Functional and Molecular Aspects of Renal Prostaglandin Receptors[1]

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ABSTRACT

The diverse intrarenal effects of the prostaglandins (PG) are mediated by distinct guanine nucleotide regulatory protein (G-protein)-coupled receptors. The cDNA for these receptors have been cloned, their signal transduction mechanisms determined, and their intrarenal distribution mapped. PGE_2, the major intrarenal prostaglandin, interacts with at least three distinct E-prostanoid (EP) receptors that are highly expressed in specific regions of the kidney. Each EP receptor not only selectively binds PGE_2, but also preferentially couples to different signal transduction pathways, including: stimulation of cAMP generation, via G_q (EP_2 and EP_4 receptors); inhibition of cAMP generation, via G_i (EP_3 receptors); and activation of phosphatidylinositol hydrolysis (EP_1 receptor), via one of the Gq family members. Activation of each these EP receptors is responsible for a distinct renal effect of PGE_2, including its well-described renal hemodynamic and transport effects along the nephron. Other intrarenal prostanoid receptors include the $PGF_{2\alpha}$ receptor (FP), the thromboxane A_2 receptor (TP) and the prostacyclin receptor (IP). Knowledge about localization of these receptors and their affinities for receptor-selective agonists and antagonists should aid in the understanding of renal disease and the development of therapeutic strategies for the use of these prostaglandin analogs in select renal diseases.

Key Words: *Receptors, prostanoid, membrane, signaling, kidney*

Prostaglandins (PG) comprise a diverse family of highly biologically active lipids derived from enzymatic metabolism of arachidonic acid by cyclooxygenase. Cyclooxygenase, also referred to as prostaglandin H synthase (PGHS), catalyzes a two-step reaction, first cyclizing arachidonic acid to form PGG_2 and then, reducing the 15-hydroperoxy group to form PGH_2 (Figure 1) (1). After its formation, PGH_2 is enzymatically converted to several oxygenated species, including prostaglandin E_2 (PGE_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), prostaglandin D_2 (PGD_2), prostacyclin (PGI_2), and thromboxane A_2 (TxA_2), known collectively as prostanoids. Whereas prostaglandin synthesis occurs in all cells and tissues, the kidney is a particularly rich source for prostanoids. Prostaglandin E_2 is a major renal metabolite, and urinary PGE_2 concentrations are typically in the nanomolar range, well above circulating picomolar concentrations (2,3). In contrast to classic circulating hormones, such as insulin or vasopressin, prostanoids act locally on the tissues in which they are synthesized, or on tissues adjacent to those in which they are synthesized. Their local production may be stimulated by several hormones including angiotensin, vasopressin, and bradykinin, and the prostanoids may thereby mediate or modulate the actions of these hormones (2,3).

As would be predicted from their autocrine action, the kidney is an important biologic target for these intrarenal prostaglandins. Prostaglandins regulate both renal hemodynamics and epithelial water and solute transport. Each prostaglandin (*e.g.*, PGE_2, $PGF_{2\alpha}$, PGI_2) has distinct effects on these renal targets. Additionally, a single prostaglandin, such as PGE_2, may have multiple and at times apparently opposing functional effects on a given target tissue. For example, although PGE_2 is a vasodilator in some vascular beds, it is predominantly a vasoconstrictor in other vascular beds (4,5). There is now firm evidence that these diverse effects are accounted for by multiple receptor subtypes for individual prostaglandins (Figure 2).

CHARACTERIZATION OF RENAL PROSTANOID RECEPTORS

Pharmacologic studies using structural analogs of prostaglandins provided the initial evidence that multiple receptors existed for each prostaglandin. These studies also suggested the existence of multiple receptors for individual prostanoids (5,6). For example, the PGE_2 analog butaprost was only active in vascular

