CHEMICAL REVIEWS

REVIEW

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Prostanoid Receptors

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1. INTRODUCTION

Prostanoids are potent oxygenated lipid molecules that contribute to a wide variety of physiological responses and pathological processes. They are generated by the metabolism of unsaturated 20-carbon fatty acids such as arachidonic acid (AA) through the cyclooxygenase (COX) pathway. Following cellular activation, AA is released from membrane phospholipids by phospholipase A_2 (PLA_2); it is then converted to the prostaglandin (PG) intermediate PGH_2 by COX-1 and COX-2.[1] COX isozymes catalyze a two-step reaction, first cyclizing AA to PGG_2 and then reducing the 15-hydroperoxy group to form PGH_2. Cell-specific PG synthases catalyze the conversion of PGH_2 to five primary bioactive prostanoids, PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2, and thromboxane A_2 (TXA_2) (Figure 1). The importance of this pathway in various diseases, including inflammation, cancer, hypertension, and thrombosis, is underscored by the use of COX-inhibiting nonsteroidal anti-inflammatory drugs (NSAIDs), which inhibit the synthesis of all these prostanoids. Each prostanoid is generated in specific cells in response to a variety of stimuli and acts as an autacoid within the tissue via specific cell-surface receptors.

Prostanoid receptors were first characterized pharmacologically based on functional studies. Studies comparing the potencies of various natural prostanoids and their synthetic analogues in bioassays involving various tissues revealed that each prostanoid has a specific site of action.[2−5] These functional studies culminated in the proposal of a general, working classification of prostanoid receptors, in which they were divided into five basic types, designated DP, EP, FP, IP, and TP, for the PGD_2, PGE_2,

Special Issue: 2011 Lipid Biochemistry, Metabolism, and Signaling

Received: January 5, 2011
Published: August 05, 2011

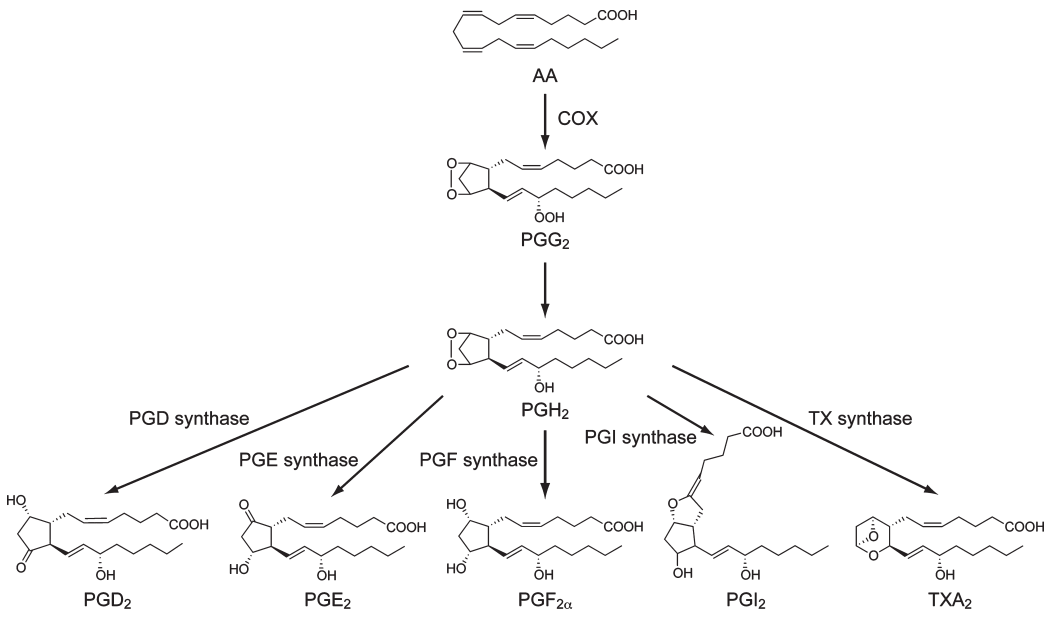

Figure 1. Biosynthetic pathways of prostanoids. The formation of PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2, and TXA_2 from AA is shown. The first two steps (AA to PGG_2 and then to PGH_2) are catalyzed by COX, and the subsequent conversion of PGH_2 to each PG is catalyzed by the respective synthases.

$PGF_{2\alpha}$, PGI_2, and TXA_2 receptors, respectively.[6,7] At each of these receptors, one of the natural prostanoids was at least 1 order of magnitude more potent than any of the other four. There is now evidence for the existence of four subtypes of EP, designated EP1, EP2, EP3, and EP4.[8] Although the original classification system for prostanoid receptors was based on functional studies with a limited range of agonists and an even more limited range of antagonists, it has entirely withstood the scrutiny of subsequent molecular biology studies. Molecular identification of these receptors revealed that this family of eight prostanoid receptor cDNAs encodes seven transmembrane G protein-coupled receptors (GPCRs), which couple to one or more signal transduction pathways.[8−10] In addition, the EP1, EP3, FP, and TP receptors have mRNA splice variants in some species.[11] A second type of PGD_2 receptor, CRTH2 (chemoattractant-receptor homologous molecule expressed by Th2 cells), was subsequently identified molecularly and belongs to the leukocyte chemoattractant receptor family.[12] With reference to its endogenous ligand, CRTH2 is also called DP2, and the first-identified DP receptor is named DP1.

Prostanoids were historically considered mediators of acute inflammation, causing symptoms such as fever, pain, and edema. Although prostanoids' roles in various diseases have been highlighted by studies on the effects of NSAIDs, elucidating the exact role of each prostanoid and its receptor in a given process has been a slow process. The generation and study of specific receptor-deficient mice has clarified the function(s) mediated by each prostanoid acting on each receptor.[13−15] Each receptor's role has also been studied using agonists and antagonists. The stable expression of each cloned receptor in cell lines has enabled high-throughput ligand binding and functional studies, which yielded nonprostanoid scaffolds and other new compounds from which potent and selective agonists and antagonists have been designed.

These studies have revealed that prostanoids play much more fundamental roles in normal physiology than had previously been thought, as well as critical roles in various pathophysiological processes. They also point to the importance of the prostanoids' physiological crosstalk with a diverse array of cytokines, chemokines, neurotransmitters, and other intercellular mediators. It is becoming increasingly clear that the signals conveyed through prostanoid receptors are integrated and coordinated with those originating from other receptors, such as cytokine and neurotransmitter receptors, in various cellular compartments. Together, these interactions constitute a complex signaling network, which regulates many physiological and pathological processes.

This review begins with a synopsis of the general features of prostanoid receptors, highlighting new discoveries about their structures, ligand binding, signaling, and membrane expression. We will then discuss each prostanoid receptor, focusing on the most recent advances in our understanding of its function (revealed mostly by the use of gene knockout mice), especially as it pertains to the immune system and nervous system. We will also discuss agonists and antagonists for each prostanoid receptor. The development of these compounds has been discussed in great detail in recent reviews,[16,17] so here we will review representative agonists and antagonists that are especially relevant to the exploration of receptor function and to therapeutic clinical applications.

2. GENERAL FEATURES OF PROSTANOID RECEPTORS

The human TXA_2 receptor TP was the first prostanoid receptor to be cloned and characterized. It was revealed to be a GPCR with seven transmembrane domains.[18] On the basis of their homology with the TP receptor, seven additional prostanoid receptors, DP1, EP1, EP2, EP3, EP4, FP, and IP, have been cloned.[19−25] The more recently discovered PGD_2 receptor DP2 is also a GPCR, but it is structurally distinct from other prostanoid receptors and belongs to the family of leukocyte chemoattractant receptors.[26]

2.1. Structures

2.1.1. Molecular Characterization. The prostanoid receptors belong to the class A, rhodopsin-like GPCRs. Figure 2 shows

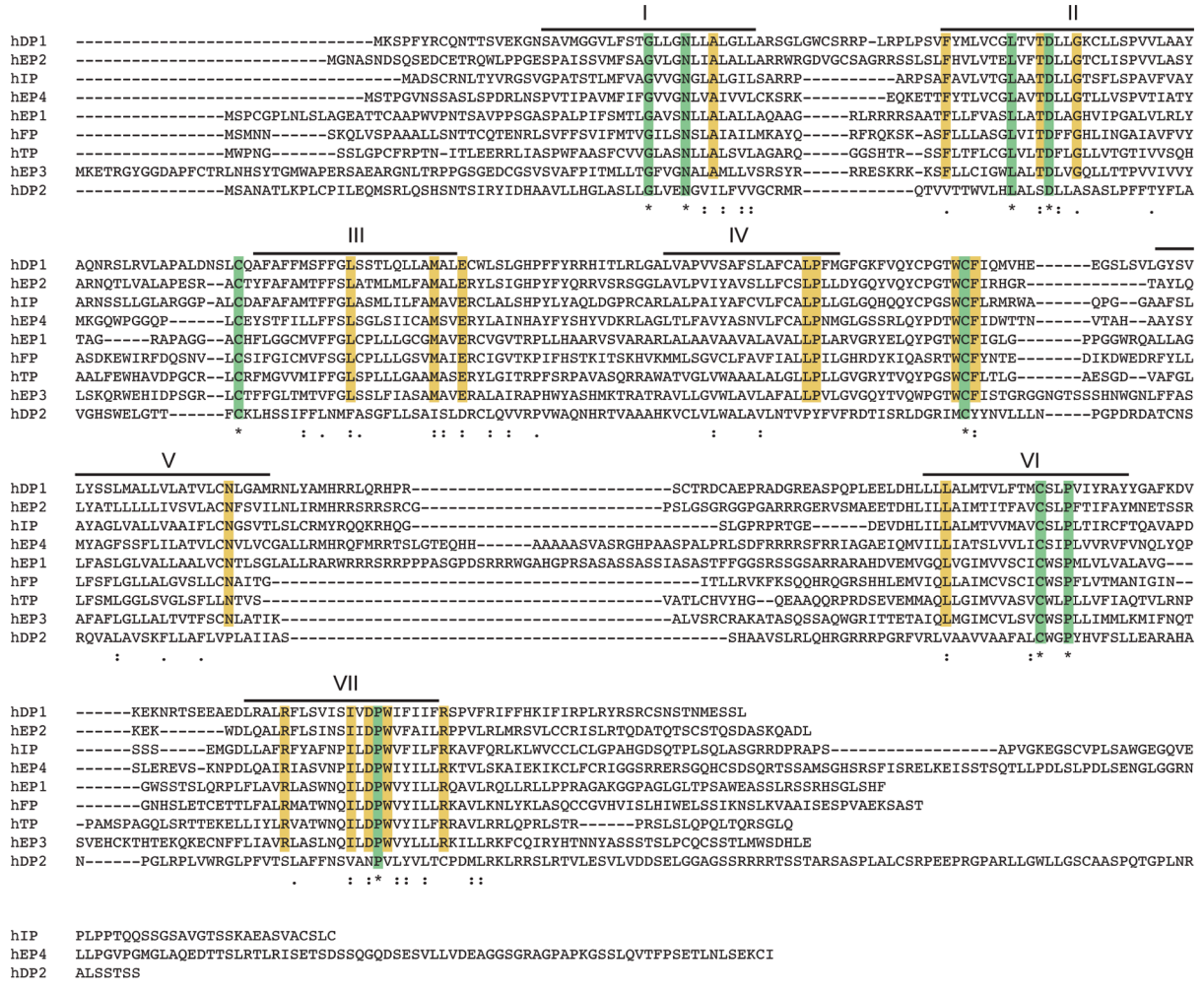

Figure 2. Alignment of the amino acid sequences of the human DP1, DP2, EP1, EP2, EP3, EP4, FP, IP, and TP receptors. Predicted transmembrane domains are shown by overlining, and gaps are indicated by dashes. Asterisks indicate identity, and single and double dots indicate semiconservative and conservative replacements, respectively. Identical amino acid residues with asterisks are also highlighted in green. Amino acid residues with complete homology except for DP2 are highlighted in yellow. The alignment was carried out with ClustalX version 2.1.[237] For the EP3, FP, and TP receptors, the EP3-I, FP_A, and TPα sequences were used, respectively.

an alignment of the primary amino acid sequences of the eight human prostanoid receptors, DP1, EP1, EP2, EP3, EP4, FP, IP, and TP, and the leukocyte chemoattractant receptor family member DP2.[18,21,27−33] The overall homology among the eight prostanoid receptors is not very high, ranging from ∼20% to 30% amino acid identity. That between the DP2 receptor and other prostanoid receptors is <20%. Only 44 amino acid residues are conserved among all the prostanoid receptors including the DP2 receptor. Of these, 9 are identical. If the DP2 receptor is excepted, the prostanoid receptors share 18 more identical amino acid residues. Most of these residues lie within the transmembrane regions, but some are in the second extracellular loop region. The homology among orthologs from different species is high: for example, the amino acid identity between the human and mouse receptors is approximately 70−90%.

