Prostacyclin signaling in the kidney: implications for health and disease

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Nasrallah, Rania, and Richard L. Hébert. Prostacyclin signaling in the kidney: implications for health and disease. Am J Physiol Renal Physiol 289: F235–F246, 2005; doi:10.1152/ajprenal.00454.2004.—The balance between vasodilator and vasoconstrictor pathways is key to the maintenance of homeostasis and the outcome of disease. In the kidney, prostaglandins (PGs) uphold this balance and regulate renal function: hemodynamics, renin secretion, growth responses, tubular transport processes, and cell fate. With the advent of cyclooxygenase (COX)-2-selective inhibitors, targeted deletions in mice (COX knockouts, PG receptor knockouts), and the discovery of intracrine signaling options for PGs (peroxisome proliferator-activated receptors and perinuclear PGE2 receptors: EP1,3,4), many advances have been made in the study of arachidonic acid metabolites. Although prostacyclin (PGI2) is a major product of the COX pathway, there is very little emphasis on its importance to the kidney. This review will discuss PGI2 biology and its relevance to different aspects of renal disease (growth, fibrosis, apoptosis), highlighting the most significant research from the past decade of PGI2 literature, what we have learned from other organ systems, while stressing the significance of cross talk between various PGI2 signaling pathways and its implications for renal health and disease.

prostaglandin I2; IP receptors; peroxisome proliferator-activated receptor-δ

PGI2 SYNTHESIS AND BIOLOGY

PROSTACYCLIN (PGI2) WAS FIRST isolated by Vane and co-workers (8) and has been implicated in many biological processes. Initially, it was most recognized for its potent vasodilatory effects and ability to inhibit aggregation of circulating platelets. Nowadays, it is quite evident that not only does it play a key role in the vasculature but it also contributes to the maintenance of homeostatic functions of many organ systems and the pathogenesis of certain diseases. In the brain, for instance, several studies confirm the role of PGI2 as a cytoprotective factor, preventing neuronal damage and even death under certain circumstances (108). It is also implicated in reproductive processes, whereby it regulates embryo implantation (76, 104). In addition, recent advances are considering its potential uses in the treatment/prevention of strokes and other cardiovascular diseases (13), pulmonary hypertension (129, 140), prevention of thrombosis, and the formation of vascular lesions (135). Interestingly, a recent study by Egan et al. (30) demonstrates a protective role for PGI2 in preventing atherogenesis in premenopausal female mice in response to estrogen. Finally, in the kidney it may be involved in ischemic renal disease (148) and chronic renal failure. There are now two recognized receptors that mediate the effects of PGI2, the cell-surface IP receptor and the nuclear peroxisome proliferator-activated receptor δ (PPARδ). Figure 1 illustrates the multiple signaling options for PGI2 in the kidney, regulating renal blood flow (RBF), glomerular filtration rate (GFR), renin secretion, glomerular and tubular growth, tubular transport processes, and cell fate.

PGI2 is one of five major products of the arachidonic acid cascade (see Fig. 2). Its production involves three steps: 1) release of arachidonic acid from membrane phospholipids by phospholipase A2; 2) catalysis of arachidonic acid by cyclooxygenases (COX-1, -2, -3) to an intermediate endoperoxide (PGH2); and 3) conversion of PGH2 to PGI2 by prostacyclin synthase (PGIS).

There are currently three known COX isoforms: COX-1, -2, and the recently identified splice variant of the COX-1 gene, COX-3 (10, 21). While COX-1 is constitutively expressed in most tissues, COX-2 is the inducible form (26). Thus it is widely believed that prostanoids (PGs) derived from COX-1 play a homeostatic role, whereas those from COX-2 would be harmful and thus could be therapeutically targeted in various diseases. However, renal studies contradict this classification, because in certain cells of the kidney COX-2 is constitutively expressed and not COX-1 (46). Various groups have investigated the expression profile of COX-2 throughout the kidney. Harris et al. (47) first showed constitutive COX-2 expression in the macula densa, juxtaplomerular apparatus, epithelial cells of the cortical thick ascending limb of Henle (cTAL), and medullary interstitial cells but no other structures [glomeruli, collecting duct (CD), arterioles]. On the other hand, a study by Ferguson et al. (32) indicates constitutive COX-2 expression in the mouse CD, which was previously not observed in the rat kidney (47). Other studies also suggest that renal localization differs somewhat among species. In a recent study of 53 normal human nephrectomy specimens, COX-2 was constitutively expressed in arterioles and glomeruli, in cTAL and macula densa, in vasa recta endothelial cells, and in CD (1). However, recent work in
Various biological processes and to the biosynthesis of PGI2. The significance of PGs to many biological functions is not so obvious in the renal system. Each enzyme produces a separate pool of PGs, and, depending on the cell type, the balance between cytoprotection and damage will determine the contribution to kidney disease.

The intermediate endoperoxides, produced by the action of COX, are acted on by PGIS to produce PGI2. Because it is a member of the cytochrome P-450 family, PGIS is also known as CYP8A (135). PGIS cDNA was cloned from different mouse and rat kidney did not detect COX-2 in distal tubules or CD (9).

