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invited review

Prostaglandin E receptors and the kidney

MATTHEW D. BREYER[1,2] AND RICHARD M. BREYER[1,3]
[1]*Division of Nephrology and Departments of Medicine,* [2]*Molecular Physiology and Biophysics, and* [3]*Pharmacology, Veterans Affairs Medical Center and Vanderbilt University School of Medicine, Nashville, Tennessee 37232*

Breyer, Matthew D., and Richard M. Breyer. Prostaglandin E receptors and the kidney. *Am J Physiol Renal Physiol* 279: F12–F23, 2000.— Prostaglandin E_2 is a major renal cyclooxygenase metabolite of arachidonate and interacts with four G protein-coupled E-prostanoid receptors designated EP_1, EP_2, EP_3, and EP_4. Through these receptors, PGE_2 modulates renal hemodynamics and salt and water excretion. The intrarenal distribution and function of EP receptors have been partially characterized, and each receptor has a distinct role. EP_1 expression predominates in the collecting duct where it inhibits Na^+ absorption, contributing to natriuresis. The EP_2 receptor regulates vascular reactivity, and EP_2 receptor-knockout mice have salt-sensitive hypertension. The EP_3 receptor is also expressed in vessels as well as in the thick ascending limb and collecting duct, where it antagonizes vasopressin-stimulated salt and water transport. EP_4 mRNA is expressed in the glomerulus and collecting duct and may regulate glomerular tone and renal renin release. The capacity of PGE_2 to bidirectionally modulate vascular tone and epithelial transport via constrictor EP_1 and EP_3 receptors vs. dilator EP_2 and EP_4 receptors allows PGE_2 to serve as a buffer, preventing excessive responses to physiological perturbations.

prostaglandin E_2 transport; hemodynamics; sodium; water

PROSTAGLANDINS COMPRISE a diverse family of autacoids derived from cyclooxygenase-mediated metabolism of arachidonic acid to PGG/H_2, generating five primary bioactive prostanoids: PGE_2, $PGF_{2\alpha}$, PGD_2, PGI_2, and thromboxane A_2 (TXA_2) (20, 116). Each of these prostanoids interacts with a unique G protein-coupled receptor (GPCR), designated EP (for E-prostanoid), FP, DP, IP, and TP receptors, respectively, for the other prostanoids (31, 132). The importance of these autacoids to systemic blood pressure and volume control is perhaps best highlighted by the deleterious side effects of cyclooxygenase inhibitors [nonsteroidal anti-inflammatory drugs (NSAIDs)], which may induce hypertension (51), Na^+ retention, and edema (91, 110), suggesting an antihypertensive role for endogenous prostaglandins. Conversely, NSAIDs reduce blood pressure in patients with hyperreninemic renovascular hypertension, suggesting that under these circumstances endogenous prostaglandins increase blood pressure (65, 70). These complex effects of NSAIDs on blood pressure are evidence for competing hypotensive and hypertensive effects of prostanoids including PGE_2, PGI_2, and TXA_2 (88), and underscores the general principal that prostaglandins have the capacity to buffer physiological processes in either positive or negative directions. Although important intrarenal effects of all these prostanoids have been described, the present review will focus on the mechanisms by which intrarenal EP receptors mediate the effects of PGE_2.

INTRARENAL PGE_2, SALT BALANCE, AND BLOOD PRESSURE

PGE_2 is a major product of cyclooxygenase-initiated arachidonic acid metabolism in the kidney and is synthesized at high rates along the nephron (20). The maintenance of normal renal blood flow and function during physiological stress is especially dependent on endogenous prostaglandin synthesis (142). In this setting, the vasoconstrictor effects of angiotensin II, catecholamines, and vasopressin in the kidney are more effectively buffered by prostaglandins than in other

Address for reprint requests and other correspondence: M. D. Breyer, Veterans Administration Medical Center, 1310 24th Ave. S, F427 ACRE Bldg., Nashville, TN 37212 (E-mail: Matthew.breyer@mcmail.vanderbilt.edu).

vascular beds, preserving normal renal blood flow, glomerular filtration rate (GFR), and salt excretion. PGE_2 not only dilates the glomerular microcirculation and vasa rectae, supplying the renal medulla (17, 115) but also modulates salt and water transport in the distal tubule (21), as discussed below. Administration of cyclooxygenase-inhibiting NSAIDs in the setting of volume depletion interferes with these dilator effects and may result in a catastrophic decline in GFR, resulting in overt renal failure (110).

Other evidence points to constrictor and prohypertensive effects of endogenous prostaglandins. Curiously, PGE_2 may initiate the production of renin and the subsequent increase in angiotensin II, the constrictor effect of which PGE_2 attenuates in certain vascular beds (61, 71). PGE_2 directly stimulates renin production in isolated juxtaglomerular apparatus (JGA) cells (69, 71), and, in conscious dogs, chronic intrarenal PGE_2 infusion increases renal renin secretion, resulting in hypertension (61). Treatment of salt-depleted rats with indomethacin not only decreases plasma renin activity but also causes blood pressure to fall, suggesting prostaglandins support blood pressure during salt depletion, via their capacity to increase renin (40, 119). Other studies suggest direct vasoconstrictor effects of PGE_2 on renal vasculature (66, 76). It is conceivable these latter effects might predominate in circumstances that expose the kidney to excessively high perfusion pressure. Thus, depending on the setting, the primary effect of PGE_2 may be to either increase or decrease vascular tone.

