

Pharmacological Characterization of a Novel Antiglaucoma Agent, Bimatoprost (AGN 192024)

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ABSTRACT

Replacement of the carboxylic acid group of prostaglandin (PG) $F_{2\alpha}$ with a nonacidic moiety, such as hydroxyl, methoxy, or amido, results in compounds with unique pharmacology. Bimatoprost (AGN 192024) is also a pharmacologically novel PGF $_{2\alpha}$ analog, where the carboxylic acid is replaced by a neutral ethylamide substituent. Bimatoprost potently contracted the feline lung parenchymal preparation (EC_{50} value of 35–55 nM) but exhibited no meaningful activity in a variety of PG-sensitive tissue and cell preparations. Its activity seemed unrelated to FP receptor stimulation according to the following evidence. 1) Bimatoprost exhibited no meaningful activity in tissues and cells containing functional FP receptors. 2) Bimatoprost activity in the cat lung parenchyma is not species-specific because its potent activity in this preparation could not be reproduced in cells stably expressing the feline FP receptor. 3)

Radioligand binding studies using feline and human recombinant FP receptors exhibited minimal competition versus [3 H]17-phenyl PGF $_{2\alpha}$ for Bimatoprost. 4) Bimatoprost pretreatment did not attenuate PGF $_{2\alpha}$ -induced Ca^{2+} signals in Swiss 3T3 cells. 5) Regional differences were apparent for Bimatoprost but not FP agonist effects in the cat lung. Bimatoprost reduced intraocular pressure in ocular normotensive and hypertensive monkeys over a 0.001 to 0.1% dose range. A single-dose and multiple-dose ocular distribution/metabolism studies using [3 H]Bimatoprost (0.1%) were performed. Within the globe, bimatoprost concentrations were 10- to 100-fold higher in anterior segment tissues compared with the aqueous humor. Bimatoprost was overwhelmingly the predominant molecular species identified at all time points in ocular tissues, indicating that the intact molecule reduces intraocular pressure.

Eicosanoids and related fatty acids have long been the subject of extensive investigation. More recently, it has become apparent that the corresponding neutral lipids exist for several fatty acids (Devane et al., 1992; Cravatt et al., 1995; Sugiura et al., 1995; Yu et al., 1997; Calignano et al., 1998; Kozak et al., 2000, 2001; Berger et al., 2001; Hanus et al., 2001; Moody et al., 2001). These neutral lipids occur in the form of an amide, ether, or ester, which replaces the invariant carboxylic acid moiety at position 1. The presence of such an electrochemically neutral substituent changes the biology from that of the corresponding fatty acid, often quite dramatically. This is illustrated by the natural ligands for cannabinoid receptors, anandamide (arachidonyl 1-ethanolamide), 2-arachidonyl glycerol, and 2-arachidonyl glyceryl ether (Devane et al., 1992; Felder et al., 1993; Zygmunt et al., 1999; Di Marzo, 2000; Sugiura et al., 2000; Hanus et al., 2001), which are pharmacologically quite distinct from the parent fatty acid. After the discovery of anandamide, further fatty

acid amides were identified as naturally occurring substances. These included oleamide, a sleep-producing substance (Cravatt et al., 1995); palmityl 1-ethanolamide, an analgesic (Calignano et al., 1998); and *N*-docosahexaenoyl ethanolamide (Bisogno et al., 1999; Berger et al., 2001).

In addition to their cannabimimetic properties, anandamide and 2-arachidonyl glycerol are substrates for cyclooxygenase-2 (COX-2). The resultant products are prostaglandin amides (prostamides) or glyceryl esters (Yu et al., 1997; Kozak et al., 2000). The pharmacology of these COX-2 products remains to be studied in detail but initial studies suggest that they are pharmacologically novel and that their activities may be unrelated to prostaglandin or cannabinoid receptor stimulation (Berglund et al., 1999; Woodward et al., 2001; Ross et al., 2002). These findings are consistent with a recent study describing the unique pharmacology of synthetic structural analogs of prostaglandin (PG) $F_{2\alpha}$, where the carboxylic acid group has been replaced by a nonionizable hydroxy or methoxy substituent (Woodward et al., 2000).

The principal subject of the studies described herein is bimatoprost (AGN 192024). Bimatoprost is also a synthetic

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ABBREVIATIONS: COX-2, cyclooxygenase-2; PG, prostaglandin; HEK, human embryonic kidney; DMEM, Dulbecco's modified Eagle's medium; HPLC, high-pressure liquid chromatography; nM $_{eq}$, nanomolar equivalent.

structural analog of PGF_{2 α} , where the charged carboxylic acid group is replaced by a neutral ethylamide substituent. Structurally, bimatoprost is therefore a prostaglandin-amide or prostamide and exhibits similar biological activity to prostamide F_{2 α} (Woodward et al., 2001). Bimatoprost is of particular interest because it is clinically the most efficacious ocular hypotensive agent reported to date, its activity exceeding that of both timolol and latanoprost (Dubiner et al., 2001; Laibovitz et al., 2001; Sherwood and Brandt, 2001; Noecker et al., 2003). In addition to *in vitro* pharmacological characterization of bimatoprost, its effects on intraocular pressure and its metabolic fate in living nonhuman primate eyes are described. This is important because it determines whether effects on intraocular pressure result from its inherent pharmacological activity or occur, at least in part, by virtue of enzymatic hydrolysis to a free acid metabolite that would behave as an authentic prostanoid FP receptor agonist (Woodward and Lawrence, 1994; Woodward et al., 2000). Thus, particular attention is devoted to studying potential 17-phenyl PGF_{2 α} formation in ocular tissues and pharmacological comparisons are made directly to 17-phenyl PGF_{2 α} .

Materials and Methods

Isolated Tissue Studies (Functional). The smooth muscle tension of isolated tissue preparations was measured isometrically with force displacement transducers (Grass FT-03) and recorded on a Grass polygraph (models 7G and 79E). The organ baths contained Krebs' solution maintained at 37°C and gassed with 95% O₂, 5% CO₂ to give a pH of 7.4. The Krebs' solution had the following composition: 118.0 mM NaCl, 4.7 mM KCl, 1.2 mM KH₂PO₄, 1.9 mM CaCl₂, 1.18 mM MgSO₄, 25.0 mM NaHCO₃, 11.7 mM glucose, and 0.001 mM indomethacin.

The cat lung parenchymal strip seems unique in that it exhibits marked responsiveness to PGF_{2 α} analogs where the carboxylic acid moiety is replaced by a nonionizable substituent. The functional and radioligand binding methods for the cat lung parenchyma are as described previously (Woodward et al., 2000). The cat lung parenchymal strip was used in conjunction with additional isolated tissue preparations to assist in characterizing the pharmacology of bimatoprost. These preparations, their principal pharmacology, and citations detailing the methodologies are listed as follows: guinea pig ileum (EP₁) (Woodward et al., 2000), chick ileum (EP₃) (Woodward et al., 2000), guinea pig vas deferens (EP₃) (Krauss et al., 1996), rabbit jugular vein (FP-endothelium intact and relaxant EP, DP, IP endothelium denuded) (Chen et al., 1995), rat aorta (TP) (Krauss et al., 1996), and rat colon (EP₃, FP) (Woodward et al., 1995). Other isolated tissue experiments undertaken where the methods have not been described previously by the authors are provided hereafter.

Rat Stomach Fundus. Rats were euthanized as described previously. The stomach was removed and cut into two longitudinal strips and the gastric mucosa was removed. Rat stomach fundus strips were suspended under 5-g tension in a 10-ml jacketed organ bath maintained at 37°C. Tissues were allowed to equilibrate for 60 min with periodic washing. After equilibration, a noncumulative dose-response curve for PGF_{2 α} was obtained. Data are expressed as percentage of PGF_{2 α} (10⁻⁶ M) response as a reference.

Mouse Ileum. Female Swiss-Webster mice (Bantin and Kingman), minimum age of 6 weeks, 28 to 42 g body weight, were killed by CO₂ gas inhalation. A 7- to 10-cm length of ileum was removed from above the caecum and sections were gently cleared of waste. Longitudinal smooth muscle segments of 1.5-cm length were suspended in Krebs' buffer with 2.8 × 10⁻⁶ M indomethacin in 10-ml jacketed organ baths, and maintained at 37°C. Ileal segments were placed under 1-g tension. The tissues were washed at least four times during the 45-min equilibration period and every 5 to 10 min during

the wash periods between doses. Noncumulative, concentration-response relationships were established. PGF_{2 α} (10⁻⁵ M) was administered to each tissue at the end of the agonist concentration-response curve to provide a reference contraction.

Gerbil Colon. Gerbils of either sex, weighing 200 to 300 g, were euthanized by CO₂ inhalation. An approximately 5-mm section of proximal ascending colon was excised and suspended under 1-g tension in a 10-ml jacketed organ bath. Tissues were allowed to equilibrate for 60 min with periodic washing. Noncumulative dose-response curves were obtained, as described for the rat colon. Data are expressed as the percentage of PGF_{2 α} (10⁻⁶ M) response.

Responses were calculated by peak height measurements. Only one concentration-response curve was generated in each tissue. The response to the test compounds was calculated as a percentage of the reference contraction in each tissue. The data are expressed as mean ± standard error of the mean of single values obtained from (*n*) different animals. Concentration (log [M])-response curves were graphed and EC₅₀ values were determined from the graph.

Cell Studies (Functional). Ca²⁺ signaling and inositol phosphate formulation studies were conducted as described previously (Woodward et al., 2000). For Ca²⁺ signal antagonism studies in Swiss 3T3 cells, the cells were pretreated for 10 min in the cuvette with bimatoprost (10⁻⁶ M) before addition of FP receptor stimulants. HEL cells constitutively express EP₁ and TP prostanoid receptor subtypes. The methods used to determine intracellular Ca²⁺ concentrations have been described previously (Woodward et al., 2000).

