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REVIEW

Prostamides (prostaglandin-ethanolamides) and their pharmacology

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The prostamides are part of a large and continually expanding series of pharmacologically unique neutral lipids. They are COX-2 derived oxidation products of the endocannabinoid/endovanniloid anandamide. Prostamide pharmacology is unique and, as in the case of the endocannabinoids anandamide and 2-arachidonylglycerol, bears little resemblance to that of the corresponding free acids. By virtue of its close relationship to the anti-glaucoma drug bimatoprost, prostamide $F_{2\alpha}$ has received the greatest research attention. Prostamide $F_{2\alpha}$ and bimatoprost effects appear independent of prostanoid FP receptor activation, according to a litany of agonist studies. Studies involving freshly isolated and separate feline iridial smooth muscle cells revealed that bimatoprost and FP receptor agonists stimulated different cells, without exception. This suggests the existence of receptors that preferentially recognize prostamide $F_{2\alpha}$. The recent discovery of prostamide antagonists has provided further support for prostamide receptors as discrete entities. The prototypical prostamide antagonists, AGN 204396 and 7, blocked the effects of prostamide $F_{2\alpha}$ and bimatoprost but not those of $PGF_{2\alpha}$ and FP receptor agonists in the feline iris. Second generation more potent prostamide antagonists, such as AGN 211334, should allow the role of prostamides in health and disease to be elucidated. From the therapeutics standpoint, the prostamide $F_{2\alpha}$ analogue bimatoprost is the most efficacious ocular hypotensive agent currently available for the treatment of glaucoma.
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Keywords: prostaglandins; prostaglandin-ethanolamides; FP receptor; prostaglandin glyceryl ester; anandamide; cyclo-oxygenase-2

Abbreviations: 2-AG, 2-arachidonylglycerol; COX-1, cyclo-oxygenase 1; COX-2, cyclo-oxygenase 2; Cyr 61, cysteine-rich angiogenic protein 61; PG, prostaglandin; prostamide, prostaglandin-ethanolamide

Introduction

The origin of prostamide (prostaglandin-ethanolamide) research resides in two quite separate lines of investigation. Unrelated studies on endocannabinoid oxygenation by cyclo-oxygenase-2 (COX-2) and the pharmacology of neutral derivatives of prostaglandins (PGs) converged to reveal a close inter-relationship. The resultant concept was that the principal endocannabinoids are oxygenated by COX-2, with the formation of pharmacologically unique prostamides and PG-glyceryl esters. PGE_2-ethanolamide was the first COX-2-derived product of anandamide to be described (Yu *et al.*, 1997). Subsequent detailed analyses of endocannabinoid oxygenation by COX-2 revealed that anandamide and 2-arachidonylglyceryol ester (2-AG) are converted to a range of products that closely approaches those formed from arachidonic acid by COX-1 and COX-2 (Kozak *et al.*, 2002; Koda *et al.*, 2004). In addition to cell culture experiments, prostamides have also been shown to be formed from anandamide in living mice (Weber *et al.*, 2004). The enzymatic formation of prostamides from anandamide was thereby established.

The PG-ethanolamides and glyceryl esters are members of an expanding network of neutral lipid mediators. The neutral lipids are already numerous but, in the majority of cases, the pharmacology is ill-defined or unknown. This is not the situation for the principal endocannabinoids anandamide and 2-AG, and rapid advances have recently occurred in the neutral PG area. From the perspective of the endocannabinoids anandamide and 2-AG, metabolic conversion equates with loss of primary activity but can result in altered pharmacology rather than de-activation, depending upon the metabolic pathway involved. For anandamide oxidation by COX-2 results in a loss of activity at cannabinoid-1 and TRPV1 receptors (De Petrocellis *et al.*, 2004) and formation of the prostamides, which are in a quite separate pharmacological category. Enzymatic hydrolysis of anandamide by fatty acid amide hydrolase (Cravatt *et al.*, 1996; Wei *et al.*, 2006) leads to arachidonic acid formation, which may

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serve as a substrate for COX-1 and COX-2, lipo-oxygenases, or *P*450 enzymes and may also be re-incorporated into the lipid pool. A schematic showing the relationship of endocannabinoid metabolic pathways is provided in Figure 1. It follows that, in the context of the endocannabinoid system, prostamide formation may be viewed as both an inactivation mechanism for anandamide and a pathway leading to pharmacologically novel, PG-like substances. By the same token, inhibitors of COX-2 could partly attenuate anandamide breakdown and selectively prevent prostamide and PG-glyceryl ester formation. The relative importance of these events with respect to the anti-inflammatory and analgesic effects of COX-2 inhibitors remains to be elucidated. Under circumstances where COX-2 is induced and anandamide is present, substantial prostamide formation may occur (Glass *et al*., 2005).

The prostamides have been pharmacologically characterized to a much greater extent than PGE_2-glyceryl ester. Prostamide $F_{2\alpha}$ has received the greatest attention, since the pharmacology of neutral $PGF_{2\alpha}$ analogues is closely tied to the antiglaucoma drug bimatoprost (Woodward *et al*., 2003; Matias *et al*., 2004). An exhaustive series of agonist studies using prostamide $F_{2\alpha}$ and bimatoprost have provided much evidence that their effects cannot be readily attributed to prostanoid FP receptor stimulation. In 2007, the first selective prostamide antagonist was reported, which provided further evidence for the prostamide receptor as a distinct entity (Woodward *et al*., 2003). This review provides an in-depth analysis of prostamide pharmacology, including a brief mention of more potent prostamide antagonists that have recently been discovered.

Prostamide biosynthesis

Anandamide may be metabolized by both hydrolytic and oxidative mechanisms. It is catabolized to arachidonic acid and ethanolamine by the fatty acid amide hydrolase enzymes. The first fatty acid amide hydrolase was discovered a decade ago (Cravatt *et al*., 1996), but a human-specific fatty acid amide hydrolase was reported recently (Wei *et al*., 2006). Anandamide is also oxidized by COX-2 and lipo-oxygenase pathways (Hampson *et al*., 1995; Ueda *et al*., 1995). A schematic for the biosynthesis of COX-2 and lipo-oxygenase products from the endocannabinoid anandamide is provided in Figure 2. The biological activity of the lipo-oxygenase products of anandamide is controversial and has received little attention, unlike the prostamides.

The first prostamide to be discovered was PGE_2-ethanolamide. It was identified as the major product when anandamide was incubated with human recombinant COX-2 or human foreskin fibroblasts cells expressing COX-2 (Yu *et al*., 1997). In marked contrast, COX-1 failed to bind and oxidize anandamide (Yu *et al*., 1997). Formation of prostamide E_2 from anandamide was subsequently confirmed in RAW 264.7 cells (Burstein *et al*., 2000). These earliest findings provided a basis for much more detailed and extensive studies, which revealed that COX-2 converted the endocannabinoids anandamide and 2-AG to a range of oxidation products that closely approached those formed from arachidonic acid (Kozak *et al*., 2002). COX-2 oxidizes anandamide to the endoperoxide intermediates prostamide G_2 and prostamide H_2 (Kozak *et al*., 2002; Koda *et al*., 2004; Yang *et al*., 2005), which are then converted by specific PG synthases to the various prostamides. Thus, it was demonstrated in RAW264.7 cells that exogenous anandamide is converted to prostamide D_2 by prostaglandin D synthase-directed isomeration of PGH_2-ethanolamide (Kozak *et al*., 2002). Similarly, anandamide was converted to prostamide E_2 and $F_{2\alpha}$ in HCA-7 cells (Kozak *et al*., 2002). Moreover, it appears that prostamide H_2 and prostamide D_2 are substrates for prostaglandin F synthase, with the resultant formation of prostamide $F_{2\alpha}$ and 11β-prostamide $F_{2\alpha}$ (Koda *et al*., 2004; Yang *et al*., 2005).