PGE_2 AND EPITHELIAL SOLUTE AND WATER TRANSPORT

In a manner analogous to its dual vascular effects, evidence suggests PGE_2 may either stimulate or inhibit epithelial solute and water transport along the nephron (4). Numerous studies have demonstrated PGE_2 directly inhibits solute absorption in in vitro microperfused thick ascending limbs (TAL), as well as water and solute absorption in the collecting duct (34, 45, 47, 58, 59, 122, 124). These findings provide a cellular basis for the well-described natriuresis and diuresis after acute intrarenal PGE_2 infusions in intact animals (61, 72, 103). Tubule microperfusion studies also demonstrate a more complex picture, because PGE_2 can either increase or decrease water absorption and cAMP generation in the collecting duct (47, 58, 117). When added to vasopressin-prestimulated collecting ducts, PGE_2 potently inhibits water absorption (60), consistent with the aforementioned in vivo diuretic effects of PGE_2 infusion (47, 72). However, when administered in the absence of vasopressin, basolateral PGE_2 actually increases osmotic water absorption (60, 72, 107). PGE_2 also simultaneously inhibits collecting duct Na^+ absorption. Thus at least three distinct effects of basolateral PGE_2 on transport have been described: stimulation of basal water absorption; inhibition of vasopressin-stimulated water absorption; and inhibition of Na^+ absorption.

In vivo studies supporting a role for endogenous PGE_2 to increase renal salt reabsorption are less widely described; however, one study shows a natriuretic effect of NSAIDs supporting this possibility (78). Taken together, these considerations support dual, opposing effects of PGE_2 on several processes, including maintenance of vascular tone, water absorption, and Na^+ absorption. The self-opposing effects of PGE_2 on both epithelial transport and vascular tone appear to be mediated by distinct EP receptors, which are the subject of the remainder of this review (31, 134).

MULTIPLE PGE_2 RECEPTORS

The vasodilator effect of PGE_2 on both arterial and venous vascular beds was one of the first recognized (29, 35, 81, 86). This is mediated, in part, by a direct relaxant effect of PGE_2 on smooth muscle that is now thought to be coupled to increased cAMP generation (81, 86). However, PGE_2 does not uniformly relax smooth muscle and has been shown to constrict trachea, gastric fundus, and ileum (30). Importantly, selected structural analogs of PGE_2 that reproduce the dilator effects of PGE_2, are completely inactive in tissues where PGE_2 is a constrictor. Conversely, analogs that reproduce the constrictor effects of PGE_2, fail to affect tissues in which PGE_2 is a dilator (30). These differential effects of PGE_2 analogs provided important initial evidence for the existence of multiple PGE_2 (EP) receptors (31).

In screening compounds for antagonist activity, SC-19220 was found to be a selective EP antagonist, only at those receptors where PGE_2 was a smooth muscle constrictor (30). These receptors were originally designated as EP_1, whereas SC-19220-insensitive dilator effects were ascribed to distinct receptors, designated EP_2. There are now at least four pharmacologically classified EP receptors. Dilator receptors are designated EP_2 and EP_4, whereas EP_1 and EP_3 receptors are constrictor receptors. Similarly, four EP receptor subtypes have been cloned and extensively studied. Some studies suggest the existence of additional EP receptor subtypes; however, molecular correlates for other subtypes have not been identified (109).

The EP receptors are members of the G protein-coupled family of receptors, possessing seven hydrophobic, membrane-spanning stretches of amino acids (134). The pharmacological and molecular characterization of the four different EP receptors have now been completed, and the affinity of multiple prostanoid analogs for each cloned receptor protein have been determined (Table 1, Fig. 1, and see Refs. 19 and 77). Although these four receptors uniformly bind PGE_2 with a higher affinity than other endogenous prostanoids, on the basis of amino acid homology, they are not as closely related to each other as to other prostanoid receptors. 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

Table 1. *EP receptor pharmacology*

Subtype	PGE_2, IC_{50}/K_d	Agonists	Antagonists	Functional Assay	Signaling	Tissue mRNA Expression
EP_1*	1–20 nM	17-Phenyl-trinor PGE_2, iloprost, sulprostone	SC-19220 SC-51322 SC-51089 AH-6809	Contracts guinea pig ileum, gastric fundus, trachea	IP3/DAG/PKC	Collecting duct, muscularis, mucosae, hypothalamus
EP_2	20 nM	Butaprost, AH-13205, 11-deoxy PGE_1		Relaxes rabbit ear, artery guinea pig ileum trachea	Increases cAMP	Uterus, arterial
EP_3*	0.3–2 nM	MB-28767, sulprostone, SC-46275, 11-deoxy PGE_1		Contracts chick ileum. Inhibits gastric acid secretion	Decreases cAMP/G_i, *rho,* and other pathways	Stomach, epithelium kidney collecting duct, thick limb
EP_4	2–11 nM	PGE_1-OH, 11-deoxy PGE_1, misoprostol	AH-23848 (weak)	Relaxes saphenous vein, jugular vein, ductus arteriosus	Increases cAMP	Ureter/bladder, kidney, thymus, intestinal, epithelia

Agonists are only relatively selective and may activate other EP receptors at higher concentrations. K_d, dissociation constant; EP, E-prostanoid; IP_3, inositol 1,4,5-trisphosphate; DAG, diacylglycerol; PKC, protein kinase C. *Splice variants exist that may have alternate signaling and functional effects.