Human platelets express three functional prostanoid receptors. Inhibition of ADP induced aggregation may be achieved by stimulation of DP or IP receptors. Aggregation is produced by TP receptor activation. The methods used in these present studies have been described in detail in Krauss et al. (1996).

Cells Stably Expressing EP₁, EP₂, EP₄, and FP Receptors. HEK-293 cells stably expressing the human or feline FP receptor, or EP₁, EP₂, or EP₄ receptors were washed with TME buffer (100 mM Tris base, 20 mM MgCl₂, and 2 M EDTA; 10 N HCl is added to achieve a pH of 7.4), scraped from the bottom of the flasks, and homogenized for 30 s using a PT 10/35 polytron (Brinkmann Instruments, Westbury, NY). TME buffer was added to achieve a final 40-ml volume in the centrifuge tubes.

The cell homogenate was centrifuged at 19,000 rpm for 20 min at 4°C using a Ti-60 rotor (Beckman Coulter, Inc., Fullerton, CA). The resultant pellet was resuspended in TME buffer to give a final 1 mg/ml protein concentration, as determined by Bio-Rad assay. Radioligand binding competition assays versus [³H]17-phenyl PGF_{2 α} (5 nM) were performed in a 200- μ l volume for 60 min. Binding reactions were started by adding plasma membrane fraction. The reaction was terminated by the addition of 4 ml of ice-cold Tris-HCl buffer and rapid filtration through glass fiber GF/B filters (Whatman, Maidstone, UK) using a cell harvester (Brandel, Inc., Gaithersburg, MD). The filters were washed three times with ice-cold buffer and oven dried for 1 h.

[³H]PGE₂ (specific activity 180 Ci mmol⁻¹) was used as the radioligand for EP receptors. [³H]17-phenyl PGF_{2 α} was used for FP receptor binding studies. Binding studies using EP₁, EP₂, EP₄, and FP receptors were performed in duplicate in at least three separate experiments. A 200- μ l assay volume was used. Incubations were for 60 min at 25°C and were terminated by the addition of 4 ml of ice-cold 50 mM Tris-HCl, followed by rapid filtration through GF/B filters (Whatman) and three additional 4-ml washes in a cell harvester (Brandel, Inc.). Competition studies were performed using a final concentration of 5 nM [³H]PGE₂ or 5 nM [³H]17-phenyl PGF_{2 α} and nonspecific binding determined with 10⁻⁵ M unlabeled PGE₂, or 17-phenyl PGF_{2 α} , according to receptor subtype studied.

Cells Transiently Expressing EP₃ Receptors. COS-7 cells were transiently transfected with pcDNA₃ containing cDNA for the EP_{3D} receptor by using lipofectin. For radioligand binding, the cells were harvested after 2 days. Plasma membrane preparation conditions for each of the transient transfectants are as follows. COS-7

cells were washed with TME buffer, scraped from the bottom of the flasks, and homogenized for 30 s using a PT 10/35 polytron (Brinkmann Instruments). TME buffer was added to achieve a final 40-ml volume in the centrifuge tubes.

The cell homogenate was centrifuged at 19,000 rpm for 20 min at 4°C using a Ti-60 rotor (Beckman Coulter, Inc.). The resultant pellet was resuspended in TME buffer to give a final 1 mg/ml protein concentration, as determined by Bio-Rad assay. Radioligand binding assays were performed in a 200- μ l volume, as described above for other EP receptors.

Cells Transiently Expressing TP Receptors. COS-7 cells were transiently transfected with pcDNA₃ containing cDNA for the TP receptor using methods as described for transient EP₃ receptor transfectants. Plasma membrane preparations for the transient transfectants and radioligand binding methods were the same as for the EP₃ receptor methods. The binding of [³H]SQ 29548 [1S-[1 α ,2 α (Z),3 α ,4 α]-7-[3-[2-[phenylamino]carbonyl]hydrozino]methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptanoic acid] (specific activity 41.5 Ci mmol⁻¹) at TP receptors was determined in duplicate in at least three separate experiments. Incubations were for 60 min at 25°C and were terminated by the addition of 4 ml of ice-cold 50 mM Tris-HCl, followed by rapid filtration through GF/B filters (Whatman) and three additional 4-ml washes in a cell harvester (Brandel, Inc.). Competition studies were performed using a final concentration of 5 nM [³H]SQ 29548 and nonspecific binding was determined with 10⁻⁵ M unlabeled SQ 29548.

Generation of cell lines stably expressing recombinant human EP₁, EP₂, EP₄, and FP in HEK-293 cells was used for preparing stable transfectants. They were grown in DMEM with 10% fetal bovine serum, G418, and 200 μ g ml⁻¹ gentamicin or penicillin/streptomycin. Selection of stable transfectants was achieved with 200 μ g ml⁻¹ hygromycin, the optimal concentration being determined by hygromycin kill curve studies.

For transfection, the cells were grown to 50 to 60% confluence on 10-cm plates. The plasmid pCEP4, incorporating cDNA inserts for the human prostanoid receptors (20 μ g), was added to 0.5 ml of 250 mM CaCl₂. HEPES-buffered saline \times 2 (2 \times HEPES-buffered saline, 280 mM NaCl, 20 mM HEPES acid, and 1.5 mM Na₂HPO₄ at pH 7.05–7.12) was then added dropwise to a total of 0.5 ml, with continuous vortexing at room temperature. After 30 min, 9 ml of DMEM was added to the mixture. The DNA/DMEM/calcium phosphate was then added to the cells, which had been previously rinsed with 10 ml of phosphate-buffered saline. The cells were then incubated for 5 h at 37°C in humidified 95% air, 5% CO₂. The calcium phosphate solution was then removed and the cells were treated with 10% glycerol in DMEM for 2 min. The glycerol solution was then replaced by DMEM with 10% fetal bovine serum. The cells were incubated overnight and the medium was replaced by DMEM/10% fetal bovine serum containing 250 μ g ml⁻¹ G418 and penicillin/streptomycin. Hygromycin B selection commenced 48 h after transfection.

Ten days after transfection, hygromycin B-resistant clones were selected and transferred to separate wells on a 24-well plate. At confluence, each clone was transferred to one well on a six-well plate and then expanded in a 10-cm dish. Cells were maintained under continuous hygromycin selection until use.

Generation of a Cell Line Stably Expressing Recombinant Feline FP Receptors. Stable cat FP receptor transfectants were prepared in a similar manner to the human stable transfectants except that lipofectin was used. Again, cells were grown to 50 to 60% confluence on 10-cm plates and the plasmid pCEP4 incorporating cyclic DNA inserts for the cat FP receptor was transfected with lipofectin. Hygromycin B selection commenced at 48 h post-transfection. Eight days after transfection, hygromycin B-resistant clones were selected and transferred to 24-well plates. At confluence, each clone was transferred to one well on a six-well plate and then expanded in a 10-cm dish. Cells were maintained under continuous hygromycin selection until use.

Human DP Receptor Luciferase Reporter Assay for hDP-HEK 293/EBNA. Supercoiled plasmid DNA was transfected into hDP-HEK 293/EBNA cells by the FuGENE 6 method. In brief, cells were washed twice and resuspended in 1 ml of DMEM and plated 24 h before transfection. Then 1 μ g of CRE-Luciferase reporter Plasmid DNA was mixed with 3 μ l of FuGENE 6 reagent in 100 μ l of DMEM and this was added dropwise to the hDP-HEK 293/EBNA cells. The cells were cultured for 24 h at 37°C. BW 245C and bimatoprost at the concentration range from 10⁻¹² to 10⁻⁶ M were added to the culture such that the cells were treated for 12 h. The cells were harvested and lysed in 100 μ l of 25 mM Tris-phosphate buffer (pH 7.5) containing 1% Triton X-100. Soluble extracts (50 μ l) were assayed for luciferase. The luciferase assay was performed with a Promega assay kit (Madison, WI). Light intensity was measured by Lumat. Relative luciferase activity was expressed as values of ratio compared with control.

Human IP Receptor Luciferase Reporter Assay for hIP-HEK 293/EBNA. Supercoiled plasmid DNA was transfected into 10⁴ hIP-HEK 293/EBNA cells by the FuGENE 6 method. In brief, cells were washed twice and resuspended in 1 ml of DMEM. Then 1 μ g of CRE-Luciferase reporter Plasmid DNA in 1 ml of DMEM containing 10 μ l of FuGENE 6 solution was mixed with the cell suspension, and the cells were cultured for 24 h at 37°C. Carbaprostacyclin and bimatoprost, at a concentration range from 10⁻¹² to 10⁻⁶ M, were added to the culture 12 h after transfection. The cells were harvested and lysed in 100 μ l of 25 mM Tris-phosphate buffer (pH 7.5) containing 1% Triton X-100. Soluble extracts (50 μ l) were assayed for luciferase. The luciferase assay was performed with a Promega assay kit. Light intensity was measured by an autolumat instrument (EG&G Berthold, Bad Wildbad, Germany). Relative luciferase activity was expressed as values of ratio compared with control.

Ocular Studies. Intraocular pressure and ocular metabolism studies were performed in monkeys as the most clinically relevant species. Intraocular pressure studies were performed in ocular normotensive monkeys and laser-induced ocular hypertensive monkeys (the "glaucomatous" monkey model).

Intraocular Pressure. Intraocular pressure studies were conducted in female cynomolgus monkeys (2–4 kg). Studies were performed in bilaterally ocular normotensive monkeys and in animals rendered unilaterally ocular hypertensive by circumferential laser treatment (Gaasterland and Kupfer, 1974; Lee et al., 1985). Intraocular pressure studies were performed in conscious animals trained to accept pneumatonometry. The animals were restrained at the time of intraocular pressure measurement by custom-designed chairs. Drugs were administered topically to the ocular surface of one eye as a 25- μ l volume drop. In the case of the ocular hypertensive monkeys, only the hypertensive eye received drug. The contralateral eye received 25 μ l of vehicle (0.1% polysorbate 80:10 mM Tris-HCl) as a control. One drop of 0.1% proparacaine was used as a corneal anesthetic for pneumatonometric measurement. Intraocular pressure was measured at 1 h before and immediately before drug administration and then at 2, 4, and 6 h postdosing. For 5-day studies, the dosing schedule was repeated on each study day.

