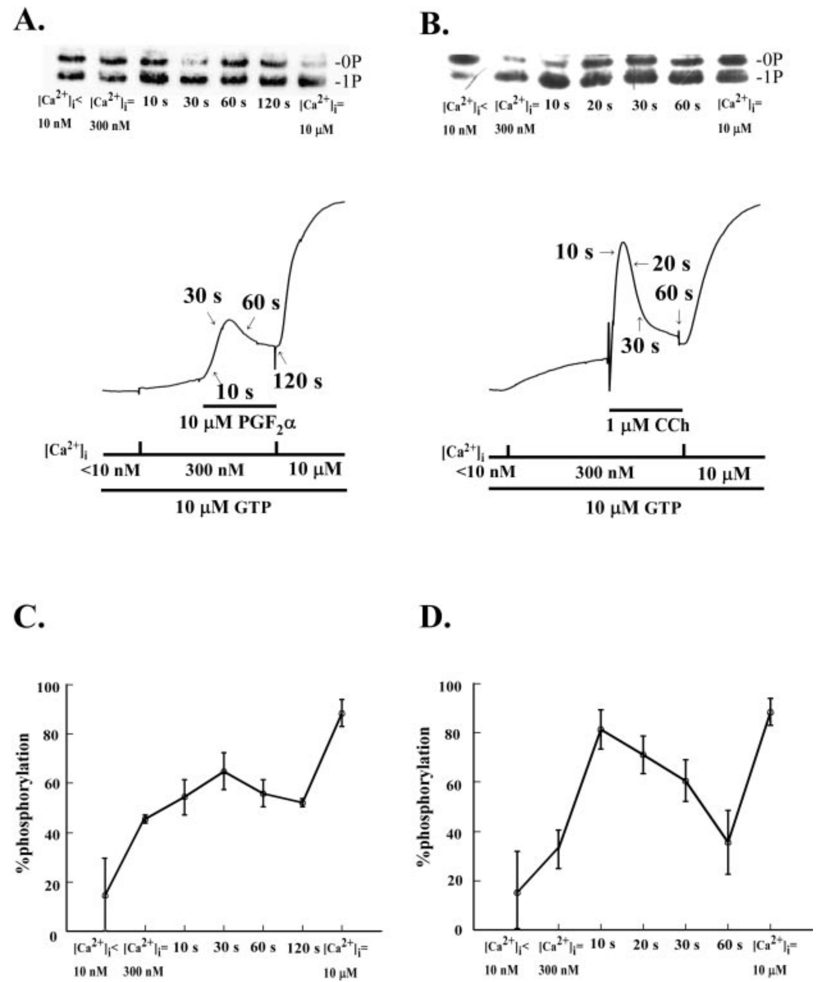

FIGURE 7. Temporal changes in MLC phosphorylation levels induced by 10 μM $PGF_2\alpha$ and 1 μM CCh in the α-toxin–permeabilized iris sphincter muscle strip. (**A**, **B**) Representative image of Western blotting for MLC phosphorylation and a representative trace that shows the specific protocol. (**C**, **D**) Quantitative analysis of temporal changes in the MLC phosphorylation levels obtained from four experiments. Relative MLC phosphorylation levels were shown as ratios of phosphorylated MLC/total MLC (pMLC/tMLC) at each time point.

DISCUSSION

In the present study, the mechanisms underlying $PGF_2\alpha$-induced contraction of the pig iris sphincter muscle were investigated, particularly in comparison with the contraction induced by latanoprost or CCh, a major constrictor agent of the iris sphincter muscle. The major findings are as follows: (1) In the intact strips, latanoprost was the least potent of the three agents for inducing contraction. (2) In the permeabilized preparation, $PGF_2\alpha$ and CCh, but not latanoprost, caused Ca^{2+} sensitization. (3) The $PGF_2\alpha$-induced enhancement of contraction at a fixed $[Ca^{2+}]_i$ was blocked only by a rho kinase inhibitor (Y27632) but not by a PKC inhibitor or a MAP kinase inhibitor. (4) Y27632 also inhibited Ca^{2+}-induced contraction. (5) Although MYPT1 was expressed, CPI-17 was not expressed in this tissue. (6) The additional tension development induced at a fixed $[Ca^{2+}]_i$ by $PGF_2\alpha$ or CCh was accompanied by an increase in MLC phosphorylation. These results indicated that $PGF_2\alpha$ and CCh induced Ca^{2+} sensitization of the pig iris sphincter muscle in an MLC phosphorylation–dependent manner through the rho–rho kinase pathway, whereas latanoprost—at the concentration that induced a contraction comparable to that of $PGF_2\alpha$ in the intact strips—had no effect on Ca^{2+} sensitivity. This is the first report describing the $PGF_2\alpha$- or CCh-induced increase in Ca^{2+} sensitivity of the myofilament and its underlying mechanism in the α-toxin–permeabilized pig iris sphincter muscle.