such as the TP and FP receptors (134). Sequence alignment of the prostanoid receptors has demonstrated that the overall homology is limited, ranging from 20 to 30%, with the highest degree of conservation lying within the seven transmembrane regions where there are 28 residues conserved across this family (9). In addition, there is a cluster of six conserved amino acid residues in the second extracellular loop. It is now evident that the GPCR superfamily contains receptor subgroups possessing distinct motifs for receptor-ligand interactions. Recent mutagenic (120, 121) and phylogenetic analyses (79) suggest prostaglandin receptors may represent a unique subfamily of receptors: although they bind small ligands these receptors share structural requirements in the extracellular sequences, similar to peptidergic GPCRs, where the extracellular loop regions play a critical role in ligand binding (121). This information will undoubtedly prove useful in designing receptor-selective prostaglandin analogs for use as pharmacological tools and therapeutic agents. The intrarenal distribution of EP receptor mRNA has also been mapped to different nephron segments, suggesting distinct functional consequences of activating each receptor subtype (22, 24, 25, 125, 127, 132).

EP_1 Receptors

The EP_1 receptor was originally described as a smooth muscle constrictor. The human EP_1 receptor cDNA encodes a 402-amino acid polypeptide with a predicted molecular mass of 41,858 kDa (42). This receptor signals via increased cell Ca^{2+}, which is accompanied by modest increases in IP_3 generation (15, 42, 137). One report suggests a COOH-terminal splice variant of the rat EP_1 receptor is present in both kidney and uterus. The existence of an EP_1 splice variant has not been confirmed for other species, but it is of interest because it does not appear to signal via Ca^{2+} and may also suppress EP_4 receptor signaling (99). Another recent report suggests EP_1 receptors are also in the nuclear envelope where they may affect nuclear Ca^{2+} entry, thereby contributing to effects of endogenous PGE_2 on gene expression (18). EP_1 receptor mRNA predominates in the kidney > gastric muscularis mucosae > adrenal gland (1, 50, 99, 137). Interpretation of EP_1 mRNA expression by Northern analysis is complicated by the presence of several size mRNA species, including ~7.0, 5, 4.4, and ~3 kb. Some of these transcripts appear to derive not from EP1 mRNA but rather from protein kinase N (PKN) mRNA, an apparently unrelated gene that is actively transcribed from the antiparallel DNA strand, possessing a sequence complementary to the EP_1 receptor (16, 19). For this reason cDNA probes, which will recognize both EP_1 and PKN transcripts, may be inadequate to quantify EP_1 mRNA. Nuclease protection or strand-specific RT-PCR may be required for specific detection of EP_1 mRNA.

Studies of EP_1 receptors may utilize one of several relatively selective antagonists that block their activation, including AH-6809, SC-19220, or SC-53122 (Table 1) (54, 55, 80). A significant impetus behind the development of clinically active EP_1 receptor antagonists derives from evidence that the EP_1 receptor plays an important role in prostaglandin-mediated pain (87) and that EP_1 receptor antagonists have analgesic properties (54, 55). These antagonists may provide a useful approach to studying the role of the EP_1 receptor in regulating renal salt and water balance in vivo. Unfortunately, not all of these antagonists are absolutely selective, and they may also variably block other receptors at higher doses.

Within the kidney, EP_1 mRNA has been mapped by in situ hybridization and is primarily in the collecting duct, increasing from the cortex to the papillae (15, 50, 127). In the collecting duct, activation of the EP_1 receptor inhibits Na^+ and water reabsorption absorption via a Ca^{2+}-coupled mechanism (50, 59, 60). The Ca^{2+} increase in the collecting duct is potently mimicked by 17-phenyl-trinor PGE_2 but not by an EP_3-selective agonist, MB-28767. Furthermore, both the PGE_2-stimulated Ca^{2+} increase and its capacity to inhibit collect-

