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PROSTANOID RECEPTORS: Subtypes and Signaling*

Richard M Breyer,[1] Carey K Bagdassarian,[2] Scott A Myers,[1] and Matthew D Breyer[3]

[1]*Division of Nephrology and Departments of Medicine and Pharmacology, Vanderbilt University, Nashville, Tennessee 37232; e-mail: rich.breyer@mcmail.vanderbilt.edu, scott.myers@mcmail.vanderbilt.edu*

[2]*Department of Chemistry, College of William and Mary, Williamsburg, Virginia 23187; e-mail: ckbagd@wm.edu*

[3]*Division of Nephrology and Departments of Medicine and Molecular Physiology and Biophysics, Vanderbilt Univesity, and Department of Veterans Affairs Medical Center, Nashville, Tennessee 37232; e-mail: matthew.breyer@mcmail.vanderbilt.edu*

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■ **Abstract** Cyclooxygenases metabolize arachidonate to five primary prostanoids: PGE_2, $PGF_{2\alpha}$, PGI_2, TxA_2, and PGD_2. These autacrine lipid mediators interact with specific members of a family of distinct G-protein-coupled prostanoid receptors, designated EP, FP, IP, TP, and DP, respectively. Each of these receptors has been cloned, expressed, and characterized. This family of eight prostanoid receptor complementary DNAs encodes seven transmembrane proteins which are typical of G-protein-coupled receptors and these receptors are distinguished by their ligand-binding profiles and the signal transduction pathways activated on ligand binding. Ligand-binding selectivity of these receptors is determined by both the transmembrane sequences and amino acid residues in the putative extracellular-loop regions. The selectivity of interaction between the receptors and G proteins appears to be mediated at least in part by the C-terminal tail region. Each of the EP_1, EP_3, FP, and TP receptors has alternative splice variants described that alter the coding sequence in the C-terminal intracellular tail region. The C-terminal variants modulate signal transduction, phosphorylation, and desensitization of these receptors, as well as altering agonist-independent constitutive activity.

INTRODUCTION

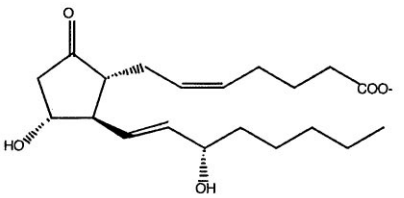
Prostaglandins (PGs) comprise a diverse family of autacoids, whose synthesis is initiated by cyclooxygenase-mediated metabolism of the unsaturated 20-carbon fatty acid arachidonic acid to PGG/H_2, generating five primary bioactive prostanoids:

PGE_2, $PGF_{2\alpha}$, PGD_2, PGI_2, and TXA_2 (1, 2). The importance of this pathway in a broad array of diseases including cancer, inflammation, and hypertension is underscored by the classic and novel uses of cyclooxygenase-inhibiting nonsteroidal anti-inflammatory drugs that nonselectively inhibit the synthesis of all of these compounds. Each prostanoid is synthesized in specific compartments within the body via the action of specific synthases. These autacoids then act within the tissue where they are synthesized via specific G-protein-coupled receptors (GPCRs), designated EP for PGE_2 receptors and FP, DP, IP, and TP for $PGF_{2\alpha}$, PGD_2, PGI_2, and TXA_2 receptors, respectively (3, 4). The chemical structure of each of the five major PGs is shown in Figure 1*A*. The energy-minimized geometries of the endogenous ligands are very similar (Figure 1*B*), and, although each PG binds with the highest affinity to its cognate receptor, considerable ligand-binding cross-reactivity can be observed between a given prostanoid and other receptors within the family (Table 1).

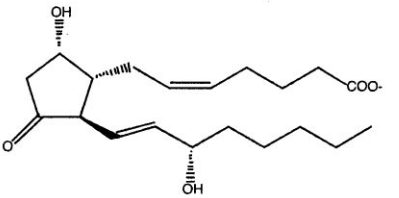
The PG receptors have the characteristic seven-hydrophobic-transmembrane-segment architecture typical of GPCRs, and several of the prostanoid receptors display alternatively spliced variants in the C-terminal sequence that can alter receptor function (Figure 2). The prostanoid receptors belong to the family A GPCRs (5). Phylogenetic studies have broken family A down into five evolutionarily conserved groups, with the PG receptors in group V (5). In addition to the prostanoid receptors, this group includes a number of receptors for autacrine, paracrine, and endocrine factors such as small tripeptides, pituitary hormones, glycoprotein hormones, opioids, and platelet-activating factor. Interestingly, the prostanoid family is most closely related to the vasopressin receptor family of peptide-binding hormone receptors, and as described below, the ligand-binding motif of the prostanoid receptors shares some similarities with this class of peptide-binding receptors rather than with other receptors that bind small-molecule ligands, for example the adrenergic receptor family (6). Most of the receptors in group V signal via stimulation of phospholipase C to produce IP_3 and di-acyl-glycerol or via inhibition of adenylyl cyclase through inhibitory guanine nucleotide-binding regulatory protein (G_i),

Figure 1 (*A*) The structure of the five principal prostaglandin metabolites. (*B*) Energy-minimized prostanoid molecular geometries. These structures result, with only small perturbations, from minimization in vacuo with either the AM1 or PM3 semi-empirical method and also from several ab initio schemes (STO-3G, 6-31G*, 6-31G**). For each prostanoid molecule, bond lengths, bond angles, and dihedral angles are systematically varied from a starting molecular geometry until a minimum energy structure is located. Ab initio schemes are the more exact since semi-empirical calculations introduce further approximations to the quantum mechanical calculations (4a). Calculations were performed with the Gaussian 94 and Molecular Simulations Inc. computational packages. Note similar three-dimensional geometries for the structurally different prostanoids. The orientation of the prostanoid structures is similar to that shown for the chemical structures in panel *A*, with the prostanoid ring on the left, the carboxyl-containing alpha side chain to the upper right, and the omega chain to the lower right. Carbon atoms are *medium lines*, oxygen atoms are *bold lines*, and hydrogen atoms are *gray* (*light*) *lines*.