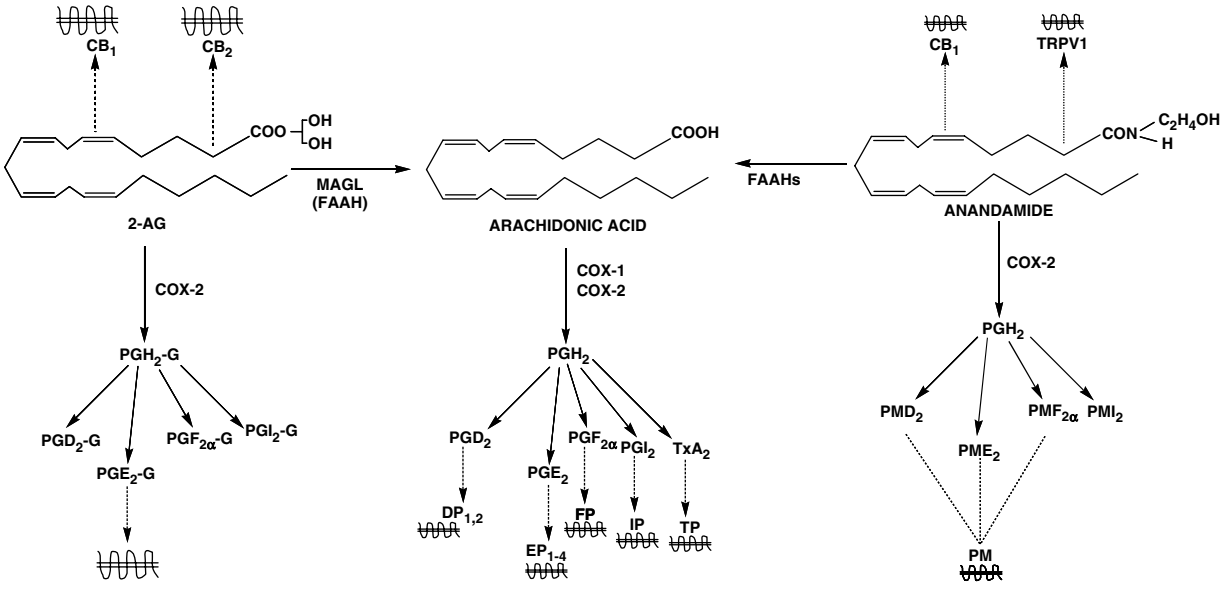

Figure 1 Pharmacology and metabolic conversion of endocannabinoids and neutral prostaglandin derivatives. G, glyceryl ester; PM, prostamide.

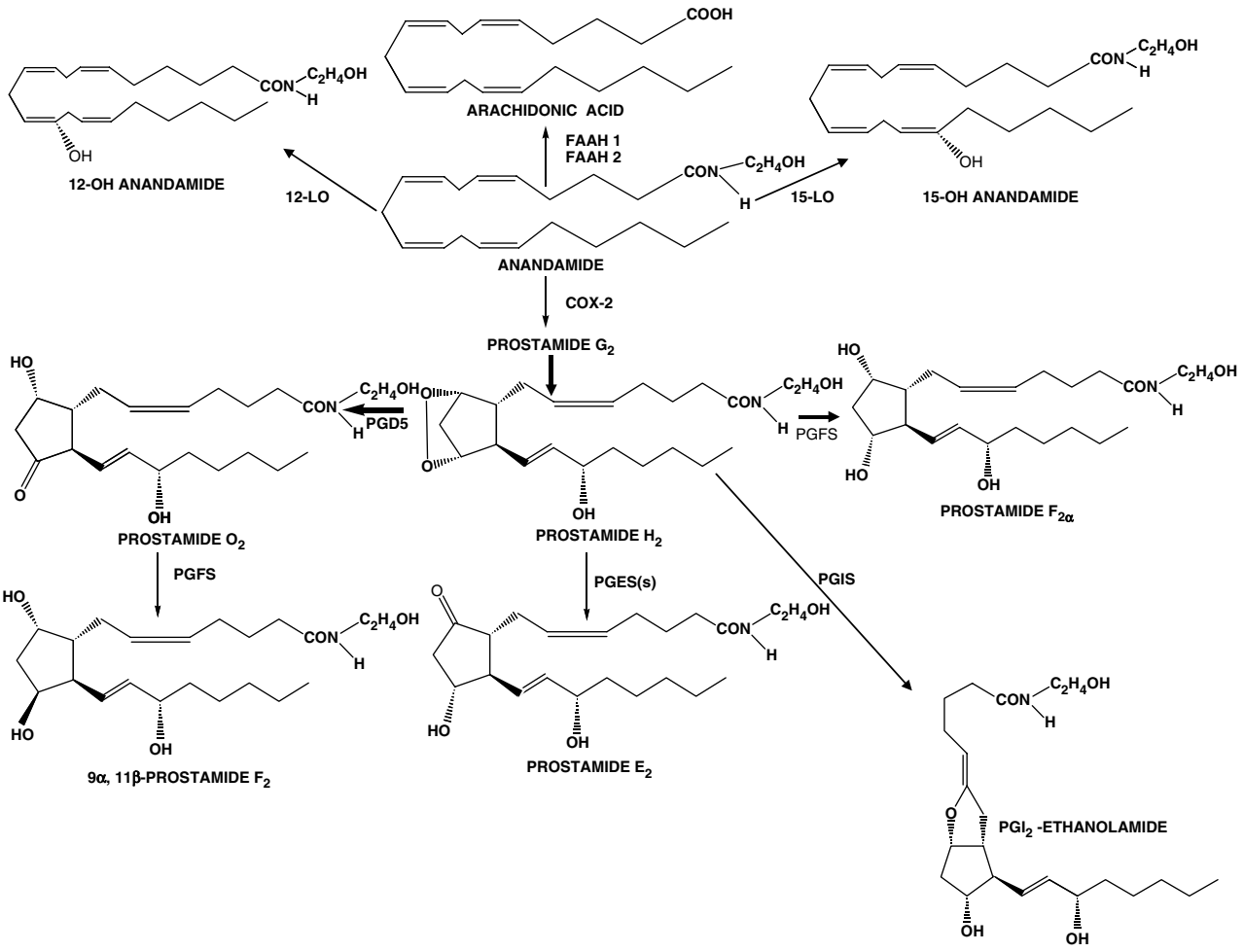

Figure 2 Anandamide conversion pathways.

Using coupled assays involving COX-2 and prostacyclin synthase, evidence was provided for the formation of PGI_2-ethanolamide (Kozak *et al*., 2002). Finally, prostamide formation from exogenously administered anandamide has been demonstrated in living animals (Weber *et al*., 2004).

Prostamide pharmacology

Agonists. At the time when the prostamides were discovered as COX-2-derived products of anandamide, virtually nothing was known about their pharmacology. Neutral $PGF_{2\alpha}$ analogues were known to exert little or no agonist activity in $PGF_{2\alpha}$-sensitive preparations (Maddox *et al*., 1978; Schaaf and Hess, 1979). Replacement of the charged carboxylate group of $PGF_{2\alpha}$ by a -$CONH_2$ group reduced activity at FP receptors by more than two orders of magnitude. Monomethyl and dimethyl functionalities produced a further reduction in agonist potency, to the point where $PGF_{2\alpha}$-$CON(CH_3)_2$ was purported to behave as an antagonist (Maddox *et al*., 1978). This activity could not be confirmed in more recent studies (Sharif *et al*., 2000; Table 1). What is more important, however, was an absence of meaningful agonist activity at prostanoid FP receptors. This has been confirmed on numerous occasions (Woodward *et al*., 2000, 2003; Liang *et al*., 2003, 2004; Krauss and Woodward, 2004; Matias *et al*., 2004; Chen *et al*., 2005). Given the structural similarity of PGs and prostamides, interaction with PG receptors was an obvious line of investigation for elucidating prostamide pharmacology but it was not the only one to be pursued.