The eight prostanoid receptors described are classified into three subfamilies according to their structural and functional similarities.[34] The phylogenetic relationship of each receptor to the others is shown in Figure 3. The DP1-EP2-EP4-IP subfamily members mediate cyclic AMP (cAMP) production, relax smooth muscles, and are termed "relaxant" receptors. Within this subfamily, the DP1 and EP2 receptors are the most closely related, and the EP2 receptor is more closely related to the IP receptor than to the EP4 receptor, even though the EP2 and EP4 receptors share an endogenous ligand, PGE_2. The EP1-FP-TP subfamily members mediate cytosolic Ca^{2+} mobilization and are termed "contractile" receptors, because they induce smooth muscle contraction. The EP3 subfamily members primarily mediate decreases in cAMP and are classified as "inhibitory" receptors. Phylogenic analyses suggest that the ancestral prostanoid receptor was an EP receptor. The leukocyte chemoattractant receptor family, which DP2 receptor belongs to, includes the leukotriene B_4 receptors BLT1 and BLT2.

Additional heterogeneity in prostanoid receptors arises through alternative splicing, which has been reported for the EP1, EP3, FP, and TP receptors.[11] The rat EP1 receptor has a variant that diverges midway through the sixth transmembrane domain.[35] The mouse EP3 receptor has three isoforms, EP3α, EP3β, and EP3γ.[36,37] Although EP3 splice variants have been cloned from many other species, including human, cow, rabbit, and rat, the number and structures of the variants differ according to species.[10] The sheep FP receptor has two isoforms, FP_A and FP_B,[38] whereas

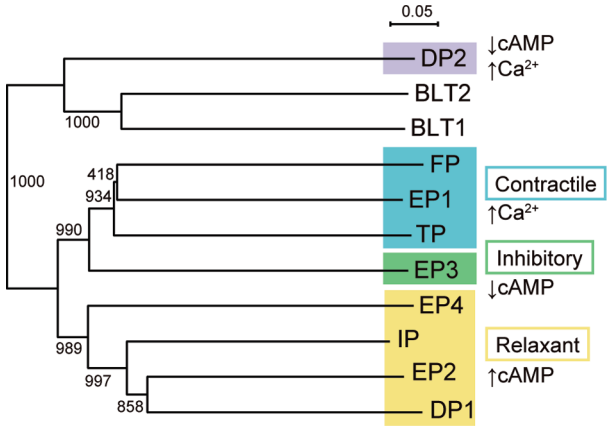

Figure 3. Phylogenetic tree of the human DP1, EP1, EP2, EP3, EP4, FP, IP, and TP receptors with the human DP2 receptor and leukotriene B_4 receptors BLT1 and BLT2. The tree was constructed with ClustalX and visualized with NJplot. Results of bootstrap analysis with 1000 replicates are indicated at branch points. The eight prostanoid receptors can be classified into three groups—relaxant, contractile, and inhibitory—by their structural similarity and the intracellular signaling they induce.

the human and bovine FP receptors have the generic FP_A isoform and an FP_S variant that diverges midway through the sixth transmembrane domain.[39,40] Several additional FP variants that diverge in the sixth transmembrane domain have been identified in human ocular tissues.[41] The human TP receptor has two isoforms, TPα and TPβ.[42] No splicing events have been reported for the DP1-EP2-EP4-IP subfamily.

Except for those of the EP1 and FP receptors, the prostanoid receptor splice variants are divergent C-terminally. These C-terminal variants generally have similar ligand-binding properties. However, these splicing events affect the receptors' G protein-coupling specificity and constitutive activity, as well as their agonist-induced desensitization and internalization.[11]

Variants that diverge in the sixth transmembrane region may not function independently. The rat EP1 variant retains the ligand-binding activity but does not induce signaling unless it is coexpressed with the EP1 or EP4 receptor. This variant appears to attenuate the PGE_2-mediated signaling via the EP1 and EP4 receptors.[35] The human FP variants do not bind ligands, but when they are complexed with the FP receptor, novel ligand-binding sites may be created, as we will see later.[39,41]

2.1.2. Three-Dimensional Structure Models. All prostanoid receptors are members of the GPCR family, whose crystallization has been extremely challenging. The crystal structure of the transmembrane domains of bovine rhodopsin has been a suitable template for constructing working models of mammalian GPCR transmembrane domains, including those of prostanoid receptors.[43] However, it is difficult to build structural models for the extracellular and intracellular loops of these receptors using the corresponding structures of bovine rhodopsin due to a number of differences, including size and sequence variations.

By two-dimensional nuclear magnetic resonance (NMR) experiments, the overall three-dimensional structure of a constrained synthetic peptide that mimics the second extracellular loop of the human TP receptor and interacts with the TP antagonist SQ29,548 was determined.[44] Because the second extracellular loop region of prostanoid receptors is highly conserved, it was hypothesized that the ligand-recognition site for the other prostanoid receptors also involves this region. Indeed, the interaction of the second extracellular loop of the human IP receptor with its agonist iloprost was confirmed by NMR techniques.[45] These studies provided evidence that the second extracellular loop of the TP and IP receptors is involved in forming their ligand-recognition site. Similarly, the NMR structures of the first and third extracellular loops of the TP receptor were determined and used to construct a solution structure.[46] The structure thus determined revealed that the second extracellular loop and the disulfide bond between the first and second extracellular loops play major roles in forming the ligand-recognition pocket. Using a similar technique, the structures of the first, second, and third intracellular loops of the TP receptor were also determined and used to refine the three-dimensional model of the receptor.[47] A G protein-binding cavity formed by the three intracellular loops was predicted by the docking of the C-terminal domain of the α subunit of the G protein G_q.

In the last several years, structures for the β_2-adrenergic receptor (β_2AR), β_1-adrenergic receptor (β_1AR), and A_{2A}-adenosine receptor, which belong to the class A GPCR family, have been reported.[48−50] More recently, the crystal structure of another class A GPCR, the chemokine receptor CXCR4, was elucidated.[51] These studies suggest that there is no general, family conserved ligand-binding pocket, and reveal that the second extracellular loop appears to play an important role in receptor activation, because it is involved in direct ligand binding, ligand recognition, or ligand entry.[52] The future crystallization of prostanoid receptors will further clarify their ligand binding and activation mechanisms and will contribute to the development and refinement of receptor-active drugs.

2.2. Ligand Binding

2.2.1. Ligand-Binding Properties. Each prostanoid receptor binds preferentially to a specific prostanoid over other prostanoids. For example, the EP receptor subtypes bind most potently to PGE_2 with K_d values in the range of 1−40 nM. The FP receptor binds most potently with $PGF_{2\alpha}$ with a K_d value of 1 nM. However, PGE_2 can also bind to the FP receptor and $PGF_{2\alpha}$ can bind to the EP3 receptor with significant affinity. Similarly, prostanoid analogues that have been used in conventional studies are usually not specific for any given receptor. For example, the IP agonist iloprost also binds to the EP1 and EP3 receptors with K_i values of about 20 nM. On the other hand, several compounds highly selective for each receptor have been developed. These agonists and antagonists will be discussed in later sections describing each receptor.

Studies on the ligand binding of prostanoid receptors have demonstrated that most of them exhibit a single class of receptor sites. Functionally, there is evidence that both transmembrane and extracellular domains are involved in ligand binding. For example, a ligand-binding pocket that includes residues in transmembrane domains V and VII has been proposed for the human TP receptor,[53] and one formed by residues in transmembrane domains II, III, and VII has been proposed for the human IP receptor.[54] As discussed earlier, the second extracellular loop region also appears to be involved in ligand binding. However, how the transmembrane and extracellular loop domains are involved in ligand binding or entry remains to be elucidated.

2.2.2. Allosteric Modulators. For decades, the development of ligands in GPCR drug discovery focused on targeting the orthosteric binding site of the receptor. This approach guided the development of classical orthosteric ligands that directly activate the target receptor (agonists) or block the actions of the

endogenous ligand (antagonists). More recently, small molecules that target allosteric sites on GPCRs have been pursued.[55,56] Ligands that bind to these allosteric sites are called allosteric modulators, because they elicit a conformational change in the receptor while still allowing, in many instances, the concomitant binding of orthosteric ligands, thus modulating the pharmacological characteristics of the latter agent.

Recently, allosteric modulators for the human EP2 receptor were described.[57] EP2 activation with the natural agonist PGE_2 stimulates adenylate cyclase (AC), resulting in elevated cAMP levels. A library of small molecules was screened for compounds that potentiate the cAMP response to a low concentration of PGE_2, which led to the identification of a series of modulators (Figure 4). These compounds share a thiophene carboxylate scaffold. The most active compound increases the potency of PGE_2 on the EP2 receptor 4- to 5-fold and shows substantial neuroprotection in an excitotoxicity model. Interestingly, these EP2 modulators form nanoparticles in aqueous solution that appear to act as active reservoirs for bioactive monomers.

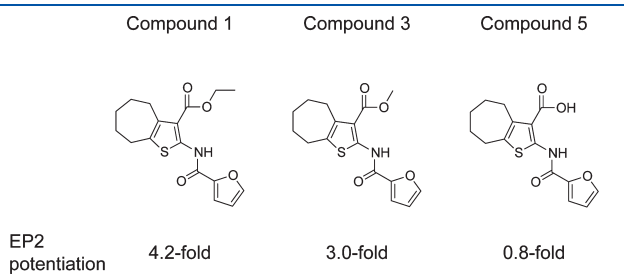

Figure 4. Structures of allosteric modulators of the human EP2 receptor.

A novel allosteric compound for the $PGF_{2\alpha}$ receptor FP, PDC113.824, has also been reported (Figure 5).[58] This compound was synthesized by refining the FP antagonist THG113, which corresponds to a peptide derived from the sequence of the second extracellular loop of the human FP receptor. It acts as a functional allosteric modulator of the FP receptor, because it exerts its effect at a site distinct from the orthosteric binding site. This compound acts both as a potent tocolytic agent in vivo and as an inhibitor of myometrial contraction in vitro and ex vivo. It is noteworthy that this compound biases $PGF_{2\alpha}$-bound receptors toward a particular intracellular signaling event, as we will see later. The clinical use of allosteric compounds is attracting increasing attention. These modulators can be used at saturating concentrations because their effects are only revealed in the presence of endogenous ligands, potentially reducing adverse effects.

2.3. Signal Transduction

In GPCR-mediated signaling, the binding of the ligand to the receptor causes the activation and dissociation of a heterotrimeric G protein, including G_s, G_i, and G_q, and the activated G protein then regulates the activities of various effectors. However, accumulating evidence indicates that GPCRs can signal independently of G proteins,[59,60] and there is now evidence that prostanoid receptor signaling involves G protein-independent pathways in addition to classical G protein-dependent pathways.

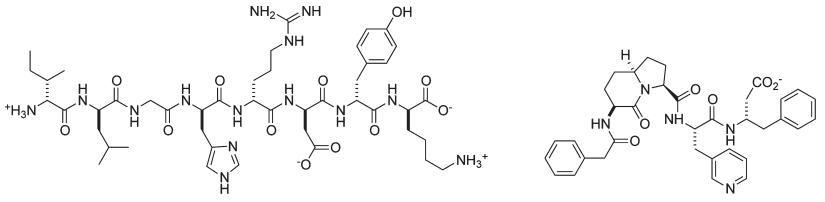

Figure 5. Structures of THG113 peptide and PDC113.824.

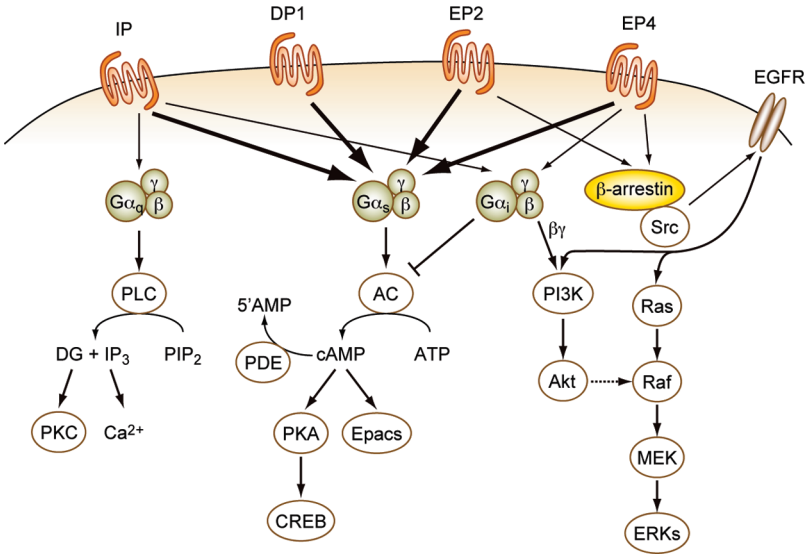

Figure 6. Signal transduction by G_s-coupled prostanoid receptors. The predominant G protein coupling is shown by bold arrows.