While separate genes encode COX-1 and COX-2, COX-3 is a splice variant of the COX-1 gene isolated from canine brain that retains intron 1. Unlike COX-1 and COX-2, this isoenzyme is highly responsive to acetaminophen (10), hence the enthusiasm surrounding its discovery. It was also detected in human cerebral cortex and heart; however, insertion of intron 1 caused a frame shift, possibly resulting in a nonfunctional protein in humans (110). Therefore, the relevance of COX-3 to human pathophysiological processes remains questionable. The main idea is that COX-3 is probably more important in the termination phase of the inflammatory response (21), hence the weak anti-inflammatory effect of acetaminophen. If so, targeting COX-3 to enhance its function may prove to be a useful anti-inflammatory tool in various diseases. The identification of COX-3 is especially promising for PGI2 biology, as suggested by Warner and Mitchell (136), because acetaminophen has been shown to reduce circulating PGI2 levels independently of actions on COX-2. Because both COX-1 and -2 are constitutively expressed in renal tissue, but also induced in disease states, it will be interesting to see whether COX-3 is present in the kidney, whether any novel renal processes may be associated with this third isoform, and, furthermore, whether an antagonistic relationship exists between COX-3 and the other COX isofoms. Generation of a COX-3-deficient mouse in the future will surely shed light on its importance to various biological processes and to the biosynthesis of PGI2.

The significance of PGs to many biological functions is clearly demonstrated by the adverse effects, especially in the gut and kidneys (25, 105), associated with the use of nonsteroidal anti-inflammatory drugs (NSAIDS), i.e., COX inhibitors. Unfavorable renal effects of NSAIDS, for example, include abnormalities in water and sodium metabolism, acute renal failure, chronic kidney injury, to name a few. Due to their gastrointestinal and renal toxicity, a great deal of research has focused on unraveling the cell biology related to individual COX enzymes. The advent of specific COX-2 inhibitors in the past decade (101) has led to substantial progress in the area of COX/PG physiology. While there does not appear to be an advantage of these over nonselective NSAIDS in terms of nephrotoxicity and loss of renal function (K+ and Na+ retention), Celecoxib and other COX-2 inhibitors are widely used nowadays (106). In the cardiovascular system, a homeostatic balance between PGI2 and thromboxane A2 (TXA2) is crucial for the prevention of hypertension, stroke, atherosclerosis, myocardial infarctions, as well as other vascular diseases (22).

Numerous studies stress the role of COX-2 in maintaining the PGI2/TXA2 ratio (13, 22, 80), thereby cautioning against the use of selective COX-2 inhibitors in susceptible individuals. Similarly, in the renal system more pronounced complications, especially in the elderly and in patients with preexisting renal disease, seem to be associated with high doses of the COX-2 inhibitor Celecoxib (37, 137). In a recent study, it was shown that COX-2-specific inhibitors play a role in the development of renal papillary necrosis (2). In addition, several groups have clearly established the importance of COX-2 for the survival of renal medullary interstitial cells (42, 103). Clearly, the idea that COX-1 produces PGs that are important for normal function, and that COX-2-derived products play a pathological role, is not so obvious in the renal system. Each enzyme produces a separate pool of PGs, and, depending on the cell type, the balance between cytoprotection and damage will determine the contribution to kidney disease.

Membrane Phospholipids

1-Phospholipase A2

CH₃(CH₂)₄(CH=CHCH₂)₄(CH₂)₂CO₂H

Arachidonic Acid

NSAIDS

COX

POX

3-Prostacyclin Synthase (PGIS)

Prostaglandin H₂

Prostacyclin (PGI₂)

Fig. 2. Synthesis of PGI2 from arachidonic acid. Biosynthesis of PGI2 involves 3 steps: 1) hormonal activation of phospholipase A2 to release arachidonic acid from phospholipids; 2) oxidation (COX activity) and reduction [peroxidase (POX) activity] of arachidonic acid by COX-1, -2, and -3 to generate an intermediate endoperoxide, PGIH₂; and 3) conversion of PGIH₂ to prostacyclin (PGI₂) by PGIS. Nonsteroidal anti-inflammatory drugs (NSAIDS) inhibit COX activity and PGI2 synthesis.
species, and in the rat it contains a 1,503-bp open reading frame encoding a protein of ~500 amino acids (126). Several studies have examined the contribution of PGIS to specific physiological processes, including formation of corpora lutea (64) and regulation of ductus arteriosus patency by inhibition of smooth muscle cell growth (23). Also, disturbances in PGIS have been implicated in different pathomechanisms such as arterial restenosis (92) and myocardial infarction (85). Cotransfection of the PGIS gene with vascular endothelial growth factor in a mouse hindlimb ischemia model was effective in improving therapeutic angiogenesis (58), and gene transfer of PGIS was beneficial in the attenuation of pulmonary hypertension in rats (119). Similarly, overexpression of PGIS decreases lung tumor incidence and multiplicity (65). Renal PGIS expression is lower than PGIS expression in other tissues, but significant expression of PGIS mRNA was noted in the inner medullary tubules and medullary interstitial cells by in situ hybridization (126). In contrast, Vitzthum et al. (134) examined mRNA expression of different prostaglandin synthases in microdissected rat nephron segments by RT-PCR, indicating that PGDS (PGD2) is found in the proximal convoluted tubule, TAL, distal convoluted tubule, cortical CD (CCD), and outer medullary collecting duct; PGES (PGE2) is restricted to the entire CD; TXA synthase (TXA2) was found only in glomeruli; however, PGIS mRNA was present in the whole kidney but not in any nephron segment examined. Yet, PGJ2 synthesis along the nephron is quite significant (31, 50, 66, 146) despite the lack of PGJ2 detection throughout the nephron. The highest regions are the glomeruli and the inner medulla, especially the inner medullary collecting duct (IMCD). However, the extent of the contribution of these regions to the overall renal PGJ2 synthesis varies among species. For example, in humans, the majority of PGJ2 is produced by the glomeruli, but in rodents the inner medulla prevails (5). Tomida et al. (125) showed that renal PGJ2 levels were altered in 5/6-nephrectomized rats. Levels were initially decreased in the cTAL, including macula densa at 1–2 wk postsurgery, but increased in these same cells as well as mesangial cells in response to a reduction of renal mass at 8 wk. Future studies will determine whether PGJ2 could serve as a useful therapeutic target in the prevention of renal disease progression.