The natural mammalian endocannabinoids are neutral arachidonic acid derivatives, and the possibility that prostamides behaved as cannabimimetics (Pinto *et al*., 1994; Berglund *et al*., 1999) or were similar to anandamide with respect to vanilloid receptor stimulation (Matias *et al*., 2004) was investigated. No cannabinoid or transient receptor potential vanilloid type 1 channels activity was observed until a 10^{-6} M concentration was exceeded and it was concluded that prostamides do not possess cannabimimetic and related activity. It should be noted that although supra-μM activity is often dismissed as pharmacologically meaningful, there is not a general consensus on this issue (Sharif *et al*., 2001). The possibility that high local concentrations of prostamides may stimulate cannabinoid receptors cannot be dismissed. Other hypotheses have been advanced to explain prostamide activity. These include activation of the peroxisome proliferation-activated receptor-γ (Rockwell and Kaminski, 2004), prostaglandin F synthase inhibition (Koda

Table 1 Pharmacology of $PGF_{2\alpha}$-amides

$PGF_{2\alpha}$ C1-substituent	*Feline iris*	*Swiss 3T3 cell*	*Rabbit jugular vein*	*Guinea-pig ileum*	*Guinea-pig vas deference*	*Human platelets (aggregation)*	*Human platelets*
	● *Prostamide*	● *FP*	● *FP* ● *EP_4* ● *DP_1*	● *EP_1*	● *EP_3*	● *TP*	● *DP_1*, ● *IP*
—CONH$_2$	21	18 000	643	NA	794	>10 000	—
—CONHCH$_3$	28	13 300	—	—	—	—	—
—CONHCH$_2$CH$_3$	35	—	250	>10 000	>10 000	—	—
—CONH(CH$_2$)$_2$CH$_3$	19	—	281	NA	>10 000	—	—
—CONH(CH$_2$)$_3$CH$_3$	60	—	17 800	NA	>10 000	—	NA
—CONH(CH$_2$)$_4$CH$_3$	158	—	17 800	NA	>10 000	—	NA
—CONH(CH$_2$)$_5$CH$_3$	258	—	20 734	—	—	—	—
—CON(CH$_2$)$_7$CH$_3$CH$_3$	771	—	3649	—	—	—	—
—CONH(CH$_2$)$_2$OH	57	4285	1277	>10 000	1590	NA	NA
—CONH(CH$_2$)$_4$OH	158	—	2860	—	—	—	—
—CON(CH$_3$)$_2$	4500	>10 000	3649	—	—	—	—

Abbreviations: NA, not active; prostamide, prostaglandin-ethanolamide.
Values are EC_{50} (nM). Experiments involved at least four replicates. Receptor subtype involvement is given (●) under the description of each preparation. Physiological responses are as follows: feline iris = contraction; Swiss 3T3 cells = Ca2+ ↑; rabbit jugular vein = relaxation; guinea-pig ileum = contraction; guinea pig vas deferens = inhibition of field stimulated contraction; human platelets (TP) = aggregation; human platelets (DP, IP) = inhibition of ADP induced aggregation.

et al., 2004; Komoto *et al.*, 2006) and substrates that indirectly reduce anandamide metabolism (Matias *et al.*, 2004).

PGE_2-ethanolamide was the first prostamide to be discovered (Yu *et al.*, 1997) and was the first subjected to pharmacological characterization as a prostanoid receptor ligand. The effects of PGE_2-ethanolamide in the guinea-pig trachea could not be readily explained by interaction with prostanoid EP receptors (Ross *et al.*, 2002). The most extensive comparative pharmacological studies with prostanoids have been conducted for prostamide $F_{2\alpha}$ and its analogue bimatoprost. In fact, bimatoprost has been the focus of many studies by virtue of its clinical status as an antiglaucoma agent (Dubiner *et al.*, 2001; Higginbotham *et al.*, 2002; Noecker *et al.*, 2003; Parrish *et al.*, 2003; Woodward *et al.*, 2004). The evolution of prostamide pharmacology is very much rooted in the pharmacological characterization of bimatoprost, with a continual cross-reference.

As in many previous cases, the elucidation of prostamide pharmacology originated from agonist studies. The earliest neutral $PGF_{2\alpha}$ analogues were $PGF_{2\alpha}$ 1-OH and $PGF_{2\alpha}$ 1-OCH_3, and their pharmacology was extensively characterized using recombinant prostanoid receptors and native receptors in cells and isolated tissues (Woodward *et al.*, 2000). A number of C-1 modifications were made, most resulted in uninteresting compounds. The amido substituents provided the most satisfactory compounds in terms of both pharmacological novelty and ophthalmological profile. The activity of $PGF_{2\alpha}$ 1-amides is provided in Table 1. The primary amide (-$CONH_2$) and N-monosubstituted analogues provided potent and selective prostamide agonists. A variety of monosubstituents provided compounds with similar activity, linear and branched alkyl substituents and ethanolamide being well tolerated. Di-substitution caused a dramatic reduction in prostamide activity. All of these prostamides exhibited no meaningful activity at prostanoid receptors, including the FP receptor. None were FP receptor antagonists. Based on the fundamental $PGF_{2\alpha}$ 1-amide structure a wide range of analogues was prepared, which included the antiglaucoma drug bimatoprost (Woodward *et al.*, 2004).

Although the pharmacology of bimatoprost and other $PGF_{2\alpha}$ amides appeared unique (Woodward *et al.*, 1994), the biological significance was obscure until the first prostamide was discovered in 1997 by Yu *et al.* At this juncture $PGF_{2\alpha}$ 1-ethanolamide was elevated from being one in an expansive collection of $PGF_{2\alpha}$ amides to a key comparator for bimatoprost. A series of comparative experiments were conducted over several years to address key questions and hypotheses that were raised. These questions/hypotheses are

listed as follows, with the background, key experiments and outcome provided.

(a) *Does prostamide pharmacology reflect differences between isolated tissue and cell studies?* The unique pharmacology of $PGF_{2\alpha}$ amides was originally revealed by comparing responses in the isolated feline iris with Ca^{2+} signalling in Swiss 3T3 cells, which is an FP receptor-mediated event (Woodward and Lawrence, 1994). The rank orders of potency were as follows:
Feline iris, 17-phenyl $PGF_{2\alpha}$ = fluprostenol ⩾ $PGF_{2\alpha}$ = prostamide $F_{2\alpha}$ = bimatoprost > PGD_2 > PGE_2 > U-46619 > sulprostone.
Swiss 3T3 cells, 17-phenyl $PGF_{2\alpha}$=fluprostenol ⩾ $PGF_{2\alpha}$ > PGD_2 > PGE_2 > U-46619 > sulprostone ⩾ prostamide $F_{2\alpha}$=bimatoprost.

Several isolated tissue preparations known to constitutively express FP receptors were found to be essentially insensitive to prostamide $F_{2\alpha}$ and bimatoprost. These included the gerbil colon, intact rabbit jugular vein, mouse uterus, rat uterus and human uterus (Woodward *et al*., 2001, 2003; Matias *et al*., 2004; Chen *et al*., 2005). They behaved in a similar manner to that observed in Swiss 3T3 cells (Woodward and Lawrence, 1994). It was concluded that only certain isolated tissue preparations uniquely recognize prostamide $F_{2\alpha}$ and its congeners: feline iris (Woodward *et al*., 2001; Matias *et al*., 2004), feline lung parenchyma (Woodward *et al*., 2003) and rabbit uterus (Chen *et al*., 2005).