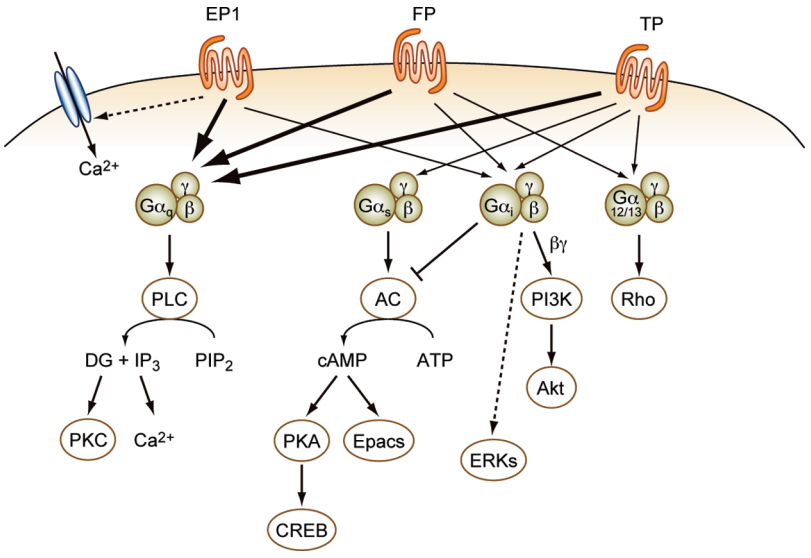

Figure 7. Signal transduction by G_q-coupled prostanoid receptors. The predominant G protein coupling is shown by bold arrows.

2.3.1. G Protein-Dependent Signaling. Prostanoid receptor signal transduction pathways have been studied by examining agonist-induced changes in second messengers such as cAMP, Ca^{2+}, and inositol phosphates, as well as the activities of downstream kinases. As previously mentioned, the DP1-EP2-EP4-IP subfamily mediates cAMP production (Figure 6). These receptors couple to G_s, which activates AC, resulting in the conversion of ATP to cAMP. Changes in cAMP levels are translated into pleiotropic intracellular effects by a panel of cAMP-binding effector proteins, including cAMP-dependent protein kinase (PKA) and exchange proteins directly activated by cAMP (Epacs).[61] Signal termination is achieved by the hydrolysis of cAMP to 5′AMP catalyzed by cyclic nucleotide phoshodiestrases (PDEs). PKA phosphorylates cAMP-responsive transcription factors such as the cAMP-response element binding protein (CREB), which leads to changes in gene expression.

The EP1-FP-TP subfamily mediates the mobilization of cytosolic Ca^{2+}. The FP and TP receptors couple to G_q and initiate phospholipase C (PLC) activation, which hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP_2) into inositol 1,4,5-trisphosphate (IP_3) and 1,2-diacylglycerol (DG), resulting in Ca^{2+} mobilization and protein kinase C (PKC) activation (Figure 7). The EP1 receptor mediates the elevation of free Ca^{2+} in Chinese hamster ovary (CHO) cells.[62] This increase is dependent on the extracellular Ca^{2+} and occurs without a detectable phosphatidylinositide (PI) response, suggesting that the EP1 receptor regulates Ca^{2+} channel gating via an unidentified G protein. However, Ji et al.[63] recently showed that PGE_2 stimulates a PI response in human embryonic kidney (HEK) 293 cells expressing the human EP1 receptor, consistent with the coupling of this receptor to G_q.

The EP3 receptor's major signaling pathway is the inhibition of AC activity via pertussis toxin (PTX)-sensitive G_i, decreasing the cAMP level (Figure 8). The DP2 receptor, which belongs to the leukocyte chemoattractant receptor family, couples to G_i in a manner similar to other chemoattractant receptors, and its activation results in PTX-sensitive decreases in cAMP levels and Ca^{2+} mobilization.[26,64] Most leukocyte chemoattractant receptors couple to G_i and activate PLC through the $G\beta\gamma$ subunits, resulting in Ca^{2+} mobilization.[65]

It should be noted that prostanoid receptors often couple to more than one G protein and signal-transduction pathway (Table 1). Among the DP1-EP2-EP4-IP subfamily, which couples primarily to G_s, the EP4 receptor appears to mediate various G_s-independent effects (Figure 6). The EP4 receptor can also activate phosphatidylinositol 3-kinase (PI3K), leading to extracellular signal-regulated kinase (ERK) phosphorylation and the induction of early growth response factor-1 (EGR-1).[66] In addition, the EP4 receptor couples to PTX-sensitive G_i, which can inhibit cAMP-dependent signaling and activate PI3K/ERK-dependent signaling.[67] Another member of this subfamily, the IP receptor, appears to couple to G_q and G_i, in addition to G_s, in a cell-type- and expression-dependent manner.[25,68]

Among the EP1-FP-TP subfamily, which mostly couple to G_q, the FP receptor has been shown to couple to $G_{12/13}$ to activate the small G protein Rho (Figure 7).[69] The FP receptor is also reported to activate ERK1/2 via a PTX-sensitive G_i protein.[70] The TP receptor couples to the largest array of G proteins, including G_s, G_i, $G_{12/13}$, G_{11}, and $G_{15/16}$.[18,71−74] It appears that $G_{12/13}$-coupled receptors generally interact with $G_{q/11}$ and, in some cases, with the G_i type of G proteins.[75] Although relatively little is known about the G proteins that couple to the EP1 receptor, a recent report indicates that EP1 also couples to PTX-sensitive G_i, which upregulates hypoxia-inducible factor-1α (HIF-1α) expression through a PI3K-signaling pathway, in HEK 293 cells expressing the human EP1 receptor.[63]

As noted earlier, the EP3 receptor has multiple alternative splice variants, which differ in their C-terminal tails and couple to different G proteins. Thus, in addition to G_i, the mouse EP3γ isoform couples to G_s, and the EP3α, EP3β, and EP3γ isoforms all appear to couple to $G_{12/13}$ and activate Rho (Figure 8).[36,37,76] The human EP3 receptor also has multiple isoforms that couple to different G proteins in addition to G_i: the isoforms EP3-II and EP3-IV couple to G_s, while EP3-I and EP3-II appear to couple to G_q.[77] The human EP3 isoforms are also reported to activate ERK1/2 via G_i with different mechanisms and outcomes.[78] Thus, a differential isoform expression pattern can provide another level of regulation of EP3-mediated signaling.

2.3.2. G Protein-Independent Signaling. It is becoming increasingly apparent that there are many ways in which GPCRs

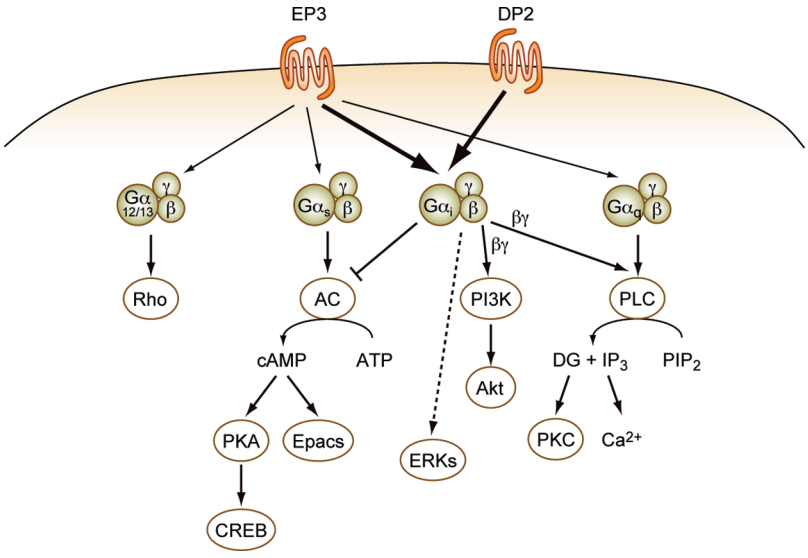

Figure 8. Signal transduction by G_i-coupled prostanoid receptors. The predominant G protein coupling is shown by bold arrows.

Table 1. Signal Transduction Properties of Prostanoid Receptors

receptor	primary G protein	additional G proteins	G protein-independent	major signaling
DP1	G_s			↑cAMP
EP2	G_s		β-arrestin	↑cAMP
EP4	G_s	G_i	β-arrestin	↑cAMP,
IP	G_s	G_q, G_i		↑cAMP
EP1	G_q or unknown	G_i		↑Ca^{2+}
FP	G_q	$G_{12/13}$, G_i		↑Ca^{2+}, PI turnover
TP	G_q	G_s, G_i, $G_{12/13}$, G_{11}, $G_{15/16}$		↑Ca^{2+}, PI turnover
EP3	G_i	G_s, $G_{12/13}$		↓cAMP
DP2	G_i			↓cAMP

can signal independently of G proteins. The first evidence of G protein-independent signaling was demonstrated for angiotensin II acting on the AT1 receptor, which activates both β-arrestin and G proteins.[79] The recruitment of β-arrestin to GPCRs is known to promote receptor internalization and desensitization, thereby silencing the G protein signaling. It is now clear that β-arrestins are not merely mediators of GPCR sensitization and endocytosis but also act as scaffolds in many intracellular signaling networks to modulate the strength and duration of signaling through diverse types of receptors and downstream effectors.[59]

Among prostanoid receptors, the EP2 and EP4 receptors have been shown to form a complex with β-arrestin to mediate G protein-independent signaling. In colorectal cancer cells, PGE_2 induces the association of an EP4/β-arrestin/Src complex, resulting in transactivation of the epidermal growth factor receptor (EGFR) and downstream Akt signaling, which is important in PGE_2-mediated cell migration.[80] Similarly, PGE_2 promotes lung cancer cell migration via an EP4/β-arrestin/Src signaling complex.[81] The EP2 receptor is also reported to form a complex with β-arrestin. An EP2/β-arrestin/Src complex is found in the papillomas of a mouse skin initiation/promotion model induced by 7,12-dimethylbenzanthracene (DMBA) and phorbol 12-myristate 13-acetate (PMA).[82] In this model, PKA, EGFR, and several effectors, including CREB, H-Ras, Src, Akt, and ERK1/2, are activated in the PMA-promoted papillomas. Treating PMA- and indomethacin-treated mice with an EP2 agonist restores the papilloma formation, and increases the activation EGFR, Src, H-Ras, ERK1/2, and Akt. Thus, the EP2 receptor activates multiple signaling pathways, including G_s-dependent AC and PKA activation, as well as the G protein-independent activation of EGFR that involves EP2/β-arresin/Src complex formation. These signaling pathways have been shown to play a role in keratinocyte proliferation.[83]

2.3.3. Ligand-Induced Selective Signaling. As discussed above, prostanoid receptors often couple to multiple G proteins and signaling pathways. EP4 signaling has G_s-mediated intracellular cAMP responses and PTX-sensitive G_i-mediated effects, and also involves β-arrestin-dependent pathways. Considerable functional selectivity has been reported among structurally related EP4 agonists.[84] PGE_2 is the most selective in activating G_s, whereas $PGF_{2\alpha}$ and PGE_1 alcohol are the most biased for activating G_i and β-arrestin, respectively. These effects can be explained by the concept that individual agonists lead to differential and independent receptor coupling to different G proteins or intracellular effectors, referred to as ligand-induced selective signaling or functional selectivity.[85] Classically, GPCRs are thought to exist in two states, an inactive state and an active state, which is stabilized by agonist binding; the receptor in the active state couples to the intracellular signaling machinery and activates a cascade of events. However, there is growing evidence that receptors can assume multiple active conformations, and each of these conformations can potentially interact with a ligand in a highly selective manner.[86,87] The specific receptor conformation also selectively interacts with a specific intracellular signaling complex.

It is noteworthy that β-arrestin recruitment to the EP4 receptor, rather than G_s-mediated signaling, is associated with colorectal cancer progression.[80] In addition, G_i-mediated signaling is implicated in the EP4-mediated increases in PI3K and ERK1/2 phosphorylation, which are often associated with cancer progression.[67] Further characterization of the response of

downstream effectors, such as G_s, G_i, and β-arrestin, to analogues of PGE_2 and other prostanoids might have implications for the analogues' use in experimental and clinical settings.

The allosteric compound for the FP receptor, PDC113.824, also appears to induce selective signaling. It biases $PGF_{2\alpha}$-bound receptors toward increased PKC-ERK1/2 signaling, while blocking cell contraction and cytoskeletal reorganization by inhibiting the G_{12}-Rho signaling pathway.[58] Thus, it appears that allosteric modulators are also able to confer functional selectivity to agonists. Allosteric ligands designed with biased signaling properties are expected to offer not only the advantage of specificity for a single receptor but also selectivity for a specific subset of signaling pathways, further reducing unwanted side effects.

