Recent Progress in Prostaglandin F\textsubscript{2α} Ethanolamide (Prostamide F\textsubscript{2α}) Research and Therapeutics


Department of Biological Sciences, Allergan Inc., Irvine, California

Abstract

Prostamide (prostaglandin ethanolamide) research emerged from two distinct lines of research: 1) the unique pharmacology of the antiglaucoma drug bimatoprost and 2) the discovery that endocannabinoid anandamide was converted by COX-2 to a series of electrochemically neutral prostaglandin (PG) ethanolamides. Bimatoprost pharmacology was found to be virtually identical to that of prostamide F\textsubscript{2α}. The earliest studies relied on comparison of agonist potencies compared with PGF\textsubscript{2α} and synthetic prostaglandin F\textsubscript{2α} (FP) receptor agonists. The subsequent discovery of selective and potent prostamide receptor antagonists (AGN 211334-6, as shown in Fig. 3) was critical for distinguishing between prostamide and FP receptor-mediated effects. The prostamide F\textsubscript{2α} receptor was then modeled by cotransfected the wild-type FP receptor with an mRNA splicing variant (altFP\textsubscript{4}). Bimatoprost is now used therapeutically for treating both glaucoma and eyelash hypotrichosis. Bimatoprost also stimulates hair growth in isolated human scalp hair follicles. A strong effect is also seen in mouse pelage hair, where bimatoprost essentially halves the onset of hair regrowth and the time to achieve full hair regrowth in shaved mice. Beyond glaucoma and hair growth, bimatoprost has potential for reducing fat deposition. Studies to date suggest that preadipocytes are the cellular target for bimatoprost. The discovery of the enzyme prostamide/PGF synthase was invaluable in elucidating the anatomic distribution of prostamide F\textsubscript{2α}. High expression in the central nervous system provided the impetus for later studies that described prostamide F\textsubscript{2α} as a nociceptive mediator in the spinal cord. At the translational level, bimatoprost has already provided therapeutics in two distinct areas and the use of both prostamide agonists and antagonists may provide other useful medicaments.

I. Introduction

Prostaglandin ethanolamide (prostamide) research evolved from two independent but convergent lines of research: 1) the discovery that anandamide was a substrate for cyclooxygenase-2 (COX)-2 (Yu et al., 1997) and 2) the pharmacology of the prostamide F\textsubscript{2α} analog bimatoprost (Woodward et al., 2008a,b). In the past...
decade, the biosynthetic pathway for prostamide F\textsubscript{2\alpha} has been elucidated, and a new enzyme prostamide/PGF synthase has been discovered (Moriuchi et al., 2008). Bimatoprost pharmacology, originally identified according to agonist potency and radioligand binding competition studies (Woodward et al., 2003), has been extended by the discovery and utilization of antagonists (Woodward et al., 2007) and structural identification of the target receptor (Liang et al., 2008). Bimatoprost was approved as a drug for reducing intraocular pressure in patients with open-angle glaucoma and ocular hypertension a decade ago. More recently, bimatoprost was approved as a drug for increasing eyelash length, thickness, and darkness in patients with eyelash hypotrichosis. This has added further impetus to prostamide/bimatoprost research, which seems likely to continue as new potential therapeutic applications continue to emerge. Extending from prostamide research, a pivotal role for anandamide is evident from knowledge of its oxygenation to the prostamides, which are pharmacologically distinct from its actions on cannabinoid and transient receptor potential vanilloid type-1 receptors (Di Marzo and De Petrocellis, 2012).

II. Biosynthesis of Prostamide F\textsubscript{2\alpha}

The biosynthetic pathways illustrated in Fig. 1. The first step in the biosynthesis of the prostamides from anandamide has been established as oxygenation by COX-2 (Yu et al., 1997; Kozak et al., 2002). Subsequent conversion of the endoperoxide intermediate (prostamide H\textsubscript{2}) leads to a diverse range of individual prostamides. The biosynthesis of only prostamides D\textsubscript{2} and F\textsubscript{2\alpha} has been the subject of studies on the actual PG synthases (Koda et al., 2004; Moriuchi et al., 2008). A key element in successful elucidation of prostamide F\textsubscript{2\alpha} biosynthesis was the development of methodologies for stabilizing the substrate prostamide H\textsubscript{2} (Moriuchi et al., 2008). Prostamide D\textsubscript{2} formation involves PGD synthase (Kozak et al., 2002), but an alternative pathway to prostamide D\textsubscript{2} has been previously reported (Koda et al., 2004). Prostamide H\textsubscript{2} and prostamide D\textsubscript{2} were both found to be substrates for PGF synthase, which is part of the aldo-keto reductase (AKR) superfamily and also known as AKR1C3 (Koda et al., 2004; Barski et al., 2008). Prostamide F\textsubscript{2\alpha} and 11\beta-prostamide F\textsubscript{2\alpha} are formed from prostamide H\textsubscript{2} and prostamide D\textsubscript{2}, respectively (Koda et al., 2004). PGF synthase (AKR1C3) is a broad-spectrum AKR. It is noteworthy that bimatoprost, a structural analog of prostamide F\textsubscript{2\alpha}, is an inhibitor of PGF synthase (Komoto et al., 2006). The distribution of PGF synthase is broad, but the physiologic significance of PGF synthase is uncertain. Knockout mouse studies have proven difficult because of the close sequence homology with other enzymes in the AKR family, and the use of bimatoprost as an inhibitor is compromised by its potent prostamide F\textsubscript{2\alpha} mimetic properties (Woodward et al., 2008a,b).