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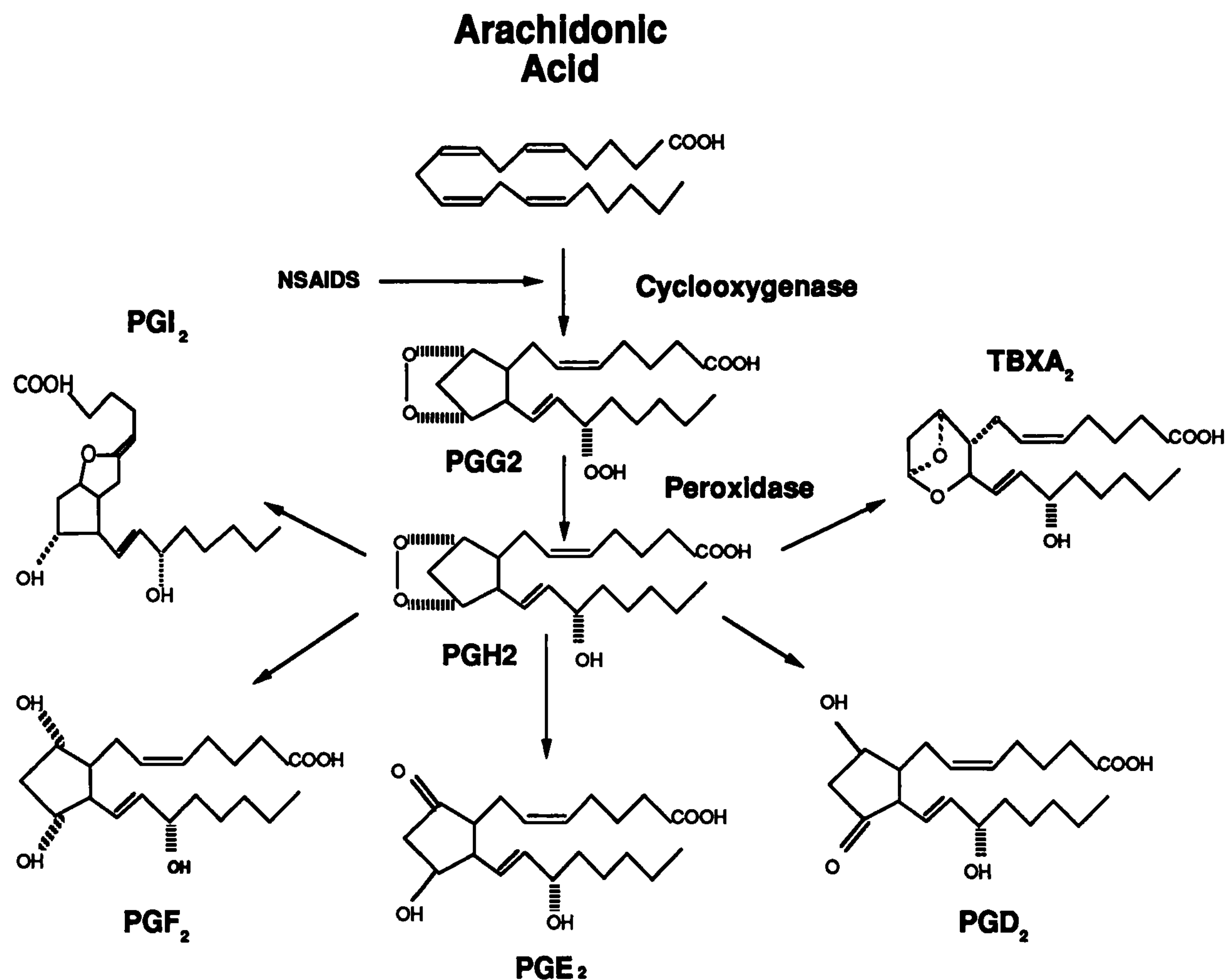

Figure 1. Cyclooxygenase metabolism of arachidonate and the chemical structures of the prostaglandins.

beds in which PGE_2 was a vasorelaxant, whereas butaprost was totally inactive in vascular beds PGE_2 was a vasoconstrictor. Conversely, the PGE_2 analog sulprostone was only active in vascular beds in which PGE_2 was a vasoconstrictor and inactive in vascular beds in which PGE_2 was a vasodilator. Based upon these studies, the existence of multiple PGE_2 receptors was proposed (5,6). The nomenclature for the prostanoid receptors has now been standardized, so that all PGE_2 receptors are referred to as "E-prostanoid" receptors or EP receptors; thromboxane receptors are "TP" receptors, $PGF_{2\alpha}$ are "FP" receptors, PGD_2 receptors are designated "DP" receptors, and PGI_2 receptors are designated "IP" receptors (Table 1) (6). Subtypes of the prostaglandin receptors are designated by subscript, so that EP_1, EP_2, EP_3, and EP_4 refer to different isoforms of PGE_2 receptors.

Amino acid sequences for each of the prostanoid receptors have now been reported (7). The family of prostanoid receptors couple to their intracellular effectors via guanine nucleotide regulatory proteins (G-proteins) (7–9). These receptors possess seven hydrophobic stretches of amino acids characteristic of G-protein coupled receptors that are believed to represent transmembrane α-helices (10). By analogy with the biogenic amine receptors (*e.g.*, adrenergic receptors), these G-protein coupled receptors are thought to bind their cognate ligands with the seven transmembrane α-helices, and couple to G-proteins by their intracellular sequences, particularly the third intracellular loop and the proximal portion of the C-terminal tail (11,12). The distal region of the C-terminal tail of G-protein linked receptors is thought to be a target for regulatory phosphorylation by protein kinase A (PKA) and receptor-specific kinases (13–15).