Ocular Metabolism. A single-dose and a multiple-dose study were performed. Male cynomolgus monkeys, weighing between 4.1 and 6.9 kg, were used. The eyes were dosed bilaterally with a 35- μ l volume drop of 0.1% [³H]bimatoprost. For the 9.5-day study the animals were dosed twice daily at 12-h intervals. Tissue collection was performed at 0.5, 2, 4, 6, 8, and 24 h postdosing for the single-dose study and at identical times after the final dose of the 9.5-day dosing study. One monkey was euthanized at each time point. The eyes were enucleated and the following ocular tissues were surgically removed: iris, ciliary body, cornea, sclera, conjunctiva, and eyelids. An aqueous humor sample was also taken from intact eyes. The tissues were weighed and placed in screw cap vials containing 2 ml of methanol. The vials were then shipped from the contract laboratory that performed the monkey studies (TSI Mason Laboratories, Worcester, MA) to Allergan, Inc. for analysis.

The metabolic fate of bimatoprost in monkey ocular tissues was determined by reverse phase high-pressure liquid chromatography (HPLC) with radiodetection. To achieve the highest concentrations of radioactivity, so that smaller peaks could be reliably quantified, the methanolic extracts from both eyes were pooled for each tissue at each time point. Determination of the total radioactivity from the methanolic extracts and combusted tissues indicated that approximately 95% of the radioactivity was extracted. Chromatographic elevations less than 2 times the noise were not quantified. The gradient HPLC system, described as follows, was used to analyze the methanolic extracts: solvent A, 20% CH₃CN-0.02 M KH₂PO₄ (pH 2.8); solvent B, 50% CH₃CN-0.02 M KH₂PO₄ (pH 2.8); pump, System Gold model 128 (Beckman, San Ramon, CA); flow rate, 1.0 ml/min; injector, model 717 plus (Waters, Milford, MA); injection volume, 20 to 100 μ l; column, ultrasphere IP, 4.6 \times 150 mm, 5 μ m (Beckman); UV detector, Spectraflow model 783 (Kratos, Ramsey, NJ) set at 200 nm; radioisotopic detector, radiomatic model 150 TR (PerkinElmer Life Sciences, Boston, MA); cocktail flow, 3.5 ml/min (Flow-Scint III (PerkinElmer Life Sciences); data collection software, System Gold version 8.10 (Beckman); and gradients, 0 to 1 min, 100% solvent A, isocratic; 1 to 17 min, 100 to 40% solvent A, 0 to 60% solvent B, (+) curved; 17 to 21 min; 40% solvent A, 60% solvent B, isocratic; 21 to 22 min, 40 to 100% solvent A, 60 to 0% solvent B, linear; and 22 to 30 min, 100% solvent A, isocratic.

Materials. Bimatoprost and all neutral PGF_{2 α} analogs were synthesized at Allergan, Inc. PGF_{2 α} , PGE₂, PGD₂, BW 245C [(4*S*)-3-[(3*R,S*)-3-[cyclohexyl-3-hydroxypropyl]-2,5-dioxo]-4-imidazolidine heptanoic acid], I-BOP [[1*S*]-[1 α ,2 α (*Z*),3 β (1*E*,3*S*),4 α]-7-[3-hydroxy-4-(4-iodophenoxy)1-butenyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptanoic acid], U-46619 [9,11-dideoxy-9 α ,11 α -methanoeperoxy prostaglandin F_{2 α}], carbaprostacyclin, SQ 29548, and 17-phenyl PGF_{2 α} were purchased from Cayman Chemicals (Kalamazoo, MI). Radiolabeled [³H]bimatoprost and [³H]17-phenyl PGF_{2 α} were custom synthesized at Amersham Biosciences, Inc. (Cardiff, UK). Radiolabeled [³H]PGE₂ was purchased from Amersham Biosciences, Inc. Radiolabeled [³H]SQ 29548 was purchased from PerkinElmer Life Sciences.

Results

The effects of bimatoprost on the feline lung parenchymal strip preparation are illustrated in Fig. 1 and are compared with the activities of PGF_{2 α} and 17-phenyl PGF_{2 α} . Bimatoprost,

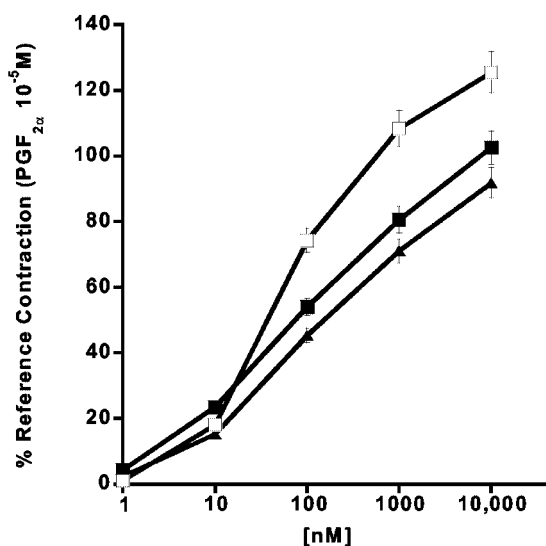


Fig. 1. Comparison of the effects of bimatoprost (\square), 17-phenyl PGF_{2 α} (\blacksquare), and PGF_{2 α} (\blacktriangle) on the cat isolated lung parenchymal preparation. Points represent mean values \pm S.E.M. of percentage of response to 10⁻⁵ M PGF_{2 α} , $n = 4$ to 6.

prost, 17-phenyl PGF_{2 α} , and PGF_{2 α} seemed to exert similar potency in contracting the cat lung parenchymal strip with the following rank order: bimatoprost \geq 17-phenyl PGF_{2 α} \geq PGF_{2 α} .

After initial experiments on peripheral strips of lung parenchymal tissue, effects were examined on more distal specimens. These studies were performed to examine potential regional variations in responsiveness to bimatoprost so that tissue specimens used for binding studies were optimal. Identical studies were performed for 17-phenyl PGF_{2 α} and the TP receptor agonist I-BOP for comparison. The effects of bimatoprost on peripheral and more medial segments of the cat lung parenchyma are depicted in Fig. 2. Two separate studies on bimatoprost were performed and are shown in Fig. 2, a and b. Bimatoprost was a potent myotropic agent in the most peripheral cat lung parenchymal specimens and EC₅₀ values of 35 nM (Fig. 2a) and 55 nM (Fig. 2b) were obtained in separate experiments. The more medial cat lung specimens were less responsive and EC₅₀ values of 379 nM (Fig. 2a) and 359 nM (Fig. 2b) were obtained. It seems that the most peripheral regions of the cat lung exhibit greater sensitivity to bimatoprost. In marked contrast, the prostanoid analogs 17-phenyl PGF_{2 α} and I-BOP showed equal efficacy in both preparations. The effects of 17-phenyl PGF_{2 α} on cat lung parenchymal tissue are depicted in Fig. 2c. EC₅₀ values for 17-phenyl PGF_{2 α} in peripheral and more medial specimens were 22 and 36 nM, respectively. When the effects of the TP agonist I-BOP on peripheral and more medial cat lung parenchymal sectors were compared (Fig. 2d), EC₅₀ values of 0.30 and 0.39 nM were obtained.

The effects of bimatoprost and 17-phenyl PGF_{2 α} on other isolated tissue preparations that are sensitive to PGF_{2 α} were studied. These preparations included the rat colon, gerbil colon, rat stomach fundus, and mouse ileum (Miller et al., 1975; Coleman, 1987; Woodward et al., 1995; Sugimoto et al., 1997). AGN 192024 exhibited no meaningful activity in these preparations compared with its activity in the cat lung parenchyma, as depicted in Fig. 3. Thus, the feline lung parenchyma seems unique in its high sensitivity to AGN 192024.

The effects of bimatoprost on Ca²⁺ signaling in Swiss 3T3 cells were also investigated, because these cells are known to exhibit characteristic FP receptor pharmacology (Woodward and Lawrence, 1994; Woodward et al., 1995). This cell preparation is also advantageous in that the transient nature of Ca²⁺ responses provides an ability to assess competition between agonist drugs measured functionally (Woodward and Lawrence, 1994). Bimatoprost exhibited no meaningful interaction with the Swiss 3T3 cell FP receptor population and no measurable elevation in intracellular free Ca²⁺ concentration occurred until a 10⁻⁵ M concentration was achieved (Fig. 4b). Pretreatment of Swiss 3T3 cells with 10⁻⁶ M bimatoprost did not affect the response to PGF_{2 α} (Fig. 4a). Thus, the presence of 10⁻⁶ M bimatoprost in the cuvette did not interact with FP receptors and thereby attenuate the response to subsequently administered PGF_{2 α} .

