MINIREVIEW

Cyclic Nucleotide Phosphodiesterase (PDE) Inhibitors: Novel Therapeutic Agents for Progressive Renal Disease

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Cyclic nucleotides are recognized as critical mediators of many renal functions, including solute transport, regulation of vascular tone, proliferation of parenchymal cells, and inflammation. Although most studies have linked elevated cAMP levels to activation of protein kinase A, cAMP can also directly activate cyclic nucleotide gated ion channels and can signal through activation of GTP exchange factors. Cyclic AMP signaling is highly compartmentalized through plasma membrane localization of adenylyl cyclase and expression of scaffolding proteins that anchor protein kinase A to specific intracellular locations. Cyclic nucleotide levels are largely regulated through catabolic processes directed by phosphodiesterases (PDEs). The PDE superfamily is large and complex, with over 60 distinct isoforms that preferentially hydrolyze cAMP, cGMP, or both. PDEs contribute to compartmentalized cyclic nucleotide signaling. The unique cell- and tissue-specific distribution of PDEs has prompted the development of highly specific PDE inhibitors to treat a variety of inflammatory conditions. In experimental systems, PDE inhibitors have been employed to demonstrate functional compartmentalization of cyclic nucleotide signaling in the kidney. For example, mitogenesis in glomerular mesangial cells and normal tubular epithelial cells is negatively regulated by an intracellular pool of cAMP that is metabolized by PDE3, but not by other PDEs. In Madin-Darby canine kidney cells, an *in vitro* model of polycystic kidney disease, an intracellular pool of cAMP directed by PDE3 stimulates mitogenesis. In mesangial cells, an intracellular pool of cAMP directed by PDE4 inhibits reactive oxygen species and expression of the potent proinflammatory cytokine monocyte chemoattractant protein 1. An intracellular pool of cGMP directed by PDE5 regulates solute transport. PDE5 inhibitors ameliorate renal injury in a chronic renal disease model. In this overview, we highlight recent studies to define relationships between PDE expression and renal function and to provide evidence that PDE inhibitors may be effective agents in treating chronic renal disease. Exp Biol Med 232:38–51, 2007

Key words: cAMP; cGMP; phosphodiesterase; progressive renal disease

Introduction

cAMP and cGMP are critical intracellular second messengers involved in the transduction of a diverse array of growth factors and physiologic stimuli. Recent studies have indicated that cAMP and cGMP regulate a variety of signaling pathways involved in the development and progression of renal disease, including mitogenesis, inflammation, and extracellular matrix synthesis (1–5).

There are a wide variety of functionally distinct G-protein coupled receptors that regulate adenylyl cyclase, the enzyme responsible for cAMP generation (6, 7). Both receptor-associated and cytosolic guanylyl cyclases direct the synthesis of cGMP (8). Effector molecules in cyclic nucleotide signaling include cAMP-dependent protein kinase A (PKA), protein kinase G (PKG, involved in cGMP signaling), cAMP-regulated guanine nucleotide exchange factors (cAMP-GEF, or EPACs), and cyclic nucleotide gated channels (9, 10).

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There is considerable evidence that disparate cell functions may be mediated through functional compartmentalization of cyclic nucleotide signaling. G-protein coupled receptors and receptor-associated guanylyl cyclases are confined to discrete portions of the plasma membrane (9). PKA, which is activated by elevated cAMP levels, is anchored *via* A kinase anchoring proteins (AKAPs) (11–16). Peptides that disrupt AKAP-anchored PKA prevent phosphorylation of target proteins (13, 15, 17).

EPACs and cyclic nucleotide gated channels also demonstrate subcellular localization, leading to compartmentalized cAMP signaling (18, 19). In renal collecting duct cells, increased cAMP in response to calcitonin activates EPAC I in a PKA-independent manner (20).

In cardiac myocytes, local elevations of cAMP have been inferred by patch clamping studies (21) or by using a cyclic nucleotide gated ion channel as a probe for cAMP (18). Spatial compartmentalization of cAMP has been demonstrated in living cells by fluorescence resonance energy transfer, using PKA or EPAC as probes for cAMP (19, 22–24).

Catabolism of cyclic nucleotides is directed by phosphodiesterases (PDEs) (25–27). In most cells the capacity for cyclic nucleotide hydrolysis far exceeds the capacity for synthesis, indicating that intracellular cyclic nucleotide levels are largely directed by PDE activity (25).

The PDE superfamily is large and complex, containing 11 highly related and structurally related gene families (1, 26–30) and over 60 distinct isoforms (Table 1). Each of the PDE families contains one to four genes, and many genes generate multiple isoforms by alternative 5′ mRNA splicing and through the utilization of different transcription initiation sites (25). PDE family members hydrolyze exclusively cAMP (PDE4, PDE7, and PDE8), exclusively cGMP (PDE5, PDE6, and PDE9), or both cAMP and cGMP (PDE1, PDE2, PDE3, PDE10, and PDE11). Members of the PDE family differ in tissue distribution, inhibitor specificity, and in mode of regulation (2, 28, 31, 32). PDEs are regulated by intracellular cyclic nucleotide concentrations, phosphorylation, interaction with regulatory proteins, subcellular compartmentalization, and binding of Ca^{2+}/calmodulin, as well as by changes in gene expression (25, 33).