More recently, an enzyme, which converts prostamide H\textsubscript{2} to prostamide F\textsubscript{2\alpha} with a high degree of substrate specificity, has been discovered (Moriuchi et al., 2008). It is termed prostamide/PGF synthase and is a member of the thioredoxin superfamily (Moriuchi et al., 2008). It is abundantly distributed in the brain and spinal cord, with preferential localization in myelin sheaths (Yoshikawa et al., 2011). Again, the physiological significance remains to be fully elucidated but, given its anatomic position in myelin sheaths, it has been suggested as playing a role in the formation and maintenance of central myelin based on increased expression during postnatal development (Yoshikawa et al., 2011). COX-2, rather than COX-1, is constitutively expressed in the white matter regions (Yoshikawa et al., 2011), but the relative roles of PGF\textsubscript{2\alpha} and prostamide F\textsubscript{2\alpha} remain to be determined.

III. Prostamide F\textsubscript{2\alpha}/Bimatoprost Pharmacology

Research to date indicates that the receptors involved in mediating the effects prostamide F\textsubscript{2\alpha} and other pharmacologically novel prostanoids involve heterodimerization of prostanoid receptors (Wilson et al., 2004; Liang et al., 2008; Woodward et al., 2011).

A. Agonists

Initial pharmacological studies on the prostamides were performed in isolated tissues. Prostamide E\textsubscript{2} was the first prostamide investigated, and its effects on the isolated guinea pig trachea preparation could not be readily attributed to interaction with prostanoid prostaglandin E\textsubscript{2} receptors (Ross et al., 2002). In isolated feline preparations, the rank order of potency was prostamide F\textsubscript{2\alpha} > D\textsubscript{2} > E\textsubscript{2} > 11\beta-prostamide F\textsubscript{2\alpha}, with the relative positions of prostamide F\textsubscript{2\alpha} and 11\beta-prostamide F\textsubscript{2\alpha} being quite different from those of PGF\textsubscript{2\alpha} and 11\beta-PGF\textsubscript{2\alpha}, in potency ranking for the corresponding prostaglandins (PGs) (Matias et al., 2004). A continuous verification of this potency rank order for enzymatically derived prostamides did not progress beyond isolated tissues to cellular, organ culture, and living animal studies. The synthetic analog bimatoprost was almost invariably selected as the compound representative of prostamide agonist properties, with reference to prostamide F\textsubscript{2\alpha} as the naturally occurring substance (Fig. 2). Bimatoprost received greater attention by virtue of being a drug used clinically to treat glaucoma and eyelash hypotrichosis. As a consequence, the agonist properties of prostamide F\textsubscript{2\alpha} are often provided by relating to bimatoprost data.

The first pharmacological evidence for a distinct receptor relied, for the most part, on the differential
pharmacology of prostamide F\(_{2\alpha}\) and bimatoprost in isolated tissue preparations expressing functional prostaglandin F\(_{2\alpha}\) (FP) receptors. In certain isolated tissue preparations, bimatoprost was essentially equi-potent with PGF\(_{2\alpha}\) and selective FP receptor agonists; these included the feline lung parenchyma and iris (Woodward et al., 2003, 2007; Matias et al., 2004) and the rabbit uterus (Chen et al., 2005). In marked contrast, intact bimatoprost exhibited no more than residual activity in other isolated FP receptors tissue preparations; these included uterine preparations obtained from rat, mouse, and human (Chen et al., 2005) and the rabbit jugular vein (Chen et al., 2005), the gerbil colon, and the mouse ileum (Woodward et al., 2003). The earliest cell studies also revealed a substantial separation in potency with respect to Ca\(^{2+}\) signaling in mouse and human fibroblast lines (Woodward et al., 2003). Moreover, pretreatment with bimatoprost did not block the Ca\(^{2+}\) response to PGF\(_{2\alpha}\) and its congeners (Woodward et al., 2003). This provided initial evidence to suggest that bimatoprost did not exert its effects by interacting with prostanoid FP receptors.