To assess receptor selectivity, bimatoprost was evaluated in a series of isolated tissue and cell pharmacology preparations that are known to be useful for determining activity at the various prostanoid receptors. These preparations (Woodward et al., 2000) included EP₁ (guinea pig ileum, Ca²⁺ signaling in HEL cells), EP₂ (rabbit jugular vein, denuded), EP₃ (guinea pig vas deferens, chick ileum), EP₄ (rabbit jug-

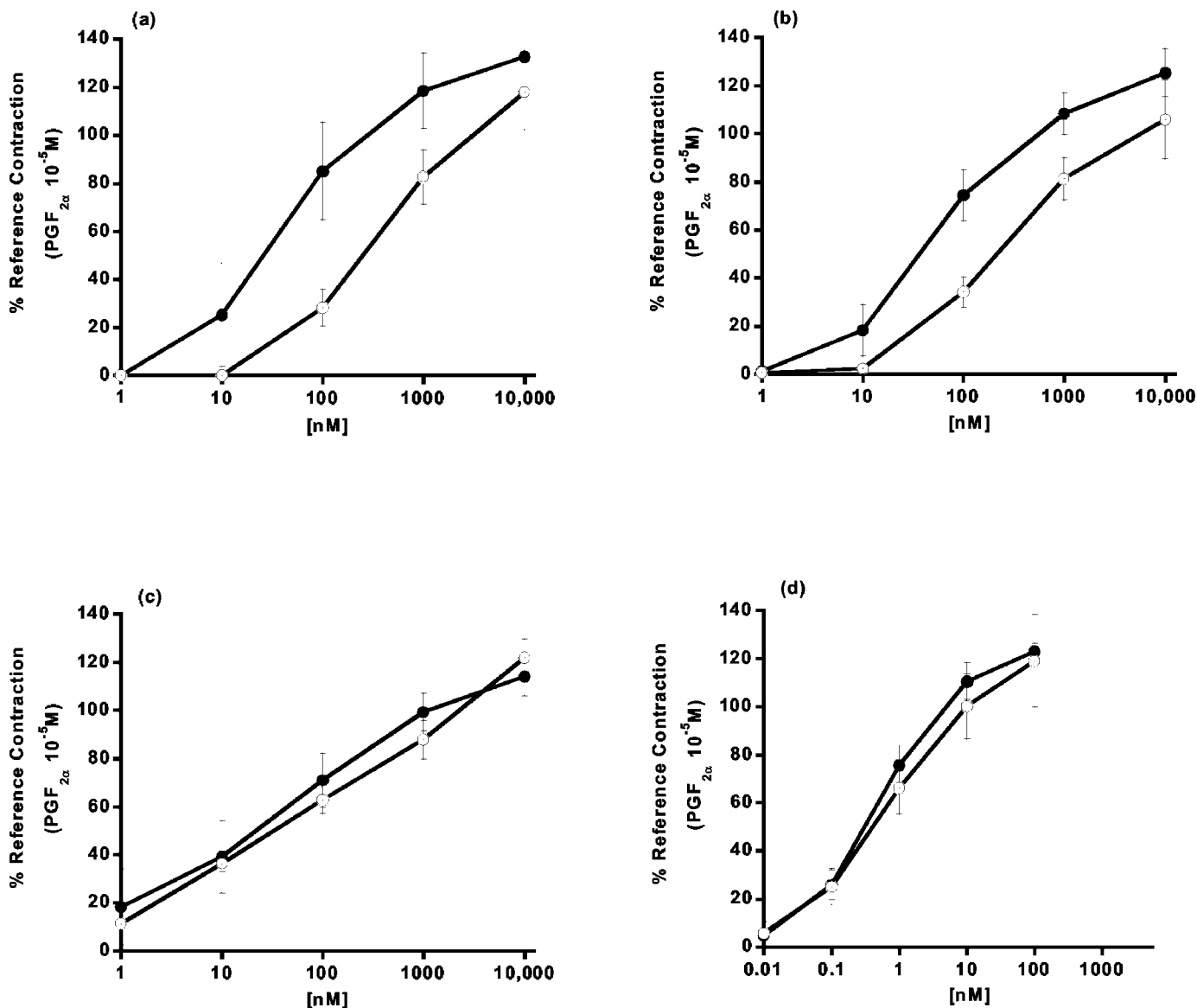


Fig. 2. Comparison of the effects of bimatoprost, 17-phenyl PGF_{2α}, and I-BOP on peripheral (●) and adjacent, more medial (○) regions of the cat lung parenchyma. a and b, results of two separate studies on bimatoprost. The effects of 17-phenyl PGF_{2α} and I-BOP are given in c and d, respectively. Points represent mean values ± S.E.M., *n* = 4 to 6.

ular vein, denuded), DP (inhibition of platelet aggregation), IP (inhibition of platelet aggregation), and TP (platelet aggregation, rat aorta). The data are summarized in Table 1.

In addition to functional assays to determine activity at natural prostanoid receptors, further studies were performed with recombinant receptors. This enabled interaction at all the human prostanoid receptors currently described by the classification (Coleman et al., 1984) to be evaluated. Radioligand binding studies were used where high-affinity radiolabeled ligands were available. Activity at recombinant DP and IP receptors was determined using CRE-Luciferase reporter assays. Bimatoprost exhibited no meaningful affinity for human prostanoid receptors, including the FP receptor. The results are presented in Table 2.

In addition to functional studies on isolated strips of cat lung parenchymal tissue, radioligand binding competition studies were performed versus [³H]17-phenyl PGF_{2α} as described previously (Woodward et al., 2000). The competition

between 5 nM [³H]17-phenyl PGF_{2α} and graded concentrations of unlabeled 17-phenyl PGF_{2α}, and bimatoprost are depicted in Fig. 5. The competition afforded by U-46619 was also examined, because the cat lung parenchyma expresses a functional TP receptor (Fig. 2). Bimatoprost seemed to be less potent than 17-phenyl PGF_{2α} in competing for 17-phenyl PGF_{2α} binding sites. IC₅₀ values obtained for 17-phenyl PGF_{2α} and bimatoprost were 6.6 and 254 nM, respectively. U-46619 exhibited minimal affinity for 17-phenyl PGF_{2α} binding sites in cat lung parenchyma tissue. It should be noted that the precision of this binding study was inferior to those involving overexpressed, recombinant receptors. Thus, specific binding was typically about 40% for cat lung parenchyma plasma membrane fractions.

Because the feline lung parenchyma was used as a key preparation in the identification of the unique and potent functional activity of bimatoprost, the activities of bimatoprost and 17-phenyl PGF_{2α} were compared at the feline re-

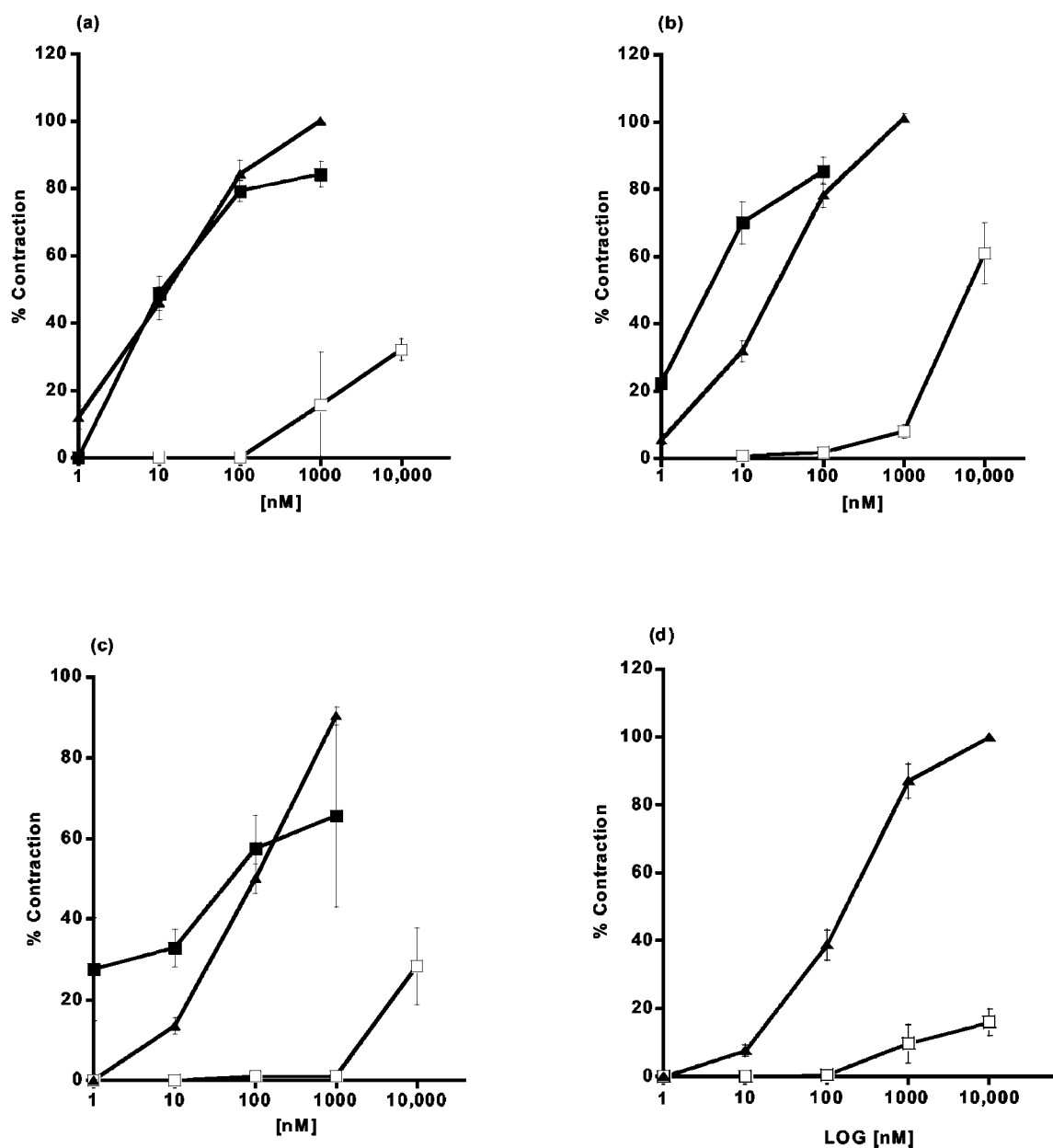


Fig. 3. Comparison of the effects of bimatoprost (□) 17-phenyl PGF_{2α} (■), and PGF_{2α} (▲) on rat colon (a), rat stomach fundus (b), gerbil colon (c), and mouse ileum (d). Points represent mean values \pm S.E.M. of percentage of response to 10^{-6} M PGF_{2α} (a–c) or 10^{-5} M PGF_{2α} (d), $n = 4$ to 7.

combinant FP receptor. These studies were performed to address the potential issue of species-specific effects. Bimatoprost was substantially less active at the recombinant feline FP receptor than 17-phenyl PGF_{2α}. IC₅₀ values for competition versus 5 nM radiolabeled 17-phenyl PGF_{2α} were 13.2 and 8900 nM for 17-phenyl PGF_{2α} and bimatoprost, respectively. Both radioligand binding competition studies and functional studies involving total inositol phosphate accumulation were performed. The data are depicted in Fig. 6.

