Real-Time Intracellular Ca\(^{2+}\) Mobilization by Travoprost Acid, Bimatoprost, Unoprostone, and Other Analogs via Endogenous Mouse, Rat, and Cloned Human FP Prostaglandin Receptors

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ABSTRACT
The ability of a number of prostaglandin F\(_{2\alpha}\) (PGF\(_{2\alpha}\)) analogs to mobilize intracellular Ca\(^{2+}\) and to compete for \([\text{H}]\text{PGF}_{2\alpha}\) binding to prostaglandin F\(_{2\alpha}\) receptors (FP) was evaluated. Radioligand binding studies measuring displacement of \([\text{H}]\text{PGF}_{2\alpha}\) by a variety of FP prostaglandin analogs yielded the following rank order of affinities: travoprost acid (17-phenyl-trinor PGF\(_{2\alpha}\); (+)-fluprostenol) > bimatoprost acid (13,14-dihydro-15-keto-20-ethyl PGF\(_{2\alpha}\)) = bimatoprost (17-phenyl-trinor PGF\(_{2\alpha}\) ethyl amide) > Lumigan (bimatoprost ophthalmic solution). In FP functional studies, travoprost acid (EC\(_{50}\) = 17.5–37 nM, \(n = 13\)), bimatoprost acid (EC\(_{50}\) = 23.3–49.0 nM, \(n = 6–12\)), unoprostone (EC\(_{50}\) = 306–1270 nM, \(n = 4–8\)), bimatoprost (EC\(_{50}\) = 3070–3940 nM, \(n = 4–9\)), and Lumigan (EC\(_{50}\) = 1470–3190 nM, \(n = 5–9\)) concentration dependently stimulated [Ca\(^{2+}\)]\(_{i}\) mobilization via the rat (A7r5 cells), mouse (3T3 cells), and cloned human ocular FP prostaglandin receptors. The rank order of potency of these compounds at the FP receptor of the three species was similar and in good agreement with the determined binding affinities. The agonist effects of these compounds were concentration dependently blocked by the FP receptor-selective antagonist, AL-8810 (11β-fluoro-15-epi-15-indanyl-tetranor PGF\(_{2\alpha}\)) \(K_i = 0.6–1.3\) \(\mu M\). These studies have demonstrated that bimatoprost, unoprostone, and bimatoprost acid possess direct agonist activities at the rat, mouse, and human FP prostaglandin receptor and that travoprost acid is the most potent of the synthetic FP prostaglandin analogs tested.

Prostaglandin F\(_{2\alpha}\) receptor (FP receptor) agonists are potent and efficacious agents that have been shown to reduce elevated intraocular pressure (IOP) in humans and nonhuman primates (Wang et al., 1990; Bito, 1997). A number of prostaglandin F\(_{2\alpha}\) (PGF\(_{2\alpha}\)) analogs are marketed for the treatment of ocular hypertension, a major risk factor for glaucoma. The ocular anti hypertensive drugs travoprost (TRAVATAN) and latanoprost (Xalatan) are well characterized prodrugs of FP receptor agonists (Hellberg et al., 2001; Stjernschantz et al., 1995). However, two additional IOP-lowering prostaglandin analogs, bimatoprost (Lumigan) and unoprostone isopropyl ester (Rescula), have recently been reported to have no observed activity at FP or other known prostaglandin receptors (Bhattacherjee et al., 2001; Woodward et al., 2001) despite being PGF\(_{2\alpha}\) analogs.

Bimatoprost is the ethyl amide derivative of 17-phenyl trinor PGF\(_{2\alpha}\), a potent prostaglandin FP receptor agonist (Woodward et al., 2001). Despite its inherent structural similarity to PGF\(_{2\alpha}\), bimatoprost is claimed not to be a prodrug of bimatoprost acid but to exert its actions via a postulated and uncharacterized “prostamide” receptor (Woodward et al., 2001). This has been the subject of some debate as recent evidence suggests that human and bovine corneal tissues convert bimatoprost to its free acid and that this hydrolysis product may account for the observed IOP reduction (Maxey et al., 2002).

Unoprostone isopropyl ester is the prodrug of unoprostone. Unoprostone is a 13,14-dihydro-15-keto analog of PGF\(_{2\alpha}\) having a two-carbon extension of the \(\alpha\) side chain (Taniguchi et al., 1996). Unoprostone (and its isopropyl ester) has recently been reported to be a docosanoid and also claimed to exert its biological activities independent of any known prostaglandin receptors (Bhattacherjee et al., 2001).