Bimatoprost is highly efficacious in lowering intraocular pressure (Woodward et al., 2003). Its ocular hypotensive effects in nonhuman primates involve increased uveoscleral outflow exclusively (Woodward et al., 2010a), but in humans, bimatoprost increases aqueous humor outflow by both the uveoscleral and trabecular meshwork pathways (Brubaker et al., 2001; Christiansen et al., 2004; Lim et al., 2008). The effects of bimatoprost have been examined on the component cells of these two pathways (Wan et al., 2007; Stamer et al., 2010) (see Fig. 2). The conventional, pressure dependent pathway via the trabecular meshwork involves both trabecular meshwork cells and the endothelial cells that line Schlemm’s canal. By use of the cellular dielectric spectroscopy technique, functional bimatoprost receptors were identified present on both human cell types (Stamer et al., 2010; see Fig. 2). By using the same technique, functional bimatoprost receptors were found on human ciliary smooth muscle cells, which are the cells responsible for increased uveoscleral outflow and the associated remodeling of the anterior portion of the ciliary body (Richter et al., 2003). Finally, bimatoprost has been shown to upregulate

---

**Fig. 1.** Prostamide biosynthetic pathway.
Cyr61 expression in human ciliary smooth muscle cells and trabecular meshwork cells (Liang et al., 2003). This gene may potentially contribute to ciliary body remodeling and the creation of organized outflow channels as a key anatomic component of increased uveoscleral outflow (Richter et al., 2003).

From these numerous agonist studies, it was not possible to discern the nature of the ligand recognition site. Two possibilities existed: 1) a receptor that recognizes prostamides F2α, which is coexpressed with the FP receptor or 2) an FP receptor subtype that recognizes prostamide F2α and PGF2α. Returning back to the feline iris preparation, this question was answered using cells isolated from this tissue and comparing bimatoprost Ca2+ signals with those produced by the FP receptor agonist 17-phenyl PGF2α (Spada et al., 2005). Bimatoprost and 17-phenyl PGF2α stimulated entirely different cells, thereby proving the existence of a distinct receptor that uniquely exhibits functional responsiveness to bimatoprost/prostamide F2α. This provided adequate information for an attempt to design antagonists for the putative prostamide receptor.

B. Antagonists

The design of prostamide antagonists was successfully accomplished by using the TP antagonist BMS 180,291 (S)-1S-(1α,2α,3α,4α)-2-[3-[4-[(n-pentylamino)carbonyl]-2-oxazolyl]-7-oxabicyclo[2.2.1]hept-2-yl]methyl]benzenepranoic acid (Webb et al., 1993) as a template, a molecule that contains an oxabicyclo moiety at its core. The prototypes were AGN 204396 and AGN 204397 (Woodward et al., 2007, 2008b). These compounds blocked the effect of bimatoprost and prostamide F2α in the feline iridial preparation but not the effects of PGF2α.
and selective FP receptor agonists (Woodward et al., 2007). Although these prototype antagonists provided definitive confirmation of a receptor that preferentially responds to prostamide F$_{2\alpha}$ and bimatoprost, with a pA$_{2}$ in the region of 5.5, their modest potency was deemed as generally less than ideal and absolutely unsuitable for living animal studies where drug bioavailability is an issue. Insertion of an O atom at position 3 of the prototypical molecules (Fig. 3) increased potency by two orders of magnitude (Woodward et al., 2008b). These compounds, designated AGN 211334, AGN 211335, and AGN 211336 were also highly selective for prostamide receptors, with no meaningful activity at all other prostanoïd receptors except TP. These compounds were used on several occasions to establish (or refute) the involvement of prostamide receptors in mediating the effects of bimatoprost. In the perfused human ocular anterior segment preparation, the effects of bimatoprost on aqueous humor outflow via the trabecular meshwork route were abolished by the prostamide antagonist AGN 211334 (Wan et al., 2007). In addition, increased hydraulic conductivity produced by bimatoprost in a monolayer of trabecular meshwork cells was also attenuated by AGN 211334 (Wan et al., 2007). Functional prostamide receptors were found to be present in all cells involved aqueous humor outflow using cellular dielectric spectroscopy. The presence of prostamide receptors on human trabecular meshwork cells was confirmed and, in addition, such receptors were identified on the endothelial cells that line Schlemm’s canal (Stamer et al., 2010). These results imply that bimatoprost influences aqueous humor drainage by interacting with both the major cell types that control pressure dependent aqueous humor outflow via the trabecular meshwork. Bimatoprost also increases pressure-independent aqueous humor outflow through the uveoscleral pathway (Richter et al., 2003; Woodward et al., 2010a), and functional prostamide receptors were found to be present on ciliary smooth muscle cells (Stamer et al., 2010). The effects of bimatoprost on trabecular meshwork and endothelial cells of Schlemm’s canal were inhibited by the prostamide antagonist AGN 211336 (Wan et al., 2007; Stamer et al., 2010). AGN 211336 was also found to abolish the increase in hair growth produced by bimatoprost in isolated human hair follicles (Khidir et al., 2013). It also reduced adipogenic responses to bimatoprost (Silvestri et al., 2013). In a study involving prostamide F$_{2\alpha}$, as opposed to bimatoprost, AGN 211336 reduced the nociceptive neuronal firing and behavioral response to spinal application of prostamide F$_{2\alpha}$ (Gatta et al., 2012). Finally, a very close congener AGN 211335, provided invaluable pharmacological support for those studies intended to clone the prostamide receptor (Liang et al., 2008).