PDE activity is found not only in the cytosol but is also localized to the plasma membrane, endoplasmic reticulum, nuclear membranes, and the cytoskeleton (34, 35). Intracellular localization of PDEs contributes to compartmentalized cyclic nucleotide signaling (36). Inhibitors selected for specific PDE families have been employed to demonstrate that PDEs play a major role in compartmentalization of cyclic nucleotide signaling (37–45). For example, we have found that cAMP hydrolysis in glomerular mesangial cells is primarily directed by PDE3 and PDE4 (39). PDE3 (but not PDE4) directs an intracellular cAMP pool that inhibits mitogenesis, whereas a PDE4-linked cAMP pool regulates reactive oxygen species (ROS) generation and monocyte chemoattractant protein-1 (MCP-1) expression (4, 5). Studies showing differential effects of PDE3 and PDE4 in cAMP signaling have been confirmed in living cells through the use of spatially restricted probes for cAMP (37, 46) or by RNA silencing studies (47).

PDE inhibitors have been evaluated as relatively nontoxic agents to treat various forms of kidney disease in animal models. This review will briefly discuss some general characteristics of PDEs and will then focus on the role of PDE inhibitors in pathophysiology of the kidney and in prevention and treatment of renal disease.

General Structure and Properties of PDE Superfamily

The structural features of the PDE superfamily have been the subject of a number of recent reviews (25, 27, 33, 48, 49) and will not be discussed in detail here. Common features among PDE families include a highly conserved catalytic core near the carboxy-terminus, a paired regulatory region positioned immediately amino-terminal to the catalytic domain, and a unique amino-terminal region, which confers isoform specificity.

The highly conserved catalytic core (~270 amino acids) is positioned close to the carboxy-terminus of the PDE molecule and exhibits much more similarity in sequence among members of one individual PDE gene family (more than 80%) than between different PDE gene families (~25%–40% identities). The catalytic core contains a histidine-rich PDE signature sequence motif $[HD(X_2)H(X_4)N]$ and two consensus Zn^{2+} binding domains. Site-directed mutagenesis of these critical histidines abrogates activity of several PDE isoforms. The catalytic domain contains common structural elements involved in hydrolysis of the cyclic nucleotide 3′,5′-phosphodiester bond as well as family-specific determinants responsible for the differences in substrate affinities and inhibitor sensitivities of the different gene families. Within the PDE3 catalytic domain is a unique 44–amino acid insertion that is not found in the other PDE gene families (2, 28, 31, 32).

The paired regulatory regions contain a number of family-specific features, including Ca^{2+}/calmodulin binding regions (PDE1), cGMP binding sites (PDE2, PDE5, and PDE6), and paired upstream conserved region modules (PDE4) (50). Various isoforms of PDE4 contain one or two upstream conserved regions (UCR1 and UCR2) within the amino-terminal portion (49). Long forms of PDE4 contain UCR1 and UCR2, whereas short forms lack UCR1 and/or portions of UCR2. These regions appear to confer different regulatory properties of different PDE4 isoforms. The unique amino-terminal region contains membrane localization sequences and sites for interactions with other signaling pathways (25, 50–52).

The amino-terminal regulatory regions of PDE isoforms play a key role in intracellular targeting (1, 33, 49, 53). For example, the amino-terminal domain of PDE3 is necessary for targeting of PDE3 to the endoplasmic

Table 1. PDE Superfamily

PDE family	Gene products	Substrates	Selective inhibitors	Regulations	Experimental and clinical applications
PDE1	1A, 1B, 1C	cAMP/cGMP	Vinpocetine Nicardipine 8-MeOM-IBMX	Ca^{2+}/calmodulin PKA/PKG	Dementia, memory loss (90, 139)
PDE2	2A	cAMP/cGMP	EHNA IC933 Bay 60–7550	Stimulated by cGMP	Sepsis Acute respiratory distress syndrome (140) Memory loss (141)
PDE3	3A, 3B	cAMP	Lixazinone Cilostamide Milrinone Cilostazol OPC-33540 Dihydropyridazinone	Inhibited by cGMP Phosphorylated by PKB	Glomerulonephritis (102) Congestive heart failure (142–144) Intermittent claudication (145) Thrombosis (146) Pulmonary hypertension (147)
PDE4	4A, 4B, 4C, 4D	cAMP	Rolipram Ro 20–1724 Denbufylline Cilomilast Roflumilast SCH 351591 V11294A AWD 12–281 L-826,141	Phosphorylated by PKA Phosphorylated by ERK	Glomerulonephritis (102, 129) Asthma, COPD[a] (148–151) Bipolar depression (152, 153) Autoimmune encephalomyelitis (154) Organ transplantation (155–157)
PDE5	5A	cGMP	Sildenafil (Viagra) Zaprinast Dipyridamole Ariflo Vardenafil Tadalafil E4021 DMPPO	Binds cGMP Phosphorylated by PKA Phosphorylated by PKG	Chronic renal failure (138) Salt retention in nephritic syndrome (99, 130) Pulmonary hypertension (158, 159) Erectile dysfunction (160) Organ transplantation (161) Memory consolidation of object information (162)
PDE6	6A, 6B, 6C	cGMP	Zaprinast Dipyridamole Vardenafil Tadalafil E4021 DMPPO	Transducin-activated	—
PDE7	7A, 7B	cAMP	Dipyridamole BRL 50481 IC242 BMS-586353 Thiadiazoles	Rolipram-insensitive	—
PDE8	8A, 8B	cAMP	Dipyridamole	Rolipram-insensitive IBMX-insensitive	—
PDE9	9A	cGMP	Zaprinast SCH 51866	IBMX-insensitive	—
PDE10	10A	cAMP/cGMP	Dipyridamole Papaverine	Unknown	—
PDE11	11A	cAMP/cGMP	Tadalafil Zaprinast Dipyridamole	Unknown	—

[a] COPD, chronic obstructive pulmonary disease.

reticulum (54). The unique amino-terminal domain targets PDE4A4/5 to members of the SRC family of tyrosyl kinases (55), PDE4D5 to the scaffolding protein RACK1 (56, 57), PDE4D3 to myomegalin (58), and PDE4A5 to the immunophilin XAP2 (59). PDE4D3 bound to AKAPs can be activated by PKA-mediated phosphorylation, leading to local decreases in cAMP levels, limiting the extent of PKA activation (42, 60).