In addition to PGIS, PGJ2 synthesis is also influenced by PGJ2-stimulating factor (PSF) in the vasculature. Yamauchi et al. (145) first cloned PSF from human diploid fibroblast cells, but only a few studies have examined the significance of this factor to PGJ2 biology, identifying possible roles in disease processes. For example, Umeda et al. (128) demonstrated increased PSF mRNA in human colon cancer sites and increased PSF protein in culture media of mucosal adenocarcinoma cells. On the other hand, a study by Hashimoto et al. (49) showed that PSF levels were diminished in vascular endothelial cells in response to PKC activation by lysophosphatidylcholine, a main product of low-density lipoprotein oxidation, providing a possible mechanism for PSF reductions associated with atherosclerosis in ischemic heart disease patients. Also, in patients with human immunodeficiency virus there appear to be diminished PSF levels, resulting in reduced arterial PGJ2, thereby altering hemostatic balance and increasing the susceptibility to cardiovascular complications like atheroembolism, infarction, and stroke (68). Similarly, PSF is also detected in the glomerular vasculature (97), regulating PGJ2 production by endothelial cells. This study also showed that PSF levels in the renal vasculature are diminished in streptozotocin (STZ)-diabetic rats, but the contribution to glomerular hemodynamic changes and the initial hyperfiltration seen in diabetes has not been established. Interestingly, Hata et al. (51) suggest that changes in PSF levels could account for biphasic (early drop followed by later increase) alterations in renal blood flow that are characteristic of diabetic retinopathy in STZ rats. How PSF contributes to overall PGJ2 levels in the kidney and PGJ2-mediated effects is not apparent at this time.

PGJ2 is very labile and is rapidly metabolized into a nearly inactive product, 6-keto-PGF1α. This rapid inactivation of PGJ2 has been a major limitation in the initial studies of PGJ2 biology, but several pharmacological analogs are now available, including cicaprost (CCP), which is a highly selective IP agonist, and iloprost (ILP), which is less selective owing to its potency for the EP receptors (140). Several other formulations are currently in use, including beraprost sodium, UT-15, and taprostene, highly recognized for their improved stability (17, 95). For instance, treprostinil infused intravenously has a half-life of 34 min compared with the 2-min half-life of PGJ2 and is considered for the treatment of pulmonary arteriolar hypertension (95). Despite their random use in various studies, new reports suggest that certain agonists can distinguish between the different IP receptors among species (14). Thus care must be taken in comparing data among species and the effects obtained from one agonist or another. Clark et al. (18) just reported the development of a series of selective, high-affinity IP receptor antagonists with analgesic properties in the rat, which will surely be useful in future studies once thoroughly characterized. Despite these drawbacks, PGJ2 analogs have proven to be assets in the various studies investigating the mechanisms of action of PGJ2 in a plethora of biological processes, and there have been many advances linking PGJ2 to certain pathological events. A few good reviews since the mid-1990s are available on PGJ2 and its cell surface IP receptor and/or nuclear target PPARδ (56, 113, 139, 140).

RENA L PG J2/IP RECEPTOR SYSTEM

PGJ2 elicits most of its cellular effects by binding to cell surface IP receptors (19, 87). The first IP receptor was cloned from mouse thymus in 1994 (86) and shortly thereafter from rat (107), human (4), and other species. The cloned cDNA in rats comprises a 1,248-bp open reading frame encoding a protein of 416 amino acids with a molecular mass of ~45 kDa (107). The mRNA is a 3.7-kb transcript detected by Northern blotting. The rat and mouse IP receptors are 94% homologous in their amino acid sequence, but IP has low homology with other prostanoid receptors. By Northern blot analysis and in situ hybridization in the mouse, IP mRNA was detected in the thymus, spleen, heart, lungs, and high levels in the vasculature and the brain (86). While IP was not detected in the mouse kidney by Northern blotting, it was localized by in situ hybridization in the vasculature and glomeruli, with faint expression in the IMCD (93). In contrast, we show a high IP expression in rat IMCD (89, 91).

There seems to be some controversy across most species with respect to IP localization in specific renal cells. For instance, high levels of IP mRNA are present in Tamm-Horsfall-positive tubules (mTAL) of the rat outer medulla (54),
but human studies indicate that both IP protein and mRNA are only found in non-mTAL tubules of the outer medulla (67). In addition, IP is present in human mesangial cells and podocytes (67) but only in rodent mesangial cells and not podocytes (3, 91, 93). In comparison, our group showed the presence of IP mRNA on rat tissue sections in a subset of cortical tubules, glomeruli (mainly clusters of cells located centrally, most likely mesangial cells), interstitial cells, and in the vasculature; in the tubular epithelial cells of the outer and inner medulla; and also in various tissue and cell preparations. The reasons for these discrepancies are not yet clear, however, several possibilities do arise: 1) technical differences depending on the probes used for detection; 2) actual physiological differences in function or role of IP receptors in specific tubule segments among species; 3) existence of IP subtypes in human or mice kidneys that are not detectable with probes against the “classic” IP; or 4) redundancies between receptor pathways such that in human or mice mTAL, for example, IP receptor function is taken over by another receptor like the PGE₂ receptor EP₄ subtype. A study by Yamashita et al. (144) reports the expression of IP receptor protein in the rat afferent arterioles and glomeruli, but no reference is made to localization in other parts of the rat nephron. We also provide some data that IP receptor protein is ubiquitously expressed in various segments/cells of the rat nephron: proximal tubule, mesangial cells, IMCD, inner medulla, and outer medulla, but not whole cortex (89). Overall, the expression pattern of IP receptors is consistent with the documented role of PGI₂ in the kidney, regulating renal and glomerular hemodynamics, renin secretion, as well as tubular transport processes. Our laboratory is the first to confirm IP receptor expression in proximal tubule cells (89), but the relevance to the function of this nephron segment in health and disease has not yet been explored. In a study by Paller and Manivel (100), both PGE₂ and PGI₂ had a protective role in primary cultures of proximal tubule epithelial cells that were subjected to hypoxia and reoxygenation; the mechanism of cytoprotection remains unclear.