C. Cloning the Receptor

The discovery of potent and selective prostamide antagonists provided definitive evidence for a separate pharmacological entity and, in turn, impetus for cloning the receptor. Clues for the identity of the receptor were provided by taking into account the existent, pertinent information at that point in time. This is summarized as follows: 1) prostamide F$_{2\alpha}$ and bimatoprost-responsive preparations also responded to
PGF₂α (although in many cases PGF₂α activation was not accompanied by responses to prostamide F₂α and its analogs); 2) bimatoprost-induced ocular hypertensive activity was abolished in FP receptor knockout mice (Crowston et al., 2005; Ota et al., 2005); 3) an FP receptor mRNA splicing variant was shown to be active (Pierce et al., 1997, Fujino et al., 2000); 4) prostanoid receptor heterodimerization was shown to create novel activation/binding sites (Wilson et al., 2004). These data suggested that the FP receptor gene was key to encoding the prostamide receptor. Thus, attention was directed toward mRNA splicing variants. A previous study on an ovine FP receptor variant had shown differential signaling with respect to receptor desensitization (Pierce et al., 1997, Fujino et al., 2000). The first human FP mRNA splicing variant had also been characterized (Vielhauer et al., 2004), and this was followed with the discovery of six further examples (Liang and Woodward, 2008). Thus, the possibility that FP receptor splicing variants accounting for prostamide receptor activity in some way was contemplated, and stable transfectants were prepared for study. This notion was reinforced by the identification of FP receptor variants in the human ciliary muscle, an important anatomic target site for the ocular hypertensive effects of bimatoprost (Richter et al., 2003; Woodward et al., 2010a).

The FP receptor variants were studied using the fluorometric imaging plate reader instrumentation to detect biologic activity as a Ca²⁺ signal. None of the human FP receptor mRNA splicing variants produced a change in intracellular Ca²⁺ concentration in response to PGF₂α, its analogs, or bimatoprost. In the context of FP mRNA splicing variants, this left FP receptor heterodimerization as the only likely explanation for the existence of a prostamide F₂α/bimatoprost sensitive receptor. It was noteworthy that the 8-epiPGE₂ receptor site was created by the formation of an IPTP receptor model heterodimer (Wilson et al., 2004). This being the case, we attempted to create a receptor using transfection of the gene products and comparing the response to that of the singular elements. This experimental strategy was successful. Cotransfection of the wild-type FP receptor and an mRNA splicing variant (altFP₄) produced a repertoire of responses, including a model for prostamide/bimatoprost pharmacology (Liang et al., 2008).

The model for prostamide F₂α/bimatoprost activity was established in two parts. The first series of studies involved comparing Ca²⁺ signaling responses to bimatoprost and PGF₂α at 10⁻⁷ M in cells transfected with the wild-type FP receptor, the altFP₄ mRNA splicing variant, or a cotransfection of both. Neither PGF₂α nor bimatoprost stimulated the altFP₄ splicing variant. PGF₂α produced a marked Ca²⁺ signal in cells transfected with the wild-type FP receptor; this comprised a Ca²⁺ spike that transitioned smoothly into a well-maintained, elevated [Ca²⁺]i. Bimatoprost produced only a very modest and transient response. In the wild-type/altFP₄ cotransfectants, the PGF₂α Ca²⁺ signaling was identical to that observed for the wild-type FP receptor. Bimatoprost responses were quite different in the cotransfectants compared with those observed for the wild-type receptor transfectants. Although bimatoprost elicited a transient Ca²⁺ spike identical to that produced by PGF₂α, in every respect, the transient Ca²⁺ signal to bimatoprost was followed by an oscillating signal with an elongated frequency. The pharmacology of this biphasic signaling was then investigated. The prostamide antagonist AGN 211335 dose-dependently inhibited the secondary, oscillating Ca²⁺ signal response to bimatoprost but did not alter the transient Ca²⁺ signal (Liang et al., 2008). AGN 211335 did not affect the response to PGF₂α in the wild-type FP/altFP₄ cotransfectants. The prostamide receptor model was then confirmed using two entirely different biologic end-points. These were myosin light chain phosphorylation and Cyr61 expression. Cyr61 was of particular interest because bimatoprost has been shown to upregulate Cyr61 in human ciliary smooth muscle cells (Liang et al., 2003), and these cells represent the drug target for lowering intraocular pressure by increasing uveoscleral outflow (Richter et al., 2003; Woodward et al., 2010a). Likewise, AGN 211335 dose-dependently blocked bimatoprost-induced Cyr61 upregulation and myosin light chain phosphorylation, indicating pharmacological uniformity with respect to the broad activity spectrum of bimatoprost.