It is now generally accepted that although smooth muscle contraction is primarily regulated by $[Ca^{2+}]_i$,[15] the modulation of Ca^{2+} sensitivity also plays an important role.[15–18] The mechanism for such an increased Ca^{2+} sensitivity (Ca^{2+} sensitization) is still unclear. However, this mechanism can be classified into two distinct mechanisms. One is increased Ca^{2+} sensitivity with increased MLC phosphorylation (MLC phosphorylation–dependent Ca^{2+} sensitization),[18] and the other is without increased MLC phosphorylation (MLC phosphorylation–independent Ca^{2+} sensitization).[25–27] The present study clearly demonstrated that the $PGF_2\alpha$- or CCh-induced contraction of the pig iris sphincter muscle involves the MLC phosphorylation-dependent Ca^{2+} sensitization because $PGF_2\alpha$ and CCh induced additional tension development with increased MLC phosphorylation at a constant $[Ca^{2+}]_i$. In addition, the tension levels induced by CCh were greater than those induced by $PGF_2\alpha$ in the intact strips, but, in the permeabilized preparation, the sustained levels of $PGF_2\alpha$-induced contraction were similar to those of the CCh-induced contraction. This observation indicated that $PGF_2\alpha$-induced contraction is more dependent on the Ca^{2+} sensitization than on the increase in $[Ca^{2+}]_i$ compared with CCh-induced contraction. In addition, we showed that thromboxane B_2 increased Ca^{2+} sensitivity in the iris sphincter muscle, which has not yet been previously described. We consider that it may thus be one of the possible causes of uncontrollable miosis, including surgically induced miosis.

The signal transduction pathway for MLC phosphorylation–dependent Ca^{2+} sensitization has recently been elucidated. The small guanosine triphosphatase rho is implicated in this type of Ca^{2+} sensitization of smooth muscle contraction. The

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GTP-bound active form of rhoA activates a downstream kinase, rho kinase, which phosphorylates the myosin-binding subunit (MBS) of myosin phosphatase to inhibit its activity.[28] Another possible pathway for MLC phosphorylation–dependent Ca^{2+} sensitization involves PKC and CPI-17. This novel protein has been reported to inhibit MLC phosphatase when phosphorylated by PKC.[29] In addition, MAP kinase has been implicated in iris sphincter muscle contraction.[30,31] Based on these considerations, we examined the effect of rho kinase inhibitor (Y27632), PKC inhibitor (GF109203X), and MAP kinase inhibitor (PD98059). We showed that the $PGF_2\alpha$-induced Ca^{2+} sensitization of the contractile apparatus in the α-toxin–permeabilized iris sphincter muscle, which is likely to depend on MLC phosphorylation, was inhibited by Y27632, but not by GF109203X or PD98059. These results indicated that the pathway of the $PGF_2\alpha$- or CCh-induced increase in Ca^{2+} sensitivity involves the rho–rho kinase system but not the PKC-CPI-17 pathway. The facts that PDBu had no effect on tension development and that CPI-17 did not exist in the pig iris sphincter muscle supported this hypothesis. In addition, Y27632 also depressed the Ca^{2+}-induced contraction of the pig iris sphincter muscle, thus indicating that the rho–rho kinase pathway may be involved in Ca^{2+}-calmodulin–dependent contraction. In close agreement with this result, Sakurada et al.[32] recently reported that membrane depolarization by 60 mM KCl and noradrenalin stimulation induced a similarly sustained contraction in the rabbit aorta, whereas both stimuli induced similar time-dependent, sustained increases in the amount of an active GTP-bound form of rhoA.

Concerning the regulation of IOP, the rho kinase inhibitor has been reported to reduce IOP through an effect on the actin cytoskeleton by interfering with the actomyosin system.[33,34] Similarly, MLCK inhibitors have been reported to increase the outflow facility so that they might reduce IOP through the inhibition of MLC phosphorylation.[35–37] It can thus be speculated that these agents, which are coupled with the rho–rho kinase or the Ca^{2+}-MLCK pathway, or both, may increase IOP. Latanoprost, a $PGF_2\alpha$ analogue, has been widely used for the treatment of glaucoma, whereas $PGF_2\alpha$ is thought to increase IOP. This discrepancy can thus be clearly explained by the present results, which show the rho–rho kinase pathway to be involved in the $PGF_2\alpha$-mediated pathway but not in the latanoprost-mediated pathway. In other words, $PGF_2\alpha$ might have a bidirectional effect on IOP, increasing IOP by increasing the effect on MLC phosphorylation and decreasing IOP by increasing the uveoscleral outflow of aqueous humor. In the case of latanoprost, the former action was weaker than $PGF_2\alpha$ and therefore was considered to play a beneficial role in the treatment of glaucoma. In the present study, we chose esterified latanoprost, which has already been clinically used. However, it should be noted that free-acid latanoprost might have a greater effect because it is reported to be 100 times more potent than the esterified latanoprost in a rabbit uterus preparation.[38,39]

In summary, $PGF_2\alpha$, but not latanoprost, was found to induce an increase in the Ca^{2+} sensitivity of the contractile apparatus in an MLC phosphorylation–dependent manner through the activation of the rho–rho kinase signaling pathway. The lack of any effect on Ca^{2+} sensitivity by latanoprost is thus considered to be beneficial in the treatment of glaucoma.

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