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Cyclic Nucleotide Phosphodiesterases: Molecular Regulation to Clinical Use

ANDREW T. BENDER AND JOSEPH A. BEAVO

Department of Pharmacology, University of Washington, Seattle, Washington

Address correspondence to: Dr. Joseph A. Beavo, Department of Pharmacology, University of Washington Medical School, Health Sciences Building, Box 357280, Seattle, WA 98195-7280. E-mail: beavo@u.washington.edu

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***Abstract*——Cyclic nucleotide phosphodiesterases (PDEs) are enzymes that regulate the cellular levels of the second messengers, cAMP and cGMP, by controlling their rates of degradation. There are 11 different PDE families, with each family typically having several different isoforms and splice variants. These unique PDEs differ in their three-dimensional structure, kinetic properties, modes of regulation, intracellular localization, cellular expression, and inhibitor sensitivities. Current data suggest that individual isozymes modulate distinct regulatory pathways in the cell. These properties therefore offer the opportunity for selectively targeting specific PDEs for treatment of specific disease states. The feasibility of these enzymes as drug targets is exemplified by the commercial and clinical successes of the erectile dysfunction drugs, sildenafil (Viagra), tadalafil (Cialis), and vardenafil (Levitra). PDE inhibitors are also currently available or in development for treatment of a variety of other pathological conditions. In this review the basic biochemical properties, cellular regulation, expression patterns, and physiological functions of the different PDE isoforms will be discussed. How these properties relate to the current and future development of PDE inhibitors as pharmacological agents is especially considered. PDEs hold great promise as drug targets and recent research advances make this an exciting time for the field of PDE research.**

I. Introduction

A. Definition of Phosphodiesterase Enzymes

The cyclic nucleotide phosphodiesterases (PDEs[1]) described in this review are a family of related phosphohydrolases that selectively catalyze the hydrolysis of the 3′ cyclic phosphate bonds of adenosine and/or guanosine 3′,5′ cyclic monophosphate. The structure of cAMP and the bond hydrolyzed is shown in Fig. 1. These enzymes are often referred to as class I cyclic nucleotide PDEs to differentiate them from class II enzymes. Class II enzymes are found in many species including mammals and will also catalyze the hydrolysis of the phosphodiester bond. However, in general, the Class II enzymes do not show the same substrate selectivity as the class I enzymes and much more is known about the class I enzymes.

B. Early Studies

Almost immediately after the discovery of cAMP by Sutherland and colleagues, cyclic nucleotide PDE activity was described (Butcher and Sutherland, 1962). With the subsequent discovery of cGMP, it was found that both cAMP and cGMP could be hydrolyzed by the same type of activity, i.e., hydrolysis of the 3′ cyclic phosphate bond. On the basis of substrate competition studies, it was clear that at least some of these activities must have the same catalytic site. In fact, many of the early studies on cyclic nucleotides were directed toward understanding PDE activity since at that time it was much easier to measure PDE activity than either cAMP or cGMP themselves or the enzymes that catalyzed their synthesis. With the advent of assays using radioactive substrate, it became clear that there were likely to be multiple forms of PDEs with different kinetic and regulatory properties (Thompson et al., 1979; Beavo et al., 1982). However, it was not until higher resolution fractionation techniques, monoclonal antibodies, and molecular cloning and sequencing procedures were applied to the PDEs that the truly large number of different gene products was fully appreciated.

[1] Abbreviations: PDE, phosphodiesterase; cAMP, adenosine 3′5′-cyclic monophosphate; cGMP, guanosine 3′5′-cyclic monophosphate; GAF, cyclic GMP, adenylyl cyclase, FhlA; CaM, calmodulin; PK, protein kinase; PKA, cAMP-dependent protein kinase; PKG, cGMP-dependent protein kinase; CNS, central nervous system; ANP, atrial natriuretic peptide; NO, nitric oxide; PDP, 9-(6-phenyl-2-oxohex-3-yl)-2-(3,4-dimethoxybenzyl)-purin-6-one; BAY 60-7750, 2-(3,4-dimethoxybenzyl)-7-[(1*R*)-1-hydroxyethyl]-4-phenylbutyl]-5-methylimidazo[5,1-*f*] [1,2,4]triazin-4(3*H*)-one; PI3K, phosphatidylinositol 3-kinase; IGF1, insulin-like growth factor 1; OPC-33450, 6-[3-[3-cyclooctyl-3-[(1*R**,2*R**)-2-hydroxycyclohexyl]ureido]-propoxy]-2(1*H*)-quinolinone; UCR, upstream conserved region; CRE, cAMP-responsive element-binding protein; RACK, receptor for activated C-kinase 1; AKAP, A-kinase anchoring proteins; ERK, extracellular signal-regulated kinase; BRL 50481, 3-(*N*,*N*-dimethylsulfonamido)-4-methyl-nitrobenzene; PAS, Period, Arnt, and Sim; IBMX, 3-isobutyl-1-methylxanthine; BAY 73-6691, 1-(2-chlorophenyl)-6-[(2*R*)-3,3,3-trifluoro-2-methylpropyl]-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidine-4-one.