Structural diversity of PDE isoforms contributes to compartmentalization of cyclic nucleotide signaling. In

living cardiac myocytes, fluorescence resonance energy transfer imaging using an acceptor fluorophore fused to the regulatory and catalytic subunits of PKA and adenoviral expression of a cyclic nucleotide gated channel have been employed as dynamic cAMP probes to demonstrate that PDE4 enzymes regulate pools of cAMP that are distinct from those regulated by PDE3 (37, 46). In COS1 cells, co-immunoprecipitation studies have demonstrated that the PDE4 isoforms PDE4D3 and PDE4C2—but not other PDE4 isoforms—form a complex with the PKA regulatory subunit PKA-RII. Knockdown of PKA-RII, but not PKA-RI, by siRNA prevents PKA-mediated phosphorylation of PDE4C2 and PDE4D3. These studies indicate that PDE4C2 and PDE4D3 regulate activation of PKA-RII, which is tethered *via* AKAP450 to the perinuclear region (13). RNA silencing studies have also been employed to demonstrate that PDE4D5 specifically interacts with β-arrestin to decrease PKA-mediated phosphorylation of the β-2 adrenergic receptor (47).

Three-dimensional structures of the catalytic domains of PDE1, PDE3, PDE4, PDE5, and PDE9 have been recently reported (61, 62). High-resolution co-crystal structure of PDE4B, PDE4D, PDE5A, and PDE1B has led to the identification of an invariant glutamine residue that dictates substrate specificity for cAMP, cGMP, or both, in an orientation-specific fashion (63). The catalytic domain of PDE4 reveals a compact alpha helical structure consisting of 16 helices divided into three subdomains (49, 64). The catalytic site contains a metal binding pocket, a solvent-filled side pocket, and a pocket containing the purine selective glutamine and a hydrophobic clamp to facilitate nucleotide binding (64). This active site is highly conserved among PDE4 family members.

This structural information provides the basis for the development of more potent and selective PDE inhibitors. A scaffold-based drug discovery paradigm has been applied to PDE4 (65). In this process, a low-molecular-weight compound library is screened to identify low-affinity inhibitors. This is followed by high-throughput co-crystallography to identify compounds that exhibit a dominant binding mode and have appropriate sites for substitution. Such compounds serve as scaffolds for optimization. This design strategy has led to the development of compounds that are more than 4000 times more potent than many of the currently employed PDE4 inhibitors.

It has been demonstrated that there is cross talk between the cAMP and extracellular signal-regulated protein kinase (ERK) signaling pathways, which is mediated by members of the PDE4 family (66–69). Long isoforms of PDE4 are activated by PKA-mediated phosphorylation (33, 53). ERK inhibits the activity of all long PDE4 isoforms, with the exception of PDE4A, through phosphorylation of the catalytic unit (66, 67, 70–72). PDE4 inhibition leads to localized increases in cAMP levels, which lead to activation of PKA. PKA then phosphorylates PDE4, relieving it from ERK-mediated inhibition (66). Phosphorylation of PDE4 by PKA may also play a role in cAMP desensitization (73).

Studies to define the phenotype of mice bearing PDE4 subfamily–specific knockouts have clearly demonstrated that, in many cases, PDE isoforms do not have redundant functions. Mice bearing homozygous deletion of the PDE1B gene show increased locomotor activity in response to dopamine agonists and impaired spatial learning (74). Female PDE3A knockout mice are viable and ovulate normally but are completely infertile as a result of arrest of oogenesis at the germinal vesicle stage (75).

The development of PDE4 subfamily–specific knockouts has provided the mechanistic basis for using PDE4 inhibitors to treat a variety of inflammatory diseases (76–81). For example, lipopolysaccharide (LPS) increases transcription of the PDE4B gene (82, 83). Leukocytes isolated from PDE4B knockout mice display a 90% reduction in LPS-stimulated tumor necrosis factor (TNF) production (78). TNF production in response to LPS is essentially normal in macrophages isolated from PDE4D and PDEE4A knockout mice (84).

Respiratory bronchial smooth muscle contraction in response to cholinergic agonists is reduced in PDE4A and PDE4B knockout mice (76). PDE4D knockout mice have impaired fertility (79) and develop a progressive cardiomyopathy (85).

PDE Isozymes in Kidney

The existence of enzymatic activity in the kidney that hydrolyzes cAMP and later cGMP was first reported in the 1960s, when the cAMP system was surveyed in various organs and tissues that were well-known target tissues for various hormones (86, 87). Subsequently, properties of PDE from whole-kidney preparations were studied (88, 89). However, since specific PDE inhibitors were unavailable at that time, results from those studies provided limited information. The concept of a well-defined PDE superfamily was described in the late 1980s, and isozyme-selective PDE inhibitors were developed in the 1990s (90–92). These developments have made it possible to elucidate the function of PDE isozymes or isoforms in kidney tissues *in vivo* or in cultured renal cells *in vitro*.