Each of the prostanoid receptors is considered below according to its signal transduction pathways: receptors coupled to diacylglycerol (DAG)/inositol phosphate turnover, including the TP, FP and EP_1 receptors; the G_i-coupled EP_3 receptor; and the G_s-coupled receptors, including EP_2, EP_4, IP, and DP receptors.

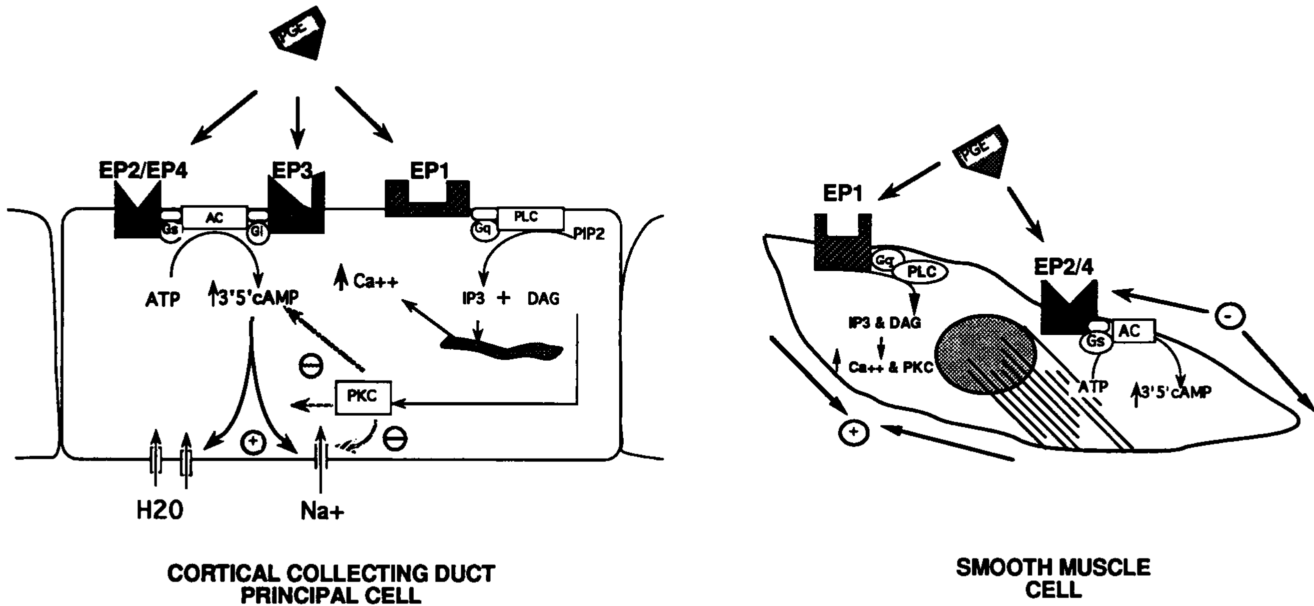

Figure 2. Signal transduction and functional effects of the various EP receptors in renal epithelial cells (the cortical collecting duct) and smooth muscle. Note that for simplicity of this figure, the G_i-coupled EP_3 receptor is not shown on the smooth muscle cell, although there is evidence that it may act as a constrictor of smooth muscle.

TABLE 1. Prostanoid-receptor pharmacology and signaling

Eicosanoid	Receptor	Agonist	Antagonist	Signaling
Thromboxane A_2	TP	I-BOP	Bay U3405	↑ IP_3/DAG
		U46619	AH19437	
		STA2	AH23848	
PGI_2 (Prostacyclin)	IP	Cicaprost		↑ cAMP
		AP-227		
		Iloprost		
PGE	EP			
	EP_1	17-phenyl-trinor PGE_2	SC19220	↑ IP_3/DAG
		Iloprost	AH6809	
		Sulprostone		
	EP_2	Butaprost		↑ cAMP
		AH13205		
		11-deoxy PGE_1		
		19(R)-OH-PGE_2 (?)		
	EP_3	MB 28767		↓ cAMP
		Sulprostone		
		11-deoxy-PGE_1		
	EP_4	?	AH23848 (±)	↑ cAMP
$PGF_{2\alpha}$	FP	Fluprostenol		↑ IP_3/DAG
PGD	DP	BW 245C	BW A868C	↑ cAMP

The TXA_2 Receptor

The receptor for TXA_2 or "TP" receptor was the first eicosanoid receptor cloned (8). Thromboxane A_2 is a highly labile arachidonic-acid metabolite and is both a potent vasoconstrictor and stimulant of platelet aggregation (16). The successful cloning of this prostanoid receptor depended upon the purification of the human platelet TP-receptor protein. Ushikubi *et al.* purified a 57 kd glycoprotein from human platelets by using the TP-receptor antagonist S145 to develop a ligand-affinity chromatography step. The purified protein displayed binding properties consistent with a TP receptor (17). The purification obtained in these studies was sufficient to allow partial amino-acid sequence analysis, which was used in turn to design oligonucleotide probes for screening cDNA libraries. A 2.9 kilobase (kb) clone encoding the full-length coding region for the TP receptor was then isolated from a human-

placenta cDNA library (8). This clone encoded a protein of 343 amino acids containing the seven hydrophobic stretches of amino acids.