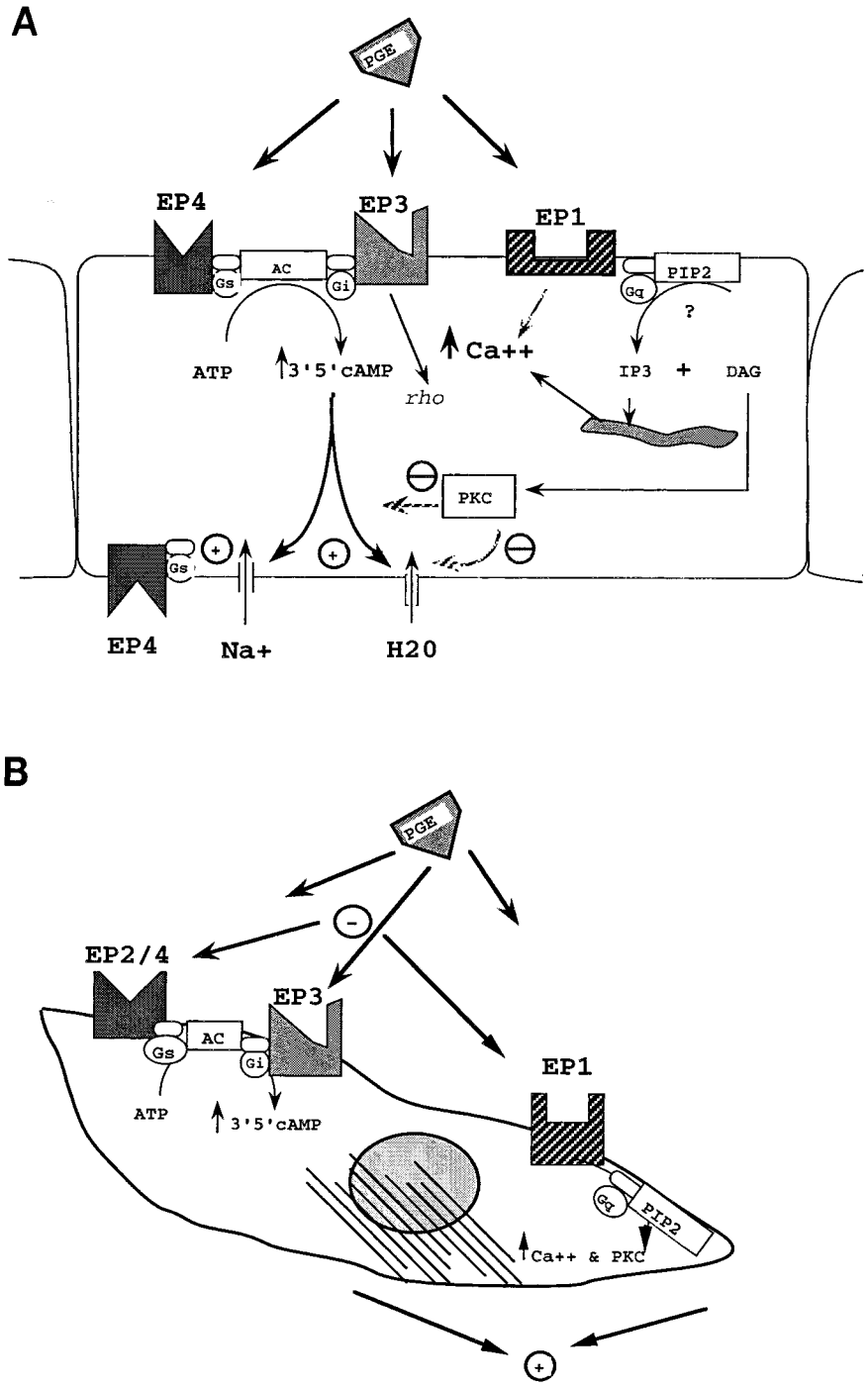

Fig. 1. Signaling mechanisms activated by 4 G protein-coupled E-prostanoid (EP) receptors. *A*: renal epithelial cell. IP_3, inositol 1,4,5-trisphosphate; PKC, protein kinase C; PIP_2, phosphatidylinositol 4,5,-bisphosphate; DAG, diacylglycerol. *B*: smooth muscle cell. AC, adenlyl cyclate.

ing duct Na^+ absorption can be blocked by using EP_1 receptor antagonists AH-6809 and SC-19220 (10 μM) (50). Taken together, these results suggest that renal EP_1 receptor activation contributes to PGE_2-dependent natriuresis by inhibiting Na^+ transport in the collecting duct. A preliminary report also suggests EP_1 receptor mRNA is present in glomeruli, where it could play a role as a vasoconstrictor; however, this has not been confirmed (68).

The net contribution of EP_1 receptor activation to regulation of renal salt and water excretion in vivo remains uncertain. Given the above considerations suggesting that the renal EP_1 receptor contributes to natriuresis, EP_1 receptor antagonists might be expected to reduce the renal capacity to excrete Na^+ and thereby increase total body salt content and possibly blood pressure. As will be discussed below, in the absence of potent and specific receptor antagonists, important information regarding the physiological roles of prostanoid receptors has been obtained by using transgenic mice with targeted disruption of these genes (76, 90, 96, 114, 128, 135). Unfortunately, the

utility of standard gene targeting approaches for the EP_1 receptor is complicated by the presence of transcription PKN from the antiparallel DNA strand (16). It has been argued that standard techniques to disrupt the EP_1 locus, which alter the 3′-untranslated region of the PKN gene in turn may affect PKN expression, thereby introducing additional physiological changes (16, 19). Thus studies utilizing knockout mice generated by standard targeted disruption of the EP_1 gene must be interpreted with this caveat in mind (135).

EP_2 Receptors

The literature is somewhat confusing regarding the EP_2 receptor's nomenclature, because before 1995, when the human EP_2 receptor was cloned, the cloned EP_4 receptor was misclassified as the EP_2 receptor (97). Authentic EP_2 receptors have now also been cloned for mice, rats, rabbits, and cows (48, 74, 95, 105). The human EP_2 receptor cDNA encodes a 358-amino acid polypeptide, which signals through increased cAMP (14, 105). Mutagenesis studies have demonstrated that the extracellular loop regions of the EP_2 receptor are critical determinants of receptor function. Introduction of point mutations (120) or creation of receptor chimeras with the EP_4 receptor (121) in certain extracellular regions results in a loss of receptor binding and signal transduction. EP_2 receptors are selectively activated by butaprost (19, 77). 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 AH-23848 (29, 105). Interestingly, a single point mutation in the seventh transmembrane domain of the EP_2 receptor resulted in a selective gain of function of the receptor for prostacyclin analogs (75). Taken together, these data suggest that both the extracellular sequences and the transmembrane regions are important for receptor-ligand interactions.

The precise tissue distribution of the EP_2 receptor has been only partially characterized, using Northern blot analysis of mRNA distribution. This reveals a major mRNA species of ~3.1 kb that is most abundant in the uterus, lung, and spleen, exhibiting only low levels of expression in the kidney (19, 74, 95, 105). The mRNA is expressed at much lower levels than EP_4 mRNA (74). Functional studies suggest the EP_2 receptor plays a role in uterine implantation (82) and a relaxant role in trachea and vasculature (30, 31). In addition, recent studies demonstrate targeted disruption of the EP_2 receptor interferes with fertility and results in salt-sensitive hypertension (76, 133). This latter finding supports an important role for the EP_2 receptor in protecting systemic blood pressure, perhaps via its vasodilator effect and effects on renal salt handling (see below).