2.4. Membrane Trafficking

Membrane GPCR trafficking is fundamental to shaping signaling networks.[88,89] Like many other membrane proteins, prostanoid receptors are synthesized and processed in the endoplasmic reticulum (ER). Post-translational modification, oligomerization, association with accessory proteins, and folding take place in this compartment and are required for export. Subsequent trafficking through the ER and Golgi intermediate compartment (ERGIC) and Golgi apparatus facilitates their maturation and correct targeting to the cell surface. At the cell surface, the receptor is able to bind its ligand, which initiates the activation of various signaling cascades. Cell-surface signaling is regulated by receptor internalization from the cell surface, which occurs primarily via clathrin-coated pits (CCPs). Internalized receptors are either rapidly targeted to the lysosome for degradation or recycled back to the plasma membrane.

2.4.1. Trafficking to the Cell Surface. At present, there is little information about the identity of the accessory proteins involved in regulating and correctly targeting the prostanoid receptors to the cell surface. However, the receptor for activated C-kinase 1 (RACK1), a scaffold protein that contains seven WD40 repeats, has been shown to interact with the human TP receptor TPβ and to regulate receptor transport from the ER to the cell surface.[90] The effect of RACK1 on receptor trafficking to the cell surface appears to be selective for some GPCRs; inhibiting RACK1 expression does not affect the cell-surface targeting of β_2AR or the DP1 receptor. Whether the cell-surface targeting of other prostanoid receptors involves RACK1 remains unclear.

2.4.2. Trafficking from the Cell Surface. At the cell surface, GPCRs bind their ligands and activate signaling cascades mostly through heterotrimeric G proteins. Once activated, the GPCRs are rapidly phosphorylated, and β-arrestin is quickly recruited from the cytosol to the phosphorylated receptor, which uncouples the receptor from its G protein and targets the receptor to CCPs for internalization. This results in rapid desensitization of the G protein signaling. GPCR internalization also occurs in the absence of agonists, although the rates are generally slow compared with agonist-induced internalization. As mentioned earlier, some prostanoid receptors have splice variants differing in their C-terminal tails. These variants may differ in their localization in response to agonist stimulation. Two isoforms of the human TP receptor, TPα and TPβ, show differences in agonist-induced receptor internalization: TPβ undergoes clathrin-dependent internalization, whereas TPα does not.[91] TPβ is palmitoylated at Cys^{347}, Cys^{373}, and Cys^{377}, and the palmitoylation at $Cys^{373,377}$ is critical for TPβ internalization.[92] The ovine FP isoform FP_A undergoes a rapid agonist-induced internalization that requires PKC and involves clathrin, whereas FP_B undergoes a constitutive, agonist-independent internalization that does not involve PKC or clathrin.[93] The DP1 and DP2 receptors undergo agonist-induced internalization, and the internalization of DP2, but not DP1, involves PKC or PKA.[94]

The internalized receptors in early endosomes are trafficked to various postendocytic compartments. They are rapidly targeted to lysosomes for degradation, resulting in complete signal termination, or recycled back to the plasma membrane, resulting in resensitization. The Rabs, a family of small GTPases, play essential roles in this trafficking. Following the receptors' internalization to early endosomes, they can recycle back to the plasma membrane via Rab4-positive vesicles or via Rab11-positive perinuclear recycling endosomes. TPβ isoform recycling is dictated by Rab11, but not by Rab4.[95,96] Similarly, IP receptor recycling is regulated by Rab11.[97,98] The IP receptor associates directly with Rab11 via its C-terminal domain. The recycling of the DP1 receptor appears to be promoted by Rab4, but not by Rab11, whereas the reverse is observed for the DP2 receptor.[94] However, the trafficking systems for most of the prostanoid receptors are still not well understood. Clarifying these systems will shed light on the spatial and temporal regulation of prostanoid receptor signaling and may provide pathway-specific targets for therapeutic intervention.

2.5. Receptor Oligomerization

Although GPCRs have conventionally been thought to exist and act as monomers, there is growing evidence that most of them exist as homodimers or even oligomers.[99,100] This was most convincingly demonstrated using fluorescence resonance energy transfer (FRET) or bioluminescence resonance energy transfer (BRET), which directly demonstrate receptor protein dimerization in living cells. Although the functional significance of this process is not completely understood, it is now accepted that GPCR oligomerization can be important for receptor expression and function, including ligand binding and signaling specificity. Different GPCRs may also form heterodimers and heterooligomers, which can affect the function of their agonists.[101]

2.5.1. Oligomerization among Prostanoid Receptors. The IP receptor primarily couples to G_s and increases intracellular cAMP. The TP isoforms TPα and TPβ both couple to G_q to activate PLC. It was reported that the TP agonists U46619 and I-BOP induce cAMP generation in HEK 293 cells coexpressing the IP and TPα receptors, as well as in aortic smooth muscle cells expressing both the IP and TP receptors, but not in cells expressing either receptor alone, or in smooth muscle cells from IP-deficient mice; moreover, the cAMP production is coincident with the formation of an IP/TPα heterodimer.[102] PGI_2 and TXA_2 are biological opposites: PGI_2 is a vasodilator and inhibits platelet aggregation and smooth muscle cell growth, whereas TXA_2 is a vasoconstrictor and stimulates platelet aggregation and smooth muscle cell proliferation. The formation of an IP/TPα heterodimer promotes a "PGI_2-like" cellular response to TXA_2, limiting the injurious actions of TXA_2. In addition, IP/TPα heterodimerization creates a novel binding site for the isoprostane iPE_2III.[102] The IP/TPα interaction also permits receptor endocytosis to be regulated via a trafficking pathway determined by the activated dimeric receptor.[103]

Similarly, the TPα and TPβ receptors heterodimerize, altering the receptor regulation and signaling response to TP agonists.[104,105] In particular, TPα/TPβ dimerization enhances the signal transduction mediated by the isoprostanes iPE_2III and $iPF_{2\alpha}III$.[106]

Heterodimerization among splice variants is also reported for the FP receptor.[41] Six FP splicing variants have been identified in human ocular tissues. Immunoprecipitation confirmed that the generic FP_A receptor dimerizes with one of the FP variants in HEK 293 cells coexpressing these isoforms. Bimatoprost, a prostamide analogue that is effective for reducing intraocular pressure, has no effect on the FP_A receptor but appears to interact with the FP heterodimer to induce alterations in second messenger signaling. Thus, heterodimerization may create an entirely unique ligand-binding site. For the IP/TPα heterodimer, a unique isoprostane iPE_2III-binding site occurs, and in the case of the FP heterodimer, prostamide responsiveness is conferred.

Receptor dimerization may underlie the impaired signaling through prostanoid receptors seen in individuals heterozygous for a specific mutation. Platelets from individuals carrying the IP^{R212C} mutation in one allele are functionally nonresponsive to an IP agonist. This contrasts with the gene dosage effect in mice heterozygous for IP deletion, in which the platelet response to an IP agonist is midway between wild-type and homozygous null mice.[107] When the mutant receptor IP^{R212C} is expressed in HEK 293 cells with the wild-type IP receptor, it exerts a dominant action on the wild-type receptor.[108] Furthermore, the TP agonist-induced cAMP generation that occurs through IP/TPα heterodimer formation is not observed when the TPα is dimerized with IP^{R212C}. This dominant-negative effect of IP^{R212C} on the wild-type IP and on TPα through dimerization is likely to contribute to the accelerated cardiovascular diseases seen in individuals carrying one IP^{R212C} allele. Although the mechanisms of receptor dimerization or oligomerization are not yet clear, Giguere et al.[109] showed the involvement of disulfide-bond formation in the IP receptor homodimerization.

2.5.2. Oligomerization with a Different Class of Receptors. Prostanoid receptors can also form heterodimers with receptors from a different class. EP1 receptor and β_2AR heterodimerization was demonstrated in airway smooth muscle cells by fluorescence microscopy, BRET, and coimmunoprecipitation.[110] The EP1-specific agonist 17-phenyl trinor-PGE_2 (17-PTP) binds to the heterodimer and uncouples β_2AR from G_s, thereby diminishing the bronchodilator response of the β_2AR agonists. Thus, although the EP1 receptor alone does not have a significant direct effect on airway tone, it acts as a β_2AR modulator. This represents a novel level of functional antagonism between bronchoconstrictors and bronchodilators.[110,111]

3. PROPERTIES AND FUNCTIONS OF PROSTANOID RECEPTOR TYPES AND SUBTYPES

As mentioned, the prostanoid receptors include DP1, EP1, EP2, EP3, EP4, FP, IP, and TP, which are related in structure, and one leukocyte chemoattractant receptor family member, DP2. Stable transfectants expressing each prostanoid receptor have enabled the high-throughput screening of compound libraries, leading to the discovery of new scaffolds. In addition, the generation and study of mice deficient in each receptor has revealed important functions of each receptor in health and disease. In this section, the properties and functions of each receptor, revealed primarily through studies using gene knockout mice and receptor-specific agonists and antagonists, will be discussed. However, it is important to recognize that species differences exist in the distribution and pathophysiological functions of prostanoid receptors, and that discoveries and results obtained using gene knockout mice cannot be assumed to translate across species without verification. Thus, the potential clinical relevance of the results of nonhuman studies discussed below needs to be carefully determined.

3.1. DP1 Receptors

The prostanoid PGD_2 is synthesized from the PG intermediate PGH_2 by either hematopoietic or lipocalin PGD synthase. PGD_2 is produced in the brain, where it is involved in regulating sleep and other central nervous system (CNS) activities, including pain perception.[112] In peripheral tissues, PGD_2 is produced most abundantly in mast cells, is released after immunoglobulin E (IgE) challenge, and has been detected in high concentrations at allergic inflammation sites. PGD_2 is also produced by macrophages, dendritic cells (DCs), and T helper 2 (Th2) cells, albeit at much lower levels. PGD_2's action is mediated by two PGD_2 receptors, DP1 and DP2, and it is now emerging that DP1 and DP2 have crucial and compensatory roles in mediating various aspects of allergic responses.[113,114]

The DP1 receptor was the first receptor identified for PGD_2.[19] The human and mouse DP1 receptors encode 359- and 357-amino-acid polypeptides, respectively.[19,27] The DP1 receptor couples to G_s, and its activation leads to elevated intracellular cAMP levels.

3.1.1. Biological Functions of DP1 Receptors. The DP1 receptor is expressed by vascular smooth muscle cells, bronchial smooth muscle cells, and platelets, and it mediates vasodilatation, bronchodilation, and the inhibition of platelet aggregation. At sites of mast cell-mediated allergic inflammation, DP1 receptor activation has an important role in increasing blood flow, leading to hyperemia and edema, which contributes to nasal congestion.

The DP1 receptor appears to have additional properties relevant to allergic diseases. DP1-deficient mice exhibit a decreased asthmatic response in an ovalbumin (OVA)-induced asthma model, suggesting that the DP1 receptor enhances allergic responses.[115] In this model, PGD_2 is thought to act on bronchial epithelial cells via the DP1 receptor, which enhances the secretion of chemokines that recruit Th2 cells. The DP1 receptor's pro-inflammatory role is also supported by a report on selective DP1 antagonists that can alleviate asthmatic responses, as will be discussed later.[116]

On the other hand, the DP1 receptor also has an anti-inflammatory role. PGD_2 acts through the DP1 receptor to inhibit the migration of airway DCs and skin Langerhans cells (LCs), thereby limiting T-cell activation.[117−119] Studies using monocyte-derived DCs also show that PGD_2 inhibits their maturation through the DP1 receptor.[120,121] Hammad et al.[122] showed that an inhaled selective DP1 agonist suppresses the cardinal features of asthma by acting on DCs and increasing $Foxp3^+$ $CD4^+$ regulatory T cells, which suppress inflammation in an interleukin-10 (IL-10)-dependent manner. Thus, triggering the DP1 receptor on DCs is an important mechanism for inducing regulatory T cells and controlling the extent of airway inflammation.