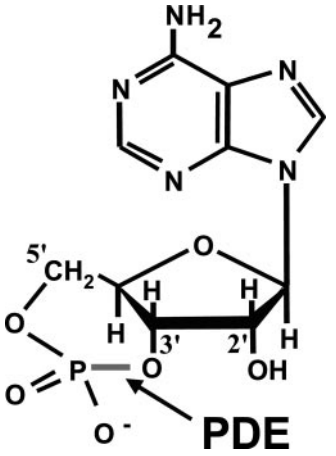

FIG. 1. Phosphodiesterases hydrolyze the 3′ cyclic phosphate bond.

C. Current Studies—Functional Pools of Cyclic Nucleotides Subserved by Specific Phosphodiesterases

The complexity of the cyclic nucleotide PDE system has forced increasingly more sophisticated and complex approaches to be adopted to understand the roles of PDEs in regulation of cAMP and cGMP in the cell. It is now very clear that any single cell type can express several different PDEs and also that the nature and localization of these PDEs is likely to be a major regulator of the local concentration of cAMP or cGMP in the cell. PDEs are regulated not only at the genetic level but also by diverse biochemical mechanisms including phosphorylation/dephosphorylation, allosteric binding of cGMP or cAMP, binding of Ca^{+2}/calmodulin, and various protein-protein interactions. The concept that a major role for many PDEs is to modulate the three-dimensional shape, the amplitude, and the temporal duration of "clouds" of cyclic nucleotide in the cell is developing. Some PDEs undoubtedly function just to keep this cloud from spreading to inappropriate areas of the cell. Others function to regulate local access to specific cAMP and cGMP receptors in the cell, which are often tethered to specific intracellular locales. It is also conceivable that not all PDEs function to control cyclic nucleotide hydrolysis but instead act as scaffolding proteins or use allosteric changes induced by binding of cyclic nucleotides to alter protein-protein interactions. In this case the hydrolytic step would then terminate the allosteric change. Although this latter concept is quite possible, no firm evidence for it has been demonstrated.

D. Why Phosphodiesterases Make Good Drug Targets

Immediately after the discovery of PDE activity, it was found that caffeine was an effective inhibitor of PDE activity and a number of nonselective PDE inhibitors including the caffeine analog, theophylline, have been in use as therapeutic agents for many years now. Thus, the principle that inhibition of PDE activity could be a valid therapeutic target is now well accepted. However, most

of the early PDE inhibitors had a very narrow therapeutic index, due at least in part to the fact that nearly all of the early inhibitors would inhibit most, if not all, PDE activity in every tissue.

One important general reason that PDEs have been pursued as therapeutic targets is related to the basic pharmacological principle that regulation of degradation of any ligand or second messenger can often make a more rapid and larger percentage change in concentration than comparable regulation of the rates of synthesis. This is true for either pharmacokinetic changes in drug levels or changes in amounts of an endogenous cellular regulatory molecule or metabolite. This intrinsic property is further enhanced if the machinery in the cell that alters degradation has an intrinsically higher V_{max} value than the machinery for synthesis. In this case, it has been known for over 40 years that almost all tissues contain at least an order of magnitude higher maximal PDE activity than cyclase activity for either cAMP or cGMP. This, of course, is not the whole story as it is unlikely that most PDEs operate under V_{max} substrate conditions in the cell. Nevertheless, this high potential activity is present, and the current idea is that in many compartments of the cell, substrate levels may be quite high.

It has been apparent for many years now that there is a rather extraordinarily large number of different forms of PDEs expressed in mammalian tissues, each of which can have a unique architecture at the active site. Moreover, there is increasing evidence that many of these PDEs are tightly connected to different physiological functions in the body and by inference also to different pathological conditions. Therefore, it has been widely believed that it should be possible to develop isoform selective inhibitors that can target specific functions and pathological conditions without a high likelihood of causing nonspecific side effects. The recent therapeutic and commercial success of agents such as sildenafil (Viagra), a selective PDE5 inhibitor, has validated the concept. These properties are discussed in more detail in later paragraphs.

Another reason PDEs are likely to be good drug targets relates to the concentrations of their substrates in the cell. It is commonly accepted that the levels of cAMP and cGMP in most cells are typically <1 to 10 μM. This means that a competitive inhibitor would not need to compete with very high levels of endogenous substrate to be effective. This fact has, for example, hindered the development of most protein kinase inhibitors, as they need to have high enough affinity to displace millimolar concentrations of ATP. At the same time, such an inhibitor must be selective among thousands of other enzymes that use ATP. However, the challenging development of protein kinase inhibitors is not impossible as selective inhibitors are beginning to appear. So the fact that PDEs are relatively unique in their substrate binding requirements and also that they use a substrate that is 100 to 1000 times lower than ATP makes them an intrinsically more attractive pharmacological target than many other enzymes that use more abundant substrates. In fact, as discussed later, recent evidence from many different companies suggests that it is relatively straightforward and feasible to identify and develop small molecules with substantially different affinities for most of the different PDE families.

E. Multiple Forms of Phosphodiesterases—Current Understanding

One of the most important reasons that the PDEs are recognized as being good drug targets is the fact that there are so many different isoforms. Only since the completion of the human and mouse genome projects has the extent of this enzyme diversity begun to be fully appreciated. Originally, PDEs were classified on the basis of their substrate specificity, modes of regulation, and elution order from DEAE ion exchange columns. As soon as primary amino acid and nucleotide sequences started to become available, they were further classified according to family relationships based on homologies in primary sequence.