When expressed in COS 7 cells, this clone displayed the characteristic ligand binding properties of the human TP receptor: S145 > TP antagonist ONO-3708 ≫ PGF_{2a}>PGE_2>PGI_2>TXB_2. Signal-transduction properties of this receptor were evaluated in *Xenopus* oocytes in which agonist stimulation opened a PI turnover-dependent Ca^{++} channel, consistent with the PI turnover observed for the endogenous receptor *in vivo*. A limited Northern blot analysis revealed significant expression of a 2.8 kb species in both placenta and lung, the two tissue types evaluated. More recently a second isoform of the TP receptor has been cloned from a human umbilical vein endothelial cell library (18). The endothelial form of the TP receptor encodes a protein of 369 amino acids, which differs only in the C-terminal sequence distal to the seventh transmembrane domain. These two isoforms are believed to be generated by alternative splicing of a common mRNA precursor. The ligand-binding and signal-transduction properties of these two isoforms were essentially identical, and the functional significance of these two splice variants has yet to be determined.

The mouse and rat homologs for the human placental TP receptor have now also been cloned (19,20). Expression of these clones demonstrated similar ligand-binding and signal-transduction properties compared with the human TP receptor. Extensive Northern blot analysis demonstrated a major mRNA species hybridized at 2.0 kb in a number of tissues and a second species of 3.0 kb was evident in thymus. Overall, the highest level of expression was observed in the thymus, followed by spleen, lung, and kidney. Lower levels of expression were observed in heart, uterus, and brain.

In the kidney, TXA_2 causes intense vasoconstriction, decreasing GFR (21) (Table 2). These effects are mediated by glomerular TP receptors coupled to phosphatidylinositol hydrolysis, protein kinase C activation, and glomerular mesangial cell contraction (22). The intrarenal distribution of the TP receptor mRNA has recently been reported. TP receptor mRNA was detected in the glomerulus and in smooth muscle cells of the renal arterioles, confirming the existence of the TP receptor in these tissues at the molecular level (22a). Important pathogenic roles for TXA_2 and glomerular TP receptors in mediating renal dysfunction in glomerulonephritis, diabetes mellitus, and sepsis have also been proposed (23–25). Roles for TP receptors in the modulation of tubule epithelial function in the kidney are poorly substantiated (26).

FP Receptor

This receptor was cloned by taking advantage of the high degree of sequence similarity among the cloned prostanoid receptors in the second extracellular loop (containing the sequence PG(T/S)WCF) and the seventh transmembrane domain (containing the sequence NQILDPWVY). Abramovitz and coworkers designed a degenerate oligonucleotide probe based upon the conserved sequence in the seventh transmembrane domain sequence and used this to isolate the cDNA encoding the $PGF_{2\alpha}$ receptor from a human kidney cDNA library (27). This cDNA, encoding a protein of 359 amino acids, the expression of which conferred $[^3H]PGF_{2\alpha}$ binding to membranes isolated from transfected COS-M6 cells yielding a K_D of 1 nM. This $[^3H]PGF_{2\alpha}$ binding was displaced by a panel of ligands with a agonist rank-order of potency typical for an FP receptor: $PGF_{2\alpha}$ = fluprostenol > PGD_2 > PGE_2 > U46619 > iloprost. When expressed in oocytes, $PGF_{2\alpha}$ or fluprostenol induced a Ca^{++}-dependent Cl^- current, strongly suggesting that this receptor signals in mammalian cells by raising $[Ca^{++}]_i$.