PGD_2 is the most abundant prostanoid in the brain, and it regulates sleep, temperature, and nociception.[112] PGD_2 is produced by lipocalin-type PGD synthase, which is localized mainly to the leptomeninges, choroid plexus, and oligodendrocytes, and circulates in the cerebrospinal fluid.[123] PGD_2 acts on the DP1 receptor on leptomeningeal cells of the basal forebrain to release adenosine as a paracrine-signaling molecule to promote sleep.[112] In addition to PGD_2, PGE_2 and $PGF_{2\alpha}$ also appear to be involved in regulating sleep.[124]

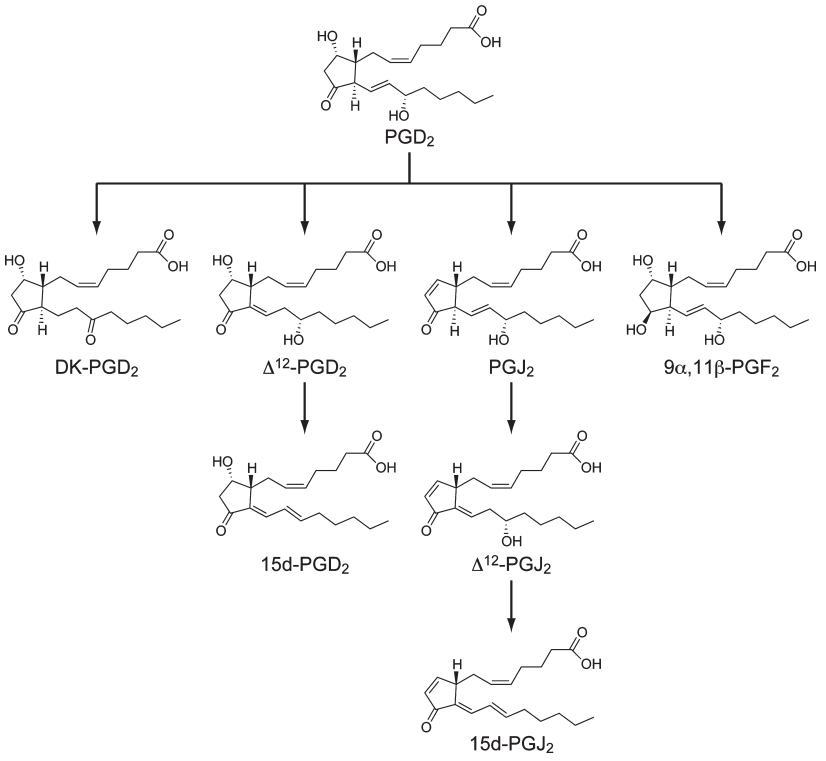

Figure 9. PGD_2 metabolites with activity on DP1 and/or DP2.

DP1 receptor involvement in neuroinflammation has also been reported. Mohri et al.[125] reported that DP1 mRNA expression is increased in Alzheimer's disease (AD) brains compared with non-AD brains. DP1 mRNA is specifically localized to microglia and reactive astrocytes within the senile plaques of AD brains, suggesting that PGD_2 may act as a mediator of the plaque-associated inflammation in AD. On the other hand, PGD_2 appears to be involved in neuroprotection. Physiological concentrations of PGD_2 rescue neurons in paradigms of glutamate toxicity in cultured hippocampal neurons and organotypic slices, in a manner dependent on cAMP signaling.[126]

3.1.2. DP1 Agonists and Antagonists. PGD_2 activates both DP1 and DP2 receptors with EC_{50} values around 2 nM. The PGD_2 metabolite PGJ_2 is equipotent with PGD_2 for the DP1 receptor but is an order of magnitude less potent for the DP2 receptor (Figure 9).[26,64] PGE_2 and $PGF_{2\alpha}$ are also moderate- to low-potency agonists for this receptor. Much of our knowledge about the role of the DP1 receptor comes from using the selective agonist BW245C and the selective antagonist BWA868C. The synthetic prostanoid BW245C, initially described as a potent and selective inhibitor of platelet aggregation, was found to be a highly potent and selective DP1 agonist, with a >100-fold selectivity compared with all other prostanoid receptors, including the DP2 receptor (Figure 10). This compound is still the most commonly used selective DP agonist. The racemic compound L-644,698 is a full DP1 agonist devoid of affinity for the DP2 receptor at a concentration of 100 μM.

The prostanoid-inspired compound BWA868C behaves as a selective, competitive DP1 antagonist on human platelets (Figure 10). More recent studies indicate that BWA868C has partial agonist activity. Screening the compound libraries at Shionogi for DP1 binding resulted in the identification of S-1452, previously known as a TP antagonist. Subsequent structure−activity relationship (SAR) studies led to the synthesis of S-5751, which is orally active in guinea pig models of allergy and inflammation.[116]

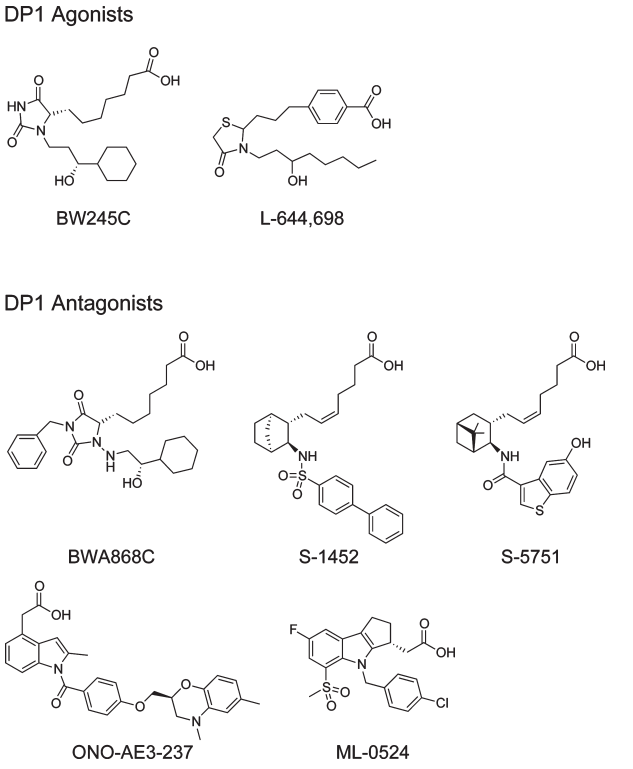

Figure 10. Structures of DP1 agonists and antagonists.

The COX inhibitor indomethacin is a nonprostanoid used to develop DP1 antagonists, although indomethacin itself has no

activity on the DP1 receptor. Optimization led to the discovery of ONO-AE3-237, a benzoxazine with an acetate unit located on C4.[127] In the Merck series of DP1 antagonists, the positions of the carboxylic group and the substituents on the benzene ring of the indole template were optimized to yield MK-0524 (Laropiprant).[128] DP1 antagonists may have merit in the treatment of asthma, rhinitis, and conjunctivitis.

3.2. DP2 Receptors

Although the DP1 receptor mediates a number of physiological responses to PGD_2, some other responses, particularly those related to the migration of inflammatory cells, could not be explained by DP1 receptor activation.[129] Several studies with selective agonists and antagonists indicated the existence of a second PGD_2 receptor. In 1999, the gene GPR44 was reported to encode a GPCR with a high similarity to chemoattractant receptors.[130] Concomitantly, Nagata et al.[131] identified a biomarker for Th2 cells, which they termed CRTH2. This proved to be identical to GPR44. Besides being selectively expressed on Th2 cells, CRTH2 is also expressed in basophils and eosinophils, and activated mast cells produce a mediator that stimulates CRTH2 to mobilize intracellular Ca^{2+}.[33] The mast cell-derived factor capable of activating CRTH2 turned out to be PGD_2, thus identifying CRTH2 as a novel PGD_2 receptor with different functions from the DP1 receptor.[26] Monneret et al.[132] independently reported the pharmacological identification of a new PGD_2 receptor on eosinophils that inhibits cAMP generation, termed DP2, and this receptor was discovered to be identical to CRTH2. Thus, this receptor is currently known under two names, CRTH2 and DP2.

The human and mouse DP2 receptors encode 395- and 382-amino-acid polypeptides, respectively.[131,133] The DP2 receptor couples to G_i and inhibits cAMP production. It also increases intracellular Ca^{2+} levels.

3.2.1. Biological Functions of DP2 Receptors. The DP2 receptor is expressed in Th2 cells, basophils, and eosinophils, and it mediates the chemotaxis of these cells in response to PGD_2. The ability of PGD_2 to promote eosinophil accumulation in the airways is mimicked by selective DP2 agonists but not DP1 agonists.[134] PGD_2 injection also enhances a DP2-dependent allergic response in mouse skin.[135] In addition to recruiting leukocytes to allergic inflammation sites, PGD_2 induces the production of Th2 cytokines, including IL-4, IL-5, and IL-13, via the DP2 receptor.[136] These observations suggest that the DP2 receptor is involved in various allergic responses.

DP2-deficient mice were generated independently by two groups, who reported apparently conflicting results. Satoh et al.[137] reported that both the hapten-specific IgE ear-swelling response and the chronic contact hypersensitivity response induced by repeated hapten challenge were reduced in DP2-deficient mice, which is consistent with pharmacological studies. In contrast, Chevalier et al.[138] unexpectedly found that OVA-immunized DP2-deficient mice showed enhanced eosinophil recruitment into the lung compared with wild-type mice; this was associated with increased IL-5 production by DP2-deficient cells. This apparent discrepancy may be related to the DP2 receptor expression pattern in mice, in which the DP2 receptor is expressed equally on both Th1 and Th2 cells. It is possible that the DP2-mediated effects on Th1 cells dominate over its effects on Th2 cells, depending on the immunization protocol.

Although the DP1 and DP2 receptors are structurally unrelated and have distinct signaling pathways, they share a common ligand, PGD_2, and it appears that these receptors work together to promote Th2-dependent allergic responses. The activated DP1 receptor promotes an environment that allows Th2 cell polarization, and the DP2 receptor mediates their recruitment and activation to produce cytokines.

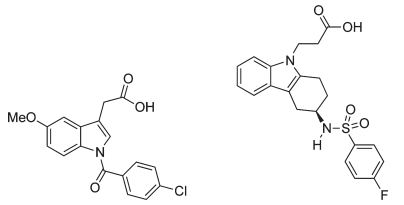

Figure 11. Structures of indomethacin and ramatroban.

3.2.2. DP2 Agonists and Antagonists. PGD_2 activates the DP2 receptor with EC_{50} values in the low nanomolar range. The enzymatically derived PG metabolite 13,14-dihydro-15-keto-PGD_2 (DK-PGD_2) is as potent as PGD_2 in activating the DP2 receptor, but it does not activate the DP1 receptor. The metabolites Δ^{12}-PGD_2, formed by isomerization by albumin, and 15-deoxy $\Delta^{12,14}$-PGD_2 (15d-PGD_2) are also potent and selective DP2 agonists. The most abundant PGD_2 metabolite in plasma, Δ^{12}-PGJ_2, and 15-deoxy $\Delta^{12,14}$-PGJ_2 (15d-PGJ_2) are also potent DP2 agonists (Figure 9).

Much effort has been directed toward developing DP2 antagonists. Indomethacin and ramatroban, which act, respectively, as an agonist and an antagonist, were the first nonprostanoid compounds to be identified as DP2 ligands (Figure 11). These two compounds have frequently served as leads for identifying new selective compounds. There is also a rapidly increasing number of nonindole compound series with DP2 antagonist properties.

Ramatroban was originally identified as a TP antagonist, but it is now recognized to bind to the DP2 receptor with moderate affinity.[139] Studies with ramatroban suggest that the DP2 receptor is important for mediating eosinophil accumulation in response to an allergic challenge. Ramatroban's effects on eosinophil recruitment are unlikely to be mediated by TP antagonism, as eosinophils do not express TP receptors, and selective TP antagonists do not influence eosinophil function.

Recently, DP2 antagonists of the phenylacetic acid and tetrahydroquinoline class have been reported,[140] and a variety of DP2 antagonists are active in models of allergic rhinitis, asthma, atopic dermatitis, and lung inflammation.[16,17]

3.3. EP1 Receptors

PGE_2 is the most abundant product of COX-initiated AA metabolism. PGE_2 has multiple—and at times, apparently opposing—functions in a given tissue or cell. The differential effects of PGE_2 analogues are important evidence of the existence of multiple EP receptors. Molecular cloning has confirmed the existence of four EP receptor subtypes, designated EP1, EP2, EP3, and EP4, each of which is encoded by a distinct gene. These multiple receptors are likely to account for the diverse effects of PGE_2. Further diversity in the EP1 and EP3 receptors is generated by alternative splicing.

The EP1 receptor was originally described as a smooth muscle constrictor. The human and mouse EP1 receptors encode 402- and 405-amino-acid polypeptides, respectively.[20,28] The rat EP1 receptor encodes a 405-amino-acid polypeptide with 96% identity to the mouse EP1 receptor. An additional variant of the

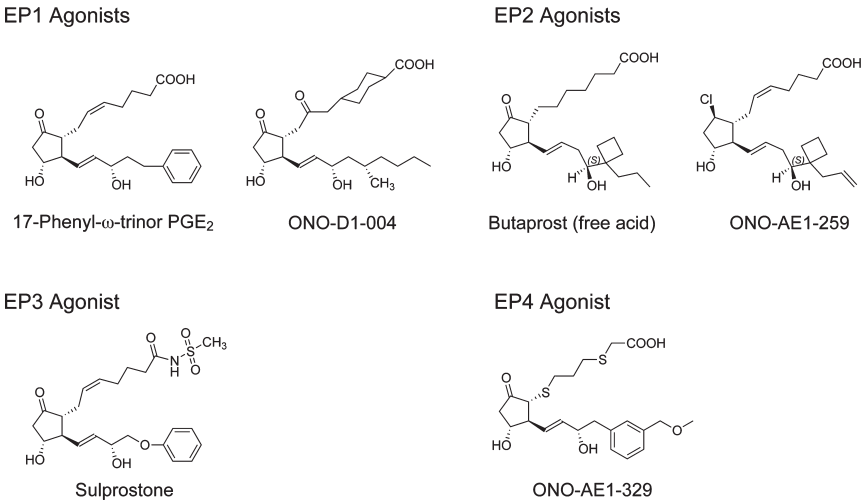

Figure 12. Structures of EP agonists.

rat EP1, encoding a 366-amino-acid protein, has also been described; this alternative splicing variant is predicted to result from a failure to splice the RNA at nucleotide position 952, in the sixth transmembrane region.[35] EP1 receptor activation primarily results in intracellular Ca^{2+} mobilization.