The effects of bimatoprost on intraocular pressure were studied in both ocular normotensive and ocular hypertensive cynomolgus monkeys. Ocular hypertension was produced by partial circumferential laser photocoagulation of the trabecular meshwork, which is recognized as a model for ocular hypertension and glaucoma (Gaasterland and Kupfer, 1974; Lee et al., 1985). In the glaucomatous monkey model the

animals are rendered unilaterally hypertensive and consequently, there is no contralateral eye that can be used to assess procedural influences on intraocular pressure. This is not a concern in ocular normotensive monkeys and, for this and other reasons, studies in ocular normotensive monkeys are important for directly comparing responses to test drug solution and vehicle.

Graded doses of bimatoprost lowered intraocular pressure in both ocular normotensive monkeys (Fig. 7) and ocular hypertensive monkeys (Fig. 8). The dose-response relationships were shallow. Satisfactory assessment of dose response on intraocular pressure is, however, greatly complicated because bimatoprost was administered twice daily in the 5-day studies in ocular normotensive monkeys and intraocular pressure was not measured beyond 6 h in the ocular hypertensive monkey studies. What is clear is that bimatoprost is

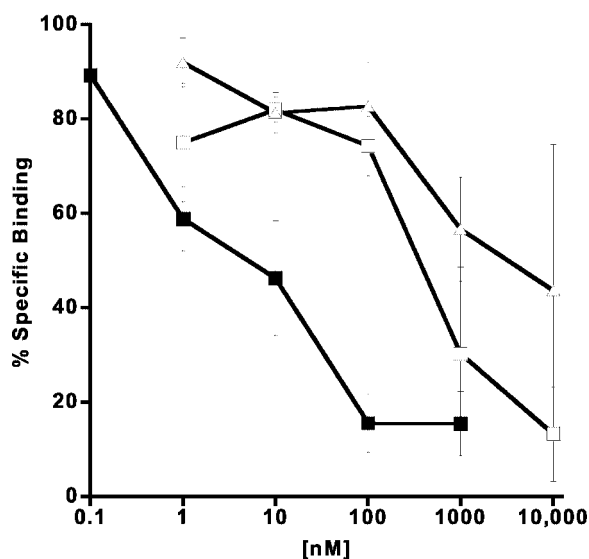


Fig. 5. Cat lung parenchymal tissue radioligand binding competition studies versus [³H]17-phenyl PGF_{2α} involving 17-phenyl PGF_{2α} (■), bimatoprost (□), and U-46619 (△). Points represent mean values ± S.E.M.; bimatoprost and U-46619, *n* = 3; 17-phenyl PGF_{2α}, *n* = 4.

Bimatoprost was rapidly absorbed and remained essentially intact in monkey ocular tissues with only trace formation of metabolites. Ocular distribution was as follows: peri-orbital tissues (eyelids, conjunctiva) > ocular surface tissues (cornea, sclera) > anterior segment tissues (iris, ciliary body) > anterior chamber (aqueous humor). *C*_{max} occurred at the 0.5- and 2-h time points in most instances. The eyelids tended to reach *T*_{max} at much later time points. *C*_{max}, *T*_{max}, and area under curve for both studies are compared in Table 3.

The time course for ocular tissue concentrations of bimatoprost and metabolite formation at predetermined time points in ocular tissues are depicted in Figs. 9 and 10. Ocular surface tissues (cornea, sclera) and anterior segment compartments (ciliary body, iris, anterior chamber) are grouped separately. The *y*-axes are converted to nanomolar equivalents (nM_{eq}) to assist direct comparison with the pharmacol-

ogy data for bimatoprost and 17-phenyl PGF_{2α}. Four minor metabolites were detected. Three structurally unidentified metabolites were designated as metabolites I, II, and III with retention times of 12.5, 13.7, and 20.7 min, respectively. Particular attention was devoted to 17-phenyl PGF_{2α} because, if present in substantial quantities, it would represent an altered pharmacophoric species relative to bimatoprost. Example chromatograms for the bimatoprost standard, a positive identification of 17-phenyl PGF_{2α} in tissue, and a tissue sample where this potential metabolite was absent are shown in Fig. 11. The HPLC chromatogram for bimatoprost exhibits a characteristic trailing shoulder (Fig. 11). This complicated the identification of trace amounts of 17-phenyl PGF_{2α} and consequently, a range of tissue retention times (16.1–17.4 min) was allowed as a positive identification. This conservative analysis, if anything, would tend to overestimate the amounts of 17-phenyl PGF_{2α} present. Because the molecular weights of the three unidentified, trace metabolites were not known, their identification is symbolized at the bottom of each panel in Figs. 9 and 10. Data for the cornea and sclera from both studies are graphically depicted in Fig. 9. Data obtained for the iris, ciliary body, and anterior segment for both studies are given in Fig. 10.

In all tissues studied and at all time points, bimatoprost was overwhelmingly the predominant molecular species identified. Thus, bimatoprost seems highly resistant to metabolism in the primate eye. Four metabolites were detected in trace amounts and few consistent formation patterns were observed. The structurally unidentified metabolites I and II were detected with the greatest frequency. Given the very low abundance of these minor metabolites, time-dependent elimination was often not apparent until the 24-h time point was reached. Metabolite III, when detected, exhibited no interpretable formation or elimination pattern. The identification of the free acid metabolite in tissues was random (Figs. 9 and 10). Considering the ciliary body, which is the site of action for bimatoprost in monkeys (Krauss et al., 2001), there seemed to be no relationship between intraocular pressure reduction and formation of 17-phenyl PGF_{2α}. These data seem consistent with a previous study on human

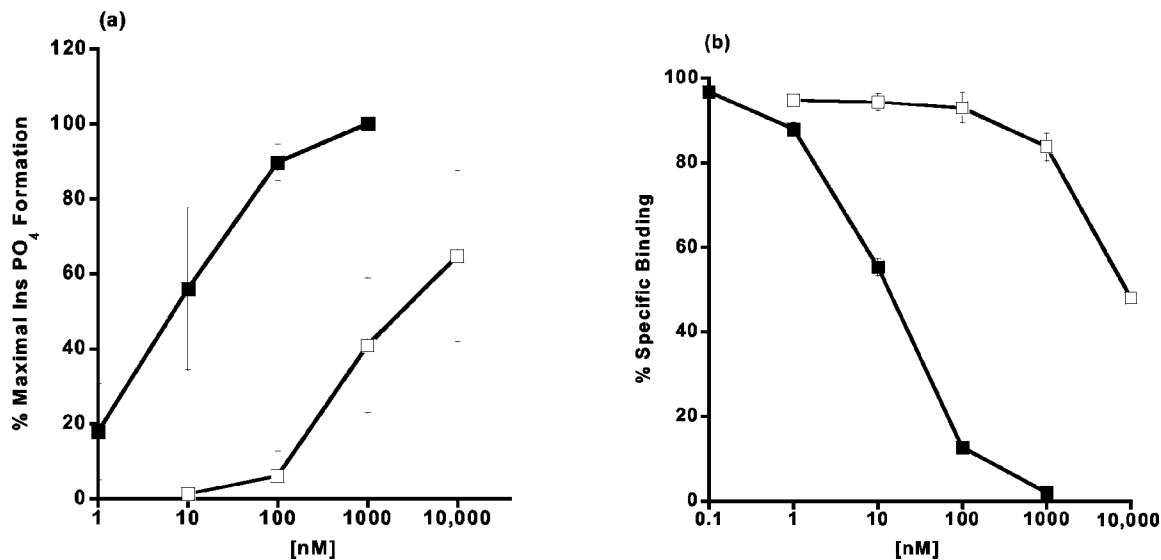


Fig. 6. Comparison of the effects of bimatoprost (□) and 17-phenyl PGF_{2α} (■) on the feline FP receptor measuring total inositol formation (a) and radioligand binding competition versus [³H]17-phenyl PGF_{2α} (b). Points represent mean values ± S.E.M., *n* = 3 to 4.