The role of FP receptors in the regulation of renal function is poorly studied. $PGF_{2\alpha}$ increases Ca^{++} in cultured glomerular mesangial cells, and there was no evidence for homologous desensitization TXA_2 or PGE_2 (28). This suggests that an FP receptor may modulate glomerular contraction. Whereas $PGF_{2\alpha}$

TABLE 2. Intrarenal distribution of prostanoid receptors[a]

Prostanoid Receptor	Glomerulus	PT	tDL	mTAL	cTAL	DCT	CCD	OMCD	IMCD	Reference
TP	+	–	–	–	–	–	–	–	–	22, 25, 26
FP	+	–	–	–	–	–	+	–	–	26, 27, 28, 29
EP										
EP_1	–	–	–	–	–	–	++	++	++	48, 49, 50, 54
EP_2	?	?	?	?	?	?	?	?	?	41
EP_3	–	–	–	++	–	++	++	++	–	47, 50, 63, 64
EP_4	++	+	+	+	?	?	+	+	?	47, 50, 52, 69, 72, 73, 74, 75
IP	+/?	–	–	–	–	–	+	–	+	78, 79, 80, 81, 82
DP	?	–	–	–	–	–	–	–	–	85

[a] +, functional evidence; ++, molecular evidence; –, no evidence; ?, insufficient data. PT, proximal tubule; tDL, thin descending limb; mTAL, medullary thick ascending limb; cTAL, cortical thick ascending limb; DCT, distal convoluted tubule; CCD, cortical collecting duct; OMCD, outer medullary collecting duct; IMCD, inner medullary collecting duct.

also raises intracellular Ca^{++} in the cortical collecting duct (CCD) (29), possibly contributing to its capacity to inhibit water permeability in this nephron segment (26), fluprostenol was without effect on intracellular Ca^{++}. Furthermore, pretreatment with PGE_2 similarly raised intracellular Ca^{++} and desensitized the CCD to the Ca^{++} effects of $PGF_{2\alpha}$ (29). Since $PGF_{2\alpha}$ can bind to the EP_1 and EP_3 receptors (30–32), the above data suggests that the effects of $PGF_{2\alpha}$ in the CCD may be mediated via an EP receptor. Thus a role for the FP receptor in renal epithelial cells remains to be established.

Prostaglandin E_2 Receptors

PGE_2 is a major renal cyclooxygenase metabolite of arachidonate and is the major prostanoid excreted in the urine (3,33–36). The kidney is also an important target for PGE_2, possessing abundant PGE_2 binding sites (37–40). At least four subtypes of the PGE_2 receptor had been proposed (5,6) on the basis of pharmacologic studies performed in smooth muscle. These E-prostanoid receptors (EP receptors) are designated EP_1, EP_2, EP_3 and EP_4. The cDNA for each EP receptor has now been reported (6,7,41).

EP_1 receptors cause smooth muscle contraction of guinea pig ileum and dog fundus, and are selectively activated by 17-phenyl-trinor-PGE_2 and sulprostone, and are antagonized by SC19220 and AH6809 (5,6). EP_2 receptors relax smooth muscle in cat trachea and guinea pig ileum. This EP-receptor subtype is activated by butaprost and AY23636 but not sulprostone. EP_3 receptors cause smooth muscle contraction in chick ileum and inhibit acid secretion in rat gastric mucosa, are activated by both sulprostone and AY23636, but are not antagonized by SC19220. Recently a fourth receptor subtype designated EP_4 has been described in pig saphenous vein, where it causes EP_2-like smooth muscle relaxation, but is not activated by the EP_2-selective agonist butaprost (42).

The existence of multiple PGE_2 receptors meshes nicely with studies demonstrating that PGE_2 also couples to multiple signaling pathways. Thus PGE_2 stimulates phosphatidylinositol turnover via a G-protein-mediated mechanism and modifies cAMP generation via G_s and G_i (43–47). These distinct signaling pathways are mediated by different receptors.

EP_1 Receptor

The EP_1 receptor was originally identified by its ability to cause smooth muscle contraction in a variety of tissues. A cDNA encoding the human EP_1 receptor was recently isolated from a human erythroleukemia cell line (30). This cDNA encoded a protein of 402 amino acids and conferred high affinity $[^3H]PGE_2$ binding (K_D = 1nM) when expressed in COS cells. The $[^3H]PGE_2$ binding could be displaced by a panel of natural and synthetic prostaglandins with a rank-order potency typical of an EP_1 receptor: $PGE_2 > PGE_1 > PGF_{2\alpha} >$ AH6809 (antagonist) > SC19220 (antagonist) > PGD_2 > butaprost. In electrophysiological assays, oocytes expressing the EP_1 clone displayed a ligand-mediated Cl^- current characteristic of Ca^{++}-coupled receptors.

The mouse EP_1 receptor, which was cloned from kidney, shares 85% sequence identity with the human clone (48). Ligand-binding and signal-transduction properties are essentially identical to the human clone, with the exception that the unlike the human clone, the mouse clone did not demonstrate binding of the EP_1 antagonist AH6809. Northern blot analysis demonstrated high levels of expression of the mouse EP_1 receptor mRNA in the kidney with lower levels of expression in the lung. No expression was observed in a variety of other tissues, including brain, thymus, heart, liver, ileum, and stomach.