In preliminary studies, we demonstrated the functional agonist activity of bimatoprost at the endogenous FP receptor of mouse Swiss 3T3 cells (Sharif et al., 2001). In the current studies, we sought to fully characterize the pharmacological activities of bimatoprost, unoprostone, and additional prostaglandin analogs through FP receptor binding using...
with 10 μg/ml gentamicin sulfate and 10% fetal bovine serum. HEK-293 cells expressing the full-length cloned human ciliary body FP receptor (HEK-FP cells) were grown in the above media supplemented with 0.4 mg/ml gentamicin. Cells were propagated at 5- to 7-day intervals by treatment with 0.05% trypsin-0.55 mM EDTA.

Materials. A7r5 smooth muscle cells derived from embryonic rat aorta and Swiss albino mouse 3T3 fibroblasts (CRL 1444) were purchased from the American Type Culture Collection (Manassas, VA). HEK-293 cells expressing the cloned human ciliary body FP receptor were obtained from Dr. G. Fitzgerald (University of Pennsylvania, Philadelphia, PA) and cultured as previously described (Kunapuli et al., 1997). Cell culture media, antibiotics, and trypsin-EDTA were obtained from Invitrogen (Carlsbad, CA). Fetal bovine serum was purchased from Hyclone Laboratories (Logan, UT) heat-inactivated at 56°C for 30 min and stored at −20°C. [3H]Prostaglandin F$_{2α}$ (150–175 Ci/mmol) was purchased from PerkinElmer Life Sciences, Inc. (Boston, MA). Bimatoprost free acid (17-phenyl trinor PGF$_{2α}$), bimatoprost (17-phenyl trinor PGF$_{2α}$ ethyl amide), unoprostone, unoprostone isopropyl ester, and other standard prostanoids were from Cayman Chemical (Ann Arbor, MI). Lumigan (0.03% bimatoprost ophthalmic solution) was obtained from Allergan, Inc. (Irvine, CA). Travoprost free acid and AL-8810 were synthesized in the Medicinal Chemistry Department of Alcon Research, Ltd. Fluorometric imaging plate reader (FLIPR) and calcium assay kit dyes were from Molecular Devices Corp. (Sunnyvale, CA).

Fig. 1. Competition curves for prostaglandin analogs displacing specific [3H]PGF$_{2α}$ binding to BCLM membranes. Data are mean ± S.E.M. from n = 3 to 7 independent experiments.

[3H]PGF$_{2α}$ and functional intracellular calcium ([Ca$^{2+}$]$_{i}$) mobilization assays.

[Ca$^{2+}$]$_{i}$ mobilization studies in real time were undertaken to identify direct agonist activity of prostaglandin analogs while precluding the potential for compound hydrolysis or other processing associated with longer assay incubation times. In particular, we sought to compare and contrast the functional activities of these compounds at the endogenous FP receptor in rat vascular smooth muscle (A7r5; Griffin et al., 1998) and mouse fibroblast (Swiss 3T3; Griffin et al., 1997) cells, and also at the cloned human ocular FP receptor (Kunapuli et al., 1997; Sharif et al., 2001) expressed in human embryonic kidney (HEK-293) cells. In addition, we used a selective FP receptor antagonist, AL-8810 (11β-fluoro-15-epi-15-indanyl tetrnor PGF$_{2α}$), (Griffin et al., 1999; Sharif et al., 2001) to demonstrate the FP nature of bimatoprost, unoprostone, and bimatoprost acid in these cells.

Materials and Methods

Cell Culture. A7r5 rat vascular smooth muscle cells and Swiss albino mouse 3T3 fibroblasts were cultured in Dulbecco’s modified Eagle’s medium containing 4.5 g/l glucose, 110 mg/l sodium pyruvate, pyridoxine hydrochloride, and Glutamax I, and supplemented with 10 μg/ml gentamicin sulfate and 10% fetal bovine serum. HEK-293 cells expressing the full-length cloned human ciliary body FP receptor (HEK-FP cells) were grown in the above media supplemented with 0.4 mg/ml gentamicin. Cells were propagated at 5- to 7-day intervals by treatment with 0.05% trypsin-0.55 mM EDTA.