PDE Isozymes in Nephron Segments. In the 1970s, Dr. Thomas Dousa's group demonstrated that in rat glomeruli, activities of cGMP-PDE are higher than activities of cAMP-PDE and that cGMP stimulates cAMP-PDE (93). Later studies using selective PDE inhibitors indicated the presence of high PDE2 activity, with lower activities of PDE3 and PDE4 in glomeruli isolated from rat kidney (94, 95). cGMP in rat glomeruli is hydrolyzed by PDE5 and to a lesser degree by PDE1 (94). The PDE profile in extracts of tubule suspensions isolated from cortex is substantially different from that of glomeruli. PDE4 has the highest activity, while the activities of PDE3 and PDE1 are much lower. cGMP in tubule is hydrolyzed equally by PDE1 and

PDE5 (94). In a murine model of nephrogenic diabetes insipidus, the same group established that increased PDE activity in collecting ducts plays a dominant role in the failure to elicit an increase in cAMP in response to stimulation by vasopressin (96). More recent studies using selective PDE3 and PDE4 inhibitors indicated the abnormally high activity of PDE4 as a key pathogenic factor in this model (97, 98).

Identification of PDE isozyme patterns in isolated nephron segments provides critical information for studies of pathophysiologic changes in cyclic nucleotide signal transduction systems in animal models. For example, in glomeruli isolated from nephrotic rats, increased cGMP-PDE activity and decreased cGMP accumulation can be corrected by the PDE5 inhibitor zaprinast, leading to restoration of volume expansion-induced natriuresis (99, 100).

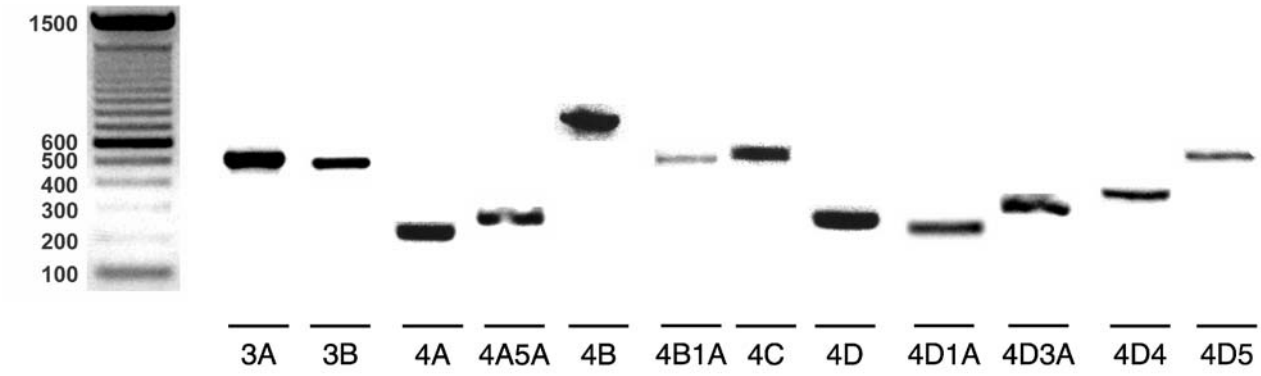
PDE Isozymes and Function in Cultured Renal Cells. Culture techniques have been employed to provide homogeneous populations of cells, which have enabled studies to define relationships between PDE isozyme profiles and cellular function. Although cultured cells may dedifferentiate or change phenotypes in the course of *in vitro* growth, cyclic nucleotide signaling and PDE isozymes appear to be well preserved in cultured cells. For example, a direct comparison of the cAMP signaling system in rat aortic smooth muscle cells in primary culture and in freshly dissected aortic tissue showed similar responses to PDE inhibitors, and responses of the cAMP signaling system differ only in time course and magnitude (101). In renal cells, *in vitro* studies to define PDE profiles and function have been verified in appropriate *in vivo* models of renal injury (94, 102).

Glomerular Mesangial Cells. Mesangial cells comprise approximately 25% of the cellular volume of glomerulus (103) and are often the focal point of pathobiologic processes involved in renal injury that results in glomerulonephritis (104). In our previous studies we demonstrated that in rat mesangial cells, cAMP is hydrolyzed by PDE4 and, to a lesser degree, by PDE3 (105). By reverse transcription–polymerase chain reaction we recently further determined the isoforms of PDE3 and PDE4 expressed in rat mesangial cells. Rat mesangial cells express both PDE3 genes (3A and 3B) and all four PDE4 genes (4A, 4B, 4C, and 4D) (4). Multiple PDE4 isoforms were characterized in mesangial cells, including PDE4A5A, PDE4B1A, PDE4D1A, PDE4D3A, PDE4D4, and PDE4D5 (Fig. 1). This information provides the basis for selecting and testing isoform-specific PDE inhibitors, which are more effective, yet allows researchers to do so with less systemic side effects in *in vitro* and *in vivo* studies. We have previously demonstrated that cGMP hydrolysis is directed by PDE1 and PDE5 in rat mesangial cells (105).