The intrarenal role of the EP_1 receptor is only partially characterized. *In situ* hybridization of an EP_1 receptor riboprobe to mouse or human kidney demonstrates selective hybridization to the CCD (49,50) (Table 2). In the rabbit CCD, PGE_2 inhibits sodium transport by a mechanism coupled to the release of Ca^{++} from intracellular stores (46,47). The ability of PGE_2 to increase intracellular Ca^{++} in the CCD is mimicked most potently by 17-phenyltrinor-PGE_2, followed by sulprostone. This action is not reproduced by either the EP_3 selective compound MB28767 or the EP_2/EP_3 active compound 11-deoxy-PGE_2 (29), and can be blocked by EP_1 receptor antagonists (49). This rank order of potency is consistent with the $[Ca^{++}]_i$-coupled inhibitory effects of PGE_2 on Na^+ transport, being mediated via an EP_1 receptor and not one of the alternatively spliced EP_3-receptor variants.

Although hybridization of the EP_1 riboprobe to the glomerulus was not observed, some data suggests existence of an EP_1 receptor in the glomerulus. While PGE_2 is thought of predominantly as a glomerular vasodilator (51–53), vasoconstrictor effects of PGE_2 in the renal circulation have also been described (54). PGE_2 has been demonstrated to increase Ca^{++} in cultured mesangial cells, suggesting the presence of an intraglomerular PGE_2 receptor coupled to phosphatidylinositol hydrolysis (28). It may be that a vasoconstrictor EP_1 receptor mediates glomerular vasoconstriction.

EP_3 Receptor

The EP_3 has now been cloned from man, mouse, rabbit, rat, and cow (9,31,55–62). This EP-receptor subtype appears to be unique among the prostanoid receptors in that multiple alternatively spliced variants exist. At least nine alternatively spliced isoforms are expressed in man, four in rabbit and cow, and three in the mouse and rat. Among these species, there exist at least 16 distinct isoforms. This suggests that either there is a large interspecies variation or alternatively, there are a very large number of receptor isoforms in each species and only a small number of the total set have been cloned for each species. For

each species, the receptor isoforms are identical throughout the seven transmembrane domain, and differ either in the C-terminal amino-acid sequence distal to the seventh transmembrane domain and/or in the 3' untranslated region.

The EP_3 receptor isoforms have essentially similar ligand-binding properties when transfected in mammalian cells lines. The rabbit EP_3 receptor transfected into COS1 cells bound PGE_2 with a K_D of 320 pM. Competition experiments with natural and synthetic prostaglandin analogs showed the following order of affinity: MB28767>sulprostone≥ PGE_2 = PGE_1 > $PGF_{2\alpha}$ > PGD_2, in good agreement with the *in vivo* functional agonist order of potency of the rabbit renal G_i-coupled EP_3 receptor (43–45).

Physiologic evidence suggests that the EP_3 receptor signals via inhibition of cAMP generation (43,44,47), and similarly, PGE_2 inhibited cAMP generation when added to cultured cells transfected with the cloned EP_3 receptors from mouse, rabbit, human, and cow (9,32). Recently, Namba *et al.* have suggested that alternatively spliced EP_3 variants differentially couple to alternate signaling systems, including phosphatidylinositol hydrolysis and stimulation of cAMP generation (9). However, it should be noted that activation of these alternate signaling pathways required micromolar agonist concentrations, in contrast to the nanomolar concentrations of PGE_2 or MB28767 required to inhibit cAMP generation. It therefore remains uncertain whether the EP_3 receptor couples to these alternate signaling mechanisms under physiologic conditions.

Renal effects of EP_3 receptor activation. The intrarenal distribution of the EP_3 receptor has been mapped both by *in situ* hybridization in rabbit kidney (Figure 3) (63) and polymerase chain reaction in microdissected rat nephron segments (64) (Table 2). These studies demonstrate high levels of EP_3 mRNA expression in medullary thick ascending limb (TAL), as well as lower levels of expression in the medullary collecting duct and the CCD. While EP_3 mRNA was detected in rat cortical TAL, no significant expression of EP_3 mRNA was detected in the rabbit cortical TAL. The differential expression of EP_3 mRNA in the cortical versus medullary TAL of the rabbit corresponds to the absence of effect of PGE_2 on ion transport in cortical *versus* medullary TAL along of the rabbit nephron. Stokes found that although PGE_2 markedly inhibited Cl^- absorption in the rabbit medullary TAL, it had no effect on rabbit cortical TAL Cl^- transport (65). This suggests that activation of the EP_3 receptor may be responsible for inhibition of NaCl absorption in the TAL.