(b) *Is bimatoprost an FP receptor agonist?* The prostanoid receptor classification designates receptors according to the ligands with which they preferentially interact (Coleman *et al*., 1984). This is a key concept for avoiding confusion, as many compounds that preferentially interact with a single receptor have off-target activity at much higher concentrations. For example, fluprostenol is widely and correctly regarded as a selective FP receptor agonist but it has measurable EP_3 activity at concentrations that exceed 10^{-6} M (Hellberg *et al*., 2001). Similarly, U-46619 is widely viewed as a selective TP receptor agonist but it also stimulates FP receptors at high concentrations (Woodward and Lawrence, 1994). It has been postulated that bimatoprost is an FP receptor agonist *per se* (Sharif *et al*., 2001, 2002, 2003). This is based on the weak interaction with FP receptors that occurs at supra-μM concentrations (Sharif *et al*., 2001; Woodward *et al*., 2001, 2003, 2004). Such a definition of bimatoprost as an FP receptor agonist does not take into account the prostamide-like activity that is observed at concentrations of at least 100-fold lower in prostamide-sensitive preparations (Liang *et al*., 2003, 2004; Woodward *et al*., 2003, 2004; Matias *et al*., 2004; Chen *et al*., 2005). Finally, bimatoprost does not block FP receptor stimulation, indicating a lack of FP receptor interaction (Woodward *et al*., 2003).

(c) *Does prostamide activity result from enzymatic conversion to the free acid (FP agonist)?* Conversion of bimatoprost and prostamide $F_{2\alpha}$ in prostamide-sensitive preparations has been investigated by both indirect (bioassay) and direct methods. Using anandamide as a positive control, no detectable conversion of prostamide $F_{2\alpha}$ or bimatoprost was detected in the isolated feline iris and ciliary body (Matias *et al*., 2004). This corresponds to the low bimatoprost conversion rates observed in isolated bovine and human ocular tissues (Maxey *et al*., 2002; Davies *et al*., 2003; Krauss and Woodward, 2004). Even the ester latanoprost undergoes low enzymatic hydrolysis in certain tissues, as indicated by the very weak activity of latanoprost in the isolated feline (Resul *et al*., 1997) or porcine iris (Hasegawa *et al*., 2006). Finally, potent stimulation of the isolated rabbit uterus was attributed to the activity of intact bimatoprost, since bioassay indicated no enzymatic hydrolysis to the free acid metabolite (Chen *et al*., 2005).

(d) *Is prostamide activity species specific?* Since prostamide activity was first discovered in feline tissues, it was originally suggested that such activity was species specific. Studies comparing responses at feline and human recombinant FP receptors demonstrated a single pharmacological identity, with no meaningful interaction with bimatoprost (Woodward *et al*., 2003) or prostamide $F_{2\alpha}$ (Matias *et al*., 2004). The rabbit uterus was later identified as exquisitely sensitive to bimatoprost but no such activity was observed in the intact rabbit jugular vein (Chen *et al*., 2005). Further confirmation that prostamide activity is species independent was provided by gene regulation studies (Liang *et al*., 2003). Comparing cysteine-rich angiogenic protein 61 (Cyr 61) and connective tissue growth factor expression in human ciliary smooth muscle cells, $PGF_{2\alpha}$ was shown to upregulate connective tissue growth factor and Cyr 61. In contrast to $PGF_{2\alpha}$ and at concentrations that do not stimulate FP receptors, bimatoprost upregulated Cyr 61 but not connective tissue growth factor. The feline iris was used as a positive control and responded in an identical manner with respect to Cyr 61 and connective tissue growth factor expression (Liang *et al*., 2003).

Issues relating to species, tissue and metabolism were thereby addressed. One salient feature of these studies was that whenever prostamide $F_{2\alpha}$ effects in cells and tissues were manifest, a response to $PGF_{2\alpha}$ was also apparent, albeit not necessarily identical. This comparative agonist activity profile for FP receptor stimulation and prostamide $F_{2\alpha}$ mimetics made further pharmacological analysis extremely difficult. Prostamide pharmacology could be explained in two ways: (1) a receptor population that preferentially recognizes prostamide $F_{2\alpha}$ and coexists with FP receptors (2) an FP receptor subclass that equally recognizes both $PGF_{2\alpha}$ and prostamide $F_{2\alpha}$. To address the latter hypothesis, one further series of agonist studies was performed in the feline iris. These involved isolated feline iris cells, with Ca^{2+} signalling monitored by fluorescence confocal microscopy (Spada *et al*., 2005). These studies revealed that bimatoprost and FP receptor agonists ($PGF_{2\alpha}$, 17-phenyl $PGF_{2\alpha}$) stimulated entirely different cells (Spada *et al*., 2005). No overlap occurred. These studies provided evidence for the existence of a population of receptors that exclusively recognize prostamides. These studies also provided the impetus to search for an antagonist.

Antagonist pharmacology

Studies with agonists were pursued to the point where it appeared that the putative prostamide receptor was dedicated to selectively interact with neutral PGs. Definitive

pharmacological characterization required, however, a selective antagonist that blocked either (1) prostanoid FP receptor or (2) prostamide activity.

Several drugs have been claimed to block prostanoid FP receptors, but subsequent experiments have failed to provide confirmation (Table 1; Sharif *et al*., 2001). AL-8810 has been reported to be a selective FP receptor antagonist (Griffin *et al*., 1999) and accordingly, its utility was investigated. The results were mixed. On studying the effects of AL-8810 on FP receptor-mediated upregulation of the orphan nuclear receptor Nur 77, it behaved as a satisfactory antagonist with little or no residual agonist activity (Liang *et al*., 2004). These results did not transition into Ca^{2+} signalling studies in cells stably expressing human recombinant FP receptors. Careful analysis of other reports on AL-8810 indicates measurable FP receptor stimulation (Griffin *et al*., 1999; Hutchinson *et al*., 2003). Unfortunately, AL-8810 behaved as a weak but high efficacy agonist in the feline iris (Woodward *et al*., 2007). AL-8810 was, therefore, unsuitable for delineating prostanoid FP and prostamide receptor pharmacology in the feline iris. This obligated the discovery of a prostamide antagonist.

The strategy for identifying a prostamide antagonist was to use antagonists for receptors in cluster 2 of the molecular evolution classification (Narumiya *et al*., 1999) and then derivatize them to the C-1 amide analogue. Cluster 2 comprises EP_1, FP and TP receptors. The TP receptor antagonists were considered as particularly useful as several potent and structurally diverse TP receptor antagonist series have been designed. Moreover, antagonists for other prostanoid receptors have originated from a TP antagonist as the starting template. The prostamide receptor antagonists were discovered based on the oxabicycloheptane analogue BMS 180,291 (Webb *et al*., 1993) and were AGN 204396 and AGN 204397 (Krauss and Woodward 2006; Woodward *et al*., 2007). Structures AGN 204396 and 204397 are 3(-2-[(1*R*,2*R*,3*S*,4*R*)-3-[-4-cyclohexyl-butylcarbarnoyl)-oxazol-2-yl]-7-oxa-bicyclo[2.2.1]hept-2-ylmethyl)-4-fluro-phenyl)-propyl-ethylamide and hydroxyethylamide, respectively. The pharmacological activity profile is provided in Table 2. They are highly selective prostamide antagonists, with no meaningful off-target activity at prostanoid receptors, including FP receptors constitutively expressed in the feline iris. AGN 204396 and 204397 are also TP antagonists but no functional TP receptors are present in most prostamide preparations, and, therefore, no complicating pharmacology exists.

Table 2 Pharmacology of prostamide antagonists AGN 204396 and AGN 204397

Receptor	*204396 K_b (nM)*	*AGN 204397 K_b (nM)*
Prostamide	2635	3099
DP_1	NA	NA
EP_1	NA	NA
EP_2	NA	NA
EP_3	NA	NA
EP_4	NA	NA
FP	NA	NA
IP	NA	NA
TP	1.5	1.5

Abbreviation: prostamide, prostaglandin-ethanolamide.
Prostamide refers to activity in the feline iris sphincter preparation; other preparations are human recombinant prostanoid receptors.