EP_3 Receptors

The EP_3 receptor generally acts as a constrictor of smooth muscle (31). As with the EP_2 receptor, mutations critical for ligand binding and signal transduction have been described in both the transmembrane and extracellular regions of the receptor (8, 9, 63, 94). This receptor is unique in that at least six alternatively spliced variants defined by unique COOH-terminal cytoplasmic tails exist in humans alone, and over 22 unique variants have been observed in rats, rabbits, mice, cows, and humans (2, 26, 67, 93, 104, 111). These splice variants encode proteins of a predicted molecular mass between 40 and 45 kDa (1, 26, 104). All the EP_3 splice variants bind PGE_2, and the EP_3 agonists MB-28767, and sulprostone, with similar affinity. Although these variants uniformly and potently inhibit cAMP generation via a pertussis toxin-sensitive G_i-coupled mechanism, additional signaling mechanisms (Fig. 1) appear to be differentially activated by these different COOH-terminal tails (7, 11, 93). One recent study suggests signaling through the small G protein *rho* (7). Each of the rabbit splice variants of this G_i-coupled receptor also appears to activate cAMP-responsive binding protein/cAMP-responsive element-mediated gene expression, under some conditions, suggesting that these receptors participate in long-term regulation of cellular events (11). Finally, 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 (57). The physiological significance of the different COOH-terminal splice variants remains uncertain. Nuclease protection and Northern analysis demonstrate relatively high levels of EP_3 receptor expression in several tissues including kidney, uterus, adrenal gland, and stomach, with Northern analysis showing major mRNA species at ~2.4 and ~7.0 kb (1, 26, 104, 111, 141).

The significance of EP_3 receptor activation to systemic physiology has been significantly advanced by the availability of mice with targeted disruption of this gene (38, 135). Mice with targeted deletion of the EP_3 receptor exhibit an impaired febrile response to PGE_2, suggesting the EP_3 receptor antagonists could be effective antipyretic agents. In contrast, despite relatively high levels of EP_3 receptor in kidney (24, 26, 126, 127), mice with targeted disruption of this receptor only display a subtle alteration in the effect of NSAIDs on urinary-concentrating ability, as described in greater detail below (38). These finding raise the possibility that some of the renal action of PGE_2 normally mediated by the EP_3 receptor has been co-opted by other receptors.

The EP_4 Receptor

Like the EP_2 receptor, the EP_4 signals through increased cAMP (14, 105). The human EP_4 receptor cDNA encodes a 488-amino acid polypeptide with a predicted molecular mass of ~53 kDa (14). Again, care must be taken in reviewing the literature before 1995, when this receptor was generally referred to as the EP_2 receptor (97). In addition to the human receptor, EP_4 receptors for mouse, rat, rabbit, and cow have been

cloned (3, 14, 19, 25, 62, 97). EP_4 receptors may be pharmacologically distinguished from the EP_1 and EP_3 receptors by their insensitivity to sulprostone and from EP_2 receptors by its insensitivity to butaprost (19, 77) and relatively selective activation by PGE_1-OH (19, 77). Furthermore, [^{3}H]PGE_2 binds to the EP_4 receptor with at least 10-fold higher affinity than the EP_2 receptor. Structurally, the EP_4 receptor has a much longer COOH-terminal sequence than the EP_2 receptor and has been shown to undergo short-term agonist-induced desensitization, which is absent in the EP_2 receptor (13, 98).

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 gland, and kidney (14, 25, 62, 108). Roles for EP_4 receptors in immune cell activation and osteoblast function have been reported (73, 89, 100, 138). Important vasodilator effects of EP_4 receptor activation in venous and arterial beds have been described (29, 31). A particular role for the EP_4 receptor in regulating closure of the pulmonary ductus arteriosus has also been suggested by the recent studies in mice with targeted disruption of the EP_4 receptor gene (96, 114). EP_4−/− mice on a 129 background had close to 100% perinatal mortality due to persistent patent ductus arteriosus (96). Interestingly, when bred on a mixed genetic background, as many as 21% of EP_4−/− mice had closure of the ductus and survived. Preliminary studies in these survivors support an important role for the EP_4 receptor as a systemic vasodepressor (10, 29, 31); however, their heterogeneous genetic background complicates the interpretation of these results, because survival may select for modifier genes that not only allow ductus closure but also alter hemodynamics. Other roles for the EP_4 receptor in controlling blood pressure have been suggested, including the ability to stimulate aldosterone release from zona glomerulosa cells (32). In the kidney, EP_4 receptor mRNA expression is primarily in the glomerulus, where its precise function is uncharacterized (22, 25, 127) but might contribute to regulation of the renal microcirculation as well as renin release.