Materials. A7r5 smooth muscle cells derived from embryonic rat aorta and Swiss albino mouse 3T3 fibroblasts (CRL 1444) were purchased from the American Type Culture Collection (Manassas, VA). HEK-293 cells expressing the cloned human ciliary body FP receptor were obtained from Dr. G. Fitzgerald (University of Pennsylvania, Philadelphia, PA) and cultured as previously described (Kunapuli et al., 1997). Cell culture media, antibiotics, and trypsin-EDTA were obtained from Invitrogen (Carlsbad, CA). Fetal bovine serum was purchased from Hyclone Laboratories (Logan, UT) heat-inactivated at 56°C for 30 min and stored at −20°C. [3H]Prostaglandin F$_{2α}$ (150–175 Ci/mmol) was purchased from PerkinElmer Life Sciences, Inc. (Boston, MA). Bimatoprost free acid (17-phenyl trinor PGF$_{2α}$), bimatoprost (17-phenyl trinor PGF$_{2α}$ ethyl amide), unoprostone, unoprostone isopropyl ester, and other standard prostanoids were from Cayman Chemical (Ann Arbor, MI). Lumigan (0.03% bimatoprost ophthalmic solution) was obtained from Allergan, Inc. (Irvine, CA). Travoprost free acid and AL-8810 were synthesized in the Medicinal Chemistry Department of Alcon Research, Ltd. Fluorometric imaging plate reader (FLIPR) and calcium assay kit dyes were from Molecular Devices Corp. (Sunnyvale, CA).

[3H]Prostaglandin F$_{2α}$ Binding Studies. Competitive prostaglandin FP receptor binding was performed using washed bovine corpus luteum membrane preparations (20 mg/ml in Krebs buffer, pH 7.4) as previously described (Sharif et al., 1998). Briefly, washed bovine corpus luteum membrane (BCLM) homogenates were incubated with [3H]PGF$_{2α}$ (1 nM) and increasing concentrations of the test compound for 2 h at 23°C. Non-specific binding was defined using 1 μM unlabeled PGF$_{2α}$ or (−)-fluprostenol. Assays were terminated by rapid vacuum filtration using Whatman GF/B glass fiber filters previously soaked in 0.3% polyethylenimine. Receptor-bound radioactivity was determined by liquid scintillation spectrometry at 50% efficiency.

Intracellular Ca$^{2+}$ Mobilization Studies. [Ca$^{2+}$]$_{i}$ mobilization studies were performed using a FLIPR as previously described (Sullivan et al., 1999; Jerman et al., 2000; Sharif et al., 2001). Briefly, A7r5, 3T3, or HEK-FP cells were seeded into black-walled, clear-bottom 96-well plates at a cell density of 25,000 to 50,000 cells/well in normal media 48 h before experiment. Cells in normal media were then loaded with a calcium kit assay dye in FLIPR buffer and evaluated in 5- and 10-point concentration-response formats. Agonist-stimulated intracellular calcium mobilization was measured using a FLIPR I system (Molecular Devices Inc.) monitoring changes in cellular fluorescence (Hanks’ balanced salt solution (Ca$^{2+}$)$_{i}$ = 1.3 mM) buffered with 20 mM Hepes, pH 7.4, containing 2.5 mM probenecid) and incubated at 23°C for 1 h. Test compounds were diluted in 10% dimethyl sulfoxide/10% ethanol and then in FLIPR buffer and evaluated in 5- and 10-point concentration-response formats. Agonist-stimulated intracellular calcium mobilization was measured using a FLIPR I system (Molecular Devices Inc.) monitoring changes in cellular fluorescence (Ca$^{2+}$)$_{i}$ = 488 nm, λem = 540 nm) before and after addition of various agonists. Calibration of the FLIPR I instrument was performed as per the manufacturer’s standard procedures. For antagonist studies, dye-loaded cells were incubated with varying concentrations of the FP

![Graph](image-url)
Fig. 2. Intracellular Ca\textsuperscript{2+} mobilization in rat A7r5 cells. A7r5 cells were loaded with a Ca\textsuperscript{2+}-sensitive dye and exposed to test compounds, and the change in fluorescence was measured over 180 s. The representative fluorescence traces show concentration-dependent mobilization of [Ca\textsuperscript{2+}]i in response to travoprost acid (A), bimatoprost acid (B), bimatoprost (C), Lumigan (D), unoprostone (free acid) (E), and PGF\textsubscript{2α} (F).
receptor-selective antagonist, AL-8810, for 15 min before addition of agonists.