In contrast, the EP_3 receptor appears to have little effect on Na^+ absorption in the isolated perfused rabbit CCD (47). Instead, the EP_3 receptor appears to be responsible for the classic effect of PGE_2 to inhibit vasopressin-stimulated water permeability in the CCD (47). As previously discussed, multiple isoforms of the EP_3 receptor, generated by alternative splicing and differing only in their C-terminal tails, appear to exist in freshly isolated rabbit CCD. Whereas some studies suggest differences in signaling or desensitization of these EP_3 isoforms when expressed in COS or CHO cells, the physiologic significance of these receptors in this native tissue remains to be characterized (31,47).

EP_2 and EP_4 Receptors

EP_2 receptors are defined by their ability to relax smooth muscle, presumably by raising intracellular cAMP concentration. Recently, a second vasodilatory EP receptor has been characterized, which is not stimulated by the classical EP_2 agonists (42). This novel vasorelaxant receptor has been termed EP_4. Honda *et al.* isolated a cDNA clone by screening a P815 mastocytoma library with an EP_3 probe at low stringency (66) that encodes a protein of 513 amino acids, which has 36% homology to the mouse EP_3 receptor. This cDNA clone and its homologs in other species were originally designated EP_2 receptors, although through a more thorough pharmacological characterization, it has now become clear that this clone represents the EP_4 subtype (67). When expressed in COS-1 cells, this cDNA clone conferred $[^3H]PGE_2$ binding and had a order of affinity consistent with an EP receptor. This receptor was also shown to increase cAMP in a dose-dependent manner. Its affinity for receptor selective analogs was, however, not consistent with the functionally defined EP_2 pharmacology: although it bound misoprostol with high affinity, it did not bind the definitive EP_2-selective ligand, butaprost.

The identification of the Honda clone as an authentic EP_4 receptor was confirmed by Regan and coworkers, who recently reported a butaprost-sensitive EP receptor cloned from a human placental cDNA library (41). This cDNA encodes a protein of 358 amino acids. Within the proposed transmembrane domains, there is 38% amino acid identity to the EP_4 receptor described above (66,68). When expressed in COS cells, this receptor stimulates cAMP generation in response to butaprost in a concentration-dependent manner. Thus, based upon pharmacological characterization, this cDNA appears to correspond to the authentic EP_2, whereas the previously discussed cAMP stimulating EP-receptor corresponds to the EP_4 receptor. The highest expression level of this authentic EP_2 receptor was seen in human placenta and lung. Only low levels of expression were observed in kidney.

In contrast to the authentic EP_2 receptor, the EP_4 receptor appears to be widely expressed by Northern blot analysis and nuclease protection. High levels of expression were detected in duodenum, ileum, spleen, thymus, and lung, and lower levels were detected in kidney. In the kidney, *in situ* hybridization shows the greatest expression of the EP_4 in the glomerulus (Figure 3), suggesting this receptor may participate in the glomerular effects of PGE_2 (50) (Table 2). PGE_2 stim-

ulates cyclic AMP generation when added to preglomerular microvasculature, where it is thought to mediate the glomerular vasodilator effect of PGE_2 (52,69,70). It has been suggested that impaired PGE_2-dependent vasodilation is a component of the hypertension in spontaneously hypertensive rats (70,71). Alterations of EP-receptor expression in this animal model of hypertension remains to be demonstrated.

There are also well-documented stimulatory effects of PGE_2 on cyclic AMP along the nephron. PGE_2 has been shown to stimulate cAMP generation in thin descending limbs in the rat (72). Other studies demonstrate significant stimulation of cAMP generation in both rabbit and rat medullary and cortical TAL (73, 74). The functional significance and molecular identity of these cAMP stimulating EP receptors in segments of the loop of Henle remains to be established (Figure 3).

PGE_2 also stimulates a receptor coupled to cAMP generation in the cortical and medullary collecting ducts (44,47,73,75). Stimulation of this EP receptor increases water reabsorption (47,75) and could conceivably stimulate Na^+ absorption by this segment of the nephron (76).

Prostacyclin (IP) Receptors

The cDNA sequence of the prostacyclin receptor (IP) has recently been reported (77,78). Like other prostanoid receptors, this cDNA encodes a protein with seven hydrophobic (presumably membrane-spanning) domains. Although expression of the IP receptor in mouse kidney was not observed, high levels of expression were observed in the human kidney (78). There is good functional evidence for the existence of IP receptors in the kidney (see below). The IP receptor is selectively activated by the analog cicaprost (5).