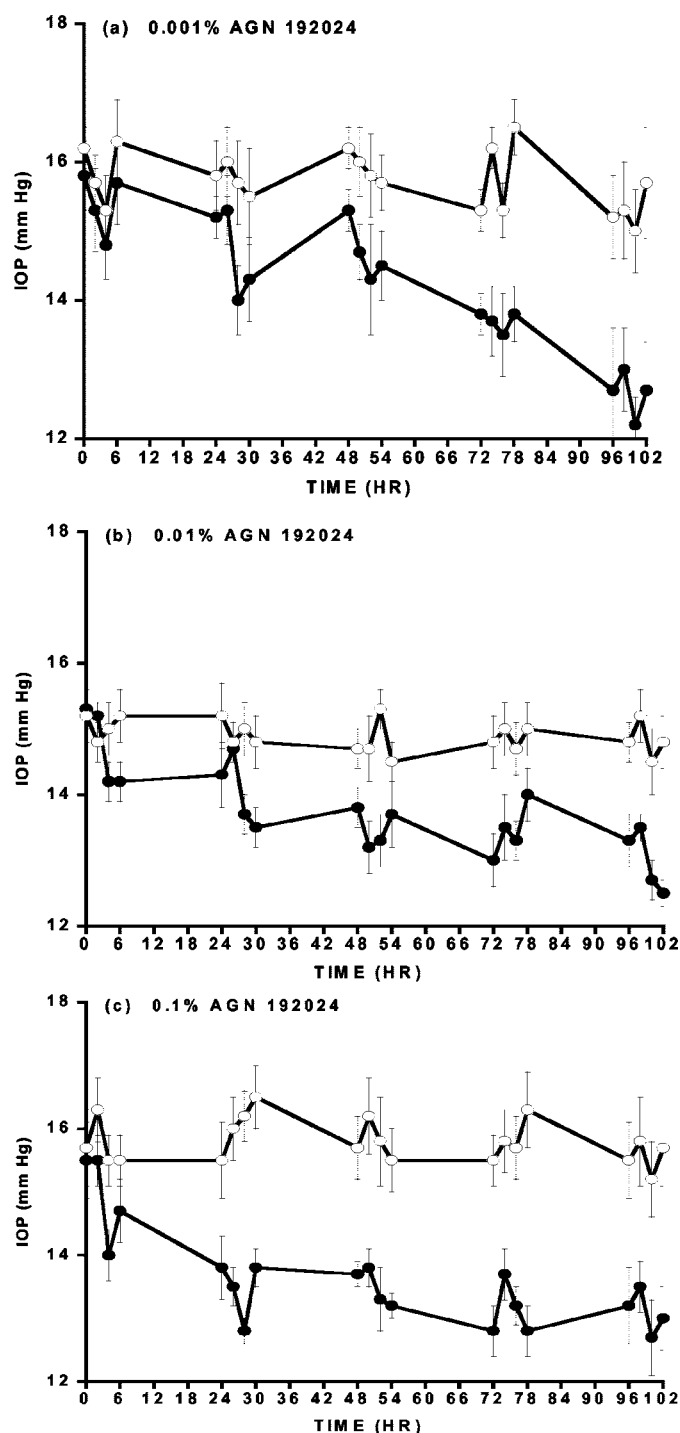


Fig. 7. Effect of graded doses of bimatoprost (●) on intraocular pressure in ocular normotensive monkeys: 0.001% (a), 0.01% (b), and 0.1% (c). Vehicle-treated contralateral eyes are represented by ○. Values are mean ± S.E.M. Bimatoprost produced significant reductions at all doses at most time points. *n* = 6.

iris-ciliary body homogenates in which no conversion of bimatoprost to a free acid metabolite was apparent over a time course where an authentic prodrug was >90% converted to its active pharmacophore (Woodward et al., 2001). In both living monkey studies, the free-acid metabolite was more consistently identified in the aqueous humor, but bimatoprost was always found present in substantially greater quantities.

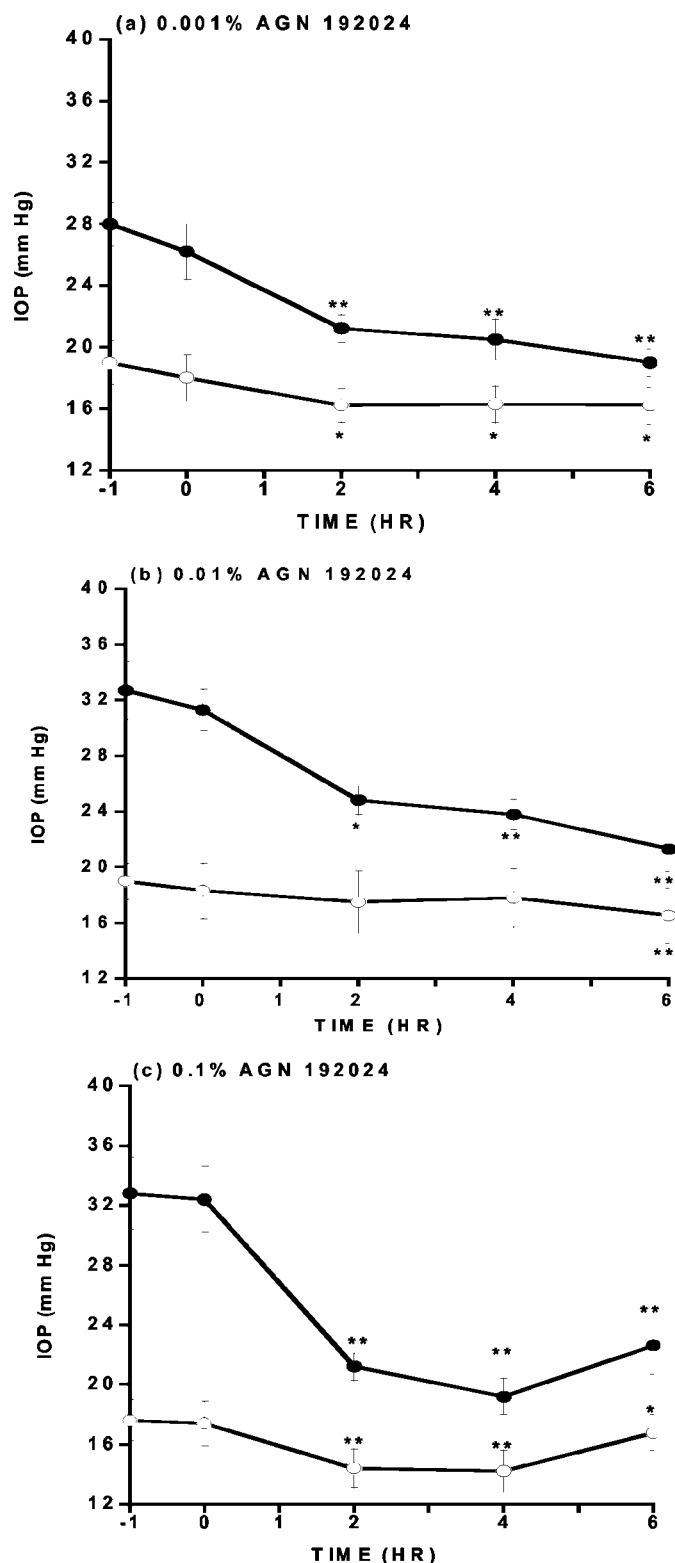


Fig. 8. Effect of graded doses of bimatoprost (●) on the intraocular pressure of laser-induced ocular hypertensive monkeys 0.001% (a), 0.01% (b), and 0.1% (c). Vehicle-treated ocular normotensive contralateral eyes are represented by ○. Values are mean ± S.E.M. *, *p* < 0.05; **, *p* < 0.01. *n* = 6

In the single-dose study, C_{max} was achieved at 0.5 h for the cornea (Fig. 9a) and 2 h for the lower sclera (Fig. 9b). Thereafter, tissue concentrations declined by 8 h to less than 1/10

TABLE 3

Pharmacokinetic parameters for 0.1% [³H]bimatoprost in monkey ocular tissues after a single dose or multiple doses of bimatoprost. C_{\max} (maximum concentration), T_{\max} (time to reach C_{\max}), AUC (area under tissue concentration-time curve).

	C_{\max}	T_{\max}	AUC
	ng/g or ml	h	ng · h/g or ml
Single dose study			
Upper eyelid	1,890	0.5	25,900
Lower eyelid	2,160	8	32,000
Upper conjunctiva	3,060	0.5	20,300
Lower conjunctiva	2,570	2	32,100
Upper sclera	1,010	0.5	5,170
Lower sclera	1,163	2	6,850
Cornea	622	0.5	2,610
Iris	445	2	1,470*
Ciliary body	352	0.5	1,450
Aqueous humor	41.8	0.5	110*
Multiple dose study			
Upper eyelid	11,800	8	162,000
Lower eyelid	30,200	8	332,000
Upper conjunctiva	6,050	2	57,100
Lower conjunctiva	5,350	0.5	44,400
Upper sclera	3,780	8	53,500
Lower sclera	8,760	2	57,200
Cornea	2,890	0.5	27,500
Iris	309	0.5	1,970
Ciliary body	1,098	0.5	5,730
Aqueous humor	14.2	6	102

* AUC values are 0 to 24 h or 0 to 8 h.

their maximum values. The multiple-dose study essentially confirmed that the T_{\max} values for the cornea (Fig. 9c) and sclera (Fig. 9d) occurred at 0.5 and 2 h postdosing, respectively. Tissue concentrations for both cornea and sclera in the multiple-dose study were higher than the values obtained for the single-dose study. These values seemed greater than could be accounted for by the twice-daily dosing regimen and suggest some accumulation in these tissues on repeated dosing. Interestingly, no evidence for substantial accumulation was apparent for the ciliary body (Fig. 10, a and d) or iris (Fig. 10, b and e). Thus, values obtained for the twice-daily, 9.5-day regimen were essentially double those obtained for the single-dose study.

Bimatoprost levels in the anterior chamber were much lower than those detected in anterior segment and other ocular tissues. For example, levels in the aqueous humor were approximately 1/10 of those in the ciliary body for the single-dose study. This difference was more pronounced in the multiple-dose study and, in some instances, 100-fold differences between ciliary body and aqueous humor levels were achieved.

Discussion

These studies suggest that bimatoprost is a prostamide analog that possesses unique pharmacological activity. It exhibits potent inherent pharmacological activity in the cat lung parenchymal strip preparation but has no meaningful activity in a diverse variety of other prostaglandin-sensitive cell and tissue preparations. The contention that the cat lung parenchyma contains a unique population of receptors that preferentially recognize bimatoprost and its congeners was supported by radioligand binding studies. Bimatoprost is also a potent and efficacious ocular hypotensive agent. It reduces intraocular pressure in both normal and ocular hypertensive monkeys and studies on graded doses have dem-

onstrated that even a 0.001% dose exerts a statistically significant ocular hypotensive effect. Effects on intraocular pressure seem to involve the intact bimatoprost molecule, according to ocular drug metabolism studies in living primates.