The development of isoform-specific PDE inhibitors has greatly facilitated studies to define the mechanisms through which intracellular cyclic nucleotides regulate a variety of critical cell functions (2). Using isoform-specific inhibitors, we previously demonstrated that rat mesangial cells possess functionally compartmentalized intracellular pools of cAMP that are differentially regulated by PDEs (Fig. 2); an intracellular cAMP pool hydrolyzed by PDE3 suppresses mitogenesis, and an intracellular cAMP pool hydrolyzed by PDE4 suppresses ROS generation (39). We recently extended our studies to define potential mechanisms underlying the differential effect of PDE3 and PDE4 inhibitors in the regulation of mitogenesis in mesangial cells. Although both PDE3 and PDE4 inhibitors activate PKA and modestly elevate cAMP levels to a similar extent, they have a differentiated effect on Raf-1 phosphorylation. PDE3, but not PDE4, inhibitors promote phosphorylation of Raf-1 on serine 43 and serine 259, sites that are associated with inhibition of RAF-1 activity. On the other hand, PDE3 inhibitors block phosphorylation of Raf-1 on serine 338, which is phosphorylated in association with Raf-1 activation. PDE4 inhibitors had no effect on serine 338 phosphorylation (106–109). PDE3, but not PDE4, inhibitors suppress Raf-1 kinase activity and ERK activation. The inhibitory effect of PDE3 inhibitors on ERK activity is abolished when constitutively active Ras, Raf, or MEK-1 constructs are expressed in mesangial cells. These studies provide evidence that MAPK signaling is regulated by functionally compartmentalized pools of cAMP in mesangial cells. The compartmentalization can be overcome by increasing the intracellular cAMP level with the potent adenylate cyclase agonist forskolin. We also found that the antiproliferative effect of PDE3 inhibitors was associated with decreased cyclin A and cyclin D levels, cyclin E activity, and increased expression of the cell cycle inhibitor p21. These studies indicate that in rat mesangial cells, PDE3 inhibitors suppress mitogenesis by inhibiting the Ras-Raf-MEK-ERK pathway. Additional targets of the PDE3-directed cAMP signaling include cyclin D, cyclin E, cyclin A, and the cell cycle inhibitor p21 (4).

Although nonselective PDE inhibitors such as IBMX have an antiproliferative effect on mesangial cells, a variety of systemic side effects limit their clinical use. In contrast, isoform-specific PDE inhibitors have been used to treat a variety of inflammatory and autoimmune diseases (2). Selective PDE inhibitors may provide therapeutic targets for modulating ERK activation in response to acute or chronic renal injury and may thereby arrest progression of a variety of chronic renal diseases.

Of the many inflammatory mediators produced following acute renal injury, MCP-1 has emerged as a critical mediator of glomerular and interstitial inflammation. Human and experimental studies have shown that MCP-1 expression correlates with interstitial infiltration of macrophages and T cells during chronic renal disease (110–114). In our recently published study (5), we demonstrated that in rat mesangial cells, transforming growth factor (TGF)-β1 induces MCP-1 expression in a time- and dose-dependent manner. The PDE4 inhibitor rolipram blocks TGF-β1–



PDE Isoforms	Accession Number	Sense Primer	Antisense primer	Fragment size
3A	U38179	CCGAATTCCCTTATCATAACAGAATCCACGCCACT	GGAAATTCGTGTTTCTTCAGGTCAGATGCC	508
3B	X22867	CCGAATTCTATCACAATCGTGTGCATGCCACAGA	CCGAATTCTTTGAGATCTGTAGCAAGGATTGC	499
4A	M26715	GCGGGACCT(AG)CTGAAGAAATTCC	CAGGGTG(AG)TCCACATCGTGG	233
4A5A	L27057	CTCCTGGCTGACCTGAAGACTATG	ACAATGTAGTCGATGAAGCCCACC	290
4B	AF202733	CAGCTCATGACCCAGATAAGTGG	GTCTGCACA(AG)TGTACCATGTTGCG	787
4B1A	J04563	AAACCTTCACGGAGCACCGAACAAGAGG	GCCACGTTGAAGATGTTAAGGCCCCATT	507
4C	XM214325	ACTGAGTCTGCGCAGGATGG	C(AC)TCCTCTTCCTCTG(CT)CTCCTC	539
4D	NM017032	CC(CT)(CT)TGACTGTTATCATGCACACC	GATC(CT)ACATCATGTATTGCACTGGC	262
4D1A	M25349	TCCGGTGAAGCGCTTAAGAACTGAGTCC	CCTGCTTGCCAGACCGACTCATTTCA	227
4D3A	U09457	CAGGTGGGCTTCATAGACTACATT	GCTCGTCAAGGGGAATTTCAGTGG	310
4D4	L20969	CAGACGTTTGGATGTGGACAATGG	GCTTTGTTGATGGATGGTTGGTTG	348
4D5	AF012073	GATGGGGAAGAAGAGGAAAGCCAG	ATTTCTGTGGCTCATCCTCCTCCT	543

Figure 1. PDE profile in rat mesangial cells. Total RNA was isolated from rat mesangial cells, treated with DNase I, and cDNA was obtained by reverse transcription. cDNA fragments of PDE3 and PDE4 isoforms were amplified by polymerase chain reaction using rat isoform-specific PDE3 and PDE4 primers.

stimulated MCP-1 expression. The inhibitory effect of rolipram is PKA dependent, and this effect occurs, at least in part, through a decrease in MCP-1 message stability. Although the Smad family of proteins has been identified as a key intracellular mediator of TGF-β signaling (115, 116), rolipram has no effect on activation of the Smad pathway by TGF-β1. TGF-β1–mediated induction of MCP-1 requires activation of ERK and p38, both of which are suppressed by rolipram. TGF-β1 stimulates ROS generation in mesangial cells. Rolipram blocks TGF-β1–stimulated ROS generation in mesangial cells. Both rolipram and ROS scavengers block TGF-β1–stimulated MCP-1 expression. These observations indicate that positive cross talk between TGF-β1 and MCP-1 signaling in mesangial cells may underlie the development of progressive renal disease. Rolipram, by preventing TGF-β1–stimulated MCP-1 expression, may offer a therapeutic approach in retarding the progression of renal disease (5).

