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Bimatoprost and its free acid are prostaglandin FP receptor agonists

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Abstract

Bimatoprost (17-phenyl-prostaglandin $F_{2\alpha}$ ethyl amide) has been reported not to exert its actions via prostaglandin receptors. Here, bimatoprost displaced [^{3}H]prostaglandin $F_{2\alpha}$ from FP receptors ($K_i = 6310 \pm 1650$ nM). Bimatoprost rapidly mobilized intracellular Ca^{2+} ($[Ca^{2+}]_i$) via cloned human FP receptors expressed in human embryonic kidney cells ($EC_{50} = 2940 \pm 1663$ nM) and via native FP receptors in 3T3 mouse fibroblasts ($EC_{50} = 2200 \pm 670$ nM). Furthermore, AL-8810 ((5*Z*, 13*E*)-(9*S*,11*S*,15*R*)-9,15-dihydroxy-11-fluoro-15-(2-indanyl)-16,17,18,19,20-pentanor-5,13-prostadienoic acid), an FP receptor antagonist, blocked the bimatoprost-induced $[Ca^{2+}]_i$ mobilization. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Bimatoprost; Lumigan™; Prostaglandin FP receptor

Bimatoprost, the ethyl amide of the potent prostaglandin FP receptor agonist 17-phenyl-prostaglandin $F_{2\alpha}$, has recently been marketed as Lumigan™ for treatment of ocular hypertension (Woodward et al., 2001). Bimatoprost is reported to exert its actions via an uncharacterized "prostamide" receptor but *not* via any prostanoid receptors (Woodward et al., 2001). We sought to independently characterize the pharmacological activity of bimatoprost (and other related compounds) using radioligand binding and intracellular Ca^{2+} ($[Ca^{2+}]_i$) mobilization assays. Contrary to the previous publication (Woodward et al., 2001), we now show for the first time that bimatoprost binds to the prostaglandin FP receptor, and directly and rapidly activates the FP receptors to mobilize $[Ca^{2+}]_i$. In addition, we show that an FP receptor antagonist can block bimatoprost's $[Ca^{2+}]_i$ mobilizing effects.

Prostaglandin FP receptor binding was conducted on bovine corpus luteum homogenates using [^{3}H]prostaglandin $F_{2\alpha}$ (1 nM final; Sharif et al., 1998). $[Ca^{2+}]_i$ was measured in Swiss 3T3 mouse fibroblasts (Woodward et al., 1990; Griffin et al., 1999) and in human embryonic kidney (HEK-293) cells transfected with the human ocular FP receptor (Kunapuli et al., 1997) on a Fluorometric Imaging Plate Reader (FLIPR) as previously described (Jerman et al., 2000). The sources of the reagents were: free acids of travoprost and bimatoprost, and AL-8810 ((5*Z*, 13*E*)-(9*S*,11*S*,15*R*)-9,15-dihydroxy-11-fluoro-15-(2-indanyl)-16,17,18,19,20-pentanor-5,13-prostadienoic acid; Alcon Research); bimatoprost (Cayman, Ann Arbor, MI); Lumigan™ (0.03% bimatoprost ophthalmic solution; Allergan, Irvine, CA); Ca^{2+}-sensitive dye kit (Molecular Devices, Menlo Park, CA); Swiss 3T3 cells from ATCC (Rockville, MD); HEK-293 cells expressing cloned human ocular FP receptors (Dr. G. FitzGerald, University of Pennsylvania); [^{3}H]prostaglandin $F_{2\alpha}$ [150 Ci/mmol] from NEN (Boston, MA).

[^{3}H]prostaglandin $F_{2\alpha}$ binding to FP receptors was displaced by travoprost acid ((+)-fluprostenol; $K_i = 47 \pm 2$ nM), a potent and highly selective FP receptor agonist (Sharif et al., 1999), and by bimatoprost (Cayman; $K_i = 6310 \pm 1650$ nM), Lumigan™ ($K_i = 4390 \pm 1060$ nM) and bimatoprost free acid (17-phenyl-prostaglandin $F_{2\alpha}$; $K_i = 83 \pm 2$ nM) (Hill coefficients = 0.8–1.0; $n \geq 3$). Bimatoprost, Lumigan™, bimatoprost acid and travoprost acid rapidly mobilized $[Ca^{2+}]_i$ in Swiss 3T3 cells (expressing native FP receptors) in less than 5 s of addition to the cells (Fig. 1). These compounds yielded the following agonist potency (EC_{50}) and intrinsic activity (IA) values: travoprost acid $EC_{50} = 29 \pm 3$ nM (IA = 0.97 ± 0.03); bimatoprost acid $EC_{50} = 39 \pm 7$ nM (IA = 0.8 ± 0.02); bimatoprost (Cayman) $EC_{50} = 2220 \pm 670$ nM (IA = 0.56 ± 0.05); bimatoprost (Lumigan™) $EC_{50} = 2700 \pm 780$ nM (IA = 0.59 ± 0.06)