Currently, it is widely accepted that there are 11 different families of PDE comprising 21 different gene products[2] (Tables 1–3; Fig. 2). However, there are many more than 21 mRNA and protein products transcribed from these genes because of the use of alternative transcriptional start sites and alternative splicing of mRNA precursor molecules. Current estimates are for >100 different mRNA products, most of which can be translated into different proteins. However, given the present difficulty of predicting transcriptional start sites and splice variants from primary genomic sequence data, it is still not known with any certainty for any species how many different PDE mRNAs are transcribed. Furthermore, it also is not yet clear whether all transcript variants are present in all species.

Given the complexity of the PDE family, the question often arises about the physiological relevance of multiple isoforms. Is this complexity just a manifestation of functional redundancy, or does it have regulatory significance in the organism? It is probably fair to say that most experts in the field are coming to the common viewpoint that although there may be some redundancy among isozymes, many, if not most, of the different PDE variants play specific physiological roles. That is, there is functional relevance to the

[2] Note that the number of functional enzymes in most species may not equal the number of gene products. For example, the major rod cell photoreceptor PDE6 exists as a heterodimer of two different gene products, PDE6A and PDE6B. The cone photoreceptor cell PDE6 consists of a homodimeric PDE6C. In some species, such as chickens, the rod enzyme may be a PDE6B homodimer. Thus, it is not clear whether each gene that contains a conserved catalytic sequence should be classified separately as a different PDE.

TABLE 1
PDE enzyme kinetic properties

Isoform	Substrate Specificity	K_m		V_{max} (purified)		References
		cGMP	cAMP	cGMP	cAMP	
		μM		*μmol/min/mg*		
PDE1A	cAMP<cGMP	2.6–3.5	72.7–124	50–300	70–450	Sharma et al. (1984); Hansen et al., (1988); Sonnenburg et al. (1995); Snyder et al. (1999)
PDE1B	cAMP<cGMP	1.2–5.9	10–24	30	10	Sharma and Wang, (1986); Bender et al. (2005)
PDE1C	cAMP=cGMP	0.6–2.2	0.3–1.1	—	—	Loughney et al. (1996); Yan et al. (1996)
PDE2A	cAMP=cGMP	10	30	123	120	Martins et al. (1982); Rosman et al. (1997)
PDE3A	cAMP>cGMP	0.02–0.15	0.18	0.34	3.0–6	Grant and Colman (1984); Harrison et al. (1986)
PDE3B	cAMP>cGMP	0.28	0.38	2.0	8.5	Degerman et al. (1987)
PDE4A	cAMP>cGMP	—	2.9–10	—	0.58	Wang et al. (1997); Salanova et al. (1998); Rena et al. (2001); Wallace et al. (2005)
PDE4B	cAMP>cGMP	—	1.5–4.7	—	0.13	Huston et al. (1997); Wang et al. (1997); Salanova et al., 1998
PDE4C	cAMP>cGMP	—	1.7	—	0.31	Wang et al. (1997)
PDE4D	cAMP>cGMP	—	1.2–5.9	—	0.03–1.56	Wang et al. (1997); Salanova et al. (1998)
PDE5A	cGMP>cAMP	2.9–6.2	290	1.3	1.0	Loughney et al. (1998); Lin et al. (2000); Zoraghi et al. (2006)
PDE6A/B	cGMP>cAMP	15	700	2300	—	Gillespie and Beavo (1988)
PDE6C	cGMP>cAMP	17	610	1400	—	Gillespie and Beavo (1988)
PDE7A	cAMP>cGMP	—	0.1–0.2	—	—	Michaeli et al. (1993); Han et al. (1997)
PDE7B	cAMP>cGMP	—	0.03–0.07	—	—	Hetman et al. (2000); Sasaki et al. (2000, 2002)
PDE8A	cAMP>cGMP	—	0.06	—	—	Fisher et al. (1998)
PDE8B	cAMP>cGMP	—	0.10	—	—	Gamanuma et al. (2003)
PDE9A	cGMP>cAMP	0.70–0.17	230	—	—	Fisher et al. (1998); Soderling et al. (1998b)
PDE10A	cAMP<cGMP	13–14	0.22–1.1	—	—	Fujishige et al., (1999a); Kotera et al. (1999b)
PDE11A	cAMP=cGMP	0.95–2.1	2.0–3.2	—	—	Fawcett et al. (2000); Yuasa et al. (2001b)

—, no reliable information available.

different mRNA and protein sequences that are transcribed, and there is functional relevance to the use of alternative start sites having different regulatory promoter regions. In the sections to follow we will try to emphasize cases for which such examples have already been shown or are strongly implicated as well as point out many other cases for which specific functions are likely, but firm supporting data have not yet been obtained.

