Prostanoid-Induced Relaxation of Precontracted Cat Ciliary Muscle Is Mediated by EP$_2$ and DP Receptors

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The pharmacology of prostanoid-induced relaxation of the precontracted cat ciliary smooth muscle was characterized using synthetic prostaglandin (PG) analogues that are selective for specific prostanoid receptors. Relaxation was studied using carbachol to precontract the isolated longitudinal ciliary muscle, followed by application of the PG agonist. Of the compounds studied, PGE$_2$ was the most potent relaxant (concentration that produced 50% of maximum relaxation, $10^{-7}$ mol/l), and its maximal effect in each preparation was used as a standard for comparison. Both PGD$_2$ and PGF$_{2\alpha}$ produced relaxations that were approximately 30- and 100-fold weaker, respectively, than those produced by PGE$_2$. Prostanoids with activity at the EP$_2$ (19-(R)-hydroxy PGE$_2$ and 11-deoxy PGE$_2$) and DP (BW 245C) receptors potently relaxed the ciliary muscle. Other EP receptor subtypes and the TP receptor were not involved as indicated by the lack of relaxant activity of sulprostone (EP$_3 >$ EP$_1$), MB 28767 (EP$_3 >$ TP), and U-46619 (TP). Although 17-phenyl trinor PGE$_2$ (EP$_1$ and EP$_3$) and PGI$_2$ (IP) had some activity, it occurred at a nonselective dose ($10^{-4}$ mol/l). The presence of DP receptors in the cat ciliary muscle was confirmed by using BW A868C, a selective DP-receptor antagonist. This drug (concentration, 1 μmol/l) displaced the relaxant effects of PGD$_2$ but had no effect on the activities of PGE$_2$ and 11-deoxy PGE$_2$. In addition, 17-phenyl trinor PGF$_{2\alpha}$ (FP) was inactive, indicating that the FP receptor was not involved in ciliary muscle relaxation. There was no evidence for prostanoid receptors that may mediate contraction of the ciliary muscle because the PGs studied had no contractile effects. These results indicate that PG-induced relaxation of the cat ciliary muscle precontracted by carbachol may be mediated by stimulation of both EP$_2$ and DP receptors. Invest Ophthalmol Vis Sci 33:3195-3201, 1992

The decreases in intraocular pressure induced by prostaglandins (PGs) in cats, monkeys, and humans may result from increased aqueous humor outflow. Additional studies have suggested that PGs increase aqueous outflow through the uveoscleral pathway and not through the conventional trabecular route. The role of PG-induced ciliary muscle relaxation in the increase in uveoscleral outflow was suggested by physiologic antagonism studies. It was found that PGF$_{2\alpha}$-induced ocular hypotension and increased uveoscleral outflow were abolished by precontracting the ciliary muscle with pilocarpine. Thus, the ocular hypotension evoked by PGs, which occurs by increasing uveoscleral outflow, may involve ciliary muscle relaxation. Therefore, the isolated ciliary muscle may represent a suitable preparation for studying the target tissue for the effects of PGs on intraocular pressure in vitro. The cat was selected as a relatively convenient species for study because the ocular hypotensive effects of natural PGs such as PGE$_2$, PGF$_{2\alpha}$, and PGD$_2$ have been studied extensively in this species.

Prostanoid-receptor characterization in vitro revealed that the natural PGs have a modest degree of selectivity for a particular receptor subtype and provided evidence for five major prostanoid receptor subclasses (EP, DP, FP, IP, and TP) in smooth muscle. Subdivision of the EP-receptor subclass also was proposed. Based on this current classification, recent work suggests that decreases in intraocular pressure in cats may be a result of stimulation of EP$_2$ and DP receptors. However, the pharmacologic basis of prostanoid-induced ocular hypotension and relaxation of the ciliary muscle have not been compared in the context of the current working classification for prostanoid receptors.