Although bimatoprost is a structural analog of PGF_{2α}, the pharmacological activity of bimatoprost seems unrelated to the PGF_{2α}-sensitive FP receptor. The structural basis of this unique activity undoubtedly resides in the replacement of the negatively charged carboxylic acid group with an electrochemically neutral ethylamide moiety. Thus, replacement of the carboxylic acid group of PGF_{2α} with a neutral amide, hydroxyl, or alkoxy substituent is known to result in a marked reduction in PGF_{2α}-like agonist activity (Maddox et al., 1978; Schaaf and Hess, 1979; Woodward et al., 2000, 2001). This phenomenon is illustrated for the purpose of these studies by comparing the activity of bimatoprost with that of the potent and selective FP receptor agonist 17-phenyl PGF_{2α} (Magerlein et al., 1975; Miller et al., 1975; Woodward et al., 1995). 17-phenyl PGF_{2α} exhibited potent activity in a variety of PGF_{2α}-sensitive isolated tissues (rat colon, fundus, rat uterus, gerbil colon), whereas bimatoprost exhibited no discernable activity until a 10⁻⁵ M concentration was achieved. In marked contrast, bimatoprost exhibited potent activity in cat lung parenchymal tissue such that bimatoprost and 17-phenyl PGF_{2α} were essentially equipotent. Thus, the activity of bimatoprost in the cat lung parenchyma seems unique and is not reproduced in PGF_{2α}-sensitive or in other prostaglandin-sensitive isolated tissue preparations (Coleman et al., 1994) reported herein (Table 1). Interestingly, regional sensitivity to bimatoprost, but not FP or TP receptor stimulation, was apparent in the cat lung parenchyma.

To investigate whether bimatoprost effects in the cat lung parenchyma were species-specific, the feline recombinant FP receptor was studied and the effects of bimatoprost and 17-phenyl PGF_{2α} were again directly compared. In total inositol phosphate formation and radioligand binding competition studies versus [³H]17-phenyl PGF_{2α}, bimatoprost was approximately 2 to 3 orders of magnitude less potent than 17-phenyl PGF_{2α}. Bimatoprost also exhibited a similar relative absence of activity at recombinant and natural human FP receptors. Moreover, pretreatment with bimatoprost did not attenuate the Ca²⁺ signal produced by PGF_{2α} in Swiss 3T3 cells. Taken together, these data argue against bimatoprost behaving as a species (feline)-selective FP agonist. Rather, the functional effects of bimatoprost in the cat lung parenchyma seem to reflect a unique pharmacology that is independent of the prostanoid FP receptor.

In addition to studies at recombinant and natural prostanoid FP receptors, binding competition studies on feline lung parenchymal plasma membrane preparations were also undertaken. Because the lung parenchyma contains a functional TP receptor, competition studies with the thromboxane mimetic U-46619 were performed as a control. The results of the radioligand binding studies indicated that bimatoprost and U-46619 exhibited less affinity than 17-phenyl PGF_{2α} for [³H]17-phenyl PGF_{2α} binding sites in the cat lung parenchyma. Given the similarity in functional potency for the prostamide analog bimatoprost and the FP receptor agonist 17-phenyl PGF_{2α}, these data also suggest that bimatoprost exerts its functional activity in the cat lung paren-

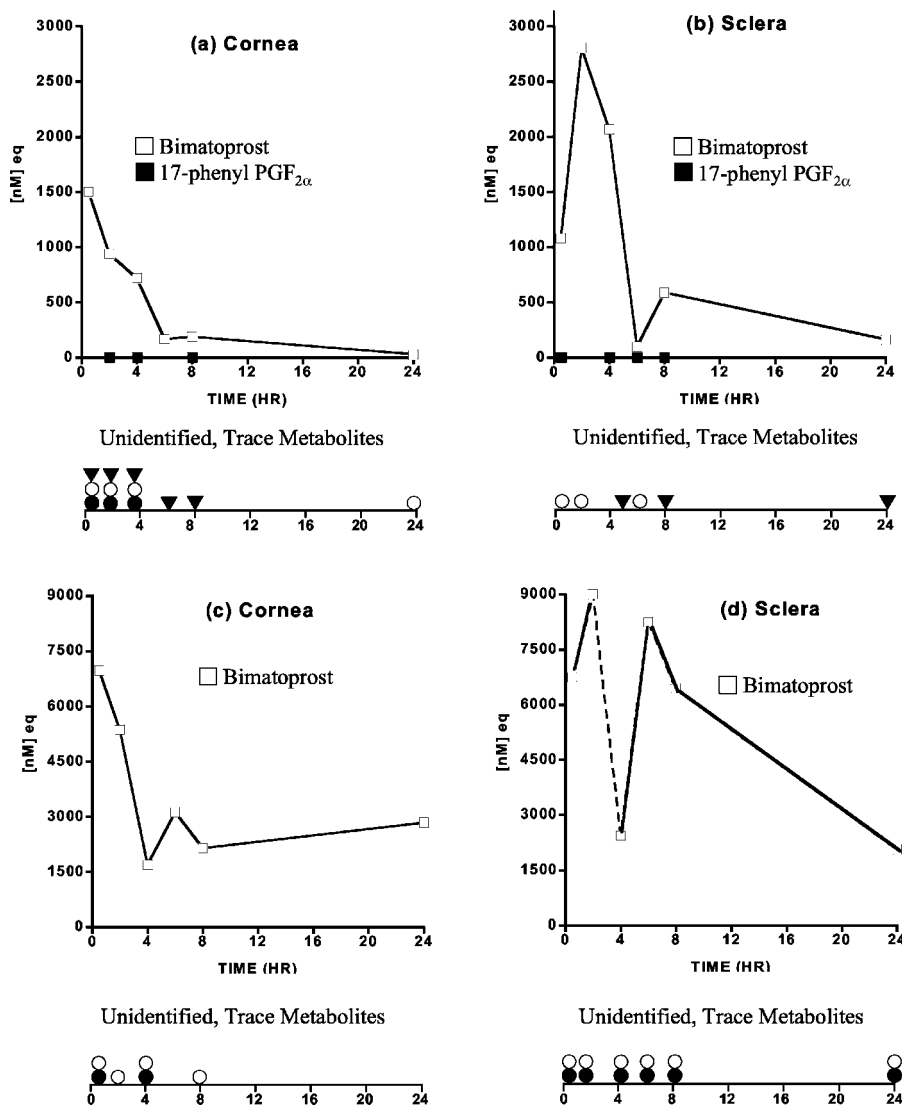


Fig. 9. Tissue concentrations of bimatoprost (□), 17-phenyl PGF_{2α} (■), and metabolites I (●), II (○), and III (▼) after a single dose (a and b) and multiple doses (c and d). Data for the cornea (a and c) and sclera (d and b) are given. Bimatoprost and 17-phenyl PGF_{2α} concentrations are given as nM_{eq}. Because the identity and molecular weight of metabolites I, II, and III are unknown, a positive identification is denoted at the bottom of each panel on a secondary x-axis. Each point represents values from one animal (nb the 2 h sclera value for the multiple-dosing study is not given in d; see Table 3).

chyma at a receptor distinct from the prostanoid-sensitive FP receptor.

Bimatoprost is a potent and highly efficacious ocular hypotensive in glaucomatous patients, with pronounced activity at doses as low as 0.003% (Laibovitz et al., 2001). The monkey intraocular pressure data described herein provided a rationale for clinical testing and are, therefore, essentially consistent with the recently reported clinical findings. Bimatoprost, administered twice daily to ocular normotensive monkeys, was a potent ocular hypotensive and even a twice-daily 0.001% dose produced statistically significant and well maintained reductions in intraocular pressure. These findings were confirmed in the laser-induced ocular hypertensive monkey model. At doses below those used clinically, the ocular hypotensive activity of bimatoprost does not differ markedly from that of the esterified FP agonist travoprost, which, in the free acid form, is the potent FP receptor agonist fluprostenol. Thus, travoprost (Hellberg et al., 2001) and bimatoprost, at similar low doses, both produce about a 25% reduction in the intraocular pressure of ocular hypertensive monkeys. Because 17-phenyl PGF_{2α} and fluprostenol are essentially equipotent FP receptor agonists (Woodward et al., 1995), their ocular hypotensive activity should be similar.

Based on the likely premise that there is little difference between the ocular hypotensive efficacy of low doses of bimatoprost and the isopropyl esters of fluprostenol and 17-phenyl PGF_{2α}, the ocular metabolism of bimatoprost is a key determinant of its ocular pharmacology according to the following reasoning. If bimatoprost was as extensively and rapidly converted to its putative enzymatic hydrolysis product 17-phenyl PGF_{2α} as a classical prostaglandin prodrug such as latanoprost (Sjöquist et al., 1999), then FP receptor stimulation would contribute to its ocular hypotensive activity. If bimatoprost metabolism in the eye was minimal or absent, it follows that its ocular hypotensive activity is attributable to the intact molecule. The pharmacological basis of this ocular hypotensive activity was, therefore, further explored by investigating the metabolic disposition of bimatoprost in the living monkey eye. A single-dose study and a twice-daily dosing 9.5-day study were performed, which provided an approximate match for the intraocular pressure study designs. In these studies, four metabolites were detected at trace levels. Three of these were unidentified molecules; one was the free acid, enzymatic hydrolysis product. Particular attention was devoted to the identification of the potential enzymatic hydrolysis product 17-phenyl PGF_{2α}, because this