IV. Biologic Functions/Therapeutics

A. Glaucoma

Glaucoma is a leading cause of blindness. It is an optic neuropathy that results in progressive deterioration of the visual field. Elevated intraocular pressure is...
a major risk factor for the development of glaucoma (Quigley et al., 1994; The AGIS Investigators, 2000; Leske et al., 2003). Results from the “Early Manifest Glaucoma” trial demonstrated that lowering intraocular pressure by even 1 mm Hg is clinically relevant for reducing the risk of development or progression of glaucoma (Leske et al., 2003). This positions bimatoprost, a highly effective ocular hypotensive agent, as a clinically important drug in the ophthalmologists’ armamentarium of antiglaucoma drugs. For this reason, the ocular hypotensive mechanism of action merits attention.

In common with the prostanoid FP receptor agonist ester prodrug latanoprost (Toris et al., 2008), bimatoprost lowers intraocular pressure by increasing outflow of aqueous humor via the uveoscleral pathway (Woodward et al., 2010a) in nonhuman primates. Uveoscleral outflow is pressure independent and occurs in the anterior portion of the ciliary body, involving the formation of organized drainage structures (Richter et al., 2003). For latanoprost, the exclusive uveoscleral outflow mechanism seen in monkeys was initially reported to transition into human subjects (Toris et al., 1993), but subsequent investigations claimed an additional effect on pressure-dependent aqueous humor outflow via the trabecular meshwork (Lim et al., 2008). An effect on trabecular outflow has been confirmed for latanoprost (Bahler et al., 2008) and bimatoprost (Wan et al., 2007) using the isolated perfused human ocular anterior segment preparation. This is a particularly useful preparation because it effectively isolates the trabecular outflow pathway for independent study.

The ocular hypotensive activity of bimatoprost has also been investigated at the cellular level. Intraocular pressure-independent effects on aqueous humor outflow are regulated by ciliary smooth muscle cells located in the anterior segment of the ciliary body (Richter et al., 2003; Woodward et al., 2010a). The pressure-dependent trabecular outflow pathway is influenced by two distinct cell types (Fig. 2). One cell type is the trabecular meshwork cells, which are arranged along support beams located in the ocular anterior chamber angle. Schlemm’s canal is the collector channel for aqueous humor; it is lined with endothelial cells that are positioned to regulate the exit of aqueous humor (Fig. 2). Bimatoprost has been shown to increase hydraulic conductivity across a monolayer of trabecular meshwork cells, an effect that is blocked by the prostamide antagonist AGN 211334 (Wan et al., 2007). The existence of prostamide pharmacology in trabecular meshwork cells has been confirmed using cellular dielectric spectroscopy (Stamer et al., 2010), an electrical impedance based assay that permits rapid, high-throughput screening for functional pharmacology. Functional, bimatoprost-sensitive receptors have been identified in trabecular meshwork, endothelial cells of Schlemm’s canal, and ciliary smooth muscle cells (Stamer et al., 2010), thereby providing a cellular basis for the effects of bimatoprost.

**Fig. 4.** Effect of the prostamide antagonist AGN 211336 on the decrease dog in intraocular pressure (IOP) produced by bimatoprost (A) and by latanoprost (B). ●, Eyes that received AGN 211336 (5% once daily). ○, Eyes that received vehicle (once daily). Both eyes received bimatoprost once daily to provide a paired comparison. Values are mean ± S.E.M., n = 6. Reproduced with permission from Woodward et al., 2009.
on both trabecular and uveoscleral aqueous humor outflow pathways as elucidated by conventional aqueous humor dynamics studies. See Fig. 2 for anatomic location of the three key ocular cells involved in controlling aqueous humor outflow.

Coming full circle, by returning to studies in the living eye, the pharmacology of bimatoprost on intraocular pressure has been investigated. By use of a paired experimental design, the prostamide antagonist AGN 211336 attenuated the ocular hypotensive response to bimatoprost but not to that of the FP receptor agonist ester prodrug latanoprost (Fig. 4). Thus, the differential pharmacological basis of the effects of bimatoprost and latanoprost on intraocular pressure were confirmed by employing a selective prostamide antagonist.

Bimatoprost effects in the eye have also been interpreted in a nonpharmacological manner. Contemporary with the introduction of bimatoprost as an antiglaucoma drug, it was claimed that bimatoprost was a prodrug and that the putative enzymatic hydrolysis product, 17-phenyl PGF2α, was a prodrug. However, the pharmacology of latanoprost and bimatoprost are also prodrugs, receptor expression, or a more diverse series of compounds including the esters latanoprost and travoprost.