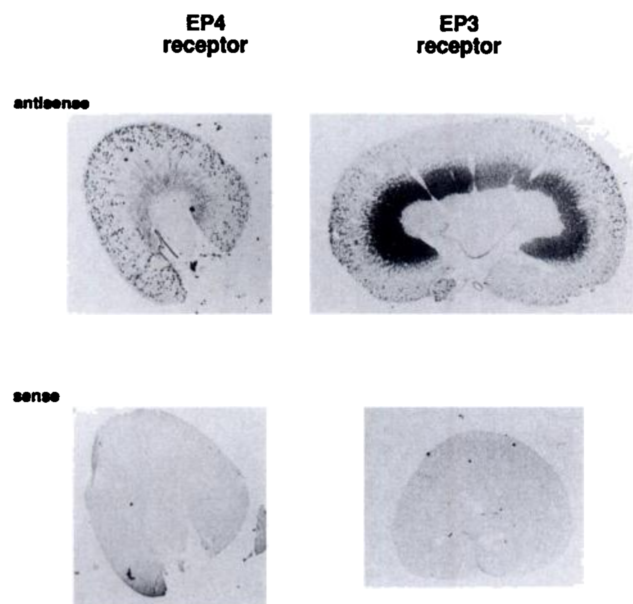

Figure 3. Intrarenal distribution of the rabbit EP_4 and EP_3 receptors. The EP_4 receptor is highly expressed in the glomerulus, with low levels of expression in the outer medulla. In contrast, the EP_3 is highly expressed in the outer medulla in thick limb and collecting duct, with some cortical expression in the collecting duct.

Most evidence suggests the PGI_2 receptor signals via stimulation of cAMP generation, however, the cloned mouse PGI_2 receptor also signaled via phosphatidylinositol hydrolysis. Notably, a 10,000-fold higher PGI_2 concentration was required to stimulate phosphatidylinositol hydrolysis (10^{-7} M) in CHO cells transfected with the IP receptor, than the concentrations required to stimulate cAMP accumulation in the same cells (10^{-10} M) (77). It remains unclear whether phosphatidylinositol hydrolysis plays any significant physiologic role in the action of PGI_2, however, one recent report suggests this signal-transduction pathway mediates the effect of prostacyclin in the rabbit CCD (79) (see below).

PGI_2 has been demonstrated to play an important vasodilator role in the glomerular microvasculature (69,80). The effects of PGI_2 and PGE_2 to stimulate cAMP generation in the glomerular microvasculature were distinct and additive (52), demonstrating that the effects of these two prostanoids are mediated via separate receptors. Prostacyclin also plays an important role in mediating renin release from the juxtaglomerular apparatus (81).

Renal epithelial effects of PGI_2 have also been reported but these are less well established. Hébert demonstrated that the PGI_2 analogs, iloprost and carbacyclin, inhibit water permeability and increase intracellular Ca^{++} in the isolated perfused CCD (79). In contrast to PGE_2, iloprost alone failed to increase basal water permeability, suggesting that, at least in the CCD, the receptor with which it interacts is not coupled to cAMP generation. Because iloprost has also been shown to be an agonist at EP_1 receptors (5), it remains unclear whether these effects prostacyclin analogs are mediated via a prostacyclin receptor coupled to IP_3 generation or via an EP_1 receptor. There is also evidence that PGI_2 stimulates cAMP generation in cultured rat inner medullary collecting ducts, however these studies have not been confirmed in freshly isolated tissue (82). In summary, while IP receptors appear to play an important vasodilator role in the kidney, their role in the regulation of renal epithelial function remains to be clearly established.

DP Receptors

PGD_2 is a major cyclooxygenase product of pulmonary mast cells (83) and possibly tracheal epithelial cells (84), however, it appears to be a minor product of intrarenal arachidonate metabolism (3). The cDNA sequence for the DP receptor has recently been reported, and it encodes a protein of 357 amino acids (85). This receptor has the greatest homology to the IP and $EP_{2/4}$ receptors. Like these receptors, the DP

receptor is thought to couple primarily to increased cAMP generation. The DP receptor mRNA was highly expressed in ileum, lung, stomach, and uterus.

The is little evidence supporting a major role for PGD_2 in the kidney. Intrarenal infusion of PGD_2 resulted in a dose-dependent increase in renal artery blood flow, urine output, creatinine clearance, and sodium and postassium excretion (86). DP-selective PGD_2 analogs, including the agonist BW 245C and the antagonist BW A868C, (5,87) should help to clarify whether these renal effects of PGD_2 are mediated by authentic DP receptors.

CONCLUSION

In conclusion, PGE_2 is the major intrarenal prostaglandin. At least three of the four EP receptors identified thus far are highly expressed in the kidney. Each EP receptor has a distinct distribution, and appears to modulate distinct renal actions. There are also important roles for IP, FP, and TP receptors, although the intrarenal distribution of these receptors remains to be mapped. The availability of receptor-selective prostaglandin analogs may offer new modes of therapy for renal disease.

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