It should be noted that the feline iris is highly responsive to both prostamides and prostanoid FP receptor agonists, which makes the feline iris a particularly useful preparation for investigating prostamide pharmacology and its relationship to prostanoid pharmacology. AGN 204396 (Woodward *et al*., 2007) and AGN 204397 (Figure 3) antagonized the contractile effects of prostamide $F_{2\alpha}$ and its analogue bimatoprost, but not those of $PGF_{2\alpha}$ and synthetic FP agonists in the feline iris (Figure 3). Consistent with previous feline iris studies, where Ca^{2+} signalling was monitored in individual cells (Spada *et al*., 2005), antagonist studies also suggest that the prostamide receptor does not meaningfully interact with $PGF_{2\alpha}$ but preferentially recognizes prostamides. The contractile effects of AL-8810 were not affected by AGN 204396 pretreatment, providing further evidence for prostamide and prostanoid FP receptors as distinct entities. Finally, the prostamide antagonist AGN 204396 did not affect contraction produced by PGE_2-glyceryl ester, a COX-2-derived metabolite of the endocannabinoid 2-AG (Kozak *et al*., 2002; Nirodi *et al*., 2004).

AGN 204396 and AGN 204397 are considered the first prototypical prostamide antagonists but are not very potent. AGN 204396 has a pA_2 of 5.64 (Woodward *et al*., 2007). Very recently, more potent antagonists have been designed (Selcia Ltd, Ongar, Essex, England). These are typified by AGN 211334 (Wan *et al*., 2007), which is more than one order of magnitude more potent than AGN 204396 and AGN 204397. AGN 211334 was used to provide more definitive evidence that the effects of bimatoprost on conventional aqueous humour outflow are prostamide receptor mediated (Wan *et al*., 2007). For the future, AGN 211334 and its congeners are likely to be sufficiently potent to be useful for pharmacological studies in living animals. The structures of the prostamide antagonists AGN 204396, AGN 204397 and AGN 211334 are provided in Figure 4.

Therapeutics

Prostamide research is in its infancy and the direct involvement of prostamides and PG-glyceryl esters in disease processes remains to be determined. Upregulation of COX-2, coupled to increased anandamide levels, may lead to the formation of prostamides as major products at sites of inflammation and infection (Glass *et al*., 2005). In previous studies using antibody-based methods, prostamides may have been misidentified as PGs, since commercial antibodies to PGE_2 and $PGF_{2\alpha}$ exhibited major cross-reactivity with the corresponding prostamides (Glass *et al*., 2005). It is possible that the therapeutic benefit of COX-2 inhibitors is, at least in part, derived from a reduction in prostamide and PG-glyceryl ester levels. The newly developed prostamide antagonists may be very helpful in testing this concept.

The prostamide area of research has, however, already yielded one successful therapeutic. This is the antiglaucoma drug bimatoprost. The unique pharmacology of bimatoprost was recognized some time ago (Woodward *et al*., 1994) but was not elucidated as a prostamide $F_{2\alpha}$ mimetic until

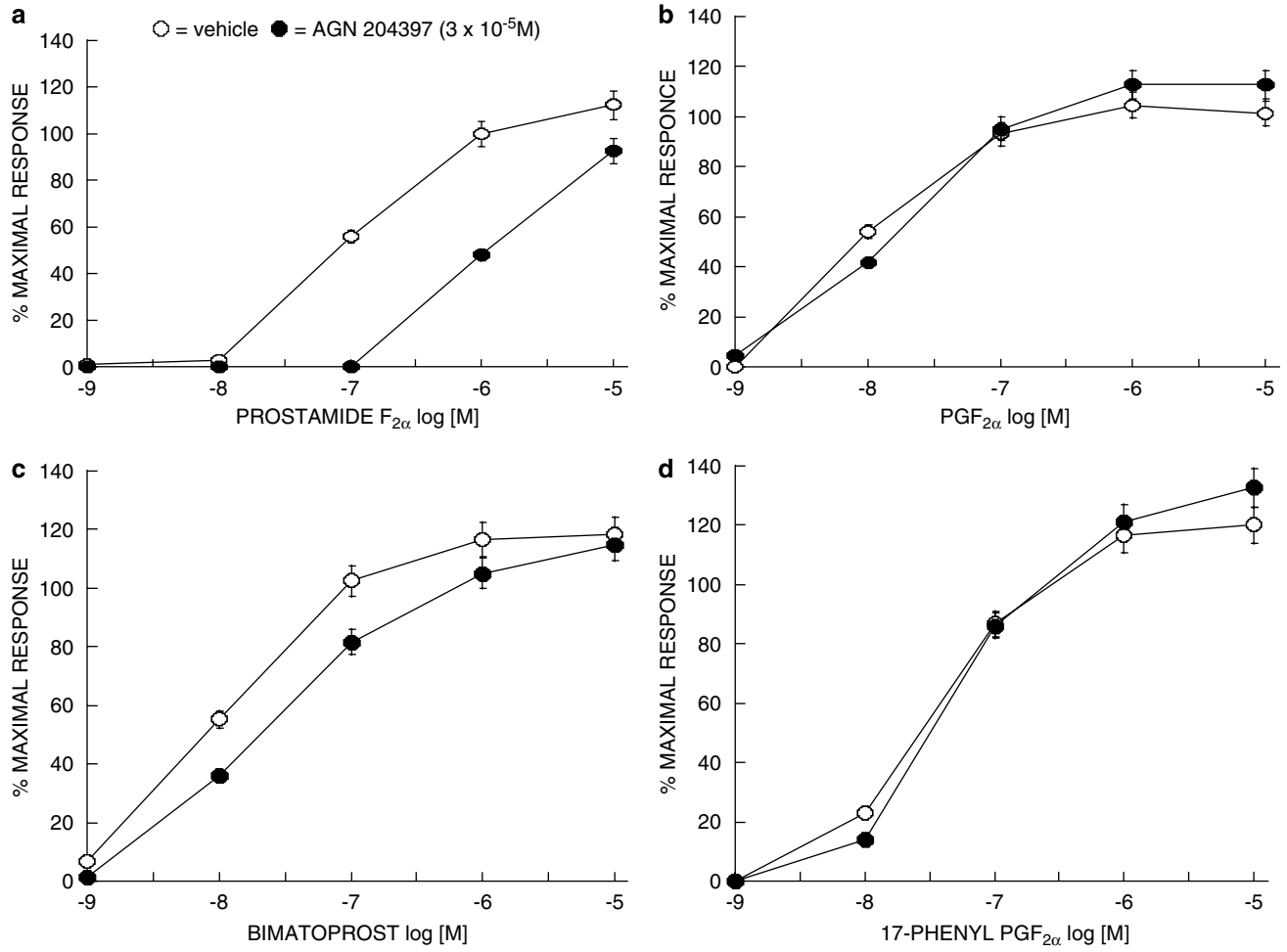

Figure 3 Effect of AGN 204397 (3×10^{-5} M) on contraction of the feline iris produced by (**a**) prostamide $F_{2\alpha}$ (**b**) $PGF_{2\alpha}$ (**c**) bimatoprost (**d**) and 17-phenyl $PGF_{2\alpha}$. Open symbols represent vehicle treated preparations, closed symbols represent preparations that received AGN 204397. Values are mean ± s.e.m.; $n = 4$.