ROLE OF EP RECEPTOR SUBTYPES IN REGULATING RENAL FUNCTION

Glomerular Microcirculation

Prostaglandins play an important role regulating the renal cortical microcirculation. Both glomerular constrictor and dilator effects of prostaglandins have been reported (17, 66, 112). In the setting of volume depletion, endogenous PGE_2 helps maintain GFR possibly by dilating the afferent arteriole (17, 37, 110). Recent studies showing cyclooxygenase 2 is localized to the macula densa (49, 56) also suggest a particular role for prostaglandins in regulating the glomerular microcirculation. Control of GFR by the macula densa via tubuloglomerular feedback (TGF) suggests both dilator and constrictor effects of prostanoids (12, 17, 112). One recent study suggests that cyclooxygenase 2-mediated synthesis is predominantly responsible for dilator prostanoids (64). The array of prostanoids produced by the macula densa remains uncharacterized, but PGE_2 is the primary product synthesized by microdissected cortical TAL (20). Nor have the prostanoid receptors mediating the downstream vasoconstrictor and vasodilator effects of prostaglandins on the glomerular microcirculation or their location been determined. Some data suggest roles for EP and IP receptors coupled to increased cAMP generation as mediating these vasodilator effects in the preglomerular circulation (28, 112, 113). Edwards (37) found PGE_2 exerted a dilator effect on the afferent arteriole but not the efferent arteriole of rabbit glomeruli, consistent with the presence of an EP_2 or EP_4 receptor in the preglomerular microcirculation. In contrast, constrictor effects of PGE_2 in the afferent arteriole of rat have been reported, suggesting an EP_1 or EP_3 receptor (66). The presence of at least two EP receptor subtypes, constrictor and dilator, in the preglomerular microcirculation seems likely. Thus the net effect of PGE_2 on the glomerular microcirculation will depend not only on the resting tone of these vessels but also on which EP receptor functionally predominates.

Renal Medullary Microcirculation

In the setting of systemic hypertension, the normal response of the kidney is to increase salt excretion, thereby mitigating the increase in blood pressure. This so-called "pressure natriuresis" plays a key role in the ability of the kidney to protect against hypertension (52, 53). Increased blood pressure is accompanied by increased renal perfusion pressure, which is associated with enhanced PGE_2 excretion (27). Inhibition of prostaglandin synthesis markedly blunts (although it does not eliminate) pressure natriuresis (106). The mechanism by which PGE_2 contributes to pressure natriuresis may involve changes in resistance of the renal medullary microcirculation (102, 106). PGE_2 directly dilates descending vasa recta, and increased medullary blood flow may contribute to increased interstitial pressure observed as renal perfusion pressure increases, leading to enhanced salt excretion (115). The identity of the dilator PGE_2 receptor controlling the contractile properties of the descending vasa recta remains uncertain, but dilator EP_2 or EP_4 receptors seem likely candidates (31). Recent studies demonstrating salt-sensitive hypertension in mice with targeted disruption of the EP_2 receptor (76) suggest the EP_2 receptor facilitates the ability of the kidney to increase sodium excretion, thereby protecting systemic blood pressure from a high-salt diet. Although EP_2-mediated effects on renal epithelial transport cannot be excluded, cAMP-coupled effects of PGE_2 on transport in the collecting duct are not mimicked by butaprost and appear more likely to be related to activation of the EP_4 receptor (107). Furthermore, EP_2 receptor mRNA has not been detected along the nephron (22). Given its defined role in vascular smooth muscle (76), these

effects of the EP_2 receptor disruption seem more likely to relate to its effects on renal vascular tone. In particular, loss of a vasodilator effect in the renal medulla might modify pressure natriuresis and could contribute to hypertension in EP_2-knockout mice. Nonetheless, a defined role for this receptor in regulating renal medullary blood flow remains to be established.

EP Receptors and Renin Release

Soon after the introduction of cyclooxygenase inhibitors, it was recognized that endogenous prostaglandins play an important role in stimulating renin release (43, 112). Treatment of salt-depleted rats with indomethacin not only decreases plasma renin activity but also causes blood pressure to fall, suggesting prostaglandins support blood pressure during salt depletion, via their capacity to increase renin (40, 119). Prostanoids also play a central role in the pathogenesis of renal-vascular hypertension, and administration of NSAIDs lowers blood pressure in both animals and humans with renal artery stenosis (65, 70, 83). PGE_2 appears to be an important prostanoid, which, like PGI_2, stimulates renin release. In conscious dogs, chronic intrarenal PGE_2 infusion increases renal renin secretion, resulting in hypertension (61). PGE_2 induces renin release in isolated preglomerular JGA cells (71). Like the effect of β-adrenergic agents, this effect appears to be through a cAMP-coupled response, supporting a role for an EP_4 or EP_2 receptor (71). PGE_2 also stimulates cAMP generation in freshly isolated preglomerular rabbit renal arterioles (28). Although localization of EP_2 or EP_4 receptors to the juxtaglomerular apparatus has not been demonstrated, EP_4 receptor mRNA is relatively abundant in the glomerulus (22, 25, 127), supporting the possibility that renal EP_4 receptor activation contributes to enhanced renin release. In contrast, regulation of plasma renin activity and intrarenal renin mRNA does not appear to be different in wild-type and EP_2-knockout mice, (133), arguing against a major role for the EP_2 receptor in regulating renin release. These considerations are tempered by the fact that the precise mechanism by which prostaglandins contribute to renin release remains elusive, and molecular identification of EP receptors in renin-secreting JGA cells is lacking. Rather, one report demonstrates EP_3 receptor mRNA is localized to the macula densa, suggesting this cAMP-inhibiting receptor may also contribute to the control of renin release (127). In conclusion, direct vasomotor effects of EP_2 and EP_4 receptors, as well as effects on renin release, may play critical roles in regulating systemic blood pressure and renal hemodynamics.