Results

[^3]H]PGF_{2\alpha} Binding. Competition binding of prostanoids to the FP receptor was performed using the natural ligand[^3]H]PGF_{2\alpha}, and native FP receptors expressed in BCLM preparations. In a typical binding experiment, specific binding of[^3]H]PGF_{2\alpha} (1 nM) to BCLM represented >80% of total binding observed. In concentration-inhibition binding studies, all prostaglandin analogs tested inhibited the specific binding of[^3]H]PGF_{2\alpha} to BCLM in a concentration-dependent manner (Fig. 1). Travoprost acid [(+)-fluprostenol], a potent and highly selective FP receptor agonist, and bimatoprost acid (17-phenyl- trinor PGF_{2\alpha}) strongly displaced[^3]H]PGF_{2\alpha} binding to FP receptors. Bimatoprost (17-phenyl- trinor PGF_{2\alpha}ethyl amide), Lumigan (0.03% bimatoprost ophthalmic solution), and unoprostone, the free acid form of the prodrug unoprostone isopropyl ester, completely inhibited[^3]H]PGF_{2\alpha} binding to FP receptors in BCLM (Fig. 1 and Table 1). These data are in good agreement with similar prostaglandin FP receptor binding studies conducted with BCLM homogenates utilizing the FP receptor-selective radioligand[^3]H]travoprost acid (Sharif et al., 2002a).

Intracellular Calcium Mobilization. All prostaglandin compounds tested, including bimatoprost and unoprostone, elicited a robust and rapid (<5 s post drug addition) increase in relative fluorescence corresponding to increased [Ca^{2+}], monitored in real time with the FLIPR via the rat, mouse, and human FP receptor. Representative traces of [Ca^{2+}], mobilization mediated by PG analogs in rat A7r5 cells are presented in Fig. 2. Interestingly, the real-time fluorescence traces for the tested prostaglandin analogs differ in their respective kinetic profiles. Travoprost acid, bimatoprost acid, and the FP natural ligand PGF_{2\alpha} elicited a rapid spike in [Ca^{2+}], followed by a prolonged plateau that failed to return to baseline within the time course of the experiment (180 s) (Fig. 2, panels A, B, and F). In contrast, bimatoprost, Lumigan, and unoprostone free acid elicited an immediate, robust spike in [Ca^{2+}], that rapidly decayed back to baseline levels (<180 s) (Fig. 2, panels C, D, and E). As expected, the FP receptor natural ligand, PGF_{2\alpha}, exhibited a concentration-dependent mobilization of calcium in A7r5 cells with a representative EC_{50} = 15.2 nM (Fig. 2, panel F). The observed [Ca^{2+}], mobilization response induced by PG analogs was concentration-dependent (Fig. 3) and the resulting agonist potencies (EC_{50}) of the compounds are shown in Table 2. The rank order of FP receptor agonist potency obtained (travoprost acid ≈ bimatoprost acid ≫ unoprostone ≫ bimatoprost/Lumigan) was comparable across the three species studied (Table 2).

In addition, the FP receptor-selective antagonist, AL-8810, inhibited the [Ca^{2+}], mobilization response mediated by travoprost acid, bimatoprost acid, bimatoprost/Lumigan, and unoprostone in the A7r5 cells. In the presence of AL-8810, there was a concentration-dependent reduction in the observed Ca^{2+} response to prostanoid agonists (Fig. 4). Pretreatment with 100 μM AL-8810 completely abolished the
The maximal response generated by bimatoprost (c141), and unoprostone (free acid) (F), Lumigan (') other cell systems involving [Ca 2+]
various prostaglandin analogs: travoprost acid (H18554), bimatoprost acid (H11006), mean
Data are means/S.E.M. from 4 to 13 experiments.

Discussion

Prostaglandin F2α (FP) receptor agonists such as travoprost (TRAVATAN) and latanoprost (Xalatan) are currently marketed as IOP-lowering agents for the treatment of ocular hypertension in patients suffering from glaucoma. In contrast, the biological actions of bimatoprost (Lumigan) and unoprostone isopropyl ester (Rescula) are claimed to be independent of classic prostaglandin receptor activation. This study was conducted to characterize the pharmacology of bimatoprost, unoprostone, and other PGF2α analogs at the endogenous FP receptor of rat A7r5, mouse 3T3 cells, and the cloned human ocular FP receptor expressed in HEK-293 cells. Prostaglandin analogs were also tested for their ability to competitively inhibit the binding of the FP receptor natural ligand [3H]PGF2α, to endogenous FP receptors present in bovine corpus luteum membranes.