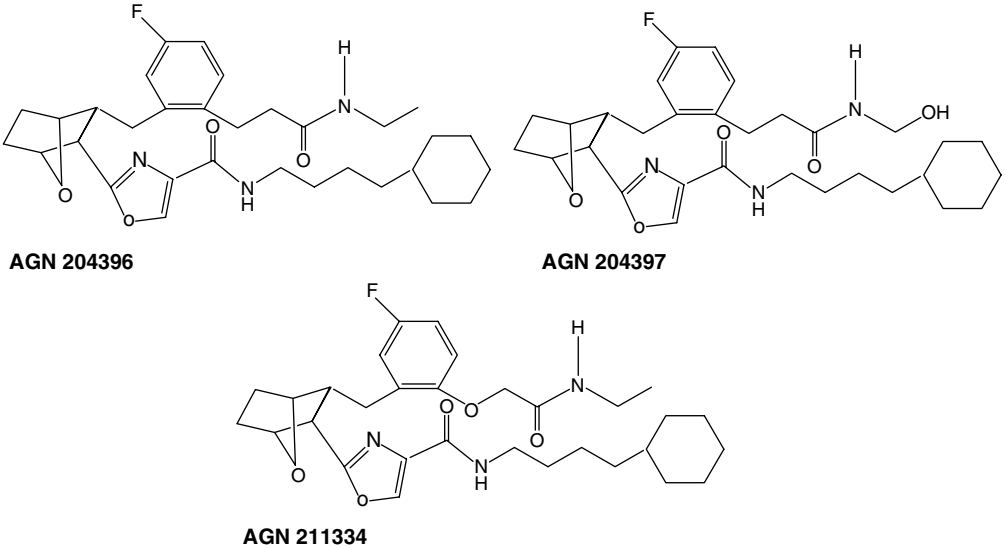

Figure 4 Structure of prostamide antagonists.

recently. The pharmacological distinction between bimatoprost and latanoprost has been made at the clinical level. Glaucoma patients refractory to latanoprost treatment were found to be susceptible to bimatoprost, which produced a pronounced lowering of intraocular pressure (Williams, 2002; Gandolfi and Cimino, 2003). In 'glaucomatous'

monkeys that responded to latanoprost, travoprost and bimatoprost, a combination of bimatoprost and latanoprost produced a greater lowering of trough and peak intraocular pressure than a latanoprost/travoprost combination (Gagliuso *et al*., 2004). Given these results, combined latanoprost/bimatoprost therapy could even be considered as a glaucoma treatment regimen.

Bimatoprost is a potent and highly efficacious ocular hypotensive. Topical application to the ocular surface produces significant decreases in intraocular pressure at doses as low as 0.001% in dogs and monkeys (Woodward *et al*., 2004). Clinical effects are similar, although the dose–response relationship is steeper in glaucomatous patients (Laibovitz *et al*., 2001). Randomized, controlled clinical studies have shown that bimatoprost is a safe, well-tolerated and highly effective antiglaucoma drug (Dubiner *et al*., 2001; Sherwood and Brandt, 2001; Higginbotham *et al*., 2002; Noecker *et al*., 2003; Parrish *et al*., 2003). Taken together, clinical evidence and experience indicate that bimatoprost is the most efficacious antiglaucoma drug currently available. The difference in absolute mm Hg is only about 0.5–1 but, when intraocular pressure approaches the normal range, each mm Hg of additional ocular hypotensive efficacy is of significant value in preventing visual field loss (Woodward and Chen, 2007). Bimatoprost efficacy may be related to a dual mechanism of action on aqueous humour outflow that involves both uveoscleral and trabecular meshwork/Schlemm's canal pathways (Brubaker *et al*., 2001; Christiansen *et al*., 2004; Wan *et al*., 2007). Effects on uveoscleral outflow have been studied directly in monkeys (Woodward *et al*., 2001) and have been correlated with morphological changes in the anterior portion of the ciliary body (Richter *et al*., 2003). In brief, bimatoprost causes a controlled remodelling of the anterior third of the ciliary body resulting in new, organized drainage channels that are partially lined with endothelial cells. This mechanism of action is common to all receptor selective ocular hypotensive PG analogues (Richter *et al*., 2003), with the exception of EP_4 receptor agonists. At the gene regulation level, Cyr 61 appears to provide a common upstream starting point for prostamides, FP agonists and EP_2 agonists (Liang *et al*., 2003). Studies in human models of the trabecular meshwork/Schlemm's canal outflow pathways have demonstrated that bimatoprost produces marked increases in hydraulic conductivity that are prostamide receptor mediated (Wan *et al*., 2007).

Generally regarded as very safe drugs, bimatoprost and prostanoid FP receptor agonists also produce ocular side effects. It should be stressed that these side effects are mostly cosmetic in quality (Stjernschantz, 2001; Hollo, 2007). They are reversible side effects (Hollo, 2007), with the exception of iridial hyperpigmentation. Again, there is pharmacological differentiation between bimatoprost and latanoprost. There is a high incidence of iridial hyperpigmentation associated with latanoprost therapy (Alm *et al*., 2004; Kitazawa, 2006), but this is a rare occurrence with bimatoprost (Sherwood and Brandt, 2001; Cohen *et al*., 2004). Side effects common to bimatoprost, latanoprost and related compounds are ocular surface hyperaemia, eyelash hypertrichosis and periorbital hyperpigmentation, albeit with differing incidence and severity (Hollo, 2007; Woodward and Chen, 2007). It should be noted that not all of these side effects are undesirable.

Luxuriant eyelash growth is regarded as a bonus that such antiglaucoma drugs provide. It is also envisaged that application of, for example, bimatoprost to the cutaneous surface and margins of the eyelids may provide a semipermanent substitute for 'eye-liner' and 'eye-shadow'. Not to mention a permanent replacement for mascara. Drugs related to bimatoprost may provide the impetus for an era of pharmacocosmetics.

Cloning the prostamide receptor

The next step, now that reasonably potent and selective prostamide antagonists have been invented, is to clone the prostamide receptor. The antagonist direction (Wan *et al*., 2007; Woodward *et al*., 2007) could be further pursued to obtain very high affinity ligands that could be used to isolate the receptor protein. This still remains a difficult and long-term strategy.

It is pertinent to note that, to date, prostamide activity has never been reported as a phenomenon completely independent of prostanoid FP receptor stimulation. Both activities are present in prostamide-sensitive preparations such as the feline lung parenchyma (Woodward *et al*., 2003), feline iris (Liang *et al*., 2003; Matias *et al*., 2004; Spada *et al*., 2005; Woodward *et al*., 2007), rabbit uterus (Chen *et al*., 2005) and human ciliary smooth muscle cells (Liang *et al*., 2003). In addition, reports from studies involving prostanoid FP receptor knockout mice claim that bimatoprost-induced ocular hypotension is attenuated (Crowston *et al*., 2005; Ota *et al*., 2005). Taken together, these results suggest that prostamide and FP receptors may be encoded by the same gene. Following this line of reasoning, the possibility that FP receptor mRNA splicing variants (Fujino *et al*., 2000; Sakamoto *et al*., 2002; Vielhauer *et al*., 2004) may account for prostamide activity appears attractive. This is an important hypothesis to test and, if correct, would unify disparate studies of mixed origin.

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Conflict of interest

The authors state no conflict of interest.

References

Alm A, Schoenfelder J, McDermott J (2004). A 5-year, multicenter, open-label, safety study of adjunctive latanoprost therapy for glaucoma. *Arch Ophthalmol* **122**: 957–965.

Berglund BA, Boring DL, Howlett AC (1999). Investigation of structural analogs of prostaglandin amides for binding to and activation of CB_1 and CB_2 cannabinoid receptors in rat brain and human tonsils. *Adv Exp Med Biol* **469**: 527–533.

Brubaker RF, Schoff EO, Nau CB, Carpenter SP, Chen K, Van Den Burgh AM (2001). Effect of AGN 192024, a new ocular hypotensive agent, on aqueous dynamics. *Am J Ophthalmol* **131**: 19–24.

Burstein SH, Rossetti RG, Yagen B, Zurier RB (2000). Oxidative metabolism of anandamide. *Prostaglandins Other Lipid Mediat* **61**: 29–41.

Chen J, Senior J, Marshall K, Abbas F, Dinh H, Dinh T *et al.* (2005). Studies using isolated uterine and other preparations show bimatoprost and prostanoid FP agonists have different activity profiles. *Br J Pharmcol* **144**: 493–501.

Christiansen GA, Nau CB, McLaren JW, Johnson DH (2004). Mechanism of ocular hypotensive action of bimatoprost (Lumigan) in patients with ocular hypertension or glaucoma. *Ophthalmology* **111**: 1658–1662.

Cohen JS, Gross RL, Cheetham JK, VanDenburgh AM, Bernstein P, Whitcup SM (2004). Two-year double-masked comparison of bimatoprost with timolol in patients with glaucoma or ocular hypertension. *Surv Ophthalmol* **49**: S45–S52.