Like other PG receptors, IP is a G protein-coupled receptor, thereby activating intracellular signaling pathways. The main signaling linked to PGI₂ binding to IP is the stimulation of adenylate cyclase via coupling to Gs protein, thereby increasing intracellular cAMP levels. To date, studies in our laboratory did not identify any IP receptor subtypes or splice variants that are homologous to the published IP cDNA in any of the kidney regions or tissue preparations examined (88, 89, 91). Nonetheless, various biochemical and functional studies in different tissues do suggest that they may exist. For example, functional studies in isolated, perfused rabbit CCD showed that PGE₂ and iloprost inhibit AVP-dependent water transport by activating two different receptors (55), possibly through an “IP₁” subtype of the IP receptor (consistent with the EP₃ receptor). Whereas in rat IMCD PGI₂ stimulates cAMP but no inhibitory response was obtained (132), the reverse occurred in rat mTAL. Both ILP and CCP inhibited AVP-dependent cAMP stimulation, but no stimulatory response with these compounds was achieved (54). Also, using different PGI₂ analogs (not CCP), conflicting binding affinities were observed in the rat central nervous system, suggesting that a different IP receptor subtype is located in specific regions of the brain such as the hippocampus (120). Furthermore, using two new central nervous system-specific IP ligands, 15R-TIC and 15-deoxy-TIC, studies indicate that an IP receptor subtype exists in the rostral region of the brain with different ligand specificity than the peripheral IP and that signaling via this subtype does not involve cAMP or calcium (108).

On the other hand, many studies indicate that IP receptors may be coupling to different G proteins and hence activating multiple signal transduction pathways (86, 130). For instance, Chu et al. (16) recently showed in Chinese hamster ovary cells that IP-receptor mediated activation of extracellular signal-regulated kinases (ERK) 1 and 2 occurs via a Gq/PKC-dependent process. Similarly, in HEK-293 cells, reports of mouse and human IP receptor switching coupling between Gi, Gq, and Gs protein were documented. However, this differential G protein-IP receptor coupling is dependent on PKA- and PKC-mediated events (81). Moreover, this variable signaling seems to be species (81) and cell type specific (15). Lefkowitz et al. (72) showed activation of phospholipase C in response to PGI₂ analogs in certain clones of HEK-293 cells but not in others. Furthermore, expression of the human IP cDNA into these same cells did not provide any Gi protein-coupled responses (81). Therefore, important differences are proposed between mouse and human IP in the mechanisms regulating the coupling to Gi and Gq protein and in their dependence on PKA and PKC.

Altogether, many regulatory mechanisms are implicated in the promiscuous coupling of IP receptors to different downstream signaling pathways. Hayes et al. (53) showed that human and mouse IP receptors contain conserved putative isoprenylation CAAX motifs in their COOH terminus and that isoprenylation does not influence ligand binding but is required for efficient coupling to adenyl cyclase and phospholipase C. O’Meara and Kinsella (96) also recently confirmed that farnesylation of IP is required for efficient intracellular signaling of this receptor. Another study by Smyth et al. (114) indicates that PKC-dependent phosphorylation of the human IP receptor is critical for homologous desensitization, uncoupling of receptor-G protein interactions, and subsequent internalization for degradation or recycling. In contrast, Sobolewski et al. (115) studied the mechanism of IP receptor desensitization in rat pulmonary artery smooth muscle cells. They showed that heterologous desensitization of CCP-cAMP (and to other G protein-coupled agonists like bradykinin) occurred within 6 h of CCP exposure and is mediated predominantly by a PKA-inhibitable isofrom of adenyl cyclase. Actually, the phosphorylation state of the IP receptor is an important determinant of G protein coupling. For instance, the switching mechanism reported for mouse IP receptors suggests that IP-adenyl cyclase activation induces PKA-dependent phosphorylation of the receptor on COOH-terminal tail serine 357, directing subsequent coupling to Gq and Gi proteins (70). In addition to phosphorylation, other posttranslational modifications such as NH₂-terminal glycosylation are also central elements in the regulation of IP receptor function in humans (114), and rats (107) and mice (107). Another interesting feature of the human IP receptor is that it can form dimers/oligomers in an agonist-independent fashion (36). A dimerization motif is located in the first transmembrane domain of the human IP receptor (113). The significance of dimerization to IP-mediated signaling has not been clarified but surely would account for some of the controversy with respect to PGI₂/IP effects in different cells or species differences observed. In a recent study by Wilson et al.
(138), a dimerization between IP and TP receptors has been demonstrated in HEK-293 cells, favoring cAMP generation linked to TP activation. They went on to suggest that this provides a mechanism by which IP receptors can limit TP-mediated effects.

Consistent with reports in the literature, our work indicates that the main signaling pathway linked to IP receptors in the kidney is the stimulation of cAMP. This was noted in mesangial cells, proximal tubule cells, as well as IMCD cells in response to both ILP and cccP. However, contrary to previous reports in isolated, perfused rabbit CCD (55, 90) or IP receptor-transfected cells (86), we did not observe any calcium signaling responses linked to IP receptors in either rat mesangial cells or IMCD. Only in freshly isolated IMCD did we observe an attenuation of AVP-stimulated cAMP in response to CcP, similar to previous work in rat mTAL in our laboratory (54) and in isolated, perfused rabbit CCD, where ILP inhibited AVP-dependent water flow in a manner independent of the EP3 receptor subtype (55). While much work is needed to characterize these mechanisms with respect to the rat IP receptor, the inhibitory cAMP response to PGI2 analogs reported in our laboratory (54, 55, 91) is the first indication that the IP-G protein switching mechanism described by Migglin and Kinsella (81) may also occur in nontransfected rabbit and rat renal epithelial cells because we could not detect an IP receptor subtype or splice variant in these cells. The fact that PGI2 activates multiple signaling pathways in renal cells diversifies its role in the kidney and highlights its significance in the renal system.