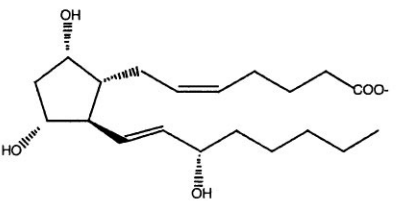
A



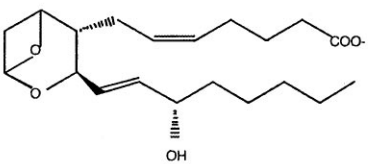
PGE_2



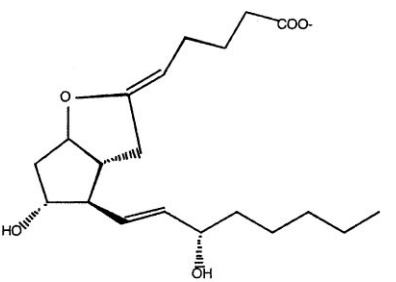
PGD_2



$PGF_{2\alpha}$



TxA_2



PGI_2

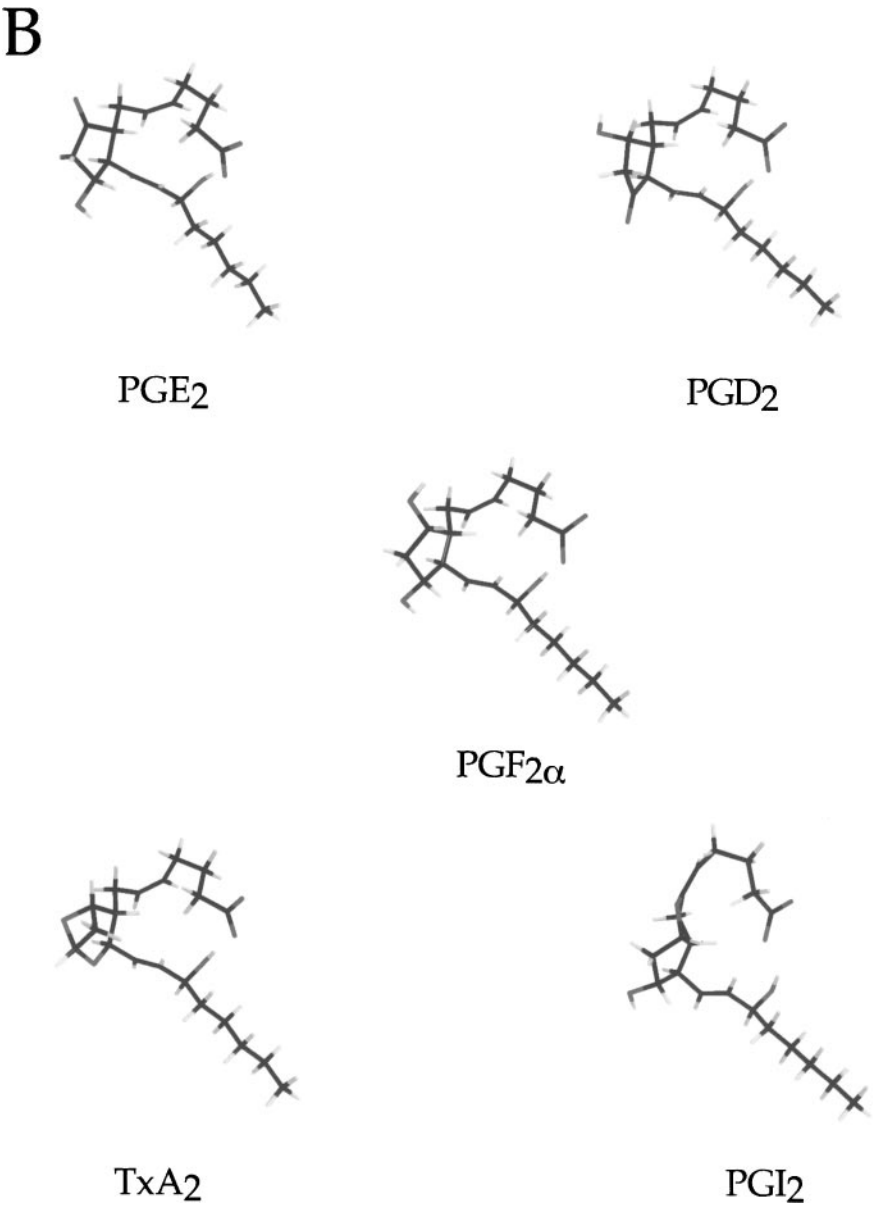


Figure 1 (*Continued*)

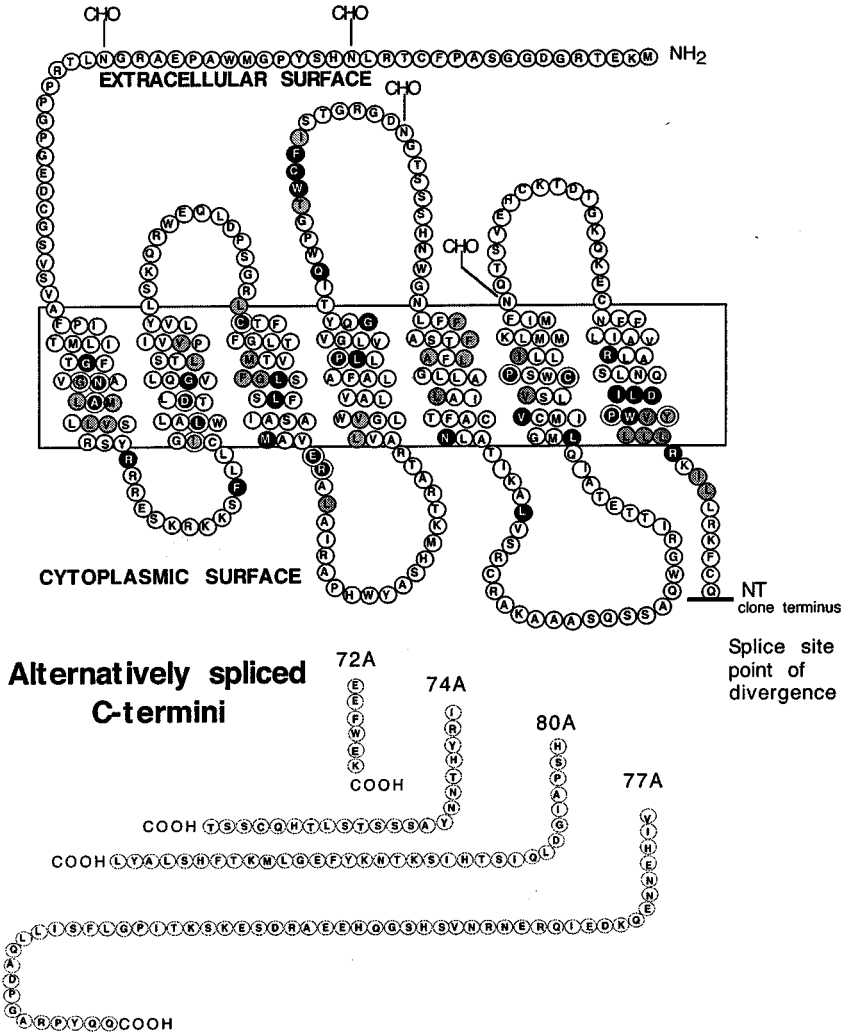


Figure 2 Identification of conserved residues in the EP_3 receptor and sequence of five rabbit EP_3 receptor splice variants differing only in their intracellular carboxyl termini. Sequence alignments of the predicted amino acid sequences for the prostanoid receptors were carried out utilizing the TMAP program (145). The sequences aligned were the human and mouse EP_1 receptors; the human EP_2 receptor; EP_3 receptors from rabbit, rat, mouse, cow, and human tissues; the EP_4 receptor from rabbit, mouse, rat, and human tissues; the TXA_2 receptor from mouse, rat, and human tissues; the FP receptor for mouse, rat, cow, and human tissues; the IP receptor from mouse and human tissues; and the DP receptor from mouse and human tissues. Conserved residues are indicated by *gray circles*, and invariant residues are indicated by *black circles*. Residues with "bulls-eye" *symbols* are conserved across the entire superfamily of GPCRs (146), those without this inset are unique to the prostanoid receptors. The predicted amino acid sequences of each splice variant are represented by the one-letter amino acid code. The carboxyl-variable tails range from 56-amino-acid residues for clone 77A to none for the NT (no-tail) clone.