Glomerular Epithelial Cells. The PDE isozyme profile in rat glomerular epithelial cells differs substantially from that of mesangial cells. We found that cAMP is hydrolyzed in glomerular epithelial cells primarily by PDE4, with little cAMP hydrolytic activity attributable to PDE3. No PDE1 or PDE2 and cGMP-hydrolyzing PDE activity were detected in glomerular epithelial cells (117).

Tubular Epithelial Cells. It is well recognized that cAMP is an essential signaling intermediate for solute transport in tubular epithelial cells. In inner medullary collecting duct (IMCD) cells isolated from rat kidney and grown in culture, PDE1, PDE3, and PDE4, but not PDE2 and PDE5, were detected. In these cells, arginine vasopressin (AVP) promotes a rapid increase in cAMP. The AVP-dependent cAMP pool appears to be metabolized primarily by PDE1. However, the PDE4 inhibitor rolipram also increases basal and AVP-stimulated cAMP accumulation. The levels of cAMP in IMCD cells, stimulated by atrial natriuretic peptide (ANP), were enhanced by PDE1 inhibitors (118).

Studies using PDE4 expression constructs have been employed to define the relative contributions of PDE1 and

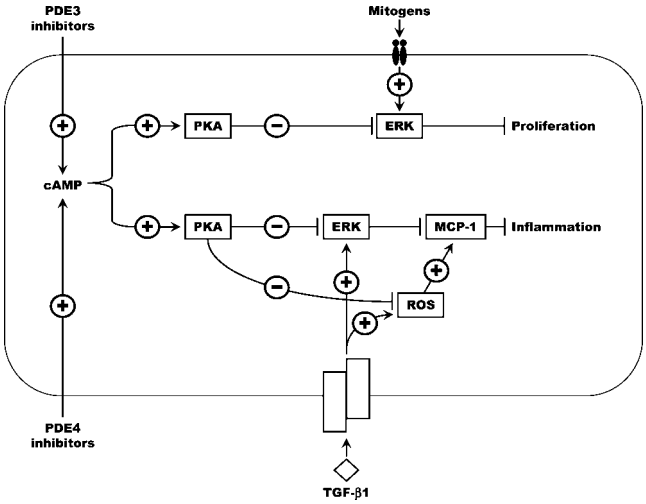


Figure 2. Scheme of signaling pathways underlying the differential effect of PDE3 and PDE4 inhibitors in regulation of proliferation and inflammation. Rat mesangial cells possess functionally compartmentalized intracellular cAMP pools that are differentially regulated by PDE3 and PDE4. An intracellular cAMP pool hydrolyzed by PDE3 suppresses rat mesangial cell proliferation by inhibiting the Ras-Raf-MEK-ERK pathway. An intracellular cAMP pool hydrolyzed by PDE4 suppresses TGF-β1–stimulated MCP-1 expression, thus suppressing rat mesangial cell inflammation. TGF-β1 stimulates MCP-1 expression through upregulating ERK activity and ROS generation. The anti-inflammatory effect of PDE4 inhibitors in rat mesangial cells is mechanistically related to inhibition of TGF-β1–stimulated ERK activation and TGF-β1–induced ROS generation.

PDE4 in regulation of AVP-stimulated cAMP levels. These studies employed LLC-PK1 cells, an established line of renal tubular cells that are frequently used for studies of cAMP signaling in renal epithelia. In wild-type LLC-PK1 cells, five PDE isozymes, PDE1 to PDE5, are expressed (119). In an LLC-PK1 cell line stably transfected with PDE4D1 (a short form of PDE4) (LLCPK1-S#16 cells), PDE4 activity was 5-fold to 7-fold higher than in sham-transfected controls. AVP or forskolin failed to stimulate cAMP accumulation. The increase of cAMP in response to AVP or forskolin was restored by the addition of the PDE4 inhibitor rolipram (120). In sham-transfected control cells, inhibition of PDE1 with the selective PDE1 inhibitor 8-Meo-IBMX enhanced AVP-stimulated cAMP accumulation, as did inhibition of PDE4 by rolipram. However, in LLCPK1-S#16 cells, only rolipram, but not 8-Meo-IBMX, restored the stimulatory effect of AVP (120). These findings indicate that both PDE1 and PDE4 are involved in the catabolism of the AVP-dependent cAMP pool; however, PDE1 inhibition cannot compensate for overexpressed PDE4, indicating that PDE4 is the key isozyme that controls AVP-dependent cAMP metabolism in LLC-PK1 cells.

Vasopressin-stimulated insertion of the aquaporin 2 (AQP2) water channel into the plasma membrane of kidney collecting duct principal cells is a key event in the urinary concentrating mechanism. It has been recognized that cAMP-mediated PKA activation is involved in vasopressin-receptor signaling, and a parallel cAMP-mediated signaling pathway also leads to AQP2 membrane insertion in AQP2-transfected LLC-PK1 (LLC-AQP2) cells and in outer medullary collecting duct principal cells *in situ* (123). AVP-stimulated solute transport is also regulated by cGMP. A recent study investigated the effect of the PDE5 inhibitor sildenafil on AQP2 membrane insertion in renal epithelial cells *in vitro* and *in vivo*. (121). Forty-five-minute exposure of the PDE5 inhibitors sildenafil citrate elevated intracellular cGMP levels and resulted in plasma membrane accumulation of AQP2. In addition, exposure to sildenafil for 60 mins induced apical accumulation of AQP2 in kidney medullary collecting duct principal cells both in tissue slices incubated *in vitro* as well as *in vivo* after intravenous injection of sildenafil into rats. These data indicate that AQP2 membrane insertion can be activated by a cGMP-mediated signaling pathway with cGMP PDE inhibitors, and pharmacologic activation of the vasopressin-independent cGMP signaling pathway may aid in the treatment of those forms of nephrogenic diabetes insipidus that are due to vasopressin-2 receptor dysfunction.