F. Nomenclature

Given the large number of different PDEs present in mammals, it is not surprising that there have been and probably will continue to be issues of nomenclature. Several years ago a number of investigators in the field arrived at a consensus system that has largely been followed by most authors since then (Beavo et al., 1994). A typical name would be as shown for the following example: HsPDE1A2. The Hs signifies the species of origin, *Homo sapiens*; PDE denotes a 3′,5′ cyclic nucleotide phosphodiesterase; the Arabic numeral 1 signifies that it is a member of the *PDE1* gene family; the capital A signifies it is the A gene (often but not always the first member of the family reported in GenBank[3]); and finally the number 2 signifies that it is the second variant reported in the databases. Recently, a slightly modified version of this nomenclature has been promoted largely by the mouse genomic community (http://www.informatics.jax.org/mgihome/nomen/gene.shtml). In this nomenclature, the same PDE would be written mostly in lower case letters in italics as *Pde1a_v2* when referring to the nucleotide and in nonitalicized capital letters as PDE1A_V2 when referring to the protein. The letter V or v stands for variant. Given the similarity in concept, it would seem that either system should be acceptable and in fact presently both are used, depending on journal preferences.

Regardless of the method used, a problem that is still apparent in either system has been how to unambiguously assign what designation should be used to identify any new variant (i.e., alternative start site variant or splice variant). In the original system it depended (in theory at least) on its order of appearance in GenBank. Unfortunately, it is not uncommon for a series of PDEs to be cloned and entered into GenBank by one investigator working on one species without knowing that another investigator working in another species has cloned, characterized, and started the deposition process of the same variant gene product. Therefore, the naming of splice or start variants has not always been consistent, particularly among species. In some ways this is inevitable and will require periodic updates of the nomenclature. The data in the tables in this review for each enzyme family are provided as an attempt to temporarily reconcile much of this discrepancy, but the reader is reminded that there will undoubtedly continue to be inconsistencies. For the foreseeable future, therefore, it is probably best for authors to refer to unique

[3] Whereas originally the A gene may have been the first reported and the B gene the second, it is often the case that the first PDE in the same family from another species reported in GenBank could have been the B gene. In such cases, the nomenclature defers to the sequence alignment for naming, not to the order of cloning or identification.

TABLE 2
Overview of PDE isoform localization

Isoform	Localization	
	Tissue/Cellular	Intracellular
PDE1A	Smooth muscle, heart, lung, brain, sperm; PDE1A1 in lung and heart, PDE1A2 in brain	Predominantly cytosolic
PDE1B	PDE1B1 in neurons, lymphocytes, and smooth muscle; PDE1B2 in macrophages and lymphocytes	Cytosolic
PDE1C	Brain, proliferating human smooth muscle, spermatids; PDE1C2 in olfactory epithelium	Cytosolic
PDE2A	Adrenal medulla, brain, heart, platelet, macrophage subsets, endothelial cell subsets; PDE2 is highly localized to unique neuronal populations and brain regions	PDE2A3 and PDE2A2 variants are membrane-bound, whereas PDE2A1 is cytosolic
PDE3A	Heart, vascular smooth muscle, platelets, oocyte, kidney; PDE3A variants have differential expression in cardiovascular tissues	Can be either membrane-associated or cytosolic, depending on the variant and the cell type it is expressed in
PDE3B	Vascular smooth muscle, adipocytes, hepatocytes, kidney, β cells, developing sperm, T lymphocytes, macrophages	Predominantly membrane-associated; Localized to endoplasmic reticulum and microsomal fractions
PDE4A	Widely expressed with mRNA found in many tissues; olfactory system, immune cells, and testis; high levels of several of the variants are distributed throughout the brain	PDE4A5 is localized to membrane ruffles through its Src homology domain; the supershort variant PDE4A1 is entirely membrane-associated; PDE4A4 associates with Src family kinases; PDE4A5 is localized by AKAP binding; PDE4s can be recruited to interact with β -arrestin; PDE4A is recruited to a lipid raft fraction in activated T cells
PDE4B	High levels of mRNA detected in a variety of tissues; notable expression in immune cells and the brain	PDE4s can be recruited to interact with β -arrestin; is recruited to a lipid raft fraction in activated T cells
PDE4C	More restricted expression compared with other PDE4 isoforms; mRNA found in lung, testis, and several cell lines mainly of neuronal origin	Predominantly cytosolic; PDE4s can be recruited to interact with β -arrestin
PDE4D	mRNA widely distributed and found in a variety of tissues; protein levels high in the brain and in several other tissues as well; expression of variants seems to be localized to specific tissues and regions; variants are found in many commonly used cell lines (HEK293, COS) and in inflammatory cells	Depending on the identity of the variant, can be found in cytosolic or particulate fractions; PDE4D3 is localized by binding to mAKAP and AKAP450; PDE4D3 is a part of the cardiac RyR2 channel complex; PDE4D5 interacts with RACK1; can be recruited to interact with β -arrestin; is recruited to a lipid raft fraction in activated T cells
PDE5A	Platelets, vascular smooth muscle, brain, lung, heart, kidney, skeletal muscle; PDE5A1 and PDE5A2 are widely expressed, whereas PDE5A3 is specific to vascular smooth muscle	Cytosolic
PDE6A/PDE6B	High expression in rod cells of the photoreceptor layer of retina; also found in pineal gland	Targeted to the membrane by isoprenylation; association with the δ subunit results in cytosolic localization
PDE6C	High expression in cone cells of the photoreceptor layer of retina; also found in pineal gland	Cytosolic by virtue of its association with the δ subunit
PDE7A	Immune cells, heart, skeletal muscle, endothelial cells; PDE7A1 protein detected in a variety of immune cells, whereas PDE7A2 protein was found only in cardiac tissue	Cytosolic; localizes with AKAP MTG in lymphocytes
PDE7B	mRNA found in brain, heart, liver, skeletal muscle, pancreas, testis; in rat, PDE7B3 expression was restricted to the heart, whereas PDE7B2 was only found in testis; PDE7B1 is expressed in multiple tissues	Cytosolic
PDE8A	mRNA found in many tissues but highest in testis, spleen, small intestine, ovary, colon, and kidney; PDE8A2-5 is expressed in much lower abundance than PDE8A1; differential localization of PDE8A1 and PDE8A2 mRNA	Found in both cytosolic and particulate fractions
PDE8B	Brain and thyroid; variant expression is differential as PDE8B1 is expressed only in thyroid, whereas PDE8B3 is expressed equally in brain and thyroid	Found in both cytosolic and particulate fractions
PDE9A	mRNA for most variants has been detected in nearly every tissue tested with highest levels in kidney, brain, spleen, various gastrointestinal tissues, and prostate; variants expressed differentially	PDE9A5 protein has been shown to be cytosolic, whereas PDE9A1 is localized to the nucleus
PDE10A	Highest expression in brain, testis, heart, and thyroid; also reported in pituitary gland and in striated and cardiac muscle; PDE10A2 mRNA expression seems to be higher than that for PDE10A1 in most tissues	PDE10A1 and PDE10A3 variants are cytosolic, whereas PDE10A2 is particulate; PDE10A2 phosphorylation triggers translocation of the enzyme from the Golgi to the cytosol
PDE11A	mRNA found in skeletal muscle, prostate, testis, salivary gland, thyroid gland, and liver; PDE11A1 is most prominent in skeletal muscle; PDE11A3 is specific to testis; PDE11A4 is highest in prostate	Cytosolic