We investigated the pharmacology of prostanoid-induced relaxation of the isolated cat ciliary smooth muscle by determining the activity of prostaglandin agonists with selectivity for specific PG receptors. Further characterization of the DP-receptor involvement was done using the selective antagonist, BW A868C (Burroughs Wellcome, Beckenham, UK).
Materials and Methods

Adult cats of either sex were killed by an overdose of sodium pentobarbital administered intravenously by one animal-care technician while another applied gentle restraint. The eyes were enucleated rapidly and placed on ice. Longitudinal ciliary muscle strips, 3–3.5-mm wide, were dissected from each eye and suspended by sutures in a 5-ml jacketed organ bath. The bath medium, which contained Krebs' buffer (composition in millimoles/liter: NaCl, 118; KCl, 4.7; KH₂PO₄, 1.2; CaCl₂ · 2 H₂O, 1.9; MgSO₄ · 7H₂O, 1.18; NaHCO₃, 25; and glucose, 11.7; pH adjusted to 7.4) with 10⁻⁶ mol/l indomethacin, was maintained at 37°C and aerated with 95% oxygen–5% carbon dioxide. A different cyclooxygenase inhibitor, ibuprofen (10⁻⁶ mol/l) was used instead of indomethacin for comparative studies using PGE₂ and PGD₂. The effect of agents on muscle tension was measured isometrically with a force-displacement transducer (Grass FT-03; Grass Instrument Co., Quincy, MA), and the responses were recorded on a polygraph (Grass Model 7D). An initial load of 250 mg was applied to the muscle strips, and during the 60-min equilibration period, the tension was maintained at 150–200 mg. Single submaximal doses of carbachol were applied to establish tissue responsiveness. To test the effects of prostanoids on relaxation, the ciliary muscle was pretreated with 3 µmol/l carbachol for 45 min before adding cumulative doses of the PGs. The relaxant effects of PGE₂ and PGD₂ also were tested after a 15-min pretreatment period with 3 µmol/l carbachol. After the last dose of each prostanoid, PGE₂ at 10⁻⁵ mol/l was administered to each tissue to obtain the maximal effect for comparative purposes. As a control, additional tissues were exposed to carbachol followed by the one dose of PGE₂ (10⁻³ mol/l) during the same period. Prostanoids also were administered cumulatively to noncontracted ciliary preparations. After washout of the tissue, a 60–90-min period was allowed for recovery of the preparation. In the DP antagonist studies, the PG-induced relaxation was determined in the absence of antagonist and repeated after 45-min preincubation with 1 µmol/l BW A868C or an equal volume (5 µl) of ethanol vehicle.

Relaxation responses were expressed as percentages of the maximal effect elicited by PGE₂ for each tissue. The potency (concentration that produced 50% of maximum relaxation, IC₅₀) values were obtained from the logarithmic concentration–response curves. All procedures conformed to the ARVO Resolution on the Use of Animals in Research.

Carbachol, indomethacin, and isoproterenol HCl were obtained from Sigma (St. Louis, MO). Prostaglandin E₂ (PGE₂), 19(R)-hydroxy PGE₂, PGD₂, PGE₂φ, PGI₂-Na⁺, 17-phenyl trinor PGE₂, 17-phenyl trinor PGF₂α, 11-deoxy PGE₁, 9,11-dideoxy-9α, 11α-methanoepoxy-prostaglandin F₂α (U-46619), and ibuprofen were supplied by Cayman Chemical (Ann Arbor, MI). Sulprostone (Berlex, Cedar Knolls, NJ) and 15α-hydroxy-9-oxo,16-phenoxo-17,18,19,20-te-