Finally, indirect evidence indicates that the pharmacology of latanoprost and bimatoprost are also differentiated at the clinical level. Bimatoprost is efficacious in patients unresponsive to latanoprost (Williams, 2002; Gandolfi and Cimino, 2003; Sonty et al., 2008); this would almost certainly not be the case if these drugs were pharmacologically identical. Bimatoprost is also additive to FP agonist prodrugs (Gagliuso et al., 2004).

B. Hair Growth

The enhancing effect of 0.03% bimatoprostophthalmic solution on eyelash growth was empirically discovered as a side effect in clinical trials for intraocular hypertension therapy (Fagien, 2010; Yeolin et al., 2010; Elias et al., 2011). Bimatoprost seems to stimulate eyelash growth in more patients (13%) than in the latanoprost treatment group (4%) (Gandolfi et al., 2001; Law, 2010). In a 6-month study on 52 patients with glaucoma or ocular hypertension, a 46.2% increase in eyelash growth was reported (Inoue et al., 2012). Marketed as Latisse by Allergan Inc., 0.03% bimatoprost was approved by the U.S. Food
and Drug Administration in 2008 for promoting eyelash growth. In a 1-year retrospective study involving 37 patients with eyelash alopecia areata, 43.24% of them showed acceptable cosmetic results (Vila and Camacho Martinez, 2010). In two different human eyelash studies, the mean eyelash length of the bimatoprost group was longer than the placebo group by approximately 0.9 mm (Wester et al., 2010; Woodward et al., 2010b). Bimatoprost seems to make eyelashes grow longer, darker, and thicker by extending the anagen phase of hair growth. It stimulates melanogenesis but with no change in melanocyte proliferation or prostaglandin-related inflammation and increases the size of the dermal papilla and hair bulb in humans (Kapur et al., 2005; Cohen, 2010). It increases eyelash number in follicles without follicle neogenesis in rodents (Tauchi et al., 2010). In a detailed morphometric analysis of bimatoprost-treated mouse eyelashes, eyelash thickening occurred but only in eyelashes of short and medium length (Tauchi et al., 2010). A significant increase in the percentages of follicles in anagen phase was apparent, with a significant decrease in follicles in the telogen and late catagen phases (Tauchi et al., 2010). In recent studies, bimatoprost dose dependently prolonged anagen in human scalp follicles in vitro and stimulated the initiation of anagen of rodent pelage follicles in vivo (Woodward et al., 2012; Khidhir et al., 2013). As a pharmacological correlation, the prostamide receptor (Liang et al., 2008) for bimatoprost was detected in the dermal papilla of human scalp follicles (Khidhir et al., 2013). In mouse skin and blood, bimatoprost was found as an intact molecule not metabolized to the free acid 17-phenyl PGF2\(_{\alpha}\) (Woodward et al., 2012), indicating its stimulatory effect on hair growth is through interaction with prostamide receptors. Bimatoprost is currently under clinical investigation for treating male pattern baldness.

C. Adipose Tissue

Much like the effects of bimatoprost on hair growth, the effect on periorbital fat was discovered serendipitously. Case reports, in which glaucoma patients treated in a single eye with bimatoprost, described a deepening of the lid sulcus and a general appearance of sunken eyes (Peplinski and Albiani Smith, 2004; Filippopoulos et al., 2008; Yam et al., 2009; Aydin et al., 2010). Additional case reports described patients in which periorbital fat pads were reduced in size and appearance, and this effect was reversible upon cessation of treatment (Peplinski and Albiani Smith, 2004; Filippopoulos et al., 2008; Yam et al., 2009; Aydin et al., 2010). Recently, a similar reduction of periorbital fat was reported with the prostaglandin analogs travoprost and latanoprost (Nakakura et al., 2011; Park et al., 2011). A general effect of PGF2\(_{\alpha}\) analogs is not completely surprising, because it was described nearly a decade and half earlier that PGF2\(_{\alpha}\) can inhibit adipocyte function and differentiation (Casimir et al., 1996; Miller et al., 1996). The mechanism was recently described for inhibition of adipocyte differentiation by PGF2\(_{\alpha}\), as being via a G\(_{q}\)-calcium-calcineurin pathway (Liu and Clipstone, 2007). These case reports are the first that suggest that this mechanism could also be active in vivo. Choi et al. (2012) compared bimatoprost with PGF2\(_{\alpha}\) and three analogs on isolated primary human orbital preadipocytes for antiadipogenic potential. All drug treatments reduced adipocyte differentiation, with bimatoprost producing the most significant reduction (Choi et al., 2012). Although this study highlights that adipocytes from the target tissue are affected similar to other adipocytes in vitro, a lack of dose-ranging studies leaves the relative effectiveness of any one drug in question.