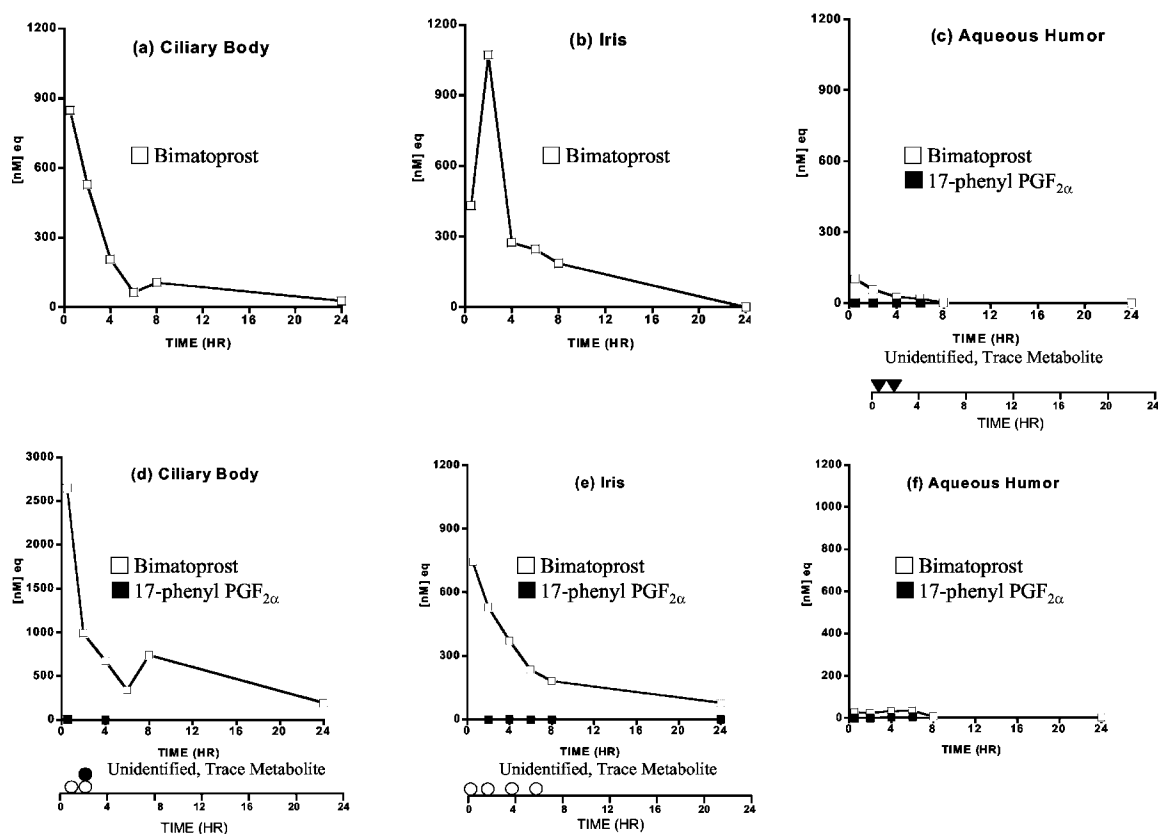


Fig. 10. Tissue concentrations of bimatoprost (□), 17-phenyl PGF_{2α} (■), and metabolites I (●), II (○), and III (▼) after a single dose (a–c) and multiple doses (d–f). Data for the ciliary body (a and d), iris (b and e), and aqueous humor (c and f) are given. Bimatoprost and 17-phenyl PGF_{2α} concentrations are given as nM_{eq}. Because the identity and molecular weight of metabolites I, II, and III are unknown, a positive identification is denoted at the bottom of each panel on a secondary x-axis where detected. Each point represents values from one animal.

is a potent FP receptor agonist (Woodward et al., 1995). One object of the ocular metabolism studies was to determine whether the ocular hypotensive effects were due entirely to the unique pharmacological activity of bimatoprost or were due, at least in part, to the formation of 17-phenyl PGF_{2α}.

The ocular metabolism studies were conducted in living monkeys and involved administration of a 0.1% dose, thereby maximizing the detection of minor metabolic species. In the single-dose study, the putative free acid metabolite of bimatoprost was not detected in the ciliary body, which is the site of ocular hypotensive action in the monkey (Krauss et al., 2001). The C_{max} for bimatoprost in the ciliary body was achieved within 30 min and closely approached an estimated 10^{-6} M concentration. Although bimatoprost levels rapidly declined thereafter, concentrations sufficient to exert a pronounced pharmacological effect were present for at least 8 h. At 24 h postdosing bimatoprost was still detected but at a level in the vicinity of its EC_{50} value in the cat lung parenchymal tissue preparation. In the multiple-dose study, the free acid metabolite was detected at only two nonconsecutive time points and in trace amounts. These metabolism studies, taken together with its potent ocular hypotensive activity, clearly indicate that bimatoprost does not need to be converted to a free acid metabolite to exert its effects on intraocular pressure. The ocular hypotensive effects of bimatoprost seem to result from its unique pharmacological activity, as identified in the cat lung parenchymal tissue preparation.

The distribution pattern of bimatoprost in ocular tissues revealed that the highest concentrations were in the perior-

bita tissues (eyelids and conjunctiva). An intermediate level was present in the ocular surface tissues, with scleral concentrations typically about double those achieved in the cornea. Because the rate of penetration of bimatoprost in the sclera is more than 4 times that reported in the cornea (Woodward et al., 2001), the higher concentrations of bimatoprost found in the sclera underscore this tissue as the preferred route of accession to the globe. Bimatoprost concentrations in the iris and ciliary body were typically less than those in ocular surface tissues. Concentrations in the aqueous humor were essentially only 1/10 of those found in the iris and ciliary body for the single-dose study. It is interesting to compare the anterior segment distribution of bimatoprost with that of the metabolically labile prodrug latanoprost (Sjöquist et al., 1999). In a single-dose study with latanoprost in monkeys, the concentrations of drug in the anterior chamber and ciliary body were similar over the 24-h study period (Sjöquist et al., 1999). For a single dose of bimatoprost, concentrations in the ciliary body were approximately 10-fold those present in the aqueous humor. This difference was even greater in the repeated dose study. This marked difference between the ocular anterior segment distribution of latanoprost and bimatoprost probably reflects their distinctly different metabolic fates in ocular tissue. Bimatoprost remains intact. Latanoprost isopropyl ester is rapidly hydrolyzed to liberate the active pharmacophore as the free acid metabolite (Sjöquist et al., 1999). Thus, a lipophilic ester is rapidly converted to an ionized species in the case of latanoprost and such a product would exhibit altered physiochemi-

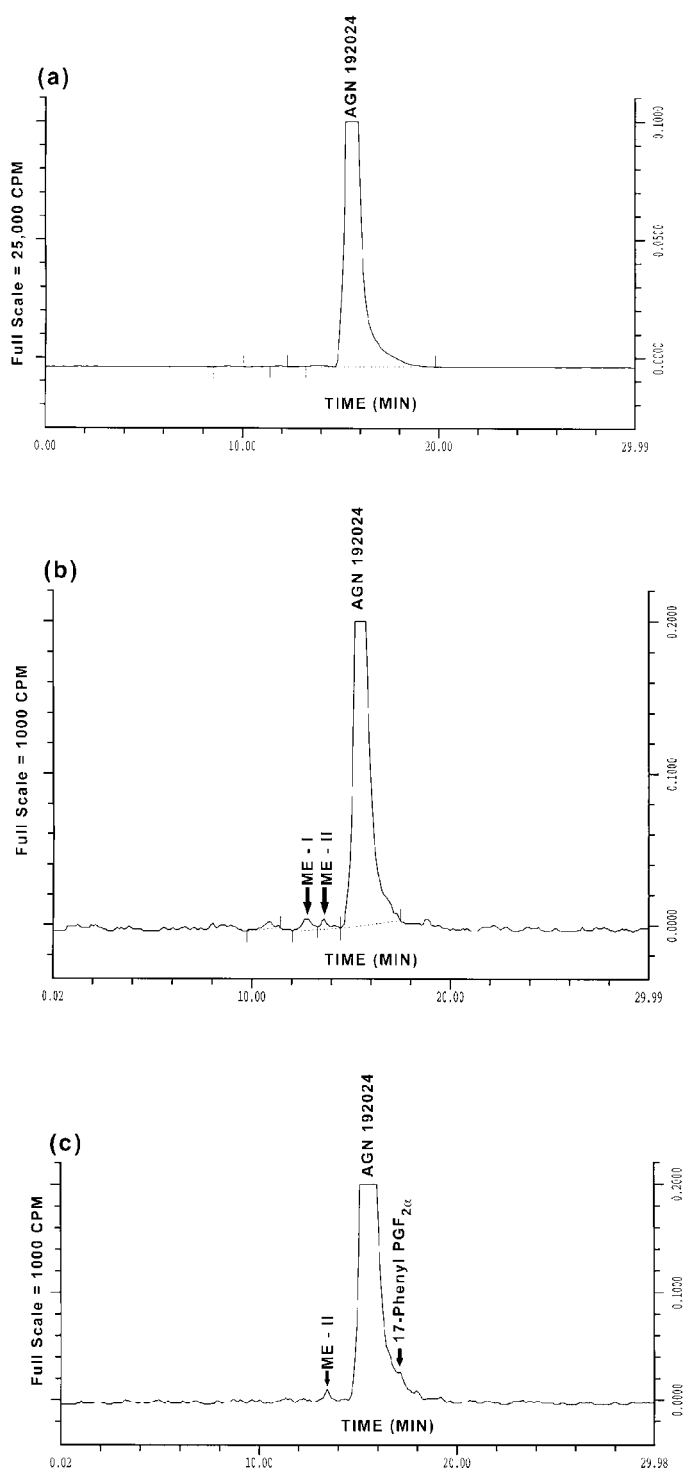


Fig. 11. Example chromatograms. a, bimatoprost standard. b, ciliary body at 2-h point of multiple-dose study. c, iris at 2-h time point of multiple-dose study. Identifications of metabolite I (b), metabolite II (b and c), and 17-phenyl PGF_{2α} (c) are depicted.

cal properties. This, in turn, would substantially influence ocular bioavailability (Camber et al., 1986).

Bimatoprost seems unique and questions remain regarding its pharmacological characterization. Certainly, further studies are necessary. It is, however, compelling to note that bimatoprost is structurally a prostamide and exhibits a pharmacological activity profile similar to that emerging for the

prostamides, which were recently discovered as molecules that are biosynthesized from anandamide by COX-2 (Yu et al., 1997; Burstein et al., 2000). Pharmacological studies are in their infancy but bimatoprost and prostamide F_{2α} are both potent and selective in stimulating cat iris sphincter smooth muscle, with no evidence for meaningful interaction with PG-sensitive or cannabinoid receptors (Berglund et al., 1999; Woodward et al., 2001). Moreover, pharmacologically active lipoxygenase derivatives of anandamide may perhaps exist (Craib et al., 2001). It seems that neutral cyclooxygenase and lipoxygenase products of anandamide may represent a distinct class of biological substances.

Acknowledgments

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