A PUTATIVE RENAL PGI2/PPAR6 SYSTEM

Until the cloning of PPAR over a decade ago, it was thought that biological activities of PGI2 were exclusively mediated by the cell-surface IP receptor. PPARs are a family of ligand-activated nuclear receptor transcription factors that heterodimerize with the retinoic acid receptor (RXR). On ligand activation, this complex binds to a peroxisome proliferator response element (PPRE) of specific target genes (78). Since the cloning of PPARα in 1990 (61), there has been an enormous body of research implicating these transcription factors in various cellular events: metabolism, differentiation, growth, apoptosis, tumorigenesis, etc. There are currently three cloned PPARs (α, γ, β/δ), all of which are expressed in different tissues at various levels. All three genes contain a DNA binding and a ligand binding domain, and each is responsive to a particular group of compounds, recently reviewed by Marx et al. (79). The fibrate receptor PPARα is highly expressed in metabolically active tissues: skeletal muscle, liver, heart, kidney, and brown adipose tissue. The glitazone receptor PPARγ is abundant in the brown adipose tissue, intestine, spleen, and is found in the kidney. Its endogenous ligand is prostaglandin J2, an active metabolite of prostaglandin D2. Finally, PPARβ/δ or NUC1 is the most ubiquitous form and is the intracrine target for PGI2.

Although PPARβ/δ is the most widely distributed member of this family, until recently its physiological functions in different organ systems have remained undefined. However, many studies have now clearly established its role in critical processes. Certain investigators have gained insight into the relevance of PPARδ using transgenic mice (73, 102). A recent report by Harman et al. (45) implicates mouse PPARδ deficiencies in promoting colon cancer growth, in contrast to a previously reported carcinogenic role of PPARδ activation. In addition, Cheng et al. (11) found a link between cre-loxP-mediated PPARδ deletion in cardiomyocytes and decreased fatty acid oxidation leading to heart disease. Another body of literature suggests that PPARδ may be an important target for preventing inflammation and atherosclerosis, acting as a regulatory switch for transcriptional repressors such as BCL-6, as well as regulating the expression of target inflammatory genes (71). In contrast, Luquet et al. (76) used a recombination approach to generate mice with muscle-specific PPARδ over-expression. These animals displayed enhanced oxidative metabolism in their muscle as well as reductions in adipose cell size, implicating PPARδ activation in the prevention of metabolic diseases such as obesity or type II diabetes. This newly recognized potential for targeting PPARδ is actually becoming quite popular, and a recent review by Guan (39) highlights the significance of the PPAR family to the renal complications of the metabolic syndrome, listing various studies implicating PPARδ in lipid metabolism, adipogenesis, insulin resistance, atherosclerosis, as well as others. To date, the literature on PPARδ in the kidney is quite scarce, but perhaps there is potential for alterations in renal metabolic properties.

A study by Guan et al. (41) investigated the distribution of different PPARs in the rabbit kidney, but PPARδ was only present at low levels, with no significant expression in the cortex or medullary CD. In contrast, Yang et al. (147) found that PPARδ mRNA was abundant in all microdissected nephron segments examined from Sprague-Dawley rats. Therefore, in rodent kidneys it appears to be the most abundant PPAR throughout the nephron (40). Although it was shown that PGI2 regulates gene expression by activating the PPARδ pathway (40, 57), very little is known about its role in the renal system. The extent to which these pathways interact with classic PG signaling mechanisms remains to be determined. For instance, Hatae et al. (52) studied the effects of PGI2/PPARδ on cell apoptosis, demonstrating that the PGI2/IP/cAMP pathway antagonizes the effects mediated by the PPARδ pathway in HEK-293 cells. In a study by Hao et al. (42, 43), it was demonstrated that COX-2-derived PGI2 activates downstream PPARδ to protect cultured medullary interstitial cells from hypertonicity-induced cell death. In unpublished studies, our work indicates high expression of PPARδ in a mouse IMCD cell line. Because both IP and PPARδ are abundant in the same cells, cross talk between cell-surface PGI2 receptors and their nuclear binding sites will influence the overall cell response. The extent to which these pathways interact with classic PG signaling mechanisms, at the cell surface, remains to be determined.

SIGNIFICANCE OF PGI2 TO RENAL FUNCTION: WHAT TARGETED DELETIONS IN MICE HAVE REVEALED

The idea that PGI2 more than other prostanooids is quite relevant for the maintenance of renal function stems from the fact that only two mouse knockouts in the COX/PG field, COX-2 (27, 83) and PGIS (148), display significant disturbances in the kidney. The described renal phenotype for these animals is remarkably similar, despite certain differences. Morham et al. (83) reported severe developmental defects in
the COX-2-deficient mice, as well as severe nephropathy: reduced number of glomeruli, immature glomeruli and tubules, tubular atrophy, and interstitial inflammation and fibrosis. Similarly, the gross morphological assessment by Yokoyama et al. (148) showed atrophy, surface irregularities, and cysts in PGIS knockouts. As well, PGIS deficiency induced renal fibrosis and vascular injury including arteriosclerosis and wall thickening in the kidney and aorta, consistent with the notable role of PGI2 in maintaining vascular homeostasis. Furthermore, fibrotic and necrotic lesions were distributed from the medulla to the cortex, along tubules and vessels, with increases in collagen IV. The fact that the renal phenotype closely resembles the one reported in the COX-2 knockout, and no major abnormalities in the COX-1 knockout (69), further confirms the preferential association between COX-2 and PGIS for PGI2 biosynthesis in various cells, dependent on a favored colocalization of COX-2 and PGIS within the cell as seen in the cardiovascular (13) and reproductive systems (74). This same association is reported in humans (6, 80), for instance, as well as in coexpression systems (127). Moreover, COX-2 inhibitors completely blocked PGI2 synthesis in endotoxin-exposed bovine smooth muscle cells, whereas only partial inhibition of PGE2 was obtained (109). A recent study by Egan et al. (30) also confirmed that PGI2 is derived from COX-2, mediating the atheroprotective effect of estrogen in premenopausal female mice.