The known FP receptor agonists, travoprost acid and 17-phenyl trinor PGF2α, (bimatoprost acid), exhibited high affinity binding to FP receptors in BCLM. Interestingly, the prostaglandin analogs, bimatoprost, Lumigan, and unoprostone also completely abolished [3H]PGF2α binding to BCLM in a concentration-dependent manner. Similar binding affinities were obtained for these compounds using the highly selective FP receptor agonist, [3H]travoprost acid (Sharif et al., 2002a).

Travoprost acid, bimatoprost acid, bimatoprost (from Cayman), Lumigan (bimatoprost ophthalmic solution, from Allergan), and unoprostone induced a rapid (in <5 s), concentration-dependent increase in [Ca 2+]i, in the three different species of cells expressing endogenous and cloned FP receptors. The time course kinetics of [Ca 2+]i mobilization mediated by the PG analogs in A7r5 cells were in some instances very different (compare Fig. 2, panels A, B, and F, to panels C, D, and E). For example, the response profiles for potent, high-affinity FP agonists travoprost acid, bimatoprost acid, and PGF2α consisted of an immediate spike with a prolonged response remaining well above baseline. Weaker FP agonists, bimatoprost/Lumigan and unoprostone, exhibited a rapid spike of [Ca 2+]i followed by a rapid return to baseline levels. Although the exact nature of these variations is unclear, it is reasonable to expect that these differences in [Ca 2+]i mobilization reflect kinetic differences between strong (high affinity) and weak (low affinity) FP agonists. Potent agonists may induce a strong, sustained signal transduction, whereas weaker agonists might trigger a more transient, short-lived response. Regardless of the different response profiles observed for the PG analogs tested, the effective abolition of these responses by the FP receptor antagonist, AL-8810, suggests that the observed [Ca 2+]i mobilization is entirely attributable to FP receptor activation (see below). The very rapid real-time mobilization of [Ca 2+]i elicited with bimatoprost and unoprostone suggests that these compounds behave as FP receptor agonists and directly activate the FP receptor in all cell types tested independent of

**TABLE 2**

Agonist activity of prostaglandin analogs at the mouse, rat, and cloned human FP prostaglandin receptor ([Ca 2+]i mobilization data)

<table>
<thead>
<tr>
<th>Compound</th>
<th>FP Receptor Potency EC50 ± S.E.M. (nM)</th>
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<tbody>
<tr>
<td></td>
<td>Swiss 3T3 Cells (mouse)</td>
</tr>
<tr>
<td>Travoprost acid [(+)-fluprostenol]</td>
<td>37.3 ± 4.5</td>
</tr>
<tr>
<td>Bimatoprost acid (17-phenyl trinor PGF2α)</td>
<td>49.0 ± 5.2</td>
</tr>
<tr>
<td>Unoprostone (acid) (UF-021)</td>
<td>407 ± 83</td>
</tr>
<tr>
<td>Lumigan (amide; bimatoprost ophthalmic solution; Allergan, Inc.)</td>
<td>2670 ± 449</td>
</tr>
<tr>
<td>Bimatoprost (amide; Cayman Chemical Co.)</td>
<td>3120 ± 729</td>
</tr>
</tbody>
</table>
Fig. 4. Inhibition of $[\text{Ca}^{2+}]_i$ mobilization by the FP receptor-selective antagonist, AL-8810. Representative fluorescence traces show the diminished $[\text{Ca}^{2+}]_i$ mobilization in response to a fixed agonist concentration in the presence of increasing concentrations of AL-8810: A, travoprost acid (TA), 100 nM; B, bimatoprost acid, 100 nM; C, bimatoprost, 3 μM; D, Lumigan, 3 μM; and E, unoprostone (free acid), 1 μM.
cellular processing, such as hydrolysis or conversion to other compounds via metabolism.

Swiss 3T3 mouse fibroblasts have previously been shown to express functional FP receptors associated with prostanoid-induced Ca\(^{2+}\) signals (Woodward and Lawrence, 1994; Griffin et al., 1997). Our studies here extend previous work showing similar functional agonist activity for bimatoprost and unoprostone at the mouse FP receptor in these cells (Table 2 and Sharif et al., 2001). Furthermore, utilizing HEK-293 cells expressing the cloned human ocular FP receptor from ciliary body (Kunapuli et al., 1997), travoprost acid, bimatoprost acid, bimatoprost, and unoprostone also rapidly mobilized [Ca\(^{2+}\)]\(i\) and exhibited a rank order potency comparable across species.