Coleman RA, Humphrey PPA, Kennedy I, Lumley P (1984). Prostanoid receptors: the development of a working classification. *Trends Pharmacol Sci* **5**: 303–306.

Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB (1996). Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature* **384**: 83–87.

Crowston JG, Lindsey JD, Morris CA, Wheeler LA, Medeiros FA, Weinreb RN (2005). Effect of bimatoprost on intraocular pressure in prostaglandin FP receptor knockout mice. *Invest Ophthalmol Vis Sci* **46**: 4571–4577.

Davies SS, Ju WK, Neufeld AH, Abran D, Chemtob S, Roberts LJ (2003). Hydrolysis of bimatoprost (Lumigan) to its free acid by ocular tissue *in vitro*. *J Ocular Pharmacol Ther* **19**: 45–54.

De Petrocellis L, Cascio MG, Di Marzo V (2004). The endocannabinoid system: a general view and latest additions. *Br J Pharmacol* **141**: 765–774.

Dubiner H, Cooke D, Dirks M, Stewart W, Vandenburg AM, Felix C (2001). Efficacy and safety of bimatoprost in patients with elevated intraocular pressure. *Surv Opthalmol* **45**: S353–S360.

Fujino H, Pierce KL, Srinivasan D, Protzman CE, Krauss AH-P, Woodward DF *et al.* (2000). Delayed reversal of shape change in cells expressing FP_β prostanoid receptor, possible role of receptor resensitization. *J Biol Chem* **275**: 29907–29914.

Gagliuso DJ, Wang RF, Mittag TW, Podos SM (2004). Additivity of bimatoprost or travoprost to latanoprost in glaucomatous monkey eyes. *Arch Ophthalmol* **122**: 1342–1347.

Gandolfi SA, Cimino L (2003). Effect of bimatoprost on patients with primary open-angle glaucoma on ocular hypertension who are nonresponders to latanoprost. *Ophthalmology* **110**: 609–614.

Glass M, Hong J, Sato TA, Mitchell MD (2005). Misidentification of prostamides as prostaglandins. *J Lipid Res* **46**: 1364–1368.

Griffin BW, Klimko P, Crider JY, Sharif NA (1999). AL-8810: a novel prostaglandin $F_{2\alpha}$ analog with selective antagonist effects at the prostaglandin $F_{2\alpha}$ (FP) receptor. *J Pharmacol Exp Ther* **290**: 1278–1284.

Hampson AJ, Hill WA, Zan-Philips M, Makriyannis A, Leung E, Eglen RM *et al.* (1995). Anandamide hydroxylation by brain lipoxyenase: metabolite structures and potencies at the cannabinoid receptor. *Biochem Biophys Acta* **1259**: 173–179.

Hasegawa Y, Nishimura J, Niiro N, Hirano K, Ishibashi T, Kanaide H (2006). Prostaglandin $F_{2\alpha}$, but not latanoprost, increases the Ca^{2+} sensitivity of the pig iris sphincter muscle. *Invest Ophthalmol Vis Sci* **47**: 4865–4871.

Hellberg MR, Sallee VL, McLaughlin MA (2001). Preclinical efficacy of travoprost, a potent and selective FP prostaglandin receptor agonist. *J Ocul Pharmacol Ther* **17**: 421–432.

Higginbotham EJ, Schuman JS, Goldberg I, Gross RL, Vandenburgh A, Chen K *et al.* (2002). One-year, randomized study comparing bimatoprost and timolol in glaucoma and ocular hypertension. *Arch Ophthalmol* **120**: 1286–1293.

Hollo G (2007). The side effects of the prostaglandin analogues. *Expert Opin Drug Saf* **6**: 45–52.

Hutchinson J, Marshall K, Senior J (2003). Preliminary studies using a putative FP-receptor antagonist, AL-8810, on isolated mouse uterus. *pA_2 Online* **1**: 038P.

Kitazawa Y (2006). An open-label multicenter study on the efficacy and safety of topical use of latanoprost for 156 weeks. *Jap J Clin Ophthalmol* **60**: 2047–2054.

Koda N, Tsutsui Y, Niwa H, Ito S, Woodward DF, Watanabe K (2004). Synthesis of prostaglandin F ethanolamide by prostaglandin F synthase and identification of bimatoprost as a potent inhibitor of the enzyme. New enzyme method by LC/GSI/MS. *Arch Biochem Biophys* **424**: 128–136.

Komoto J, Yamada T, Watanabe K, Woodward DF, Takusaga F (2006). Prostaglandin $F_{2\alpha}$ formation from prostaglandin H_2 by prostaglandin F synthase (PGFS): crystal structure of PGFS containing bimatoprost. *Biochem* **45**: 1987–1996.

Kozak KR, Crews BC, Morrow JD, Wang LH, Ma YH, Weinander R *et al.* (2002). Metabolism of the endocannabinoids, 2-arachidonylglycerol and anandamide, into prostaglandin, thromboxane and prostacyclin glycerol esters and ethanolamides. *J Biol Chem* **277**: 44877–44885.

Krauss AH-P, Woodward DF (2004). Update on the mechanism of action of bimatoprost: a review and discussion of new evidence. *Surv Ophthalmol* **49**: S5–S11.

Krauss AH-P, Woodward DF (2006). Prostamide receptor antagonists. US Patent 7,045,634.

Laibovitz RA, Van Den Burgh AM, Felix C, David R, Batoosingh A, Rosenthal A *et al.* (2001). Comparison of the ocular hypotensive lipid AGN 192024 with timolol. Dosing, efficacy and safety evaluation of a novel compound for glaucoma management. *Arch Ophthalmol* **199**: 994–1060.

Liang Y, Li C, Guzman VM, Chang WW, Evinger A, Pablo JV *et al.* (2004). Upregulation of orphan nuclear receptor Nur 77 following $PGF_{2\alpha}$, bimatoprost and butaprost treatments. Essential role of a protein kinase C pathway involved in the EP_2 receptor activated Nur 77 gene transcription. *Br J Pharmacol* **142**: 737–748.

Liang Y, Li C, Guzman VM, Evinger AJ, Protzman CE, Krauss AH-P *et al.* (2003). Comparison of $PGF_{2\alpha}$, bimatoprost (prostamide) and butaprost (EP_2 agonist) on Cyr 61 and CTGF gene expression. *J Biol Chem* **278**: 27267–27277.

Maddox YT, Ramwell PW, Shiner CS, Corey EJ (1978). Amide and 1-amino derivatives of prostaglandins as prostaglandin antagonists. *Nature* **273**: 549–552.

Matias I, Chen J, De Petrocellis L, Bisogno T, Ligresti A, Fezza F *et al.* (2004). Prostaglandin ethanolamides (prostamides): *in vitro* pharmacology and metabolism. *J Pharmacol Exp Ther* **309**: 745–757.

Maxey KM, Johnson JL, Labreque J (2002). The hydrolysis of bimatoprost in corneal tissue generates a potent prostanoid FP receptor agonist. *Surv Ophthalmol* **47**: 34–40.

Narumiya S, Sugimoto Y, Ushikubi F (1999). Prostanoid receptors: structure, properties, and functions. *Pharmacol Rev* **79**: 1193–1226.

Nirodi CS, Crews BC, Kozak KR, Morrow JD, Marnett LJ (2004). The glyceryl ester of prostaglandin E_2 mobilizes calcium and activates signal transduction in RAW 264.7 cells. *Proc Nat Acad Sci USA* **101**: 1840–1845.

Noecker RS, Dirks MS, Choplin NT, Bernstein P, Batoosongh AL, Whitcup SM (2003). A six-month randomized clinical trail comparing the intraocular pressure lowering efficacy of bimatoprost and latanoprost in patients with ocular hypertension or glaucoma. *Am J Ophthalmol* **135**: 55–63.

Ota T, Aibara M, Narumiya S, Araie J (2005). The effects of prostaglandin analogues on IOP in prostanoid FP receptor-deficient mice. *Invest Ophthalmol Vis Sci* **46**: 4159–4163.