As opposed to travoprost and latanoprost, bimatoprost is a prostamide and has a distinct receptor (as

![Fig. 5. Comparison of bimatoprost and PGF2\(_{\alpha}\) effects on human preadipocytes (A) and mature adipocytes (B) using cellular dielectric spectroscopy instrumentation (dZeic). Values are mean ± S.E.M.; n = 3.](image)
discussed in section III) (Liang et al., 2008). Therefore, there is some question whether there are shared signaling pathways. By use of the cellular dielectric spectroscopy technique described in Stamer et al. (2010) for investigating bimatoprost treatment of ocular cells, we examined human preadipocytes treated with bimatoprost or PGF2α (Fig. 5A). Both bimatoprost and PGF2α are active on human preadipocytes but much of the potency of bimatoprost is lost in mature adipocytes (Fig. 5B), although PGF2α potency is much less affected. Furthermore, bimatoprost suppresses early adipogenic markers peroxisome proliferator-activated receptor γ and CCAAT enhancer-binding protein α in mouse as well as differentiating human adipocytes (Silvestri et al., 2013). In addition, late markers of adipocyte maturation such as fatty acid-binding protein 4 and adiponectin, as well as lipid accumulation, were impaired (Silvestri et al., 2013). Bimatoprost exerts its antiadipogenic effect through its specific receptor FP/FPalt4 heterodimer, because it can be blocked by a prostamide receptor antagonist (Silvestri et al., 2013). In addition, a mitogen-activated protein kinase inhibitor can inhibit the antiadipogenic effect of both bimatoprost and PGF2α (Silvestri et al., 2013). These data suggest the signaling pathway leads to mitogen-activated protein kinase activation, which is required for the antiadipogenic effect of both compounds. We also observed that PGF2α, ethanolamidine has similar antiadipogenic activity, suggesting a potential de novo role for prostamides in the regulation of adipose tissue development and/or maintenance (Silvestri et al., 2013). Although fat atrophy has been described in published case reports, leading to speculation that bimatoprost and prostaglandin analogs affect mature adipocytes lipolysis or lipogenesis, we have not observed any effect of prostamides on mature adipocytes in vitro (unpublished observations). Additionally, the FP and prostamide receptor are downregulated upon adipocyte differentiation, which suggests that mature adipocytes are not the major target of these lipid species (Silvestri et al., 2013). This suggests that mature adipocytes may undergo turnover in adipose tissue in vivo. In fact, several studies show that adipocyte turnover in vivo is greater than originally appreciated (Rigamonti et al., 2011; Tchoukalova et al., 2012). The precise mechanism by which prostamides play a role in this process and under which conditions this regulation occurs in vivo remain to be fully elucidated.

D. Central Nervous System

The discovery of prostamide/PGF synthase and its distribution (Moriuchi et al., 2008) was not only instrumental in defining prostamide F2a as an endogenous prostamide (Gatta et al., 2012), it also provided direction for further investigation of biologic roles. In adult mice, prostamide/PGF synthase was found to be widely expressed in the brain, predominantly in the white matter (Yoshikawa et al., 2011). Likewise, in the spinal cord, prostamide/PGF synthase was preferentially expressed in white matter structures, whereas in the gray matter areas only the nerve fibers expressed this enzyme. The functional significance of prostamide F2a in the spinal cord was determined according to studies on intrathecal administration (Gatta et al., 2012). Intrathecal prostamide F2a exerted proalgesic effects in the paw withdrawal latency test associated with knee inflammation; this was attenuated by the prostamide antagonist AGN 211336 but not the FP receptor antagonist AL-8810 (Griffin et al., 1999). Consistent with these results, prostamide F2a increased spinal nociceptive neuronal firing, which was abolished by the prostamide antagonist AGN 211336 but again not prevented by the FP antagonist AL-8810 (Gatta et al., 2012). Local knee inflammation resulted in a marked increase in prostamide F2a, whereas spinal cord levels of anandamide were not significantly altered (Gatta et al., 2012).

Finally, there is a preliminary report that a prostamide antagonist inhibited capsaicin-induced ocular surface pain/discomfort (Poloso et al., 2012). Comparative corneal levels of anandamide and prostamide F2a remain to be reported. Because FP receptor antagonism is highly effective in producing analgesia in this model, it seems of dubious clinical relevance because PGF2α and prostamide F2a analogs do not cause discomfort when applied topically to human eyes.