TABLE 3
PDE enzyme function(s)

PDE Family	Function(s)
PDE1	PDE1A probably serves to regulate vascular smooth muscle contraction and may play a role in sperm function; PDE1B is involved in dopaminergic signaling as well as immune cell activation and survival; PDE1C is required for vascular smooth muscle cell proliferation and may also regulate sperm function and neuronal signaling
PDE2	PDE2 frequently mediates cross-talk between cGMP and cAMP pathways; it regulates aldosterone secretion from the adrenal gland, cAMP and PKA phosphorylation of Ca^{+2} channels in the heart, cGMP in neurons, long-term memory, and barrier function of endothelial cells under inflammatory conditions
PDE3	PDE3A regulates cardiac contractility, platelet aggregation, vascular smooth muscle contraction, oocyte maturation, and regulation of renin release; PDE3B mediates insulin signaling, especially its antilipolytic effects; PDE3B also regulates cell cycle/proliferation and mediates the inhibitory effects of leptin and other signals on insulin secretion and renin release
PDE4	At least one form is expressed in most cells, and PDE4s play roles in a wide array of processes, including brain function, monocyte and macrophage activation, neutrophil infiltration, vascular smooth muscle proliferation, fertility, vasodilation, and cardiac contractility
PDE5	PDE5 is a well-documented regulator of vascular smooth muscle contraction, especially in penis and lung; it is involved in NO-cGMP signaling in platelets to control aggregation and may also play a role in regulation of cGMP signaling in the brain
PDE6	PDE6 is involved in signal transduction of the photoresponse in the eye; it may also regulate melatonin release from the pineal gland
PDE7	PDE7 is implicated to play a role in T-cell activation and activation of other inflammatory cells
PDE8	PDE8 may play a role in T cell activation, sperm, or leydig cell function
PDE9	The function of PDE9 is currently unknown, but it has been postulated to regulate NO-cGMP signaling in the brain
PDE10	PDE10A is thought to be a regulator of cGMP in the brain and may play a role in learning and memory
PDE11	PDE11 possibly has a role in sperm development and function

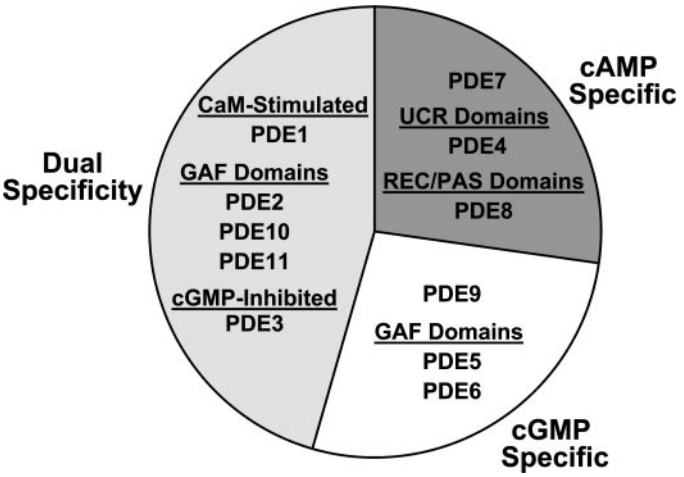

FIG. 2. PDE family classification. The 11 PDE families can be grouped into three categories based on their substrate specificity. They can also be classified by the mechanisms regulating their activity (i.e., calmodulin stimulation or cGMP inhibition) or their N-terminal regulatory domains such as the GAF domain-containing PDEs.