TABLE 1 Inhibitor constant (nM) of prostanoid binding to cloned G-protein-coupled prostanoid receptors[a]

Ligand class	Ligand[b]	FP human	FP mouse	TP human	TP mouse	EP_1 human	EP_1 mouse	EP_2 human	EP_2 mouse
EP1	PGE2	119 12	100 (73–140)	*	*	9.1 1.5	20 (15–26)	4.9 0.5	12 (9.2–15)
	17-phenyl PGE2		60 (47–77)		*		14 (11–18)		*
	SC51322/antag	*		507 47		13.8 0.7	7.9	*	
	SC51089/antag	*		*		1332 187		*	
	AH6809/antag	*		4325 232		1217 98		1150 36	
EP2	Butaprost FA	*		*		*		91 + 12	
	Butaprost ME	*	*	*	*	*	*	3513 291	110 (83–140)
	AH13205		*		*		*		240 (150–400)
EP3	Sulprostone	198 10	580 (360–930)	*	*	107 15	21 (17–25)	*	*
	MB28767	510 74	124 (123–124)	343 11	1300	419 75	120 (110–150)	988 85	*
	GR63799	1241 131	>10,000	*	*	329 38	*	*	*
	16,16 dimethylPGE2		350 (250–480)		*		*		17 (13–23)
	Misoprostol FA	9382 56510		*		*		34 5	
	Misprostol ME	*	*	*	*	*	120 (94–150)	*	250 (190–340)
EP4	1(OH)-PGE1		*		*		*		*
DP	PGD2	6.7 0.5	47 (34–66)	6602 541	*	5820 1801	*	2973 100	*
	BW 245C	*	1700	*	*	*	*	219 19	*
	ZK110841	*		1121 201		1.8 0.2		6.0 0.6	
FP	PGF2	3.2 0.3	3.4 (2.8–4.2)	8700 670	*	547 104	1300	964 64	*
	Fluprostenol	2.3 0.2	3.8 (3.1–4.8)	*	*	3833 9415	*	*	*
	Cloprostenol	0.47 0.05		6123 702		815 149		*	
	Latanoprost (FA)	2.8 0.2		*		1750 205		*	
	Latanoprost (IE)	555 98		*		8540 1304		*	
IP	Iloprost	619 105	*	6487 29	*	11 1	21 (17–26)	1870 176	1600
	Cicaprost	>1340	*	>1340	*	>1340	*	>1340	1300
	Beraprost		*		*		*		*
	Carbacyclin	427, 290	*	*	*	23 10	*	942 123	1600
TP	SQ29548/antag	*	*	4.1 0.4	13 (9.6–18)	*	*	*	*
	I-BOP		100 (73–140)		0.56 (0.44–0.72)		*		220 (160–310)
	S-145 antag		*		0.68 (0.47–0.97)		*		*
	U46619	241 8	1000 (560–1600)	35 5	67 (48–93)	*	*	*	*

(*Continued*)

TABLE 1 (*Continued*) Inhibitor constant (nM) of prostanoid binding to cloned G-protein-coupled prostanoid receptors[a]

Ligand class	Ligand[b]	EP_3 human	EP_3 mouse	EP_4 human	EP_4 mouse	DP human	DP mouse	IP human	IP mouse
EP1	PGE2	0.33 0.3	0.85 (0.69–1.1)	0.79 0.07	1.9 (1.5–2.5)	307 106	*	*	*
	17-phenyl PGE2		3.7 (2.8–4.9)		1000		*		*
	SC51322/antag	698 122		*		*		*	
	SC51089/antag	*		*		*		*	
	AH6809/antag	1597 140		*		1415 104		*	
EP2	Butaprost FA	1643 113		*		*		*	
	Butaprost ME	*	*	*	*	*	*	*	*
	AH13205		82 (57–120)		*		*		*
EP3	Sulprostone	0.35 0.11	0.6 (0.44–0.81)	7740 1130	*	*	*	*	*
	MB28767	0.14 0.02	0.68 (0.53–0.87)	10 3	500 (300–850)	*	*	*	*
	GR63799	4.77 0.16	1.9 (1.6–2.4)	149 27	480 (320–720)	*	*	*	*
	16,16 dimethylPGE2		1.9 (1.5–2.5)		43 (32–58)		*		*
	Misoprostol FA	7.9 1		23 2		*		*	
	Misprostol ME	319 15	67 (53–89)	5499 1102	67 (45–99)	*	*	*	*
EP4	1(OH)-PGE1		330 (240–460)		190 (120–280)		*		*
DP	PGD2	421 60	280 (180–430)	1483 189	*	1.7 0.3	21 (17–28)	*	*
	BW 245C	*	*	132 26	*	0.4 0.1	250 (160–380)	*	*
	ZK110841	402, 604		41 7		0.3 0.1		2138 270	
FP	PGF2	38 6	75 (53–110)	288 27	*	861 139	*	*	*
	Fluprostenol	708 65	*	*	*	*	*	*	*
	Cloprostenol	4.4 0.2		9137 920		*		*	
	Latanoprost (FA)	6503 1017		*		*		*	
	Latanoprost (IE)	>100,000		*		*		*	
IP	Iloprost	56 6	22 (17–30)	284 9	2300	1035 171	*	11 1	11 (8.7–15)
	Cicaprost	255 68	170 (130–210)	44 10	*	>1340	*	17 4	10 (8.2–13)
	Beraprost		110 (91–130)		*		*		16 (13–21)
	Carbacyclin	14 4	22 (17–30)	352 78	2300	132 16	*	17 4	110 (85–130)
TP	SQ29548/antag	*	*	*	*	*	*	*	*
	I-BOP		100 (84–130)		*		*		*
	S-145 antag		*		*		*		*
	U46619	*	*	3013 149	*	3970 390	*	*	*

[a]" " indicates standard errors as taken from Abramowitz et al (8). "*" indicates $K_i > 10{,}000$ nM. Blanks are "not tested."

[b]numbers in "()" represent 95% confidence intervals as taken from Kiriyama et al (10) FA, free acid; ME, methyl ester.

and various eicosanoid receptors have been demonstrated to signal through each of these pathways as described in detail below.

Within the prostanoid family, the receptors are typically related in sequence by 20%–30%, and overall there are 65-amino-acid residues conserved among the prostanoid receptors. Of these residues, 34 are identical across the prostanoid receptor family (Figure 2). Of the 34 absolutely conserved residues, 14 are conserved across the entire family A of GPCRs [e.g. Pro-339 in transmembrane 7 (TMVII)], leaving 20 residues that are conserved among, and unique to, the prostanoid receptor family. The majority of these prostanoid receptor "signature" residues lie within the transmembrane regions, although a significant stretch of conserved amino acids exists in the second extracellular loop region (7). Functionally, there is evidence that both transmembrane and extracellular regions of the prostanoid receptors are involved in ligand binding. Most ligand-binding studies on the prostanoid receptors have demonstrated a single class of receptor sites (8–10). Unlike the biogenic amine receptors, for example the β_2 adrenergic receptor, the addition of GTP analogs causes little if any shift in affinity for agonists in the prostanoid receptor family, and some evidence suggests that the G-protein-coupled form of the receptor has a lower affinity for ligand binding than does the uncoupled form, that is, in the presence of GTPγS (11). Another striking feature of the prostanoid receptor family is the existence of alternatively spliced messenger RNA (mRNA) variants described for four of the eight PG GPCRs, the TP, FP, EP_1, and EP_3 receptors. In each case, the alternative splicing occurs in the intracellular C-terminal region of the receptor. Alternative splicing does not appear to affect ligand-binding properties of the receptors, but it does have impact on G-protein coupling specificity and constitutive activity, as well as agonist-induced receptor phosphorylation, desensitization, and/or internalization.