Parrish RK, Palmberg P, Sheu W-P, for the XLT Study Group (2003). A comparison of latanoprost, bimatoprost, and travoprost in patients with elevated intraocular pressure: a 12-week randomized, masked evaluation multicenter study. *Am J Ophthalmol* **135**: 688–703.

Pinto JC, Potié F, Rice KC, Boring D, Johnson MR, Evans DM *et al.* (1994). Cannabinoid receptor binding and agonist activity of amides and esters of arachidonic acid. *Mol Pharmacol* **46**: 516–522.

Resul B, Stjernschantz J, Selen G, Bito L (1997). Structure–activity relationships and receptor profiles of some ocular hypotensive prostanoids. *Surv Ophthalmol* **41**: S47–S52.

Richter M, Krauss AH-P, Woodward DF, Lütjen-Drecoll E (2003). Morphological changes in the anterior eye segment after long term treatment with different receptor selective prostaglandin

agonists and a prostamide. *Invest Ophthalmol Vis Sci* **44**: 4419–4426.
Rockwell CE, Kaminski NE (2004). A cyclooxygenase metabolite of anandamide causes inhibition of interleukin-2 secretion in murine splenocytes. *J Pharmacol Exp Ther* **34**: 683–690.
Ross RA, Craib SJ, Stevenson LA, Pertwee RG, Henderson A, Toole J *et al*. (2002). Pharmacological characterization of the anandamide cyclooxygenase metabolite: prostaglandin E_2 ethanolamide. *J Pharmacol Exp Ther* **301**: 900–907.
Sakamoto K, Ishii Y, Onodera T, Sugano T (2002). Cloning and characterization of the novel isoforms for $PGF_{2\alpha}$ receptor in the bovine corpus luteum. *DNA Seq* **13**: 307–311.
Schaaf TK, Hess H-J (1979). Synthesis and biological activity of carboxyl-terminus modified prostaglandin analogs. *J Med Chem* **22**: 1340–1346.
Sharif N, Crider JY, Davis TL (2000). AL-3138 antagonizes FP prostanoid receptor-mediated inositol phosphates generation: comparison with some purported FP antagonists. *J Pharm Pharmacol* **52**: 1529–1539.
Sharif N, Kelly CR, Crider JY (2002). Agonist activity of bimatoprost, travoporst, latanoprost, unoprostone isopropyl ester and other prostaglandin analogs at the cloned human ciliary body FP prostaglandin receptor. *J Ocular Pharmacol Ther* **18**: 313–324.
Sharif NA, Kelly CR, Williams GW (2003). Bimatoprost (Lumigan) is an agonist at the cloned human ocular FP prostaglandin receptor: real-time FLIPR-based intracellular Ca^{2+} mobilization studies. *Prostaglandins Leukot Essent Fatty Acids* **68**: 27–33.
Sharif NA, Williams GW, Kelly CB (2001). Bimatoprost and its free acid are prostaglandin FP receptor agonists. *Eur J Pharmacol* **432**: 211–213.
Sherwood M, Brandt J (2001). Six-month comparison of bimatoprost once-daily and twice daily with timolol twice-daily in patients with elevated intraocular pressure. *Surv Ophthalmol* **45**: S361–S368.
Spada CS, Krauss AH-P, Woodward DF, Chen J, Protzman CE, Nieves AL *et al*. (2005). Bimatoprost and prostaglandin $F_{2\alpha}$ selectively stimulate intracellular calcium signaling in different cat iris sphincter cells. *Exp Eye Res* **80**: 135–145.
Stjernschantz JW (2001). From $PGF_{2\alpha}$-isopropyl ester to latanoprost: a review of the development of Xalatan. *Invest Ophthalmol Vis Sci* **42**: 1134–1145.
Ueda N, Yamamoto K, Yamamoto S, Tokunaga T, Shirakawa E, Shinaki H *et al*. (1995). Lipoxygenase-catalyzed oxygenation of arachidonylethanolamide, a cannabinoid receptor agonist. *Biochem Biophys Acta* **1254**: 127–134.
Vielhauer GA, Fujino H, Regan JW (2004). Cloning and localization of hFPs: a six-transmembrane mRNA splice variant of the human FP prostanoid receptor. *Arch Biochem Biophys* **421**: 175–185.
Wan Z, Woodward DF, Cornell C, Fliri H, Martos J, Pettit S *et al*. (2007). Bimatoprost and conventional drainage. *Invest Ophthalmol Vis Sci* **48** (in press).
Webb ML, Liu ECK, Monschizadegan H, Hedberg A, Misra RN, Goldenberg H *et al*. (1993). Binding and function of a potent new thromboxane receptor antagonist, BMS 180,291, in human platelets. *J Pharmacol Exp Ther* **264**: 1387–1394.
Weber A, Ni J, Ling KH-J, Acheampong A, Tang-Liu DD-S, Cravatt BF *et al*. (2004). Formation of prostaglandin 1-ethanolamides (prostamides) from anandamide in fatty acid amide hydrolose knockout (FAAH −/−) mice analyzed by high performance liquid chromatography with tandem mass spectrometry. *J Lipid Res* **45**: 757–763.
Wei BQ, Mikkelsen TJ, McKinney MK, Lander ES, Cravatt BF (2006). A second fatty acid amide hydrolase with variable distribution among placental mammals. *J Biol Chem* **281**: 36569–36578.
Williams RD (2002). Efficacy of bimatoprost in glaucoma and ocular hypertension unresponsive to latanoprost. *Adv Ther* **19**: 275–281.
Woodward DF, Andrews SW, Burk RM, Garst ME (1994). Non-acidic cyclopentane heptanoic acid, 2 cycloalkyl or arylalkyl derivatives as therapeutic agents. US Patent 5,352,708.
Woodward DF, Chen J (2007). Fixed-combination and emerging glaucoma therapies. *Expert Opin Emerging Drugs* **12**: 313–327.
Woodward DF, Krauss AH-P, Chen J, Gil DW, Kedzie KM, Protzman CE *et al*. (2000). Replacement of the carboxylic acid group of prostaglandin $F_{2\alpha}$ with a hydroxyl or methoxy substituent provides biologically unique compounds. *Br J Pharmacol* **130**: 1933–1943.
Woodward DF, Krauss AH-P, Chen J, Lai RK, Spada CS, Burk RM *et al*. (2001). The pharmacology of bimatoprost (Lumigan™). *Surv Ophthalmol* **45**: S337–S345.
Woodward DF, Krauss AH-P, Chen J, Liang Y, Li C, Protzman CE *et al*. (2003). Pharmacological characterizations of a novel antiglaucoma agent, bimatoprost (AGN 192024). *J Pharmacol Exp Ther* **305**: 772–785.
Woodward DF, Krauss AH-P, Wang JW, Protzman CE, Nieves AL, Liang Y *et al*. (2007). Identification of an antagonist that selectively blocks the activity of prostamides (prostaglandin-ethanolamides) in the feline iris. *Br J Pharmacol* **150**: 342–352.
Woodward DF, Lawrence RA (1994). Identification of a single (FP) receptor associated with prostanoid-induced Ca^{2+} signals in Swiss 3T3 cells. *Biochem Pharmacol* **47**: 1567–1574.
Woodward DF, Phelps RL, Krauss AH-P, Weber A, Short B, Chen J *et al*. (2004). Bimatoprost: a novel anti-glaucoma agent. *Cardiovasc Drug Rev* **22**: 103–120.
Yang W, Ni J, Woodward DF, Tang-Lui DD-S, Ling J (2005). Enzymatic formation of prostamide $F_{2\alpha}$ from anandamide involves as newly identified intermediate metabolite, prostamide H_2. *J Lipid Res* **46**: 2745–2751.
Yu M, Ives D, Ramesha CS (1997). Synthesis of prostaglandin E_2 ethanolamide from anandamide by cyclooxygenase-2. *J Biol Chem* **272**: 21181–21186.