The FP receptor antagonist, AL-8810, is considered to be of great utility as a pharmacologic and diagnostic tool for investigating FP receptor-mediated biological responses (Sharif and Griffin, 2002). In fact, AL-8810 has previously been shown to selectively inhibit fluprostenol-mediated PI turnover in A7r5 cells (Griffin et al., 1999) and to displace [\(^{3}\)H]PGF\(_{2\alpha}\) from BCLM (Sharif et al., 2000). Based on previous Schild analyses using PI turnover, AL-8810 behaves as a competitive inhibitor at the endogenous FP receptors of A7r5 and 3T3 cells (Griffin et al., 1999). In contrast, another analog of PGF\(_{2\alpha}\), 11-deoxy-16-fluoro PGF\(_{2\alpha}\) (AL-3138), exhibited properties consistent with a noncompetitive mechanism of antagonism in these same cell and assay systems (Sharif et al., 2000).

Several recent studies have shown that fluprostenol and travoprost acid [(\(\pm\)]-fluprostenol) are more selective agonists at the FP receptor than the natural FP ligand, PGF\(_{2\alpha}\), which displays considerable activity at prostaglandin \(E_2\) receptors (Sharif et al., 1998; Abramovitz et al., 2000; Hellberg et al., 2001; Unger et al., 2001). Further validation of the specificity of AL-8810 toward the FP receptor is shown here by the effective abolition of travoprost acid-induced [Ca\(^{2+}\)]\(i\) mobilization in A7r5 cells. Indeed, AL-8810 effectively inhibited the agonist-induced mobilization of calcium observed with all prostanoids tested in a concentration-dependent manner. The antagonist potency determined for AL-8810 inhibition of [Ca\(^{2+}\)]\(i\) mobilization in A7r5 cells using travoprost acid, bimatoprost, Lumigan, or unoprostone (free acid) was quite similar in this study. These values are in very good agreement with inhibitory potencies of AL-8810 obtained in PI turnover at the cloned human ciliary body FP receptor with several of these same agonists (Sharif et al., 2002b). The complete inhibition of calcium mobilization by AL-8810 confirms the FP receptor nature of the responses elicited by bimatoprost and unoprostone and suggests that the observed agonist activity of these prostaglandin analogs is entirely attributable to FP receptor activation.

Although travoprost and latanoprost are known to mediate their pharmacological actions via the FP prostaglandin receptor (Stjernschantz et al., 1995; Hellberg et al., 2001), the biological actions of bimatoprost and unoprostone have been the subject of some debate. In contrast to our findings presented here, Woodward et al. (2001) did not observe significant binding of bimatoprost at any of the classical prostaglandin receptors, including the FP receptor. The reason for this discrepancy is unclear;
however, it is difficult to reconcile these contradictory findings since the radiolabeled ligand and tissue used by Woodward et al. (2001) were not specified and the functional testing was undefined. In our hands, bimatoprost readily bound to the FP receptor and displaced [3H]travoprost acid (Sharif et al., 2001) and [3H]PGF2α (this study).

Bhattacharjee et al. (2001) found no specific binding of unoprostone isopropyl ester or unoprostone using [3H]unoprostone isopropyl in bovine corpus luteum membranes and competing with unlabeled unoprostone isopropyl. However, given our findings that unoprostone isopropyl and unoprostone free acid have micromolar binding affinities, we feel it would be difficult to detect appreciable specific binding using nanomolar concentrations of this weak radioligand. Bhattacharjee et al. (2001) also reported that unoprostone failed to mobilize [Ca2+]i in primary human ciliary muscle cells. We have found that [Ca2+]i mobilization in response to FP prostaglandins in human ciliary muscle can be quite weak and donor dependent, perhaps suggesting that low receptor expression and/or poor signal coupling in these primary cultures may contribute to the lack of response observed with weak FP agonists.

In conclusion, we have clearly demonstrated that both sources of bimatoprost (from Cayman Chemical Co. and as Lumigan from Allergan, Inc.) as well as unoprostone 1) specifically bind to FP receptors in BCLM; 2) directly activate endogenous FP receptors of rat A7r5 cells and mouse 3T3 fibroblasts, and also stimulate functional responses via the cloned human ocular FP receptors; and 3) can have their actions inhibited by a selective, competitive FP receptor antagonist.

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References


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