Madin-Darby Canine Kidney (MDCK) Cells. Polycystic kidney diseases (PKD) are characterized by excessive proliferation of renal tubular epithelial cells, development of fluid-filled cysts, and progressive renal insufficiency. It has been reported previously that cAMP inhibits proliferation of normal renal tubular epithelial cells but stimulates proliferation of renal tubular epithelial cells derived from patients with PKD. MDCK cells, a renal epithelial cell line widely used as an *in vitro* model of cystogenesis, also proliferate in response to cAMP (122–124). However, a potential role of PDE isozymes in cAMP-mediated MDCK cell proliferation has not previously been defined. As in several other renal cell types, cAMP hydrolysis in MDCK cells is directed primarily by PDE4 (85% of total activity) and PDE3 (15% of total activity). Although PDE4 inhibitors are more effective than PDE3 inhibitors in elevating intracellular cAMP levels in MDCK cells, only PDE3 inhibitors, but not PDE4 inhibitors, stimulate proliferation of MDCK cells. The proliferative effect of PDE3 inhibitors is associated with activation of the B-Raf-ERK signaling pathway, as well as with activation of the cell cycle regulatory proteins cyclin D and cyclin E. These results indicate that MDCK cell proliferation is regulated by a functionally compartmentalized intracellular cAMP pool directed by PDE3. We propose that therapeutic intervention directed toward augmentation of PDE3 activity may inhibit the aberrant proliferation of tubular epithelial cells and, thus, may retard the progression of PKD (125).

Interstitial Myofibroblasts. The presence of interstitial myofibroblasts has been universally recognized as a marker of progressive renal disease. In an *in vitro* study using myofibroblasts obtained from normal rat kidney, the nonspecific cAMP-PDE inhibitor pentoxifylline significantly suppressed serum-stimulated population growth and proliferation. Basal and TGF-β1–stimulated collagen secretion was decreased by pentoxifylline treatment. Pentoxifylline

may provide a valuable therapeutic strategy to reduce matrix production and/or deposition by myofibroblasts (126). More recently the same group of investigators sought to determine whether the functions of fibroblasts, isolated from a rat unilateral ureteral obstruction model, could be modified *in vitro* with dipyridamole, another PDE inhibitor. Dipyridamole significantly decreased procollagen alpha1(I) mRNA expression and total collagen secretion. Mitogenesis and cell-population growth was decreased by dipyridamole. Effects of dipyridamole on population growth were prevented by coincubation with a protein kinase G inhibitor peptide. Inhibitors of PKA (H-89) and protein kinase C (bisindolylmaleimide I) were without effect. These studies demonstrate that dipyridamole inhibits proliferation and matrix production by renal fibroblasts through a cGMP-PKG pathway (127).

Studies of PDE Inhibitors on Experimental Kidney Disease

Observations of the effect of PDE inhibitors on kidney cells *in vitro* provided the basis for studies to determine *in vivo* efficacy of PDE inhibitors in experimental renal disease models.

Glomerulonephritis. Based on *in vitro* observations that PDE3 inhibitors suppress proliferation, whereas PDE4 inhibitors suppress ROS generation in cultured mesangial cells, we determined whether administration of the PDE3 inhibitor lixazinone together with the PDE4 inhibitor rolipram could attenuate acute mesangial proliferative glomerulonephritis induced in rats by anti-rat thymocyte serum (ATS) injection. The cocktail of PDE3 and PDE4 inhibitors prevented the development of proteinuria, a major functional change in the acute phase of glomerulonephritis. Histologic and immunohistochemical examination of the kidneys showed marked suppression of pathologic changes in glomeruli of glomerulonephritis rats treated with the PDE inhibitors, reduced number of proliferating cells, less macrophage infiltration, and decreased myofibroblast transformation (102).

In the acute ATS glomerulonephritis model, other investigators have demonstrated that the nonselective cAMP-PDE inhibitor pentoxifylline reduced proteinuria, macrophage infiltration, mesangial cell proliferation, and sclerosis, compared with the results obtained for vehicle-treated nephritic rats. Pentoxifylline decreased MCP-1 and intercellular adhesion molecule-1 levels and glomerular expression of (I), α1 (III), and α1 (IV) collagen and fibronectin mRNA (128).

In a rat crescentic glomerulonephritis model induced by injection of nephrotoxic serum, administration of rolipram had both a preventive and a therapeutic effect. In the preventive study, rolipram treatment was started 2.5 hrs before injection of nephrotoxic serum. Rolipram abrogated glomerular injury (significant reduction in albuminuria and fibrin deposition). In the delayed treatment, rolipram was started 4 days after injection of nephrotoxic serum. Rolipram reduced albuminuria, fibrin deposition, and crescent formation. The protective effect of rolipram was associated with the reduced expression of TNF-α in glomeruli and renal tubules (129).