TP RECEPTORS

Thromboxane is a potent mediator of platelet shape change and aggregation as well as smooth muscle contraction and proliferation. Increased thromboxane synthesis has been linked to cardiovascular diseases including acute myocardial ischemia (12), heart failure (13), and renal diseases (14, 15), making TP receptor antagonists potential therapeutic agents for these diseases. A point mutation (Arg-60 $\rightarrow$ Leu) in the first cytoplasmic loop of the TXA_2 receptor was identified in a dominantly inherited bleeding disorder characterized by defective platelet response to TXA_2 (16).

The human TxA_2 receptor designated "TP" was the first eicosanoid receptor cloned (17) and encoded a protein of 343 amino acids (37.4 kDa) containing the seven hydrophobic stretches of amino acids. Full-length mouse, rat, and monkey TP receptor complementary DNAs (cDNAs) have also been isolated (18–20). Two alternatively spliced variants of the human thromboxane receptor have been described (21). These variants differ in the carboxyl-terminal tail of the receptor

distal to Arg-328. Similar patterns of alternative splicing have been described for both the EP_3 receptor and the FP receptor (see below). The original placental derived clone, encoding a 343-amino-acid receptor, has been designated α, and the subsequent 407-amino-acid splice variant cloned from endothelium is designated β. Although splice variants have not been described for the TP receptor in other species, the lack of homology in the variant region between the mouse TP receptor C terminus and either of the human splice variants has been proposed as evidence for the possible existence of further undescribed splice variants (4). Northern analysis of mouse tissues revealed that the highest level of TP mRNA expression is in the thymus, followed by spleen, lung, and kidney with lower levels of expression in heart, uterus, and brain (18).

Although the endogenous ligand TXA_2 is 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 (3). Competition radioligand-binding studies have demonstrated that the rank order of potency on human platelet TP receptor is I-BOP > SQ29,548 > STA_2 > U-46619 (22). This is consistent with the comprehensive assessment of >25 ligands tested in binding assays using the recombinant α splice variant (8). Recent studies have also suggested that the TP receptor may bind nonenzymatically derived isoprostane analogs at higher concentrations, and thus the TP receptor may be mediating some of the effects of this class of compounds (23, 24).

Mutational analysis of the human TP receptor has identified Trp-299 as a residue in the seventh transmembrane domain that is critical for ligand-binding selectivity. A Trp-299 $\rightarrow$ Leu mutation resulted in a receptor that bound the agonists I-BOP and U-46619 with high affinity but lost the ability to bind the antagonist SQ29,548 (25). This finding is the first in a series of studies suggesting an important role for the TMVII in ligand binding by the prostanoid receptors. It is of interest that, although a Trp is found in both the human and mouse TP receptors as well as the mouse EP_1 and FP receptors, a leucine naturally occurs in the corresponding position in the EP_3 receptor (Figure 2). Mutagenesis of the universally conserved Arg-295, one helical turn away from this Trp-299 in TMVII, led to a loss of ligand binding and signal transduction of the human TP receptor (25), a finding that has been observed in other prostanoid receptors (7, 26, 27). Mutagenesis of seven cysteine residues in the human TPα receptor revealed that several of the Cys residues, particularly those in the first and second extracellular loop regions, are critical determinants of ligand binding, perhaps through the formation of essential disulfide bonds in the receptor structure (28). Chiang & Tai (29) have mutated the two putative glycosylation sites in the extracellular sequences of the TP receptor and found that, although mutation of either one of the sites had little or no effect on ligand binding, simultaneous mutation of both sites led to a loss of ligand binding. Similar results were achieved by treatment of the Sf9 insect cell expression system with tunicamycin. Whether the glycosylation is critical for ligand binding per se or loss of glycosylation merely

impairs protein folding and/or receptor trafficking to the cell surface remains unclear.

Thromboxane receptors are classically characterized by signaling via the G_q G-protein activating Ca^{2+}/DAG signaling pathways. More recently, it has been appreciated that the TP receptor can couple via G_{11}, G_{12}, G_{13} (23, 30, 31), and the novel G-protein transglutaminase G_h (32). Although no differences were observed in the ligand binding and coupling of the TP receptor α and β splice variants, there were significant differences in their ability to internalize in response to agonist exposure (33). The β splice variant internalizes to a much greater extent than the α splice variant upon exposure to agonist. Dominant negative mutants were used to demonstrate that the internalization of the TP_β is dynamin, G-protein receptor coupled kinase, and arrestin dependent when expressed in HEK293 cells, suggesting the involvement of receptor phosphorylation and clathrin-coated pits in internalization of this receptor (33). Together these data suggest a role for alternative splicing of the TP receptor in phosphorylation, arrestin binding, and receptor internalization. A physiologic role for the alternative splicing of the TP receptor has been suggested by Walsh et al (34), who demonstrated that the TPα but not TPβ splice variant is a target for prostacyclin-activated protein kinase A phosphorylation/desensitization, suggesting that the TPα variant may be involved in the balance between thromboxane/prostacyclin-mediated vascular homeostasis.

FP RECEPTORS

Expression of the FP receptor in corpora lutea is critical for normal birth, and homozygous disruption of the murine FP receptor gene results in failure of parturition in females, apparently due to failure of the normal preterm decline in progesterone levels (35). The cDNA encoding the $PGF_{2\alpha}$ receptor was cloned from a human kidney cDNA library (36) and encodes a protein of 359-amino-acid residues. The FP receptor has also been cloned from mouse, cow, rat, and sheep cells (37–40). Alternative splicing of the sheep FP receptor was observed by Pierce et al (40), who identified two splice variants of the FP receptor cloned from a sheep corpus luteum library. Differences between these proteins, designated FP_A and FP_B, begin nine amino acids into the C-terminal cytoplasmic region, with the FP_A receptor continuing on for an additional 46 amino acids and the FP_B splice variant having only one additional amino acid distal to the splice site. The FP_A splice variant C terminus shares significant homology with the C terminus of the human FP receptor. Transfection of the human receptor into COS-M6 cells conferred preferential ^{3}H-labeled $PGF_{2\alpha}$ binding with a K_D of 1 nM. ^{3}H-labeled $PGF_{2\alpha}$ binding was displaced by a panel of ligands with a rank order potency as follows: $PGF_{2\alpha} =$ fluprostenol $>$ PGD_2 $>$ PGE_2 $>$ U46619 $>$ iloprost (8, 36). A similar agonist order of affinity was observed for the sheep FP receptor 17-phenyl-trinor-$PGF_{2\alpha}$ $>$ $PGF_{2\alpha}$ $>$ fluprostenol $>$ $PGD_2 = PGE_2 \gg 8$ iso-$PGF_{2\alpha}$ (40). It is notable that $PGF_{2\alpha}$ can also bind to EP_1 and EP_3 receptors with significant affinity, and some

reported effects of $PGF_{2\alpha}$ may be mediated via an EP receptor (10, 41, 42). Substitution of His-81 in transmembrane 2 (TMII) of the rat FP receptor with a number of different amino acids led to either a loss of ligand binding or alterations in the pH optimum of the receptor ligand interaction. The authors propose that His-81 interacts with the conserved Arg in TMVII and might play a direct role in ligand binding (43), although the effects of these mutations may also be caused by global alterations in receptor structure and folding.