Urinary Concentration and Dilution

The EP_3 receptor was the first E-prostanoid receptor cloned, but an important role for a G_i-coupled prostaglandin E receptor in regulating water and salt transport along the nephron was defined well before its molecular identification. PGE_2 directly inhibits salt and water absorption in both the in vitro microperfused TAL and collecting duct (46, 59, 60, 122). PGE_2 directly inhibits Cl^- absorption in the mouse or rabbit medullary TAL from either the luminal or basolateral surfaces (33, 122). It was subsequently demonstrated that PGE_2 also inhibits hormone-stimulated cAMP generation in TALs (92, 129). Because cAMP stimulates TAL transport, inhibition of cAMP generation through a G_i-coupled PGE_2 receptor likely contributes to the inhibitory effects of PGE_2 on TAL transport (140). The mRNA for the G_i-coupled EP_3 receptor is localized in discrete segments of the nephron and is most highly expressed in the TAL and collecting duct (22, 24, 127, 130). Good and colleagues (45, 46) demonstrated that PGE_2 modulates ion transport in the rat TAL by a pertussis toxin-sensitive mechanism (45, 46). Interestingly, these effects also appear to involve protein kinase C activation, possibly reflecting activation of a novel EP_3 receptor-signaling pathway, corresponding to pathways of recognized cultured cells (11, 93). Taken together, these data support a role for the EP_3 receptor in regulating transport in the TAL.

In the collecting duct, PGE_2 inhibits both vasopressin-stimulated osmotic water absorption and cAMP generation (58, 117, 118). Furthermore, PGE_2 inhibition of both water absorption and cAMP generation is blocked by pertussis toxin, suggesting effects mediated by the inhibitory G protein G_i (23, 60, 117, 118). These functional data fit well with in situ hybridization studies, which demonstrate high mRNA expression of the G_i-coupled EP_3 in human and rabbit collecting duct (22, 24). This distribution has been confirmed by RT-PCR in microdissected rat and mouse collecting ducts (130, 132). It is likely that PGE_2-mediated antagonism of vasopressin-stimulated salt absorption in the TAL and water absorption in the collecting duct contributes to its diuretic effect (72). Furthermore, blockade of endogenous PGE_2 synthesis likely contributes to enhanced urinary concentration in the setting of NSAID use (5).

On the basis of the preceding functional considerations, one would expect $EP_3^{-/-}$ mice to exhibit inappropriately enhanced urinary concentration. Surprisingly, $EP_3^{-/-}$ mice exhibited a comparable urinary concentration after dDAVP, similar 24-h water intake, and similar maximal minimal urinary osmolality (38). The only clear difference was that, in mice allowed free access to water, indomethacin increased urinary osmolality in normal mice but not in the knockout mice. These findings suggest compensatory mechanisms are in place that allow normal renal water excretion in $EP_3^{-/-}$ mice. The investigators hypothesized that the remaining EP_1 receptor might take over the function of the EP_3 receptor, thereby obscuring this phenotype. This remains to be formally tested. Other studies suggest the EP_3 receptor may play an important role as a vasoconstrictor receptor (103); however, no difference was seen in either GFR or renal plasma flow between anesthetized wild-type vs. EP_3 receptor-knockout mice. Further studies examining the potential role of the EP_3 receptor as systemic vasoconstrictor should yield important information.

Renal Sodium and Potassium Excretion

Administration of cyclooxygenase inhibitors is commonly associated with Na^+ retention, edema, hypertension, and/or hyperkalemia resulting from loss of intrarenal prostaglandin synthesis (91, 110). Intrarenal infusion of PGE_2 is natriuretic, and although its effects on intrarenal hemodynamics undoubtedly play an important role, direct effects of PGE_2 on epithelial transport are equally important. Despite a few reports suggesting effects of PGE_2 on transport in the proximal tubule (36, 39), its effects on the distal nephron including the thick limb and collecting duct are more clearly established. The inhibitory effects of PGE_2 on NaCl absorption in the thick ascending limb have already been discussed above and undoubtedly contribute to its natriuretic effects (46, 122). PGE_2 also inhibits Na^+ transport in microperfused collecting ducts by ~50% (59, 60). In contrast to PGE_2-mediated inhibition of vasopressin-stimulated water absorption, its capacity to inhibit Na^+ absorption is insensitive to pertussis toxin (59). Instead, PGE_2 inhibits convoluted collecting duct Na^+ absorption via a Ca^{2+}-dependent mechanism (58, 59, 84). Primarily on the basis of the lack of effect of MB-28767, a potent EP_3 agonist, on collecting duct calcium, Guan et al. (50) suggested that this effect primarily involves an EP_1 receptor rather than an EP_3 splice variant. Nonetheless, the possibility that EP_3 receptor activation also influences electrogenic ion transport in the collecting duct via pertussis-insensitive mechanisms has not been completely excluded (7, 11, 93).

As in the case with the vasculature, there is also evidence that PGE_2 can interact with a receptor that stimulates ion absorption. The capacity of PGE_2 to increase Na^+ absorption in toad bladder has been known for more than 25 years (85), so a similar capacity in renal epithelia would not be surprising. Recently, a separate effect of luminal PGE_2 has been reported in the collecting duct (6, 107). Luminal PGE_2 stimulates basal water absorption and also transiently stimulates an amiloride-sensitive current, suggesting urinary PGE_2 may also regulate salt and water excretion (107). Although NSAIDs typically reduce Na^+ excretion in anesthetized animals, one intriguing study showed meclofenamate or carprofen, when administered to conscious dogs undergoing a water diuresis (78), markedly increased urine Na^+ excretion without any change in urine volume or renal hemodynamics. These studies suggest, under particular circumstances, endogenous prostaglandins can enhance Na^+ absorption along the nephron. It is of note in this regard that PGE_2 is thought to enter the urine in the loop of Henle and thus have access to cAMP-stimulating luminal EP receptor distal nephron segments (41, 139). The possibility that enhanced distal Na^+ absorption contributes to certain forms of prostaglandin-dependent hypertension (65) remains unsubstantiated.