3.3.1. Biological Functions of EP1 Receptors. PGE_2 production during an inflammatory insult plays a role in inducing symptoms such as tissue edema and hyperalgesia. Intrathecal administration of an EP1 antagonist reduces the hyperalgesia associated with carrageenan-induced paw inflammation in rats.[141] EP1-deficient mice are healthy and fertile, without any overt physical defects.[142,143] However, their pain-sensitivity responses, tested in two acute PG-dependent models, were reduced by ~50%.[143] As will be discussed later, IP-deficient mice also show reduced pain responses,[107] suggesting that both the EP1 and IP receptors are important in mediating pain perception. Consistent with these results, the pH- and heat-induced pain sensation mediated by the capsaicin receptor is augmented by PGE_2 and PGI_2 acting on the EP1 and IP receptors, respectively.[144] In addition, the EP2 receptor is reported to mediate spinal inflammatory hyperalgesia,[145] which will be discussed later.

The EP1 receptor may also play a role in cardiovascular homeostasis. Systolic blood pressure is significantly reduced in EP1-deficient mice and is accompanied by increased renin-angiotensin activity, especially in males.[143] The effects of PGE_2 on sodium excretory mechanisms in the kidney are probably the critical determinant of its regulatory effects on blood pressure.[146] Blood pressure in the spontaneous hypertensive rat is reduced by the EP1 antagonist SC-51322, and EP1 deficiency blunts the acute pressor response to angiotensin II and reduces chronic hypertension, supporting the possibility of targeting the EP1 receptor for antihypertensive therapy.[147]

PGE_2 has long been considered immunoinhibitory. However, its effects are diverse and complex. Depending on the cell type, its activation or differentiation state, and the local environment, the outcome of PGE_2 signaling in immune responses may be immunostimulatory or immunoinhibitory.[148,149] The diverse effects of PGE_2 may be accounted for in part by the existence of the four EP receptors. Analyses of the four receptors' expression patterns have revealed their presence on most major subsets of cells involved in immune responses, including T and B cells.[150] Nagamachi et al.[151] showed that contact hypersensitivity was attenuated in EP1-deficient mice due to a reduced Th1 response, and this effect could be recapitulated by administering an EP1 antagonist to wild-type mice. Conversely, an EP1 agonist facilitated the Th1 differentiation of naive T cells in vitro. These results suggest that PGE_2, acting on the EP1 receptor on naive T cells, modulates the immune response by promoting Th1 differentiation.

An intriguing role for the EP1 receptor may be found in the CNS, where stress responses are regulated.[152,153] Stress is a state of physiological and psychological strain caused by adverse stimuli, and it commonly evokes biological responses such as fever, glucocorticoid secretion, activation of the sympathetic nervous system, and emotional behaviors. In animal models, administering lipopolysaccharide (LPS), a bacterial endotoxin, and proinflammatory cytokines, such as IL-1β, can reproduce illness-related stress behaviors, such as fever and activation of the hypothalamic−pituitary−adrenal axis.[153] Both EP1 and EP3 receptors are equally important in the adrenocorticotropic hormone (ACTH) release in response to LPS via activation of the neurons in the paraventricular nucleus of the hypothalamus that release corticotropin-releasing hormone.[154] EP1-deficient mice also show impulsive aggression and deficits in social behavior in response to psychological stress.[155] The impulsive behaviors can be corrected by dopamine receptor antagonists, implicating dopaminergic neurons in the underlying circuitry. A further study showed that the EP1 receptor enhances GABA-mediated dopaminergic neuron inhibition in the substantia nigra pars compacta, and regulates dopamine levels in the dorsal striatum.[156]

3.3.2. EP1 Agonists and Antagonists. The PGE_2 analogues that have been used in conventional studies are not specific for any given EP receptor; 17-phenyl PGE_2 has modest EP1/EP3 selectivity, while ONO-D1-004 is a more selective EP1 agonist (Figure 12). The 6a-Carba analogues of prostacyclin, such as carbacyclin and iloprost, are unexpectedly potent EP1 agonists. Ungrin et al.[157] examined an extensive panel of PG analogues in a high-throughput screening using a calcium-responsive, aequorin-based reporter assay to perform a SAR analysis for the cloned human EP1 receptor. As reviewed by Breyer,[158] the study revealed that one of the most sensitive positions for this agonist activity is the hydroxy group at carbon 15. This is notable because PGE_2's conversion to its 15-keto derivative is one of the primary pathways of its metabolic inactivation in vivo. Moreover, the EP1

receptor is more sensitive than other EP receptors to 15 OH oxidation. This enhanced sensitivity of EP1 to PGE_2 metabolism suggests that there may be differential inactivation of the signaling response at the EP receptors. C1 carboxylate modifications have been well-demonstrated to decrease agonist affinity for the EP2, EP3, and EP4 receptors. Similarly, sensitivity to modification of the C1 carboxylate by esterification has been observed for the EP1 receptor.

There are limited antagonists available for the EP receptors (Figure 13). The first EP1 antagonist was SC-19220, which is a dibenzoxazepine hydrazide.[159] It has low affinity but proved useful in the early characterization of EP receptor pharmacology. Modification of SC-19220 resulted in SC-51089 and SC-51322.[160,161] These antagonists appear to have analgesic activity in inflammatory pain models. The EP1 receptor's role in nociception has been confirmed by genetic studies, prompting the search for clinically active drugs that would reduce pain without causing the gastric and renal side effects of NSAIDs. Other EP1 antagonists include ONO-8713 and GW-848687.

3.4. EP2 Receptors

The EP2 receptor was originally characterized by its ability to cause smooth muscle relaxation. The human and mouse EP2 receptor cDNAs encode 358- and 362-amino-acid polypeptides, respectively.[21,162] EP2 receptor activation leads to increased cAMP levels via G_s, but the involvement of β-arrestin-mediated signaling has also been reported.

3.4.1. Biological Functions of EP2 Receptors. EP2-deficient mice exhibit various phenotypes, including impaired ovulation and reduced fertilization, as well as hypertension when fed a high-salt diet.[163−165] In the immune system, PGE_2 is produced by many cell types, including macrophages, DCs, fibroblasts, and endothelial cells, and exerts various inhibitory effects on DCs, such as inhibiting TNF-α production and inducing IL-10 expression. Among the four EP receptors, these effects are generally mediated by the EP2 and EP4 receptors.[166−168] Although the mechanisms by which PGE_2 inhibits TNF-α production are not completely clear, Koga et al.[169] showed that cAMP, which is elevated upon EP2/EP4 stimulation, suppresses TNF-α production via the phosphorylated c-Fos protein.

In addition to indirectly affecting T-cell priming and proliferation by modulating DC function, PGE_2 can bind EP receptors expressed on T cells to regulate T-cell proliferation and differentiation directly. PGE_2 has long been known to suppress T-cell mitogenesis, possibly by inhibiting IL-2 synthesis. Mixed lymphocyte reactions using lymphocytes from mice deficient in specific EP receptors have shown that the antiproliferative effect is mediated primarily by the EP2 receptor but also by the EP4 receptor.[166] On the other hand, Yao et al.,[170] using mouse T cells, showed that PGE_2 enhances Th1 differentiation in vitro under strengthened costimulation conditions via the EP2 and EP4 receptors, and that EP2 and EP4 signals facilitate Th17 expansion in vitro. These effects were confirmed in vivo in experimental autoimmune encephalomyelitis (EAE) and contact hypersensitivity models. Blocking both EP2 and EP4 receptors potently decreased Th1 and Th17 cell accumulation in regional lymph nodes and suppressed disease progression.[170,171] Similar Th17-promoting effects of PGE_2 via EP2 and EP4 receptors have been observed for human T cells.[172]

The EP2 receptor's role in the CNS varies in different injury types.[173] EP2 signaling mediates significant neuroprotection in acute models of cerebral ischemia and excitotoxicity, where the

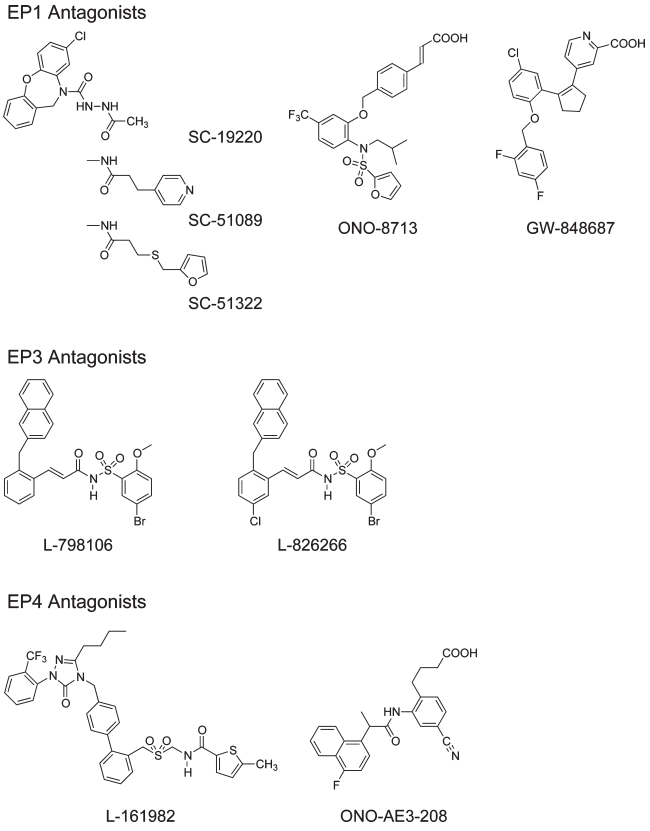

Figure 13. Structures of EP antagonists.

neuronal EP receptor mediates protection by a PKA-dependent mechanism.[174] In contrast, in models of chronic inflammation and neurodegeneration, microglial EP2 may lead to secondary neurotoxicity from increased reactive oxygen species (ROS)-producing enzymes and pro-inflammatory cytokines.[175] The EP2 receptor also plays a role in cognitive and emotional behaviors.[176,177] EP2-deficient mice exhibit impaired long-term potentiation at hippocampal perforant synapses[176] but not at the Schaffer collateral-CA1 synapse, where they show instead a deficit in long-term depression.[177]

As mentioned earlier, the EP1 and IP receptors are reported to be involved in mediating pain perception. After a peripheral inflammatory stimulus, EP2-deficient mice exhibit short-lasting peripheral hyperalgesia but lack a second, sustained hyperalgesic phase of spinal origin.[145] As these mice exhibit almost normal early hyperalgesia in a model of peripheral inflammation, it appears that the EP2 receptor mediates spinal hyperalgesia, contributing to prolonged inflammatory pain sensitization. Electrophysiological studies identified a diminished synaptic inhibition of excitatory dorsal horn neurons as the dominant source of the EP2-mediated hyperalgesia.[145] A specific glycine receptor subtype (GlyR α3) is reported to be a target for this EP2-mediated hyperalgesia; PGE_2 acting on the EP2 receptor stimulates AC and triggers increases in cAMP, which leads to PKA-dependent phosphorylation and inhibition of GlyR α3.[178] Thus, while the EP1- and IP-mediated mechanisms in primary nociceptive neurons (dorsal root ganglion neurons) may be of major relevance early in the development of inflammation and mostly peripheral in nature, the EP2-mediated mechanism in the spinal cord dorsal horn may be more relevant in chronic stages of

inflammation. Such a scenario indicates that the blockade of a single prostanoid receptor may not be sufficient for adequate analgesic therapy.[179]

3.4.2. EP2 Agonists and Antagonists. Misoprostol, in which the 15-hydroxy group of PGE_1 is displaced to C16, shows modest selectivity for EP2 and EP3 receptors. The related agonist butaprost is selective for EP2; tissue-dependent hydrolysis of its C1 ester is required for full bioactivity. Retaining the 16S configuration and replacing the 9-ketone with a β-chloro group has generated highly potent and selective EP2 agonists.[180] One of these, ONO-AE1-259, has been valuable for characterizing inhibitory EP2 systems.

There are no potent and selective EP2 antagonists available. AH6809 remains the most useful compound in this class, despite its significant activity at EP1 receptors.