When the human FP receptor was expressed in oocytes, $PGF_{2\alpha}$ or fluprostenol induced a Ca^{2+}-dependent Cl^- current, consistent with the FP receptor signaling via increased $[Ca^{2+}]_i$ (36). Both ovine FP splice variants caused an agonist-dependent increase in IP accumulation with similar 50% effective concentration values (40) and have also been demonstrated to couple to a Rho-mediated pathway (44). Interestingly, both splice variants displayed significant constitutive activity, and the FP_B variant displayed twice the constitutive activity of the FP_A variant when transiently expressed in COS-7 cells (40). The longer FP_A splice variant has multiple protein kinaseC phosphorylation sites, and this splice variant was selectively phosphorylated when expressed in cell culture (45). The authors suggest that this differential phosphorylation leads to selective desensitization of the FP_A variant but not the FP_B variant.

MULTIPLE E-PROSTANOID RECEPTORS

PGE_2 is a major product of cyclooxygenase-initiated arachidonic acid metabolism. PGE_2 may have multiple and at times apparently opposing functional effects on a given target tissue. For example, the vasodilator effects of PGE_2 have long been recognized in both arterial and venous beds (46–49). Smooth muscle relaxation by PGE_2 is, however, not uniformly observed, and PGE_2 is a potent constrictor in other smooth muscle beds, including trachea, gastric fundus, and ileum (50). Importantly, some structural analogs of PGE_2 are capable of reproducing the dilator effects of PGE_2, but are inactive on tissues where it is a constrictor. Conversely, analogs that reproduce the constrictor effects of PGE_2 may fail to affect tissues in which PGE_2 is a dilator (50). The differential effects of PGE_2 analogs are important functional evidence for the existence of multiple PGE_2 receptors (EP receptors) (3). Molecular cloning has now confirmed the existence of multiple PGE_2 receptor subtypes, each encoded by distinct genes. These receptors are designated EP_1, EP_2, EP_3, and EP_4 (10, 51), and they likely account for the diverse effects of PGE_2. Further diversity among EP receptors is generated in both the EP_1 and EP_3 receptors by alternatively spliced C-terminal variants as discussed below.

Some studies suggest that additional EP receptor subtypes could exist in sperm (52) and erythroleukemia cells (53); however, molecular correlates have not been identified. Although the four cloned EP receptors uniformly bind PGE_2 with a higher affinity than other endogenous prostanoids, they are not as closely related to each other as to other prostanoid receptors based on amino-acid homology

(4, 54). Thus, the relaxant/cAMP-coupled EP_2 receptor is more closely related to other relaxant prostanoid receptors such as the IP and DP receptors, whereas the constrictor/Ca^{2+}-coupled EP_1 receptor is more closely related to the other Ca^{2+}-coupled prostanoid receptors such as the TP and FP receptors (54). The EP receptor mRNAs also exhibit differential expression in a number of tissues, with distinct functional consequences of activating each receptor subtype (37, 42, 55–58). The characteristics of each EP receptor subtype are reviewed below.

EP_1 Receptors

The EP_1 receptor was originally described as a smooth muscle constrictor. The cloned human EP_1 receptor cDNA encodes a 402-amino-acid polypeptide (41). The mouse EP_1 receptor has also been cloned and encodes a protein of 405 amino acids (59). EP_1 receptor mRNA is expressed most highly in the kidney, followed by gastric muscularis mucosae and then adrenal tissue (59–62). Cloning of the rat EP1 receptor has also been reported. It is 96% homologous to the mouse receptor and 83% homologous to the human EP_1 receptor (41). An additional variant of the rat EP_1 receptor was also described that encodes a 366-amino-acid protein encoding an alternative sequence of 49 amino acids from the middle of TMVI to the COOH terminus, at the position of the intron/exon boundary of the two coding exons of this receptor.

There exist selective agonists that bind to the EP_1 receptor; however, these also have significant affinity for other receptor subtypes: the $EP_{1/3}$-selective agent sulprostone and the EP_1/IP-selective agonist iloprost. Several selective EP_1 antagonists have been described, including SC51089 or SC53122, which can aid in characterizing effects mediated by this receptor subtype (63–65). These antagonists appear to have analgesic activity prompting the search for clinically active drugs that would reduce pain without causing the gastric and renal side effects of nonsteroidal anti-inflammatory drugs (63, 64). Activation of the human EP_1 receptor leads to signals via IP_3 generation and increased cell Ca^{2+}. Narumiya and coworkers have noted that, although agonist stimulation of the cloned mouse EP_1 receptor causes a robust increase in $[Ca^{2+}]_I$, there was only a very modest increase in IP_3 generation (59), and the authors suggest that the increase in $[Ca^{2+}]$, therefore, might not be mediated by the G_q G-protein (4).

Okuda-Ashitaka et al (60) have described distinct signaling for the alternative EP_1 receptor mRNA variant. Although this variant binds ligand in a manner similar to that of the rat EP_1 receptor, it does not elicit detectable signal transduction. When the rEP_1 variant receptor was stably coexpressed with the longer rEP_1 receptor in CHO cells, the Ca^{2+} mobilization mediated by the EP_1 receptor was significantly suppressed, suggesting that the shorter variant antagonized rEP_1 signaling. Furthermore, when the rEP_1-variant receptor was expressed in CHO cells, cAMP formation by activation of the endogenous EP_4 receptor was strongly blocked. These authors suggest that the rEP_1-variant receptor may affect the efficiency of signal coupling of PGE receptors and attenuate the action of PGE_2

on tissues (60). Although the mechanism of action of this alternative variant of EP_1 receptor is unclear, recent evidence suggests that GPCRs may form dimers (65a,b,c). If this EP_1 receptor variant associates with other EP receptors, it might form nonproductive dimers and thus act as a dominant-negative regulator of signal transduction.

EP_2 Receptors

The nomenclature in the early literature is somewhat confusing regarding the molecular identity of the EP_2 receptor, since, prior to 1995 when the human EP_2 receptor was cloned, the cloned EP_4 receptor was referred as the EP_2 receptor (66, 67, 68). The authentic human EP_2 receptor cDNA encodes a 358-amino-acid polypeptide that signals through increased cAMP (68). EP_2 receptors have now also been cloned from the mouse, rat, rabbit, and cow (68–71). The precise tissue distribution of the EP_2 receptor has been only partially characterized, using northern blot analysis of mRNA distribution, which has revealed a major mRNA species of $\sim$3.1 kb, which is most abundant in the uterus, lung, and spleen, exhibiting only low levels of expression in the kidney (51, 68, 70, 71). EP_2 mRNA is expressed at much lower levels than EP_4 mRNA in most tissues (70). Functional studies suggest the EP_2 receptor plays an important role in uterine implantation (72–74) and a relaxant role in bronchioles, suggesting that EP_2 agonists could be used to treat asthma and chronic pulmonary disease (3, 4, 74, 75). In addition, recent studies have demonstrated that targeted disruption of the EP_2 receptor interferes with fertility and results in salt-sensitive hypertension (72, 74, 76). It remains to be determined whether polymorphisms in the EP_2 receptor are associated with infertility and/or hypertension in humans as well.

The EP_2 receptor was originally characterized by its ability to cause smooth muscle relaxation in cat trachea (77). Moreover, relaxation of the trachea could be stimulated with the agonist TR4979 (later designated "butaprost"), although this compound did not mediate smooth-muscle contraction in beds known to constrict in response to PGE_2. EP_2 receptors are selectively activated by butaprost, and butaprost activation is considered diagnostic for characterization of EP_2 receptors (10, 51). The EP_2 receptor may also be distinguished from the EP_4 receptor, the other major relaxant EP receptor, by its relative insensitivity to the EP_4 agonist PGE_1-OH and insensitivity to the weak EP_4 antagonist AH23848 (47, 68). The agonist order of affinity for the EP_2 receptor is $PGE_2 >$ 11-deoxy-$PGE_2 >$ butaprost $>$ AH13205 $=$ 19 R OH $PGE_2 \gg$ sulprostone, PGE_1 OH (68).