The involvement of prostamide F2a in pain/hyperalgesia cannot be adequately assessed without considering anandamide and its analgesic properties. Anandamide is converted by COX-2 to prostamide F2α, which is also proalgesic (Gatta et al., 2012; Poloso et al., 2012). Thus, COX-2 inhibitors may exert analgesic properties by inhibiting prostamide formation but also by increasing anandamide levels. The enzyme fatty acid amide hydrolase (FAAH) also hydrolyzes anandamide, and inhibition of FAAH is known to increase endogenous anandamide levels and produce analgesia (Lichtman et al., 2004). FAAH inhibitors would also provide more anandamide substrate for COX-2, which may mitigate their analgesic properties by increasing prostamide production. The balance between these events in clinical disease is uncertain. The analgesic mechanisms for COX-2 are obviously more expansive than FAAH inhibition; COX-2 inhibitors prevent conversion of arachidonic acid to PGs. However, COX-1 also subserves PG production, and the particular use of COX-2 inhibitors in treating pain does not argue for a single mechanism. Within the fatty acid amides context, COX-2 inhibitors may exert analgesic effects by preventing both anandamide breakdown and prostamide biosynthesis. Thus, COX-2 inhibitors may produce analgesia by acting at five
different levels to produce their overall effects, as demonstrated in Fig. 6. COX-2 inhibitors would attenuate biosynthesis of proalgesic prostamide F\textsubscript{2α} and PGE\textsubscript{2}-glyceryl ester, increase endogenous endocannabinoid levels, i.e., anandamide and 2-arachidonoylglycerol, and inhibit PG biosynthesis. It is also pertinent to note that analgesic (R)-profens have been reported to maintain endocannabinoid tone by preventing oxygenation by substrate-selective inhibition of COX-2 (Duggan et al., 2011). These findings further underscore the importance of endocannabinoid levels in algesia/analgies.

FAAH has been reported to exert analgesic activity in animal models, which was associated with increased anandamide levels in the central nervous system (Lichtman et al., 2004; Ahn et al., 2011; Caprioli et al., 2012). Early clinical reports do not, however, support these findings, and the FAAH inhibitor PF-04457845 (Ahn et al., 2011) did not reduce pain in patients with osteoarthritis (Di Marzo, 2012; Di Marzo and De Petrocellis, 2012; Huggins et al., 2012). Consideration of Fig. 6 may provide a possible explanation for this unanticipated failure of a highly potent, orally bioavailable FAAH inhibitor in early clinical trials. Monoacylglycerol lipase (MAGL), a major enzyme involved in hydrolytic conversion of 2-AG (Di Marzo, 2008), would be expected to remain at least partly operative during FAAH inhibition (Fig. 6). In the brain, MAGL couples endocannabinoid and PG pathways by contributing to the arachidonic acid pool for PG biosynthesis (Nomura et al., 2011). It appears that MAGL exerts principal control of arachidonic acid and PG levels in brain (Nomura et al., 2011). It follows that 2-AG, arachidonic acid, and levels of its COX-1/COX-2 products are unaffected by FAAH inhibition (Fig. 6), allowing no means of substantially increasing analgesic 2-AG levels or reducing proalgesic PG levels. Inhibition of anandamide hydrolysis by a FAAH inhibitor would likely provide increased anandamide levels but, in turn, increase proalgesic biosynthesis of prostamide F\textsubscript{2α} to counteract any benefit from increased anandamide concentrations (Piscitelli and Di Marzo, 2012).

V. Summary and Conclusions

Much remains unknown and much remains to be done, especially at the basic research level. At the translational research level, the prostamides, in the form of the prostamide F\textsubscript{2α} analog bimatoprost, have been therapeutically successful. Bimatoprost is a potent and highly efficacious antiglaucoma drug, and the side effect of ocular surface redness produced by the original eye drop dosage form has been removed by reformulation and altering the dose without any loss in ocular hypotensive efficacy (Craven et al., 2010). Bimatoprost is also used to treat eyelash hypotrichosis and to pharmaco-cosmetically enhance eyelash growth and appearance. The prognosis for treating scalp disorders that result in hair loss seems good, because bimatoprost stimulates hair growth in isolated, human scalp hair follicles (Khidir et al., 2013). Local application of bimatoprost could also be used to reduce fat deposits, for example under the chin and jowls. A more ambitious objective would be reduction of torso fat or upper limb fat. Other therapeutic possibilities are feasible, and clues may be found in the detailed morphometric study on the effects of bimatoprost in the nonhuman primate eye (Richter et al., 2003).

At the pharmacological level, the earliest studies used isolated tissue preparations (Woodward et al., 2003; Matias et al., 2004; Chen et al., 2005) and suggested the existence of receptors that not only preferentially recognized prostamide F\textsubscript{2α} but also accepted prostamides D\textsubscript{2} and E\textsubscript{2}. Later studies were performed using cells and organ culture (Liang et al., 2003; Spada et al., 2005; Wan et al., 2007; Stamer et al., 2010), and the primary focus was bimatoprost, as a prostamide F\textsubscript{2α} analog. To date, the effects of prostamide D\textsubscript{2} and E\textsubscript{2} remain to be characterized. It follows that the concept of a single receptor that recognizes all prostamides per se remains to be confirmed.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Woodward, Wang, Poloso.