Fig. 1. (A) Example trace shows the contraction of the isolated cat ciliary muscle by 3 µmol/l carbachol (carb) for 85 min followed by PGE₂ at 10⁻⁵ mol/l. Example traces show the relaxation of the cat ciliary muscle precontracted with 3 µmol/l carbachol for 45 min or 45 min elicited by (B) PGE₂ (45 min carb); (C) PGE₂ (15 min carb); (D) PGD₂ (45 min carb); (E) PGD₂ (15 min carb); (F) isoproterenol (isop; 45 min carb). Final concentrations (Log M) are shown. PGE₂ at 10⁻⁵ mol/l was administered after the last dose of PGD₂ (D, E) and isoproterenol (F) to obtain maximal effect.
tranorprost-13-trans-enoic acid (MB 28767; Rhone-Poulenc, Dagenham, UK) were gifts. Both 5-(6-carboxyhexyl)-1-(3-cyclohexyl-hydroxypropyl)-hydantoin (BW 245C) and 3-benzyl-5-(6-carboxyhexyl)-1-(2-cyclohexyl-2-hydroxyethylamino)-hydantoin (BW A868C) were gifts from Burroughs Wellcome.

Carbachol and isoproterenol were prepared in normal saline. The prostaglandin agonists, excluding 19(R)-hydroxy PGE2, sulprostone, and PGI2, were dissolved in 2% Na2CO3, and dilutions were made with normal saline. The 19(R)-hydroxy PGE2 in methyl acetate solution was evaporated under nitrogen, and the residue was dissolved in 0.1% polysorbate-80 in 10 mmol/l Tris buffer. This stock solution was diluted in distilled water. Sulprostone and PGI2 were prepared in normal saline and Tris base, respectively. The BW A868C was dissolved in absolute ethanol to prepare a stock solution. Drug solutions were placed on ice during the duration of the experiments.

Results

Carbachol produced a dose-related increase in contraction of the ciliary muscle over the 0.01–100 μmol/l concentration range (concentration that produced 50% of maximum contraction, 6.4 × 10^-7 mol/l; n = 6). The 3-μmol/l dose of carbachol evoked a contraction of 80 ± 4% of maximal effect and was selected as a suitable dose for examining the relaxant effects of prostanoids. On carbachol-treated control tissues, PGE2 at 10^-5 mol/l produced a 60 ± 2% relaxation (n = 14). A trace illustrating this is shown in Figure 1A. Traces showing relaxation of the carbachol precontracted ciliary muscle by PGE2, PGD2, and for comparative purposes, isoproterenol are presented in Figures 1B-F. There was no difference in the effects of PGE2 or PGD2 after either 15- or 45-min carbachol pretreatment times, indicating that the effects of these prostanoids are direct rather than a consequence of fatigue or other indirect phenomena. The use of different cyclooxygenase inhibitors, indomethacin or ibuprofen, in the bath medium resulted in no apparent differences in the effects of prostanoids. The activities of PGE2 and PGD2 are shown in Figures 2A–B. The effects of PGE2, 19(R)-hydroxy PGE2, and 11-deoxy PGE2 are shown in Figure 3A. Both PGE2 (IC50, 1 × 10^-3 mol/l) and the selective EP2-receptor agonist, 19(R)-hydroxy PGE2 (IC50, 2.4 × 10^-7 mol/l), potently relaxed the cat ciliary muscle. The EP2-receptor agonist, 11-deoxy PGE1, produced dose-related relaxations but was less active (IC50, 5.4 × 10^-6 mol/l). The effect of PGE2 was compared with those elicited by synthetic PG analogues with selectivity for the EP1 and EP3 receptor subtypes (Fig. 3B). Both MB 28767 (EP3 > TP) and sulprostone (EP3 > EP1) were ineffective in relaxing the ciliary muscle. We found 17-phenyl trinor PGE2 (EP1 and EP3) relaxed the tissue by 64% of the PGE2 maximal effect at such a high concentration (10^-4 mol/l) that its effects were likely to be nonselective. Ciliary muscle relaxations induced by BW 245C and PGD2 are compared with those induced by PGE2 in Figure 3C. The selective DP-receptor agonist BW 245C was effective in relaxing the ciliary muscle and was approximately 0.5 log10 unit less potent (IC50, 6.6 × 10^-7 mol/l) than PGE2; PGD2 was less active (IC50, 2.7 × 10^-6 mol/l) than BW 245C and achieved a 73% of maximal PGE2 effect at the 10^-4 mol/l concentration. The effects of natural PGs, PGE2, PGF2a, and PGI2 are shown in Figure 3D. PGF2a produced dose-related relaxations of the ciliary muscle but was 100-fold weaker (IC50, 1 × 10^-5 mol/l) than PGE2. Both the FP-receptor agonist, 17-phenyl trinor PGF2α, and the TP-receptor agonist, U-46619, were inactive (data not illustrated). We found PGI2 elicited a weak relaxant effect (IC50, 6.6 × 10^-7 mol/l), achieving 60% of PGE2 maximal effect at a 10^-4 mol/l concentration.