The EP_2 receptor sequence is most closely related not to the EP_4 receptor but rather to the DP and IP receptor subtypes (54, 68). Mutagenesis studies on the EP_2 receptor of Leu-304 $\rightarrow$ Tyr in TMVII resulted in a gain of function mutation that conferred binding of the IP/EP_1 analog iloprost to the EP_2 receptor (78). This result suggests that TMVII plays an important role in receptor-ligand interaction and selectivity. Mutation of a conserved Arg-302 adjacent to this leucine led to a loss of ligand binding as has been observed for the TP and EP_3 receptors, confirming

the importance of this region in ligand binding. EP_2/EP_4 chimera generation and mutagenesis studies have demonstrated that the extracellular sequences, particularly in the second extracellular loop region, are critical determinants of EP_2 receptor structure and/or ligand binding (79, 80). For the chimeric EP_2/EP_4 receptors, the nonfunctional chimeras did not traffic to the cell surface, and thus the loss of function might be attributed either to the direct binding of the extracellular regions to the ligand, to receptor misfolding, or to the lack of trafficking to an appropriate membrane compartment. Nonetheless, taken together, these studies suggest that, unlike other small-molecule binding receptors, both the extracellular and transmembrane sequences are important for receptor structure and function.

Activation of the EP_2 receptor leads to an increase in cAMP levels, consistent with its ability to relax smooth muscle in vivo (68, 70); however, it has been suggested that the EP_2 and EP_4 receptors, which differ in the length of their C-terminal sequence, have differing sensitivities to phosphorylation and desensitization. Nishigaki et al demonstrated that the EP_4 receptor underwent short-term agonist-induced desensitization. No such desensitization was observed for the EP_2 receptor (81).

EP_3 Receptors

The EP_3 receptor was originally identified as a constrictor of smooth muscle (3). Nuclease protection and northern analysis demonstrated relatively high levels of EP_3 receptor expression in several tissues including kidney, uterus, adrenal gland, and stomach tissues, with Northern analysis showing major mRNA species at $\sim$2.4 and $\sim$7.0 kb (9, 61, 82–84). Mice with targeted deletion of the EP_3 receptor exhibit an impaired febrile response to PGE_2, suggesting that EP_3 receptor antagonists could be effective antipyretic agents (85). In contrast, despite relatively high levels of EP_3 receptor in kidney tissue (9, 42, 55, 86), mice with targeted disruption of this receptor display a subtle alteration in the effect of nonsteroidal anti-inflammatory drugs on urinary concentrating ability (87), manifested by insensitivity to enhanced urinary concentration after indomethacin treatment.

This receptor is unique among the prostanoid family in that multiple alternatively spliced variants defined by unique C-terminal cytoplasmic tails exist (9, 82, 83, 86, 88–91; Figure 2 and Table 2). These splice variants encode proteins of a predicted molecular mass between 40 and 45 kDa (9, 61, 82).

All EP_3 splice variants bind PGE_2, and the EP_3 agonists bind MB28767 and sulprostone with similar affinity. The rank order of affinity for the mouse EP_3 receptor is as follows: sulprostone = M&B28767 = PGE_2 = PGE_1 > 11-deoxy PGE_1 > GR63799X > 17-phenyl-PGE_2 > misoprostol > AH13205, $\gg$ 1-OH-PGE1 (10). The human EP_3 receptor has a similar agonist order of affinity: sulprostone = M&B28767 = PGE_2 > GR63799X > 17-phenyl-PGE2 > misoprostol-free acid = enprostil = carbacyclin > misoprostol methyl ester (8). Although not tested in the above studies, one of the most selective of the EP_3 agonists is SC-46275, which has been shown to be selective and active in the dog, guinea

pig, and mouse in vivo (92–94). The EP receptors have demonstrated a marked preference for analogs that have a free acid at the C-1 carbon; however, many of the synthetic PG analogs are synthesized as methyl ester derivatives (95). Although the methyl esters are rapidly cleaved in vivo or in cell culture by endogenous esterases (96), this may not be true when performing radioligand-binding assays on purified membrane fractions, and thus this may lead to discrepancies between these two types of assay systems. It has been proposed that the C-1 carboxylate of prostanoids interacts with the conserved arginine in TMVII for other prostanoid receptors. Mutagenesis studies on this conserved arginine in the EP_3 receptor have suggested that there is indeed a nonionic interaction between the C-1 carboxylate and Arg-329, perhaps via a charge-stabilized hydrogen bond (7, 26, 27, 97). One of the distinguishing features of the prostanoid receptor family is the conserved amino acid sequence present in the second extracellular loop (Fig. 2). Site directed mutagenesis of this region has demonstrated that it plays a role in ligand-binding selectivity (97). Substitution of Trp-199 or Thr-202 with alanine resulted in receptors with increases in affinity for prostanoid compounds with a C-1 methyl ester but wild-type affinities for natural prostanoid ligands that have a carboxylate moiety at the C-1 position. The alteration in interaction with C-1 methyl esters by changes in the second extracellular loop was unexpected in light of the proposed interaction between the C-1 carboxylate of PGE_2 and the conserved Arg residue in TMVII. Substitution of Pro-200 with serine caused a loss of selectivity of $\leq$20-fold for naturally occurring prostanoid agonists as compared with the wild-type EP_3 receptor, further supporting a role of the second extracellular loop in determining ligand-binding selectivity.

As noted above, the most distinctive feature of the EP_3 receptors is the diversity generated by multiple alternative splice variants that generate alternate sequences in the C-terminal tail of this receptor subtype. A fundamental question addressed by a number of studies is the functional significance of these alternative splice variants. Proposed functional differences include alternate signal transduction pathway usage, receptor phosphorylation and desensitization, and intracellular trafficking. Several of these phenotypes may be interrelated, for example, the intracellular localization may determine the signal transduction pathway activated. Although these variants generally inhibit cAMP generation via a pertussis toxin-sensitive G_i-coupled mechanism, additional signaling mechanisms including G_s and Ca^{2+} release appear to be differentially activated by different C-terminal tails (Table 2) (89, 91, 98, 99). Recent studies suggest that the EP_3 receptor signals through the small G-protein Rho (98, 100). Activation of the bovine EP_3 splice variant induces neurite retraction in PC12 cells (100) via a tyrphostin A25-sensitive tyrosine kinase upstream of Rho, a genistein-sensitive tyrosine kinase downstream of Rho (98), and the p160 RhoA-binding kinase ROKα (101). This Rho-dependent signal transduction pathway can be initiated by constitutively active mutants of $G_{\alpha 12}$, $G_{\alpha 13}$, or $G_{\alpha q}$, suggesting that one or more of these G-proteins may be activated by agonist-stimulated EP_3 (102). The EP_3 receptor also activates protein kinase C (103, 104)- and cAMP-independent cAMP-response-element-mediated