In comparison, no major renal pathology was documented in mice with targeted deletions for any of the prostaglandin receptors. Murata et al. (84) produced a recombinant mouse lacking the IP receptor. As expected, this mouse displayed certain characteristic features, including an increased susceptibility to pain, due to a recognized interaction between PGI2 and the “serotonin” pathway in the brain. It was also more prone to thrombus formation, due to the potent antiaggregatory action of PGI2 on platelets, as well as enhanced inflammatory responses. However, these mice were normotensive and fertile and displayed no obvious renal pathology. On further characterization, Yahata et al. (143) reported that IP knockout mice exhibit salt-sensitive hypertension and enhanced renin release following water deprivation, although COX-2-derived PGI2 is recognized as a stimulator of renal renin secretion. The mechanisms underlying these observations need further investigation. However, in a recent study by Fujino et al. (33), the PGI2/IP system was shown to be an important regulator of renovascular hypertension and a critical mediator of renin release. In IP knockout mice, blood pressure, plasma renin activity, and renal renin mRNA were lower than in wild-type littermates in response to renal artery stenosis. Despite the abundance of IP receptors in the kidney, the mild renal phenotype in the IP knockout compared with the PGIS-deficient mice (148) suggests that PGI2 interactions in the kidney are a great deal more elaborate and PGI2 acting on IP-independent pathways, like activation of PPARδ or other signaling systems, may play a more dominant role in renal PGI2 biology. This hypothesis requires further investigation but surely will provide interesting insight into the interaction between these other pathways and the PGI2/IP system in renal disease.

**PGI2 and Renal Disease Processes**

Chronic renal diseases are characterized by a steady decline in renal function associated with detrimental changes in glomerular, vascular, and tubular compartments, leading to disruption of homeostatic processes: altered matrix composition and deposition, cellular reorganization, etc. PGI2 can potentially influence numerous pathomechanisms implicated in various aspects of renal disease, including renal hemodynamic changes, changes in GFR, oxidative stress, inflammatory processes, etc. A detailed discussion of all these possibilities is beyond the scope of this review. However, the past decade has focused on three key components common to most progressive renal diseases, i.e., altered growth responses, induction of fibrotic responses, increased cell death (apoptosis), and these three aspects of renal disease are highlighted below.

**PGI2 and Renal Growth**

Altered growth responses (proliferation and hypertrophy) contribute to changes in renal function characteristic of various nephropathies. A clear link between renal hypertrophy and changes in renal function has been established, suggesting that hypertension and hyperfiltration are associated with one another (122, 123), both dependent on the production of growth factors, cytokines, and inflammatory mediators, presenting a self-perpetuating cycle leading to disease progression. Furthermore, renal growth results in alterations in tubular transport, resulting in Na+ retention, for instance, as a manifestation of hyperfunction, which can lead to the development of hypertension, a common complication associated with diabetic nephropathy (28). Regulation of cell cycle progression is dependent on specific cell cycle regulatory proteins. For example, proliferation requires the activation of cyclin-dependent kinases (CDK) by cyclins for G1-phase progression. On the other hand, hypertrophy depends on G1-phase arrest, for instance CDK inhibitors like p27KIP1 (p27) and p21CIP1/WAF1 (p21) that disrupt CDK/cyclin complexes and inhibit cell proliferation (82). A review by Wolf and Ziyadeh (141) highlights the importance of many factors implicated in the regulation of diabetic renal cell growth including PKC, the renin-angiotensin system, p27 and/or p21, and transforming growth factor-β (TGF-β).

Different PGI2 analogs are effective regulators of growth responses in many cells. A great deal of emphasis has been placed on the regulation of vascular smooth muscle cell growth by PGI2 and its implications for cardiovascular disease. A proposed mechanism for these effects is the regulation of the cell cycle inhibitor p27, a cyclin-dependent kinase inhibitor that causes G1 phase arrest. PGI2 seems to alter proteins involved in p27 degradation (116), thereby inhibiting cell proliferation. This effect, however, seems to be cell type dependent and may vary depending on the PGI2 analog in question. For example, our work indicates that CCP decreases p27 levels in rat mesangial cells (88) with no change in thymidine incorporation, but Togawa et al. (124) did show an inhibitory effect of beraprost sodium on rat mesangial cell proliferation that is mediated by induction of mitogen-activated protein kinase phosphatase (MKP-1), which inhibits the MAPK pathway. Furthermore, in a murine carcinoma cell line using another PGI2 analog (carba-PGI2) no effect on cellular proliferation or thymidine incorporation was noted (7). Moreover, Clapp et al. (17) argue that CCP is a weaker antiproliferative agent than ILP or UT-15 for human pulmonary artery smooth muscle cells. Therefore, growth responses to PGI2 will depend on the species, cells, and pharmacological analog used.
It is of interest that recent reports suggest a more complicated role for p27 in the cell depending on compartmental localization, serving to regulate apoptotic events, cell transformation, and act as a transcriptional cofactor (20). Thus the regulation of p27 by PGI2 may be more significant for non-growth-related aspects of renal cell responses.