The prostanoids were tested for effects on the ciliary muscle to reveal the presence of receptors that might mediate contraction. All PGs we examined had no contractile activity over identical dosing ranges (data not shown, n = 4).
Fig. 3. Log (M) concentration-response curves for PGE2 (●) and (A) 19(R)-hydroxy PGE2 (●), 11-deoxy PGE1 (▲); (B) 17-phenyl trinor PGE2 (●), MB 28767 (▲), sulprostone (●); (C) BW 245C (●), PGD2 (●); (D) PGF2a (●), PGI2 (▲) on the isolated cat ciliary muscle. Points are mean values as percentage of PGE2 maximal relaxation for each prostanoid ± standard error of the mean, n = 6 (A-D), except n = 5 (19(R)-hydroxy PGE2).

The relaxant effect of PGD2 was antagonized by the DP-antagonist, BW A868C (Fig. 4A). The rightward shift was approximately two orders of magnitude. This drug was ineffective in displacing the EP-receptor agonists PGE2 (Fig. 4B) and 11-deoxy PGE1 (Fig. 4C). The effects of the prostanoids pre- and post-vehicle treatment were virtually identical (n = 4-5 for each prostanoid).

Discussion

Our studies provide evidence for the presence of two distinct prostanoid receptor types in the cat ciliary smooth muscle that mediate relaxation: the EP2 and DP subtypes.

Three subtypes of the PGE2-sensitive (EP) receptor mediate contraction (EP1 and EP3) or relaxation (EP2) in smooth muscle preparations.14,19,20 PGE2 and relatively selective EP2-receptor agonists caused dose-related relaxations of the cat ciliary muscle in the same rank order of potency (PGE2 > 19(R)-hydroxy PGE2 > 11-deoxy PGE1) as in an EP2-receptor preparation, such as the cat trachea.21,22 Synthetic PG analogues with selectivity for the EP1 and EP2 receptor subtypes also were evaluated. The PG agonists with high affinity for EP3 receptors, sulprostone (EP3 > EP2)13,20 and MB 28767 (EP1 > TP)20,22 had no relaxant effects. It was found 17-phenyl trinor PGE2 had mainly EP1- and EP3-receptor agonist activity in isolated guinea-pig preparations.20 The high dose at which 17-phenyl...
trinor PGE₂ elicited a relaxation response (IC₅₀, 5 × 10⁻⁵ mol/l) suggests that its relaxant action was not related to EP₁- and EP₂-receptor stimulation. The possibility of thromboxane A₂-sensitive (TP) receptor involvement in the activity of MB 28767 and other prostanoids was explored using U-46619, a potent and selective TP-receptor agonist. This drug was unable to relax the ciliary muscle, indicating that the TP receptor was not involved. Interestingly, PGE₂ and 11-deoxy PGE₁ produce ocular hypotension in cats, but the EP₁, EP₂, and TP receptor agonists have minimal or no effect on intraocular pressure. Thus, there appears to be a correlation between the ability of these prostanoids to lower intraocular pressure and their relaxant effects on the isolated ciliary muscle of the cat.