3.5. EP3 Receptors

The EP3 receptor was originally identified as a smooth muscle constrictor. This receptor is unique in that it has multiple splice variants differing in their C-terminal tails. The human EP3-I and mouse EP3α receptors encode 390- and 366-amino-acid polypeptides, respectively.[29,36] The mouse EP3 isoforms, α, β, and γ, contain C-terminal tails of 30, 26, and 29 amino acids, respectively, that do not share any structural motifs or hydrophobic features.[14]

3.5.1. Biological Functions of EP3 Receptors. Fever is elicited by both noninfectious inflammatory insults and by exogenous pyrogens, which are cellular components (such as LPS) of infectious organisms. Both stimulate the production of cytokines, such as IL-1β, which act on the brain as endogenous pyrogens. Fever can be suppressed by NSAIDs. EP3-deficient mice fail to show a febrile response to PGE_2 or to either IL-1β or LPS, indicating that EP3 signaling mediates fever generation in response to both exogenous and endogenous pyrogens.[142]

As with the EP1 receptor, the EP3 receptor has been implicated in regulating water and salt transport along the nephron.[146] EP1 and EP3 mRNA expression predominates in the collecting duct and thick limb, respectively, where their stimulation reduces sodium and water absorption, promoting natriuresis and diuresis.[146]

So far, the data reported suggest that the EP3 receptor mostly functions to inhibit immune responses. In an OVA-induced asthma model, EP3-deficient mice develop more pronounced inflammation than do wild-type mice,[181] and an EP3 agonist suppresses the inflammation. This suppression is effective when the agonist is administered 3 h after antigen challenge and is associated with the inhibition of allergy-related genes, such as chemokines. Similarly, EP3 stimulation inhibits skin inflammation in a contact hypersensitivity model.[182]

3.5.2. EP3 Agonists and Antagonists. Phenoxy substitution at C16 on the PGE template promotes EP1, EP3, and FP agonism modestly, while markedly attenuating TP agonism. The compound 11-deoxy-16-phenoxy PGE_1 shows reasonably good EP3 versus EP1 selectivity but is still a moderately potent TP agonist. Sulprostone, in which the C1 carboxylate is converted to the acidic methylsulfonamide, has modest EP3 versus EP1 selectivity and minimal TP agonism. A search for EP3 antagonism in a series of biaryl acylsulfonamides found two compounds, L-798106 and L-826266, that are highly lipophilic EP3 antagonists with a slow onset in tissues.[183]

3.6. EP4 Receptors

The human and mouse EP4 receptor cDNAs encode 488- and 513-amino-acid polypeptides, respectively, and have the longest intracellular C terminus among prostanoid receptors.[23,30] The human EP4 C-terminal tail has 38 serine and threonine residues that may serve as multiple phosphorylation sites. As with the EP2 receptor, the EP4 receptor signals though G_s to increase cAMP levels, although its coupling to G_s is much weaker than EP2's.[184] As described earlier, the EP4 receptor also signals through G_i and β-arrestin.

3.6.1. Biological Functions of EP4 Receptors. Vasodilator effects of EP4 receptor activation have been described in venous and arterial beds. The EP4 receptor may also help to regulate the perinatal closure of the ductus arteriosus.[185,186] The EP4 receptor is also involved in furosemide-induced salt-losing tubulopathy, a model for hyperprostaglandin E syndrome/antenatal Bartter syndrome.[187] The EP4 receptor mediates PGE_2-induced renin secretion and, together with the EP1 and EP3 receptors, contributes to PGE_2-mediated salt and water excretion in this model.

As with the EP2 receptor, the EP4 receptor mediates both pro-inflammatory and anti-inflammatory functions, often acting cooperatively with the EP2 receptor. The EP4 receptor has also been ascribed a role in the migration of LCs, a type of DC in the skin. PGE_2 acts on the EP4 receptor on LCs to enhance their migration to regional lymph nodes and to promote their maturation.[188] EP4 signaling is also required for DCs stimulated with an antibody against CD40 to produce IL-23.[170] The EP4 receptor is abundantly expressed on T cells, and together with the EP2 receptor, it enhances Th1 differentiation and Th17 expansion, as described earlier.[170]

The EP4 receptor also has a role in maintaining intestinal homeostasis. Kabashima et al.[189] found that EP4-deficient mice develop a severe colitis with 3% dextran sodium sulfate (DSS) treatment, which induces only marginal colitis in wild-type mice. This phenotype can be mimicked by administering an EP4 antagonist to wild-type mice. Conversely, administering an EP4 agonist to wild-type mice ameliorates the colitis induced by 7% DSS. It appears that PGE_2—EP4 signaling in the intestinal epithelium is important for the maintenance of the mucosal barrier against the injurious stimulus of DSS. An EP4 agonist tested in patients with ulcerative colitis had a beneficial effect. A clinical trial has shown the beneficial effects of an EP4 agonist in patients with mild to moderate uncreative colitis.[190]

In the CNS, the EP4 receptor, like the EP2 receptor, may function to confer neuroprotection in excitotoxic or hypoxic paradigms.[191] In addition, the EP4 receptor, along with the EP2 receptor, appears to be induced in endothelium after ischemia and during reperfusion, as well as to play a role in modulating cerebral blood flow dynamics.[173] As discussed earlier, not only PGD_2 but also PGE_2 promote sleep,[124] an effect that is mediated by the EP4 receptor at or near the subarachnoid space of the PGD_2-sensitive sleep-promoting zone.[192] In addition, PGE_2 has a wakefulness-augmenting effect mediated by EP1 and EP2 receptors around the third ventricle.[192]

3.6.2. EP4 Agonists and Antagonists. EP4 receptors may be pharmacologically distinguished from EP1 and EP3 receptors by their insensitivity to sulprostone and from EP2 receptors by their insensitivity to butaprost and relatively selective activation by PGE_1—OH. Selective EP4 agonists typically contain a 16-phenyl group. ONO-AE1-329, with an *m*-methoxymethyl substituent, has emerged as a highly selective and full EP4 agonist.[193]

The first EP4 antagonist reported, AH23848, played a pivotal role in the early pharmacological definition of the EP4 receptor.[194]

The use of AH23848 has since been overtaken by that of more selective and potent compounds. Many of these compounds, including L-161982, incorporate an acylsulfonamide as a key component of the scaffold.[195] ONO-AE3-208 has an amide moiety in place of the acylsulfonamide.

The potential uses of EP4 antagonists are diverse, including the treatment of inflammatory diseases, hyperalgesia, glaucoma, nephritis, and osteoporosis.[16,17] Recently, a novel EP4 antagonist, ER-819762, was reported to inhibit Th1 differentiation and Th17 expansion in vitro and to suppress disease in arthritis models in vivo.[196]

3.7. FP Receptors

The human and mouse FP cDNAs were cloned in 1994 and encode proteins of 359 and 366 amino acids, respectively.[24,31] Several splice variants that diverge in the sixth transmembrane domain have been identified for the human FP receptor.[39,41] As described above, the FP receptor is coupled to G_q and mobilizes cytosolic Ca^{2+}, but it is also coupled to $G_{12/13}$, and possibly to G_i, in a cell context-dependent manner.

3.7.1. Biological Functions of FP Receptors. $PGF_{2\alpha}$ is produced during the menstrual cycle by the secretory endometrium and plays a critical role in mammalian reproduction. FP receptor expression in the corpora lutea is critical for normal birth. FP-deficient mice exhibit parturition failure, apparently because luteolysis is prevented and progesterone levels do not decline preterm, resulting in reduced oxytocin receptor expression.[197] However, when an ovariectomy is performed on day 19 of pregnancy, circulating progesterone levels drop, oxytocin receptor expression is restored, and FP-deficient mice deliver successfully at term. Increased FP expression and $PGF_{2\alpha}$ signaling has been implicated in endometrial adenocarcinoma growth.[198] $PGF_{2\alpha}$ also plays a role in renal function, cardiac hypertrophy, and the regulation of intraocular pressure.[199]

FP-deficient mice also show attenuated fibrosis in bleomycin-induced pulmonary fibrosis, an animal model of idiopathic pulmonary fibrosis (IPF).[200] $PGF_{2\alpha}$ is abundant in the bronchoalveolar lavage fluid of human subjects with IPF and stimulates lung fibroblast proliferation and collagen production via the FP receptor, independent of transforming growth factor (TGF)-β, which is well-known to be crucial for fibrosis.[200] Thus, $PGF_{2\alpha}$—FP signaling may be a therapeutic target for IPF.

FP receptor expression has not been observed in the spleen, thymus, or immune cells, and thus there is little evidence to support a role for $PGF_{2\alpha}$—FP signaling in inflammatory or immunological processes.

The FP receptor's role in the pathogenesis of ischemic-reperfusion brain injury was investigated in a unilateral middle cerebral artery occlusion model of focal cerebral ischemia in mice.[201] After reperfusion, FP-deficient mice had less neurological deficit and smaller infarct volumes than wild-type mice, suggesting that $PGF_{2\alpha}$—FP signaling enhances cerebral ischemic injury.

3.7.2. FP Agonists and Antagonists. $PGF_{2\alpha}$ can also bind to EP1 and EP3 receptors with significant affinity, and some effects reported for $PGF_{2\alpha}$ may be mediated via an EP receptor. The 16-*m*-trifluoromethylphenoxy analogue of $PGF_{2\alpha}$ (fluoprostenol) has a high FP selectivity, and the 16-*m*-chlorophenoxyl analogue of $PGF_{2\alpha}$ (cloprostenol) is the most potent FP agonist reported (Figure 14). The 13,14-dihydro-17-phenyl analogue of $PGF_{2\alpha}$ (latanoprost-free acid) also has high FP selectivity, showing

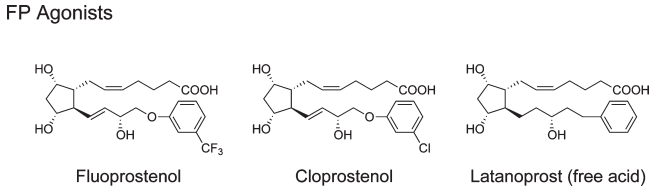

Figure 14. Structures of FP agonists.

22-fold less EP1 agonism than 17-phenyl $PGF_{2\alpha}$.[157] These FP agonists are used to treat ocular hypertension and glaucoma.[202,203]

THG113 is a specific, noncompetitive, reversible peptide FP receptor inhibitor that blocks the receptor's interaction with G_q, preventing increases in intracellular Ca^{2+} (Figure 5). THG113 effectively delays the preterm birth induced by administering LPS to pregnant mice on day 19 of pregnancy. In control mice, preterm birth occurs within 18 h of LPS administration in 100% of cases. When THG113 was administered 4−6 h after LPS, after the PG effect of luteolysis was past, the preterm birth was delayed more than 40 h.

3.8. IP Receptors

PGI_2 (prostacyclin) is the primary prostanoid produced by endothelial cells, and it plays an important role in vascular homeostasis through its potent vasodilatory and antithrombotic effects. PGI_2 functionally opposes the effects of TXA_2. The human and mouse IP receptor cDNAs encode proteins of 386 and 415 amino acids, respectively.[25,32] The IP receptor is coupled to G_s, primarily, and also to G_q and G_i.

3.8.1. Biological Functions of IP Receptors. The role of PGI_2 in cardiovascular homeostasis is well established. IP-deficient mice exhibit increased propensities toward thrombosis, intimal hyperplasia, and restenosis, as well as reperfusion injury.[107,204,205] More recently, the IP receptor was shown to have an atheroprotective effect in premenopausal females.[206] IP deficiency accelerates atherogenesis in ApoE-deficient mice, which are prone to atherosclerosis.[207] The worldwide withdrawal of a selective COX-2 inhibitor drug in 2004 was due to the increased incidence of cardiovascular events, including myocardial infarction and thrombotic strokes that were probably caused by its suppression of COX-2-derived PGI_2 and its cardioprotective effects, but not of COX-1-derived TXA_2.[208]

In addition to its role in cardiovascular homeostasis, PGI_2 is an important mediator of the edema and pain accompanying acute inflammation. In IP-deficient mice, the potentiation of bradykinin-induced microvascular permeability by PGI_2 is abolished, and carrageenan-induced paw edema is reduced.[107] IP-deficient mice also show reduced nociceptive responses in an acetic acid-induced writhing model, indicating that the IP receptor facilitates pain sensation. Thus, as discussed earlier, the EP1 and IP receptors are the dominant receptors mediating peripheral pain sensation.