**PGI2 and the Fibrotic Response**

In the kidney, the amount of fibrosis is correlated with the extent of disease progression (75, 152, 153). It is quite clear that TGF-β is the main factor involved in the initiation and perseverance of renal fibrotic responses in many renal diseases (111, 141, 151, 152). Regardless of the initiating factor, renal fibrosis involves activation of tubular epithelial cells and infiltrating mononuclear cells, followed by increases in the number of matrix-secreting fibroblasts. Actually, the conversion of tubular epithelial cells to myofibroblasts, termed epithelial-to-mesenchymal transition, has become of interest in the study of renal fibrogenesis, as recently reviewed by Zhang et al. (152), playing a critical role in the pathogenesis of renal tubulointerstitial fibrosis (38). Zeisberg et al. (151) provide a comprehensive review of the various components of renal fibrosis stressing the role of TGF-β as a key profibrogenic stimulus in renal cells (29).

Several studies have identified a putative role for PGI2 in the fibrotic process in nonrenal tissue. For example, in cardiac fibrosis bradykinin modulates collagen expression by increasing PGI2 synthesis by cardiac fibroblasts, which, in turn, attenuates collagen gene expression (35). This has important implications for the therapeutic potential of angiotensin-converting enzyme inhibitors, which enhance bradykinin pathways. Stratton et al. (117) report a possible mechanism by which PGI2 inhibits the development of sustained fibrosis. They suggest that IP receptor-mediated activation of PKA results in the blocking of the Ras/MEK/ERK pathway, which is required for SMAD-mediated induction of connective tissue growth factor in fibroblasts, an important fibroproliferative factor downstream of TGF-β (152). A reverse relationship was recently described by Tan et al. (121) with the PPARγ system, suggesting that TGF-β/Smad3 in the late phase of wound healing decreases PPARγ expression, stimulated in the initial inflammatory phase. PGI2 could also prevent renal fibrosis via antioxidant means, a characteristic of PGI2 recently revealed by Egan and co-workers (30) in aortic smooth muscle cells. Because a prominent feature of the PGIS knockout mouse is the development of interstitial fibrosis, it is possible to postulate that activation of both IP/cAMP/PKA as well as PPARγ is involved in the renal fibrotic response, contributing to the conversion of epithelial cells to myofibroblasts, the regulation of fibrosis genes, or the synthesis of matrix proteins. In this regard, we reported a significant induction of matrix metalloproteinase (MMP)-2 in response to the PGI2 analog CCP in transformed rat mesangial cells as well as a reduction in the expression of fibronectin (88), and Cheng et al. (12) recently demonstrated that MMP-2 is both necessary and sufficient for the induction of epithelial-to-mesenchymal transition.

**PGI2 and Cell Fate**

Cell loss plays an important role in renal disease progression. Cell fate is determined by the balance between cell survival signals and death signals. There are two distinct forms of cell death, apoptotic and necrotic. Apoptosis is a highly conserved and widely recognized form of programmed cell death. In many instances, both apoptosis and necrosis occur simultaneously, and discriminating between the two can provide essential insights for therapeutic intervention in many diseases. Padanilam (99) provides an excellent review of the contribution of each of these death processes to renal events. While apoptotic cell loss plays an important physiological role in the immature kidney, it can also contribute to homeostasis in the adult kidney by coordinating cell death and mitosis to maintain cell number (98). Therefore, derangements in cell death may lead to excessive proliferation of cells and their abnormal accumulation. For example, mesangial hypercellularity, a characteristic of proliferative glomerulonephritis (149, 150), increased fibroblast number in renal fibrosis (63) or cyst formation in polycystic kidneys (44, 131, 142).

On the other hand, a depletion of renal cells can contribute to the pathogenesis of any chronic, progressive renal disease. Tubulointerstitial injury, characterized by interstitial fibrosis and tubular atrophy, is a major feature of most renal diseases, including diabetic nephropathy. For instance, in renal fibrosis, apoptosis contributes to progressive renal damage rather than maintaining homeostatic cell numbers. Evidence suggests that the accumulation of interstitial fibroblasts results from altered regulation of cell survival, with a low rate of fibroblastic apoptosis contributing to their accumulation. Interestingly, it has been shown that the injured kidney converts epithelial cells to fibroblasts (63) capable of secreting TNF-α and Fas ligand, cytokines that induce apoptosis. The increased apoptotic environment and abnormal extracellular matrix are detrimental to epithelial cell survival, leading to atrophy. Thus chronic renal atrophy and interstitial fibrosis are directly associated with one another (94), and activation of apoptotic pathways contributes to the extensive cell loss characteristic of progressive tubulointerstitial diseases.

Another primary feature of many progressive renal diseases, resulting in renal failure, is glomerulosclerosis. Sclerotic glomeruli are characterized by progressive expansion of the extracellular matrix, which replaces glomerular cells (mesangial, podocytes, endothelial). Interestingly, a form of apoptotic death, known as anoikis, is a death pathway that is triggered by a disruption of matrix-cell interactions (77, 118). It has recently been observed that mesangial cells are susceptible to matrix-induced apoptosis. Changes in matrix composition are seen, with accumulation of collagen type IV, fibronectin, and other proteins. Our work indicates that fibronectin levels are significantly diminished in response to activation of IP receptors by PGI2 in rat mesangial cells (88), and MMP-2, which is an important regulator of matrix turnover in the kidney, is increased, thus suggesting that targeting the PGI2/IP system could prove to be a useful tool in preventing the accumulation of matrix underlying progressive glomerular disease.