A previous report showed that the decrease in intraocular pressure in cats elicited by PGF₂α was not a FP receptor-mediated response because FP receptor-selective agonists, fluprostenol and 17-phenyl trinor PGF₂α, were ineffective as ocular hypotensives. Our studies using PGF₂α and 17-phenyl trinor PGF₂α indicated that relaxation of the ciliary muscle also was not mediated by the receptor designated as the FP receptor (according to the current prostanoid receptor classification). It has been reported that PGF₂α has activity at the EP, FP, and TP receptors in isolated smooth muscle preparations. In view of the absence of FP and TP receptor-mediated relaxation of the cat ciliary muscle and the similarity of activity of PGF₂α and 11-deoxy PGE₁ we found in our preparation, it follows that the relaxation responses to PGF₂α may be attributed to stimulation of the EP₂ receptor.

The natural prostanoid PGI₂ produced only a weak response in the isolated ciliary smooth muscle. At high doses, the response to PGI₂ may be mediated by EP receptors because PGI₂ and many of its analogues have pharmacologic activity at both IP and EP receptors. In vivo studies, PGI₂ had no effect on cat intraocular pressure when applied topically at a dose that produced an ocular hypotensive effect for PGE₂.

The drug BW 245C, which has a high degree of selectivity and potency for the DP receptor, was slightly less active than PGE₂ in relaxing the cat ciliary muscle. In our preparation and other DP-sensitive systems, PGD₂ was less active than BW 245C. The antagonism of the action of PGD₂, but not that of PGE₂ and 11-deoxy PGE₁, by BW A868C provides substantial support for the presence of DP receptors.
in the cat ciliary muscle. In view of this finding, it is not surprising that BW 245C\textsuperscript{13} and PGD\textsubscript{2} lower intraocular pressure in cats.

The prostanoids that relaxed the cat ciliary muscle with at least a 10\textsuperscript{-6} mol/l threshold concentration were also ocular hypotensives in this species. It is unlikely, however, that ciliary muscle relaxation is the only mechanism of action for prostanoid-induced ocular hypotension. In addition, PGD\textsubscript{2} has been shown to decrease aqueous humor formation.\textsuperscript{28} Alteration in the extracellular matrix of the ciliary muscle bundles has been observed with extended PGF\textsubscript{2\alpha} dosing regimens, and this is believed to decrease resistance to aqueous humor outflow in the uveoscleral pathway.\textsuperscript{29} However, these findings were not confirmed by an alternative 2-wk study with PGF\textsubscript{2\alpha}, which also was done in cynomolgus monkeys.\textsuperscript{30} It is uncertain whether single doses of the less potent prostanoids applied topically to the eye would produce a sufficient concentration at the ciliary muscle to cause relaxation. Pharmacokinetic studies with \textsuperscript{3}H-PGF\textsubscript{2\alpha}, administered as a single topical dose to the ocular surface of a rabbit,\textsuperscript{31} suggest that only very large topical doses of PGF\textsubscript{2\alpha} free acid could provide a sufficient concentration to decrease ciliary muscle tone. Physiologic antagonism studies, however, indirectly indicate that PGF\textsubscript{2\alpha} may decrease ciliary muscle tone in vivo (shown after a continuous dosing regimen in monkeys).\textsuperscript{3} The ability of pilocarpine to reverse the ocular hypotensive effect of PGF\textsubscript{2\alpha} has been attributed to closing uveoscleral outflow as a result of ciliary muscle contraction,\textsuperscript{3} implying that PGF\textsubscript{2\alpha} causes a decrease in ciliary muscle tone.

In summary, the EP\textsubscript{2} and DP prostanoid receptors have been identified as being involved in relaxation of the isolated cat ciliary muscle. There appears to be a correlation with receptor subtypes involved in PG-induced ocular hypotension in the same species. These studies, therefore, provide an indirect pharmacologic correlation for the hypothesis that PGs may increase uveoscleral outflow by relaxing the ciliary muscle.

Key words: ciliary muscle, prostanoid receptor, prostaglandins, cat, DP-receptor antagonist

References

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