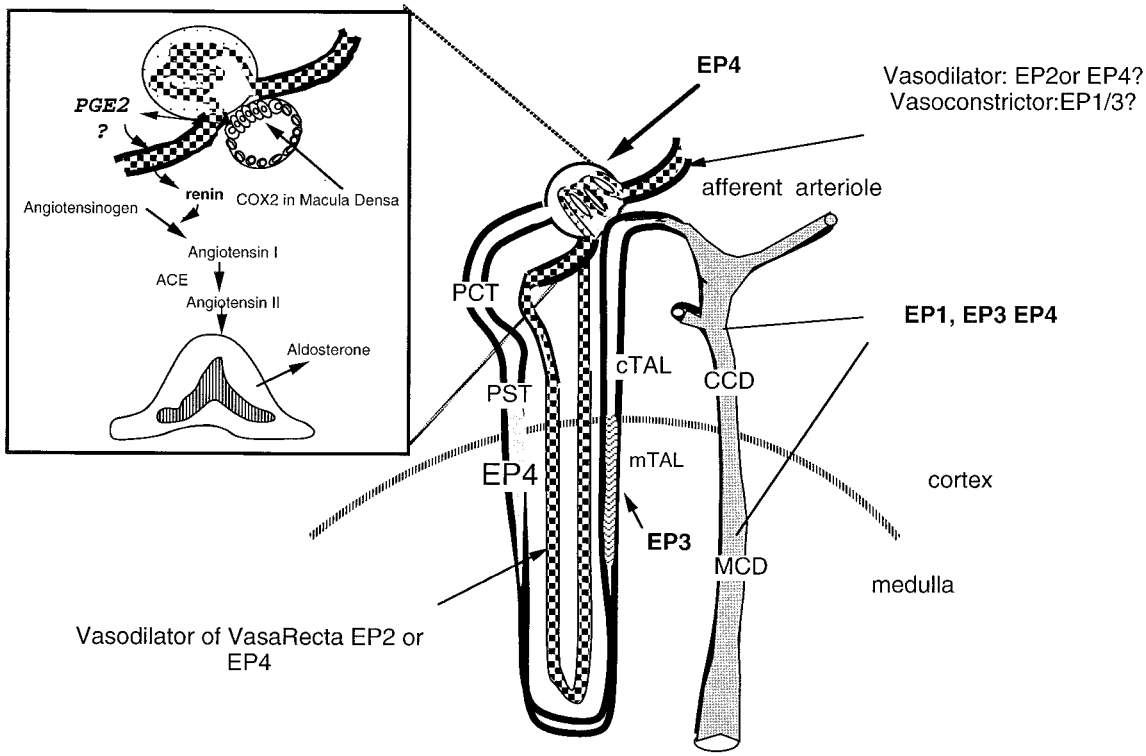

Fig. 2. Intrarenal localization and consequences of EP receptor activation along the nephron. PGE_2 stimulates renin release by juxtaglomerular apparatus (JGA) cells through a receptor coupled to cAMP generation. Dilator and constrictor PGE_2 receptors also modulate glomerular vascular tone as well as tone constrictor tone of the vasa rectae. PGE_2 also directly inhibits NaCl absorption by the thick ascending limb (TAL) and collecting duct via effects on EP_1 and EP_3 receptors. COX-2, cyclooxygenase-2; ACE, angiotensin-converting enzyme; PCT, proximal convoluted tubule; PST, proximal straight tubule; cTAL and mTAL, cortical and medullary TAL, respectively; CCD, cortical collecting duct; MCD, medullary collecting duct.

Effects of prostaglandins on renal K^+ transport have also been described; however, NSAID-associated hyperkalemia appears to be primarily secondary to effects on aldosterone rather than epithelial effects. Prostaglandin E may stimulate aldosterone secretion by both direct effects on zona glomerulosa cells (25, 32) and effects on renal renin release (61, 71). The effects on adrenal aldosterone secretion appear to be mediated by a cAMP-coupled EP_2 or EP_4 receptor (32). NSAID administration suppresses these effects, leading to hyporeninemic hypoaldosteronism (44, 101, 131). Diminished aldosterone release inhibits distal K^+ secretion, leading to hyperkalemia. In contrast, PGE_2 itself appears to directly inhibit K^+ secretion in the collecting duct (123, 136); thus loss of this inhibitory action would promote K^+ secretion, mitigating rather than exacerbating hyperkalemia. Additional studies of effects of prostanoids on K^+ handling in other nephron segments are required for a full understanding of the role of prostaglandins in K^+ handling.

In summary, EP_1, EP_3, and EP_4, receptors appear to exist in vascular glomeruli and individual nephron segments including the TAL and collecting duct (Fig. 2). EP_1 and EP_3 receptors may contribute to the natriuretic and diuretic action of PGE_2. In contrast, intrarenal EP_4 receptors may affect glomerular function as well as activate cAMP-stimulated salt and water absorption along the nephron. Finally, EP receptors also appear to play an important role in regulating renin release. Together with the other prostanoid receptors including TP, IP, and FP receptors, the EP receptors provide novel targets for modulating renal salt and water excretion as well as systemic blood pressure. It seems likely the present limited clinical utility of prostaglandin analogs will be transformed by the availability of truly selective receptor antagonists.

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