GenBank sequence identifiers when referring to a specific PDE isozyme at least once in their manuscripts. Given the nature of science and scientists, it is to be expected that inconsistencies such as this will continue to crop up as more variants are characterized in the coming years, and some method of reconciliation will need to be agreed upon and applied.

G. Crystal Structures

Within the last 4 years crystal structures for the catalytic domains from representative members of seven different PDE families have been solved. Several excellent reviews (Card et al., 2004; Zhang et al., 2004b; Jeon et al., 2005) have appeared describing the details of the structure-function relationships discovered from these studies so only a few of the highlights will be discussed here. The solved structures include those for PDE1B (Zhang et al., 2004b), PDE3B (Scapin et al., 2004), PDE4B (Xu et al., 2000, 2004; Card et al., 2004, 2005), PDE4D (Lee et al., 2002a; Huai et al., 2003a,b, 2004a; Card et al., 2004a, 2005), PDE5A (Sung et al., 2003; Card et al., 2004; Huai et al., 2004a; Zhang et al., 2004b), PDE7A (Wang et al., 2005), and PDE9A (Huai et al., 2004b). In addition, the structure of the regulatory tandem GAF domains of PDE2A has also been published (Martinez et al., 2002). However, no high-resolution structure for any PDE holoenzyme has been reported, so we know little about the molecular details of how the regulatory domains influence catalysis. Low-resolution structures determined by electron microscopy to ~29 Å resolution have been reported for PDE5A and PDE6 holoenzymes.

1. Catalytic Domain Structures. Remarkably, the overall folds and functional structural elements for each of the catalytic domains are very similar despite the fact that sequence from any one catalytic domain family exhibits only approximately 25 to 35% amino acid sequence identity with any other. Some important general lessons have been learned from the crystal structures of the catalytic domains. First, all of these catalytic domains contain three subdomains composed largely of 16 helices (Fig. 3A). The active site is formed at the junction of the helices by residues that are highly conserved among all the PDEs. At the bottom of the substrate binding pocket are two divalent metal binding sites. The metals, zinc or magnesium, are coordinated by residues located on each of the three different domains. The metal binding site that binds zinc has two histidine and two aspartic acid residues that are absolutely conserved among all PDEs studied to date. These residues form part of the signature recognition sequence for cyclic nucleotide PDEs, which is itself a subset of the larger HD

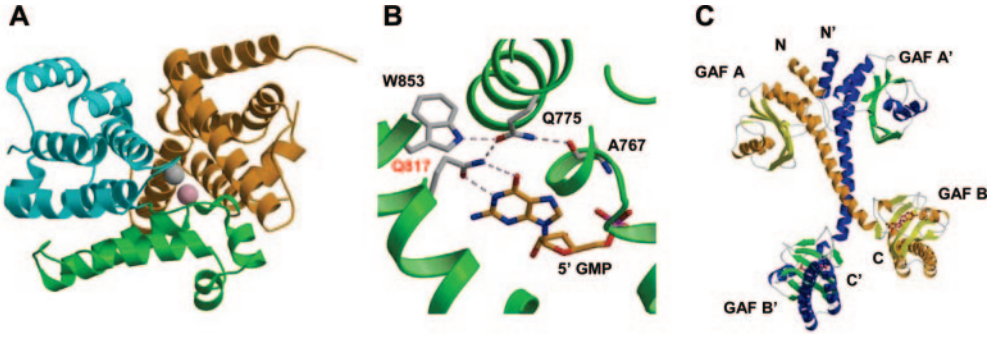

FIG. 3. PDE X-ray crystal structures. A, catalytic domain of PDE4B2D (PDB ID 1F0J) contains 17 α helices that are organized into three subdomains (Xu et al., 2000) that are colored blue, green, and brown in order from N to C terminus. The active site contains a zinc atom (gray) and a magnesium atom (violet). B, structure of the catalytic domain of human PDE5A (PDB 1T9S) shows Q817 using its amide side chain to hydrogen-bond to the guanine ring of the reaction product 5′-GMP (Zhang et al., 2004b). The authors hypothesize that steric constraints prevent the rotation of a glutamine side chain, thereby providing substrate selectivity for cGMP over cAMP. In PDE4A, the side chain is flipped, allowing selectivity for cAMP, and a different set of side chains prevents rotation into the other conformation. In the dual substrate PDEs, the glutamine is not fixed and is thought to be able to rotate freely and therefore bind either cAMP or cGMP. C, the structure of the GAF-AB dimer from mouse PDE2A (Martinez et al., 2002) is shown with one monomer colored with orange helices and yellow β strands, whereas the other has blue helices and green β strands. Loops are in gray. The bound cGMP in each GAF-B domain is in red. All figures were made using MOLSCRIPT (Kraulis, 1991).

(histidine/aspartate) domain structure that is found in a superfamily of enzymes with a predicted or known phosphohydrolase activity. These phosphohydrolase enzymes are all thought to be involved in nucleic acid metabolism, signal transduction, and possibly other functions in bacteria, archaea, and eukaryotes. As the highly conserved residues in the HD superfamily are histidines or aspartates, it is thought that they all can act to coordinate divalent cations and are therefore necessary for the activity of these proteins. This is certainly true for the phosphodiesterases. The HD domain is found in >3000 different proteins now in the SMART database (http://smart.embl-heidelberg.de/).