Nephrotic Syndrome. Valentin *et al.* (100) investigated whether cGMP-mediated responses of glomeruli to atrial natriuretic peptide (ANP) may be impaired in experimental nephrotic syndrome induced in rats by Adriamycin injection, a condition marked by sodium retention. Circulating ANP levels and ANP binding to renal receptors were not diminished. However, when glomeruli from nephrotic kidneys were incubated *in vitro* with ANP, the accumulation of cGMP was decreased, and the addition of the selective cGMP-PDE inhibitor zaprinast restored ANP-dependent cGMP accumulation to a normal range (100). Similar resistance of the glomerular cGMP system to ANP, which was also corrected by zaprinast, was observed in rats with nephrotic syndrome that develops in Heymann nephritis, a model of human membranous nephropathy (130). These observations clearly show that resistance of nephritic glomeruli to ANP is a postreceptor defect due to high cGMP-PDE activity.

To determine whether the cAMP-PKA signaling pathway coupled to specific PDE isozymes modulates renal epithelial cell proliferation, we investigated the effect of selective PDE inhibitors on renal tubular epithelial cell proliferation induced in rats by injection of folic acid. In extracts of renal cortical tubules, cAMP was metabolized predominantly by PDE4; PDE3 activity was about three times lower. By Mib-1 immunostaining, we determined that the folic acid–induced proliferation of renal cortical tissue was limited to tubular epithelial cells. Administration of the PDE3 inhibitors cilostazol or cilostamide together with the PDE4 inhibitor rolipram blocked folic acid–stimulated proliferation. Folic acid induced proliferating cell nuclear antigen (PCNA) expression by more than 10-fold; administration of the potent PDE3 inhibitor lixazinone or, to a lesser degree, cilostazol, suppressed PCNA expression; whereas rolipram alone had no effect. These results indicate that folic acid–stimulated proliferation of renal tubular epithelial cells is downregulated by activation of a cAMP-PKA signaling pathway linked to PDE3 isozymes (94).

Renal Ischemia. In a renal ischemia mouse model (131), continuous delivery of rolipram during reperfusion reduced renal injury in a dose-dependent manner. The protective effect of rolipram was associated with a significant reduction in inflammatory cell accumulation and oxidative burst. ATL-146e, an A_{2A}-adenosine receptor agonist, reduced renal ischemia-reperfusion injury when administered either before or immediately after the ischemic period (132). Because ATL-146e and rolipram act through different pathways to increase intracellular cAMP levels, combined treatment with both compounds would produce greater tissue protection than treatment with either compound alone. As predicted, co-administration of rolipram

and ATL-146e was more effective than administration of either agent alone in inhibiting neutrophil oxidative activity of human neutrophils and in inhibiting ischemia-reperfusion injury in mouse kidneys (131).

Acute Renal Failure. Endotoxin-induced acute renal failure has a prominent vascular component. Administration of the PDE4 inhibitor Ro 20–1724 prior to endotoxin injection prevented the decrease of renal blood flow, lowered renal vascular resistance, and prevented the decline of glomerular filtration rate (133). In rats with already-developed endotoxin-induced acute renal failure, the administration of Ro 20–1724 attenuated renal vasoconstriction, reduced the decline of renal blood flow and glomerular filtration rate, and improved overall survival rate (134). The mechanisms underlying the beneficial effect of Ro 20–1724 on endotoxemic acute renal failure have not been defined. The reported suppressive effect of PDE4 inhibitors on proinflammatory cytokine production and the effect of cAMP as a vasodilator of renal microvessels might be the basis of the observed protective effect of this PDE4 inhibitor.

cGMP also protects renal function in acute ischemic renal failure. The cGMP-PDE inhibitor zaprinast accelerated recovery from established acute renal failure induced by clamping of the renal arteries. Infusion of zaprinast dramatically improved postischemic glomerular filtration rate and increased cortical and outer medullary blood flow (135). The therapeutic effect of zaprinast was attributed to its unique ability to stimulate renal circulation, especially in the medulla, and to its role in vasorelaxation (136).

Chronic Renal Disease. Recent studies have shown that some PDE inhibitors could attenuate the progression of chronic renal damage in rats with remnant kidney. Previous studies have indicated that pentoxifylline attenuates experimental mesangial proliferative glomerulonephritis (128). Using the rat model of 5/6 subtotal nephrectomy, the same group of researchers recently reported that pentoxifylline treatment ameliorated proteinuria, glomerulosclerosis, interstitial inflammation, and fibrosis. This renoprotective effect of pentoxifylline was independent of changes in blood pressure, but was associated with reduced upregulation of MCP-1, reduced expression of various mitogenic and profibrogenic genes, including platelet-derived growth factor, fibroblast growth factor-2, TGF-β1, connective tissue growth factor, Types I and III collagen, and decreased numbers of interstitial myofibroblasts in the cortex of remnant kidney (137).

Another study using the same renal ablation model demonstrated that early treatment with the PDE5 inhibitor sildenafil retarded the progression of renal injury (138). The authors found that when they started therapy immediately after 5/6 subtotal nephrectomy, sildenafil prevented hypertension and deterioration of renal function; reduced histologic damage, inflammation, and apoptosis; delayed the onset of proteinuria; and preserved renal capillary integrity. However, delayed sildenafil treatment (treatment started 4 weeks after 5/6 nephrectomy) failed to improve proteinuria and glomerulosclerosis, but ameliorated hypertension and azotemia in cases involving established renal disease.

Conclusion and Perspectives

Although studies in recent years have revealed that PDE isozymes play an important role in the pathogenesis of renal failure of different origins and that PDE inhibitors could be effective in treating acute renal injury, there is very limited information regarding whether these agents are effective in preventing progressive renal disease. Unfortunately, many human renal diseases do not become clinically apparent until some element of chronic renal damage has been established. Therefore, more studies are needed to determine whether PDE inhibitors are effective therapeutic agents in treatment of human chronic renal disease and to establish the mechanisms by which these agents prevent or ameliorate chronic renal disease.

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