A role for PGI2 in regulating apoptotic responses in renal cells has been established by Hataei et al. (52) demonstrating that activation of PPARγ by ILP is proapoptotic, whereas the PGI2/IP/cAMP system is antiapoptotic in human embryonic kidney (HEK-293) cells. While the PGI2/PPARγ pathway was linked to apoptotic cell death in these cells, PGI2 derived from COX-2 in renal medullary interstitial cells promotes cell viability by activating PPARγ (42, 43). Similarly, Ishaque et al.
PGI₂ and Diabetic Nephropathy

Diabetic nephropathy, a leading cause of end-stage renal disease, comprises all three above-mentioned aspects of progressive renal disease, with increased renal growth, glomerular and tubulointerstitial fibrosis, as well as loss of renal cells (recently reviewed in Refs. 75 and 153). This form of renal disease is surely the most studied in terms of disturbances in PGI₂ biology in disease is surely the most studied in terms of disturbances in PGI₂ biology in disease. This imbalance results in a disturbance in matrix synthesis and/or degradation resulting in changes in matrix composition and enhanced matrix deposition. Therapeutically, targeting IP may rectify this balance and prevent matrix accumulation characteristic of progressive diabetic kidney disease.

(59) reports that PGI₂ derived from COX-2 plays an important antiapoptotic role against TNF-α-mediated apoptosis of glomerular mesangial cells characteristic of rat glomerular nephritis. Therefore, the pro- or antiapoptotic nature of the PGI₂ system is highly dependent on the cell context, as well as the specific contribution of IP receptors and/or PPARδ pathways in a given cell.

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CONCLUDING REMARKS

Although many years have been devoted to unraveling the role of PGI₂ in the kidney, very little has been accomplished with respect to its distinct contribution to various nephron functions in health and disease. The past decade of research has clearly established a significant role for the PGI₂/IP receptor system, a major renal prostanooid pathway, in altering glomerular as well as tubular cell signaling responses. Also, although there is ample evidence to link PGI₂ signaling to individual cell processes contributing to progressive kidney injury, including and its contribution to mesangial changes in diabetes. We observed that the PGI₂/IP system is attenuated in response to high glucose and to STZ-induced diabetes. Our group and others (60) consistently show a diminished response to PG-mediated cAMP response to elevated glucose in mesangial cells. While the exact mechanism for attenuation of the PGI₂/IP system in our cells has not been elucidated, the inhibition of IP-mediated signaling is partly due to changes at the receptor protein level (88). Although our work does not support a direct effect of PGI₂/cAMP/PKA on the fibronectin gene, and reveal whether fibronectin is a target gene in the PGI₂/PPARδ cascade, these are other options worth exploring. Altogether, PGI₂ and its analogs seem to serve a general function to prevent matrix accumulation regardless of the cell type, thereby protecting against the development of glomerulosclerosis. As depicted in Fig. 3, we propose that a defect in IP receptor-mediated signaling in the later stages of diabetes may underlie the changes in matrix seen in diabetic glomeruli, due to disruption of the balance between vasodilatory and vasoconstrictor pathways (thromboxane/TP receptor, PGE₂/EP receptor) and that specifically targeting IP may be a useful therapeutic strategy to rectify this balance and prevent matrix accumulation.

Fig. 3. Targeting a defect in PGI₂/IP may prevent matrix accumulation in diabetes. This schematic illustrates a defect in IP receptors in diabetes, which shifts the balance between vasodilator and constrictor prostaglandin pathways. This imbalance results in a disturbance in matrix synthesis and/or degradation resulting in changes in matrix composition and enhanced matrix deposition. Therapeutically, targeting IP may rectify this balance and prevent matrix accumulation characteristic of progressive diabetic kidney disease.

Fig. 4. PGI₂ may regulate growth, fibrotic, and apoptotic responses via IP and PPARδ pathways. The flow chart illustrates examples of the putative PGI₂ pathways and downstream effectors regulating growth (proliferation, hypertrophy, DNA synthesis), fibrotic [epithelial-to-mesenchymal transdifferentiation (EMT), matrix synthesis, and degradation], and apoptotic (pro vs. antiapoptotic signals) responses in cells, mediated by IP vs. PPARδ. These include ANG II; calcium and protein kinase C (Ca²⁺/PKC); cAMP and protein kinase A (PKA); connective tissue growth factor (CTGF); matrix metalloproteinases (MMP-2); mitogen-activated protein kinases (MAPK); nuclear factor-κB (NF-κB); p27 (cyclin-dependent kinase inhibitor); and transforming growth factor-β (TGF-β).
alterations in renal growth, fibrosis/sclerosis, as well as apoptosis, insufficient emphasis has been placed on deciphering these mechanisms and targeting any defects in PGI2 signaling pathways for reduction of these renal complications. Because PGI2 is an important vasodilatory renal prostaglandin, PGI2 signaling is essential to oppose vasoconstrictor pathways for maintenance of renal homeostasis. Although this review only covered three aspects of renal pathomechanisms, i.e., growth, fibrosis, and apoptosis, we cannot undermine the contribution of hemodynamic and vascular effects of PGI2 in the kidney (vascular tone, inflammation, oxidative stress, etc.), which undoubtedly influence the evolution of many progressive renal diseases. Figure 4 shows a flow chart summarizing the possible PGI2 pathways regulating growth, fibrotic, and apoptotic responses mediated by IP vs. PPARδ.

The renal role of PGI2 is complicated by the existence of an alternate signaling pathway for this prostaglandin that is perhaps more important for renal disease processes, such as the putative PGI2/PPARδ system in the kidney. This is highlighted by the fact that PGI2 is a major product of the COX-2 isoform and that both COX-2 and PGIS deficiencies in mice lead to severe nephropathy, yet targeted deletions of the cell surface target for PGI2, the IP receptor, has little effect on the healthy renal system. It is also quite disappointing that a PPARδ deficiency does not appear to mimic the nephropathy reported for PGIS or COX-2 deficiencies. To date, consistent with its ubiquitous distribution, insufficient emphasis has been placed on deciphering these mechanisms and targeting any defects in PGI2 signaling.

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