2. Glutamine Switch. One of the more interesting ideas to come from the structural studies is a proposal for the molecular mechanism of cyclic nucleotide specificity. In each of the PDEs for which a structure has been solved, there seems to be an invariant glutamine that stabilizes the binding of the purine ring in the binding pocket (Fig. 3B) (Zhang et al., 2004b). For appropriate hydrogen bonds to form to both cAMP and cGMP, this glutamine must be able to rotate freely. At the resolution now available, it seems that for all the PDEs that hydrolyze both cyclic nucleotides with relatively high affinity this free rotation is possible. For PDEs that are highly selective for cAMP at low substrate levels, this glutamine is constrained by neighboring residues into the favored orientation for cAMP binding. Conversely, for those PDEs that prefer cGMP, the glutamine is constrained into the other cGMP favoring position. One possible exception to this tenet has been noted recently for PDE5, which may have additional interactions that are important for specificity (Zoraghi et al., 2006). So, for those PDEs that hydrolyze both cAMP and cGMP, the glutamine is free to rotate, and this "glutamine switch" hypothesis seems to form the molecular basis for much of the substrate selectivity noted between different phosphodiesterases.

3. Regulatory Domain Structure. Only one structure for a regulatory domain of a class I PDE has appeared. This is the parallel tandem GAF domain of PDE2A (Fig. 3C) (Martinez et al., 2002). This PDE is "activated" by binding of cGMP to an allosteric binding site somewhere on the N-terminal half of the PDE. These binding domains are now known to be part of a much larger group of small molecule binding domains called GAF domains that are found in nearly all phyla. The acronym GAF originates from the first three such domains that were identified (mammalian c*G*MP binding PDEs, *Anabaena* *a*denylyl cyclases, and plant *F*hlA transcription factors). Several PDE family proteins other than PDE2 also contain N-terminal GAF domains. With the PDE2A GAF domain crystal structure we now know that the GAF-B and not the GAF-A domain contains bound cGMP. Interestingly, the signature NKxxFDxxE sequence found in all of the mammalian GAF domains is not actually in the cyclic nucleotide binding pocket but rather seems to be important for closing a helix across the mouth of the open pocket. Recently, the structure of a cAMP binding tandem GAF domain in the *Anabaena* adenylyl cyclase was also solved (Martinez et al., 2005). Whereas the structure of the mammalian PDE GAF domains is a parallel dimer, the *Anabaena* cyclase GAF domain is an antiparallel dimer. Despite this difference, it is known that the mammalian GAF domains can substitute for those in the *Anabaena* adenylyl cyclase (Kanacher et al., 2002). With these new structures, it may now be possible to begin to use structure-aided drug design techniques to target these allosteric regulatory nucleotide-binding sites on the five families of PDEs that contain them.

4. Inhibitor Specificity. Several of the crystal structures published have contained bound PDE inhibitors. A third particularly interesting general concept to appear from these structural studies is the idea that although PDE inhibitors bind at the active site, three different modes of binding were found to occur (Card et al., 2004;

Jeon et al., 2005). Crystal structures for PDE4B, PDE4D, and PDE5A with inhibitors revealed that the compounds interact with the enzymes either through hydrogen bonds with residues involved in nucleotide binding, through interactions with hydrophobic residues lining the active site channel, or with metal ions mediated through water (Card et al., 2004). This variety of mechanisms of inhibitor binding has encouraged medicinal chemists in their quest for increasingly selective drugs. Elucidation of more PDE structures in the absence or presence of inhibitors should facilitate the discovery of more potent and selective PDE inhibitors in the future.

II. Phosphodiesterase Families

A. Phosphodiesterase 1 Family

1. Overview. Calcium- and calmodulin-dependent phosphodiesterases, now known as PDE1s, were one of the first families to be identified (Cheung, 1970) and have been extensively studied. The name derives from both the fact this family had one of the first modes of regulation identified and also the fact that in several tissues members of this family elute from chromatographic separation columns as the first peak of PDE activity. Several recent reviews on PDE1s have appeared (Sonnenburg et al., 1998; Kakkar et al., 1999; Goraya and Cooper, 2005), and the reader is directed to these manuscripts for more detailed information than could be included in this review. The distinguishing feature of this PDE family is their regulation by Ca^{+2}/calmodulin (CaM). The binding of one Ca^{+2}/CaM complex per monomer to binding sites near the N terminus stimulates cyclic nucleotide hydrolysis. The three PDE1 isoforms, PDE1A, PDE1B, and PDE1C, are usually expressed in different cell types within a tissue or regions within a cell and therefore can help to differentially regulate a diverse number of cyclic nucleotide-dependent physiological processes in a calcium-dependent manner.