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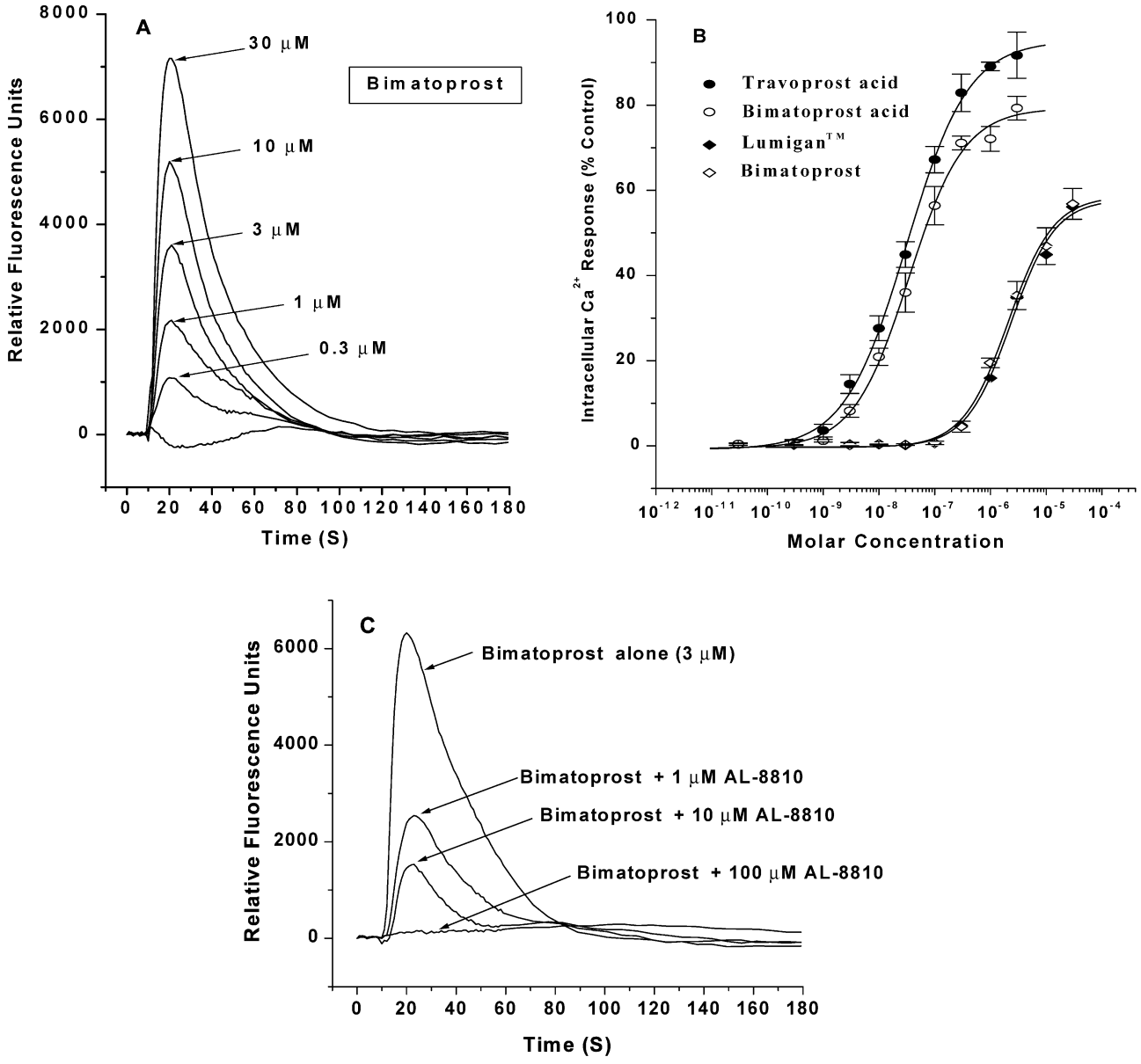

Fig. 1. Intracellular Ca^{2+} mobilization in Swiss 3T3 mouse fibroblasts. The cells were loaded with the Ca^{2+}-sensitive dye, exposed to the test compound and the change in fluorescence measured over 180 s. Top left panel (A) shows the $[Ca^{2+}]_i$ mobilization traces in response to bimatoprost; the top right panel (B) shows the concentration-dependent mobilization of $[Ca^{2+}]_i$ by travoprost acid, bimatoprost acid, bimatoprost and Lumigan™; the bottom panel (C) shows the antagonism of bimatoprost-induced functional response by AL-8810, an FP receptor antagonist. The cells were pre-incubated with AL-8810 for 15 min prior to the addition of bimatoprost.

(means ± S.E.M., $n \geq 3$; Fig. 1). Bimatoprost was also an agonist at the cloned human ocular FP receptors expressed in HEK-293 cells ($EC_{50} = 2940 \pm 1663$ nM; IA = 0.58 ± 0.03). The effects of bimatoprost (Cayman), bimatoprost (Lumigan™), bimatoprost acid and travoprost acid could be blocked (e.g. IC_{50} less than 1 μM vs. bimatoprost; Fig. 1) by the FP receptor-selective antagonist AL-8810 ((5*Z*, 13*E*)-(9*S*,11*S*,15*R*)-9,15-dihydroxy-11-fluoro-15-(2-indanyl)-16,17,18,19,20-pentanor-5,13-prostadienoic acid; Griffin et al., 1999). AL-8810's antagonist potency here corresponded well with our previous observations using phosphoinositide turnover assay paradigms (Griffin et al., 1999).

In conclusion, we have shown that, contrary to the previous publication (Woodward et al., 2001), bimatoprost from both sources (Cayman and Lumigan™ 0.03% Ophthalmic solution) binds to and acts as a direct agonist at the endogenous (mouse 3T3 cells) and cloned human ocular FP prostaglandin receptor. This conclusion was further substantiated by the concentration-dependent antagonism of the bimatoprost-induced $[Ca^{2+}]_i$ mobilization by the FP receptor antagonist, AL-8810.

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