TABLE 2 Signal transduction by EP_3 receptor splice variants

Species and clone name	cDNA source	Unique sequence	Signal transduction	Expression cell line	Reference(s)
Human					
III	Uterus	•EEFWGN	↓cAMP, ↑Ca^{2+}	CHO	91
E	Intestine	•EEFWGN	↓cAMP	JEG-3	82
b	Uterus	•EEFWGN	↓cAMP, ↑Ca^{2+}	BHK-12	83
III	Kidney	•EEFWGN	Not determined		147
III	Kidney	•EEFWGN	↓cAMP	COS-7/CHO	148
Rabbit 72A	Kidney	•EEFWEK	↑CRE	HEK293	9, 99
Human					
d	Uterus	•MRKRRLREQEEFWEGN	↓cAMP	BHK-12	83
IV	Uterus	•MRKRRLREQEEFWEGN	↓cAMP (weak), ↑Ca^{2+}	CHO	91
IV	Kidney	•MRKRRLREQEEFWEGN	↓cAMP ↑cAMP	COS-7/CHO	148
F	Intestine	•MRKRRLREQ	Not determined		82
e	Uterus	•MRKRRLREQLICSLRTLRYRGQLHIVGKYKPIVC	↓cAMP	BHK-12	83
f	Uterus	•MRKRRLREQAPLLPTPTVIDPSRFCAQPFRWFLD LSFPAMSSSHHPQLPLTLASFKLLREPCSVQLS	↓cAMP	BHK-12	83
Rabbit 74A	Kidney	•IRYHTNNYASSSTSLTHQCSST	↑CRE	HEK293	9, 99
Human					
I	Uterus	•IRYHTNNYASSSTSLPCQCSSTLMWSDHLER	↓cAMP, ↑Ca^{2+}	CHO	91
I	Kidney	•IRYHTNNYASSSTSLPCNCSSTLMWSDHLER	Not determined		147
I	Kidney	•IRYHTNNYASSSTSLPCQCSSTLMWSDHLER	↓cAMP ↑IP_3	COS-7/CHO	148
A	Intestine	•IRYHTNNYASSSTSLPCQCSSTLMWSDHLER	↓cAMP	JEG-3	82
			MAP kinase	COS-7	149
a	Uterus	•IRYHTNNYASSSTSLPCQCSSTLMWSDHLER	↓cAMP, ↑Ca^{2+}	BHK-12	83
Mouse α	P815	•IRDHT-NYASSSTSLPCPGSSALMWSDQLER	↓cAMP	CHO	86
Rat					
α	Hepatocyte	•IRDHT-NYASSSTSLPCPGSSVLMWSDQLER	↓cAMP	HEK293(EBNA)	51

(*Continued*)

TABLE 2 (*Continued*) Signal transduction by EP_3 receptor splice variants

Species and clone name	cDNA source	Unique sequence	Signal transduction	Expression cell line	Reference(s)
A	Kidney	•IRDHT-NYASSSTSLPCPGSSVLMWSDQLER	↓cAMP No change in Ca^{2+}	TKC2 COS-7	150, 151
Mouse β	P815	•MMNNLKWTFIAVPVSLGLRISSPREG	↓cAMP	CHO	88
Rat β	Hepatocyte	•MMNNLKRSFIAIPASLSMRISSPREG	↓cAMP	CHO	152
Cow					
a	Adrenal	•LLKGHSYGLDTEGGTENKDKEMKENLYISNL SRFFILLGHFTEARRGRGHIYLHTLEHQ	↓cAMP	CHO	89
b	Adrenal	•ASPRSMWDPSSPTRDRTRVPCIGSTES	↑cAMP Rho activation	CHO PC-12	89 100
c	Adrenal	•HVGS	↑cAMP	CHO	89
Human					
II	Kidney	•VANAVSSCSNDGNKGNPISLSNEIIQTEA	Not determined		147
II	Uterus	•VANAVSSCSNDGQKGQPISLSNEIIQTEA	↓cAMP, ↑Ca^{2+}	CHO	91
II	Kidney	•VANAVSSCSNDGQKGQPISLSNEIIQTEA	↓cAMP ↑cAMP ↑IP_3	COS-7/CHO	148
D	Intestine	•VANAVSSCSNDGQKGQPISLSNEIIQTEA	↓cAMP	JEG-3	82
c	Intestine	•VANAVSSCSNDGQKGQPISLSNEIIQTEA	↓cAMP, ↑Ca^{2+}	BHK-12	83
Pig —	Heart	•VANAVCSCSKNGQKVQTISLSHEITQTEA	↓cAMP, NFκ-B	CHO	153
Cow d	Adrenal	•VANAVSSYFNDGPKVPTISLSNEITQTGA	↓cAMP, ↑cAMP, ↑IP_3	CHO	89
Mouse γ	P815	•VANAVSSCSSDGQKGQAISLSNEVVQPGP	↓cAMP, ↑cAMP	CHO	90
Rat					
B	Kidney	•VANAVSSCSSDQQKGQAISLSNEVVHPGP	↓cAMP	HEK293(EBNA)	51
B	Kidney	•VANAVSSCSSDQQKGQAISLSNEVVHPGP	No change in cAMP ↑Ca^{2+}	TKC2 COS-7	150, 151
Rabbit					
77A	Kidney	•VIHENNEQKDEIQRENRNVSHSGQHEEAR DSEKSKTIPGLFSILLQADPGARPYQQ	↓cAMP, ↑CRE	HEK293	79, 99
NT	Kidney	None	↑CRE	HEK293	99
80A	Kidney	•HSPAIGDLQISTHISKTNKYFEGLMKTF HSLAYL	↑CRE	HEK293	9, 99

gene transcription in HEK293-transfected cells (99). Differences in agonist independent activity have been observed for several of the splice variants, suggesting that they may play a role in tonic regulation of intracellular metabolism (105, 106).

Chemtob and coworkers have localized EP receptors to the nuclear envelope (107, 108) in addition to the more traditional view that these receptors are expressed on the plasma membrane. More recently, Hasegawa et al have shown that there are different patterns of receptor localization in Madin-Darby canine kidney cells, depending on which mouse EP_3 splice variant was expressed, suggesting that splice variants may direct receptors to nuclear vs plasma membranes in vivo (109). Despite the extensive characterization of the EP_3 receptor splice variants in cell culture systems, physiologic significance of these different C-terminal splice variants remains uncertain.

EP_4 Receptors

As with the EP_2 receptor, the EP_4 signals through increased cAMP (67, 68). The human EP_4 receptor cDNA encodes a 488-amino-acid polypeptide with a predicted molecular mass of ~53 kDa (67). As described above, prior to 1995 this receptor cDNA was generally referred to as the EP_2 receptor (66). In addition to the human receptor, EP_4 receptors for the mouse, rat, rabbit, and cow have been cloned (51, 57, 66, 67, 110, 111). EP_4 receptor mRNA is relatively highly expressed compared with the EP_2 receptor and widely distributed, with a major species of ~3.8 kb detected by Northern analysis in thymus, ileum, lung, spleen, adrenal, and kidney tissues (57, 67, 111, 112). Important vasodilator effects of EP_4 receptor activation have been described in venous and arterial beds (3, 47). A particular role for the EP_4 receptor in regulating the peri-natal closure of the pulmonary ductus arteriosus has also been suggested by the recent studies of mice with targeted disruption of the EP_4 receptor gene (113, 114). The EP_4 receptor ligands may prove useful in promoting closure or maintaining patency of the ductus arteriosus in newborns with congenital heart disease.

The human EP_4 receptor has the following agonist order of affinity: $PGE_2 = PGE_1 > M\&B\ 28767 >$ misoprostol-free acid $\gg$ iloprost $> PGF_{2\alpha} > PGD_2$ (8, 67). Like the EP_3 receptor, the EP_4 receptor has a preference for analogs with a C-1 carboxylate that is >50-fold higher than that observed for the corresponding methyl ester (8, 57). In contrast to the EP_3 receptor, where mutation of the conserved Thr-202 in the second extracellular loop leads to a loss of preference for the methyl ester analogs, the mutation of the corresponding Thr-168 $\rightarrow$ Ala led to a loss of detectable ligand binding and agonist activation (79).