In the immune system, PGI_2 has anti-inflammatory functions, and an immunosuppressive effect on Th2-mediated inflammation is reported.[209,210] The anti-inflammatory activity of PGI_2 appears to be mediated by the IP receptor on DCs, which suppresses DC activation, maturation, and T-cell stimulatory function.[211] On the other hand, the IP receptor on T cells appears to promote Th1 differentiation, as shown by a significantly decreased contact hypersensitivity response in IP-deficient mice.[212] Lymph node cells from sensitized IP-deficient mice

exhibit decreased IFN-γ production and a smaller T-bet$^+$ subset compared with cells from control mice. Th1 differentiation is enhanced by an IP agonist in vitro, and this enhancement is nullified by a PKA inhibitor, suggesting that IP signaling is promoted through a cAMP−PKA pathway. However, once the CD4$^+$ T cells are differentiated, IP agonists have an immune-suppressive effect on them, inhibiting the production of both Th1 and Th2 cytokines.[213] Thus, as seen with the DP1, EP2, and EP4 receptors, the function of the IP receptor is context-dependent and plays both pro- and anti-inflammatory roles.

3.8.2. IP Agonists and Antagonists. IP agonists include cicaprost, iloprost, and carbacyclin (Figure 15). Orientation of the α-carboxyl terminus away from the ω-chain is crucial for potent IP agonism. A 16-methyl/18,19-triple-bond structure is often favored for the ω-terminus. Iloprost and carbacyclin potently activate the IP receptor, but they activate the EP1 receptor as well; cicaprost is more selective. PGI_2's vasodilatory action has been used clinically to reduce pulmonary vascular resistance in patients suffering from primary pulmonary hypertension.[214]

On the basis of the potential role of PGI_2 in mediating pain, IP antagonists have been developed.[215] In a series of chemical library screenings, two structurally distinct classes of selective IP antagonists emerged.[216] The 2-(phenylamino)imidazoline series is represented by RO-1138452, whereas the N-substituted phenylalanine series is represented by RO-3244019 and its difluoro analogue RO-3244794 (Figure 15).[216,217] RO-1138452 binds to human platelet IP receptors and recombinant IP receptors with high affinity, but it also binds to platelet activating factor (PAF) and imidazoline receptors. RO-1138452 and RO-3244794 significantly reduce carrageenan-induced mechanical hyperalgesia and edema formation, and RO-3244794 also significantly reduces the chronic joint discomfort induced by monoiodoacetate.[216]

Although pain has been the initial focus for IP antagonists, they have also been examined for their potential use in treating bladder disorders. RO-3244019 decreases bladder contraction frequency, increases the micturition threshold and voiding interval,[218] and is also effective in treating the neurogenic detrusor overactivity arising from spinal cord injury in rats.[219]

3.9. TP Receptors

TXA_2 is important in regulating cardiovascular homeostasis and in mediating platelet shape change and aggregation as well as smooth muscle contraction and proliferation. The human TP receptor was the first prostanoid receptor to be cloned. The human and mouse TP receptor cDNAs encode proteins of 343 and 341 amino acids, respectively.[18,220] An alternatively spliced variant (TPβ) has been identified that differs from the original TP receptor (TPα) in the C-terminal tail.[42,71]

3.9.1. Biological Functions of TP Receptors. PGI_2 and TXA_2 are major prostanoids in the cardiovascular system, and they mediate opposite functions on vasculature and platelets. Increased TXA_2 synthesis has been linked to cardiovascular diseases, including acute myocardial ischemia and heart failure, and to renal diseases. Indeed, TP-deficient mice show increased bleeding tendencies and are resistant to cardiovascular shock induced by AA or the TP agonist U-46619.[221] The interplay between PGI_2 and TXA_2 was demonstrated by an augmented response of IP-deficient mice in a vascular injury model, which was abolished by additional TP deficiency.[204] The roles of the TP receptor in the pathogenesis of cardiovascular diseases are detailed in a recent review.[222]

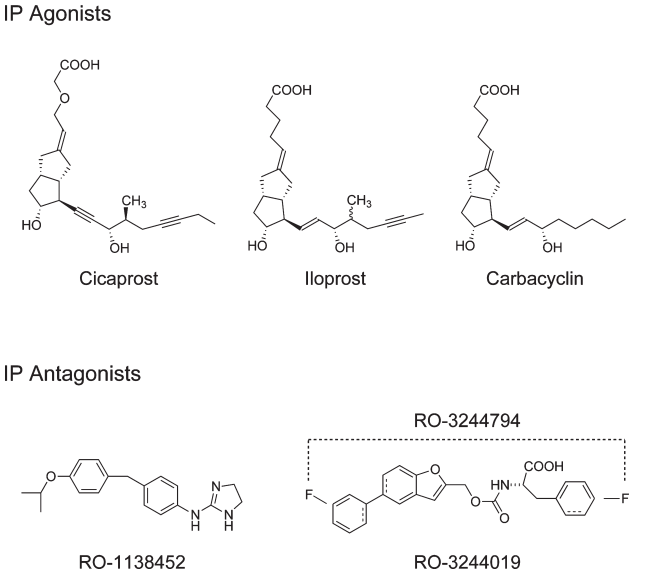

Figure 15. Structures of IP agonists and antagonists.

As TP mRNA is abundantly expressed in lymphoid organs such as the thymus and spleen, the TP receptor has been expected to play a role in immune responses. Kabashima et al.[223] found that TXA_2 generated in DCs acts on T-cell TP receptors to inhibit DC−T-cell interactions, thus negatively regulating immune responses. On the other hand, the TP receptor has also been reported to enhance cellular immune responses through pro-inflammatory activity.[224] The authors found that both mitogen-induced responses and cellular responses to alloantigen were substantially reduced in TP-deficient spleen cells, and immune-mediated tissue injury following cardiac transplant rejection was reduced by the absence of TP. The TP receptor also appears to regulate the host response against infection. During a parasitic *Trypanosoma cruzi* infection, parasite-derived TXA_2 modulates the host response through somatic cell TP receptors, to limit parasitism.[225]

In the nervous system, the TP receptor may be expressed on astrocytes, oligodendrocytes, and hippocampal neurons.[226−228] The exact function of TXA_2 remains unknown. It has been shown that isoprostanes, free radical-catalyzed AA products, are elevated in AD patients and in the Tg2576 mouse model of AD.[229] Isoprostanes act at the TP receptor, and as noted earlier, TPα/TPβ heterodimerization enhances isoprostane-mediated signaling. When the isoprostane iPGF$_{2\alpha}$-III is delivered into the brains of Tg2576 mice, the brain amyloid-β (Aβ) levels and plaque-like deposits increase, and this increase is blocked by a TP antagonist. These results suggest that TP activation mediates the effects of iPGF$_{2\alpha}$-III.

3.9.2. TP Agonists and Antagonists. TXA_2 and its precursor endoperoxide PGH_2 both activate TP receptors. Both are too unstable for use in receptor binding and signal transduction assays. A number of synthetic agonists and antagonists are available for this receptor, including the agonists I-BOP, STA_2, and U-46619 and the antagonists SQ29,548 and S-145 (Figure 16).

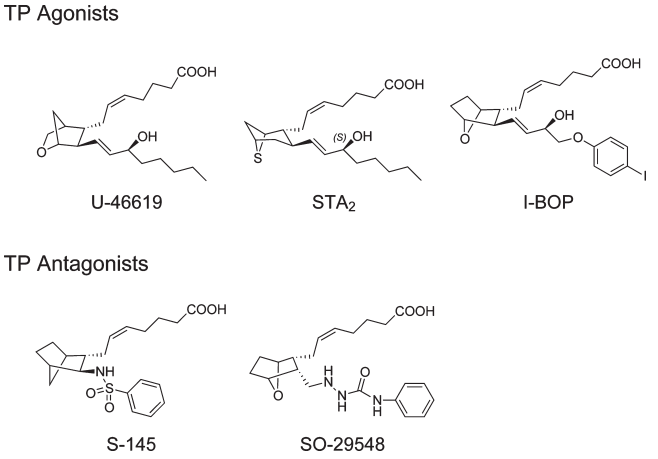

Figure 16. Structures of TP agonists and antagonists.

In addition, as described above, the TP receptor also binds isoprostanes at higher concentrations. Isoprostanes are also products of AA metabolism but are formed via a free radical-mediated mechanism, not by the COX-dependent pathway. In contrast to the classic prostanoids, which are generated from free AA, the isoprostanes are formed in situ from the fatty acid backbone esterified in membrane phospholipids, where they are also stored. Depending on which of the labile hydrogen atoms of the fatty acid is first abstracted by the free radical attack, four isoprostane regioisomers can be formed. Isoprostanes are released in response to cellular activation through a PLA_2-mediated mechanism. These products serve as a reliable marker of oxidant injury in vitro and in vivo.[230,231] The TP receptor appears to mediate some of the effects of this class of products.[73] In an experimental setting where TXA_2 is suppressed, the coincidental blockade of the TP receptor increases the beneficial anti-inflammatory action of COX-1 inhibition and results in an additional antiatherogenic effect.[232]

Although TP antagonists were initially developed for the purpose of cardiovascular therapy, another early indication for TP antagonists was asthma, which met with some clinical success, albeit limited.[233] Other early indications that do not appear to have met with any clinical success include glomerulonephritis, allergic rhinitis, inflammatory bowel disease, and diabetes.[16] There are other potential indications that have emerged more recently. The TP antagonist seratrodast appears to possess antitussive properties,[234,235] and ramatroban is reported to attenuate cough in subjects with cough-variant asthma,[236] an effect that could be attributable to seratrodast's blockade of both TP and DP2 receptors.

4. CONCLUSION

Prostanoids and their receptors control many cellular processes. Research during the last two decades has identified each prostanoid receptor molecularly and has established each receptor's function(s) by gene-deletion studies. The evidence now indicates that prostanoids play versatile roles in health and disease. In addition to their classically described role in acute inflammation, they play critical roles in immunity, cancer, and neurologic diseases. The involvement of prostanoids in many disease processes makes them important targets for therapy.

Although some agonists and antagonists for prostanoid receptors are in clinical use, developing more selective ligands or ligands with targeted polypharmacology may be beneficial. Such rational drug design will benefit from an elucidation of the crystal structure of prostanoid receptors. Although the challenges of crystallization remain formidable, solving the prostanoid receptors' structures will provide insights into their common and unique structural features involved in ligand binding, receptor activation, and interactions with signaling proteins. Understanding the structural changes that occur within the receptors will open new approaches to drug design.

Another important issue that needs to be resolved is how prostanoid receptor signaling is spatially and temporally integrated into the complex signaling network within a cell. It is becoming apparent that prostanoid receptors act cooperatively or sequentially to initiate or sustain disease states, and that their signaling converges with that of cytokines and neurotransmitters to regulate an intracellular signaling network. Such crosstalk has an important role in regulating many physiological and pathophysiological processes. The discovery of a multitude of intracellular protein interactions with prostanoid receptors points to the potential for modulating their expression, ligand selectivity, and signaling. Clarifying these interactions and their cell context-driven functions will help paint a detailed picture of the signaling network necessary to produce a cellular response, and will provide clues toward more specific drug development.

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Shuh Narumiya received his M.D. degree from Kyoto University in 1973 and his Ph.D. degree in Medical Chemistry from Kyoto University Graduate School of Medicine in 1979. He then did postdoctoral work in Pharmacology with Sir John Vane in the Wellcome Research Laboratories, England. He returned to Japan in 1981 and, since then, has been working in Kyoto University Graduate School of Medicine, being promoted to Professor in Pharmacology in 1992. During this period, he and his group first purified thromboxane A_2 receptor from human blood platelets in 1989, then cloned cDNAs for the family of eight types and subtypes of prostanoid receptors, generated knockout mice deficient in each of these receptors individually, and elucidated physiological and pathophysiological roles that each receptor plays in the body. Their works stimulated a variety of research efforts to establish a research field on prostanoid receptors. Dr. Narumiya also discovered an ADP-ribosyl transferase in *Clostridium botulinum* now known as botulinum C3 exoenzyme and identified small GTPase Rho as its specific substrate. C3 exoenzyme was then widely used to dissect functions of Rho protein, and these studies revealed that Rho functions as a molecular switch in cell morphogenesis, adhesion, and motility by inducing specific types of actin cytoskeleton. Dr. Narumiya then identified and cloned several effector proteins for Rho such as ROCK and mDia and elucidated the pathway from Rho to the actin cytoskeleton. For these achievements, he was awarded the Osaka Science Prize in 1998, the Takeda Prize for Medicine in 1999, the Erwin von Baelz Prize in 1999, the Lorenzini Giovanni Gold Medal in 2000, the Uehara Prize in 2002, the Imperial Prize and the Japan Academy Prize in 2006, the Ulysses Medal (The University College Dublin) in 2008, and The Inflammation Research Lifetime Achievement Award in 2009. Between 2005 and 2007, he was the Dean of Kyoto University Graduate School of Medicine.

ACKNOWLEDGMENT

We are grateful to Dr. Tomoyuki Furuyashiki for comments on the manuscript and Yosuke Toyoda and Kazuko Iwasa for help with the chemical structure drawings. Research in our laboratory is supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan, by a grant from ONO Research Foundation, and by the Special Coordination Funds for Promoting Science and Technology of the Japanese Government and Astellas Pharma in the Formation of Innovation Center for Fusion of Advanced Technologies Program.

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