EP_4 receptors may be pharmacologically distinguished from the EP_1 and EP_3 receptors by the EP_4 receptor insensitivity to sulprostone and from EP_2 receptors by EP_4 insensitivity to butaprost (10, 51) and relatively selective activation by PGE_1-OH (10, 51).

One striking structural difference between the two G_s-coupled EP receptors is the length of the C-terminal tail: the EP_4 receptor has a long (156-amino-acid residues) C-terminal sequence and contains 38 serine and threonine residues that might serve as multiple phosphorylation sites, whereas the EP_2 receptor has a shorter tail sequence. This suggests that the EP_4 receptor would be a target for agonist-dependent phosphorylation and desensitization and that the EP_2 receptor might be relatively insensitive to this regulatory effect. This hypothesis has been borne out in studies on the mouse EP_2 and EP_4 receptors that revealed that the mouse EP_4 receptor underwent rapid agonist-induced desensitization, whereas the EP_2 receptor did not (81). Deletion of successive sequences of the human EP_4 receptor C terminus identified a stretch of six serine residues in the tail, one or more of which might serve as a target for phosphorylation and subsequent desensitization (115, 116). Thus, the EP_2 and EP_4 receptors may play variable physiologic roles based on the persistence of the signal generated by the receptor upon ligand activation.

IP RECEPTORS

The biological effects of prostacyclin are numerous and include nocioception, antithrombosis (117), and vasodilator actions, which have been targeted therapeutically to treat pulmonary hypertension (118, 119). Recently, aerosolized iloprost was used to effectively treat pulmonary hypertension in humans (118). The cDNA for the IP receptor encodes a protein with seven hydrophobic regions (120, 121). In situ hybridization shows IP receptor mRNA predominantly in neurons of the dorsal root ganglia and vascular tissue, including aorta, pulmonary artery, and renal interlobular and glomerular afferent arterioles (122). The expression of IP receptor mRNA in the dorsal root ganglia is consistent with a role for prostacyclin in pain sensation, and mice with IP receptor gene disruption exhibit a predisposition to arterial thrombosis, diminished pain perception, and inflammatory responses (117). PGI_2 has been demonstrated to play an important vasodilator role in the kidney (123–125) as well as regulate renin release (126, 127). Prostacyclin was originally identified as a vascular derived dilator substance that inhibited platelet aggregation (128). This substance is very unstable in aqueous solution; however, stable analogs have been developed that specifically reproduce the biological activity of prostacyclin (129).

The cDNA for the human and mouse IP receptors encode proteins of 386 and 417 amino acids, respectively (120, 121, 130, 131). IP receptor mRNA is highly expressed in mouse thymus, heart, and spleen (120) and in human heart, aorta, kidney, liver, and lung (121, 131) tissues. The labile endogenous ligand is not used in receptor assays. The cloned human IP receptor binds the IP agonists iloprost $\geq$ cicaprost > carbacyclin > $PGE_2 \gg PGF_{2\alpha}$, PGD_2. Among the prostanoid receptors, the IP receptor is the least selective in discriminating among the prostanoid ring

substitutions. Unlike the EP receptors, the IP receptor does have a much higher affinity for PGE_1 analogs than is observed for the PGE_2 analogs. This latter property can be used diagnostically to distinguish between IP receptor activation and EP receptor activation by PGE analogs. The IP receptor is most closely related in sequence to the DP receptor, although in contrast to the IP receptor, the DP receptor is highly selective for PGD_2 over other prostanoid ring substitutions. Kobayashi et al have generated a large series of chimeric IP-DP receptors to define the regions of these receptors that confer ligand-binding selectivity (133). They concluded that PGE_1 vs PGE_2 selectivity of the IP receptor is determined by residues in the TMVI and TMVII regions, whereas residues conferring differences in selectivity of the prostanoid ring between the IP and DP receptors are resident in the TMI and the first extracellular loop region (133).

The IP receptor is selectively activated by the analog cicaprost (10, 50). Iloprost and carbacyclin potently activate the IP receptor but activate the EP_1 receptor as well (10). Most evidence suggests that the PGI_2 receptor signals via stimulation of cAMP generation; however, the cloned mouse PGI_2 receptor also signaled via PIP_2 (120), although a 10,000-fold-higher agonist concentration was required to stimulate PIP_2 hydrolysis (10 μM) in CHO cells transfected with the IP receptor than the concentrations required (10^{-10} M) to stimulate cAMP accumulation in the same cells (120). It remains unclear whether PIP_2 hydrolysis plays any significant physiologic role in the action of PGI_2.

DP RECEPTORS

The DP receptor was the most recent of the prostanoid receptors to be cloned and is perhaps the least well characterized. PGD_2 is the major prostanoid released from mast cells after challenge with immunoglobulin E (134), and it has also been shown to affect the sleep-wake cycle (135) and body temperature (136). Peripherally, PGD_2 has been shown to mediate vasodilation and vasoconstriction, as well as inhibition of platelet aggregation (137). DP receptor mRNA is expressed at low levels in most tissues; however, it is highly expressed in leptomeninges, retina, and mucus-secreting cells of the gastrointestinal tract (138–141). The DP receptor has been cloned from mouse, rat, and human (138, 140, 141). The human DP cDNA encodes a protein of 359 amino acids and binds PGD_2 with a high-affinity binding of 300 pM, and a lower-affinity site of 13.4 nM (8, 141). When expressed in cell culture, the DP receptor has an agonist order of affinity as follows: BW245C $>$ $PGD_2 \gg$ carbacyclin $>$ $PGE_2 \gg PGF_{2\alpha} =$ iloprost $=$ U46619. DP/IP chimeras for examining the role of specific sequences in generating DP receptor selectivity were described in the preceding section on the IP receptor subtype.

Like the IP and $EP_{2/4}$ receptors, the cloned human and mouse DP receptors each increase cAMP generation (140, 141). The cloned mouse DP receptor displays similar pharmacology to that observed for the human receptor (140). DP-selective ligands, including the agonist BW 245C (3, 142), are available to selectively activate

the DP receptor. The DP-selective ligand BW A868C was originally described as an antagonist (137), although more recent studies have suggested that it is a partial agonist (143).

SUMMARY

In summary, cloning of the PG receptor family has allowed molecular definition of this important class of autacoid receptors. Although they bind small-molecule ligands, they share ligand-binding motifs similar to receptors that bind peptide hormones, utilizing both transmembrane and extracellular-loop regions in generating ligand-binding selectivity (144). In addition to the eight prostanoid receptors, each encoded by a distinct gene, additional receptor diversity is generated through alternative splicing of several of the receptor subtypes. These splice variants affect receptor signal transduction as well as agonist-induced receptor phosphorylation and desensitization. Although a number of differences in signal transduction and biochemical properties of the various PG receptor splice variants have been identified, the physiologic significance of the alternative splice variants merits further investigation. Identification of the relevant signal transduction pathway activated in vivo in various physiologic and pathophysiologic conditions remains unclear.

Characterization of the PG receptors has been hampered by the lack of subtype-selective receptor antagonists, although this has been addressed to some extent by the generation of knockout mice for each of the eight PG receptor subtypes. The precise physiologic role of each receptor remains only partially characterized. However, given their apparently diverse effects, the development of receptor specific antagonists should offer significant advantages and flexibility over nonsteroidal anti-inflammatory drugs that nonselectively inhibit the synthesis of all PGs.

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