2. Biochemistry/Structure. The different PDE1 isoforms all have the same overall structural arrangement. As shown in Fig. 4, the PDE1s consist of two N-terminal CaM binding domains that span an inhibitory sequence. These domains are followed by a conserved C-terminal catalytic domain. Ca^{+2}/CaM stimulates activity by causing an increase in the V_{max} with little effect on the K_m (Kincaid et al., 1985). All PDE1 enzymes can hydrolyze both cAMP and cGMP, although the affinity for each nucleotide varies by isoform (Table 1). The PDE1As are highly specific for cGMP as substrate with a much lower K_m for cGMP (5 μM) than for cAMP (112 μM). However, at higher substrate levels, hydrolysis rates are much closer (the V_{max} for cAMP and cGMP are similar). The PDE1B enzymes also prefer cGMP (K_m 2.4 μM) to cAMP (K_m 24 μM) as substrate, whereas the PDE1Cs hydrolyze the two cyclic nucleotides equally well. The absolute

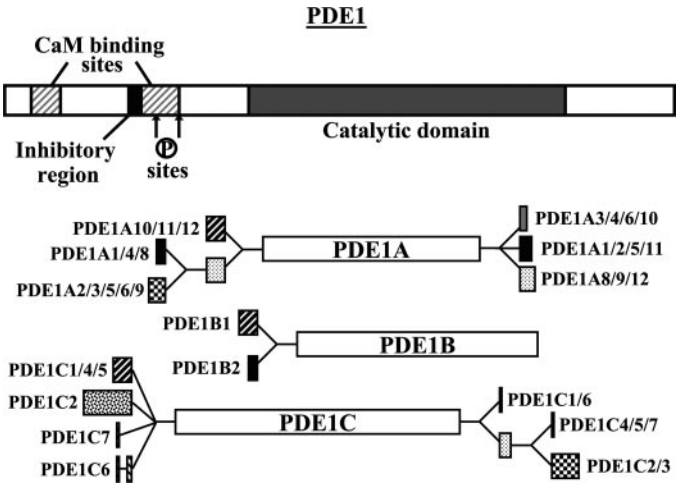

FIG. 4. The Ca^{2+}/CAM-stimulated PDEs. All PDE1s have a domain structure very similar to that depicted for PDE1A in the top part of the figure. It includes two calmodulin-binding sites and an intervening inhibitory domain. The approximate locations of the PKA phosphorylation sites are marked. The bottom three diagrams show a representation of the various alternative start and splice variants for each of the *PDE1* genes. Only the human variants of PDE1A are depicted.

V_{max} values for the different PDE1 gene products vary, with the highest being >200 μmol/min/mg with cGMP as substrate for PDE1A (Hansen et al., 1988). Values of ~20 to 30 μmol/min/mg have been reported for PDE1B (Sharma and Wang, 1986; Hashimoto et al., 1989), whereas a reliable value for pure PDE1C has not been reported. Generally the V_{max} values for cAMP and cGMP are similar for any one isoform with the V_{max} ratios for cGMP/cAMP being typically between 0.5 and 2.

In the presence of Ca^{+2}/CaM, the PDE1s are thought to be tetrameric, consisting of two catalytic monomers and two molecules of calmodulin. There is as yet no crystal structure of any PDE1 holoenzyme either with or without calmodulin in complex, so the orientation of how the calmodulin molecules are bound, how the inhibitory region interacts with the catalytic region, or how the binding of calmodulin activates the enzyme is not known with any precision. However, the crystal structure of the PDE1B catalytic domain has been solved (Zhang et al., 2004b), and in general it is very similar to the other five PDE catalytic domain structures (Card et al., 2004; Ke, 2004). Biochemical data suggest that binding of Ca^{+2}/CaM relieves an inhibition of activity (Sonnenburg et al., 1995). Myosin light chain kinase is an example of another CaM-activated enzyme for which this occurs and has been demonstrated to contain an autoinhibitory substrate-like sequence (Hoeflich and Ikura, 2002).

Significant differences in affinity for Ca^{+2}/CaM exist between different PDE1 enzymes. This is a consequence of different amino-terminal sequences that exist for many of the different variants. In general, the PDE1 enzymes have a fairly high affinity for Ca^{+2}/CaM. The EC_{50} for activation by calcium has been found to vary from 0.27 (PDE1A1) to 3.02 μM (PDE1C1). The affinity

for Ca^{+2}/CaM can also be affected by phosphorylation. Phosphorylation of PDE1A2 by PKA increases the EC_{50} for activation by CaM from 0.51 to 9.1 nM (Sharma and Wang, 1985). Similarly, phosphorylation of PDE1B by CaM kinase II reduces the affinity of PDE1B for CaM by 6-fold (Hashimoto et al., 1989). In both cases phosphorylation can be reversed by the phosphatase, calcineurin. PDE1C also has been reported to be a target for phosphorylation, and its activity is inhibited by PKA (Ang and Antoni, 2002).

3. Genetics/Splicing. The three PDE1 isoforms are products of separate genes and all three have unique variants produced by alternative splicing or alternative transcriptional start sites. Thus, genetic regulation results in the production of a multitude of diverse PDE1 proteins. The different protein products are diagrammed in Fig. 4. The PDE1A isoform has the largest number of variants. Only the human proteins are diagrammed. Some of the unique amino-terminal sequences have been found to confer different functional properties on the variants. As discussed above, differences have been noted in CaM affinity as the unique N-terminal sequences are in or near the CaM binding domains. Significant differences in other kinetic properties among the variants of each isoform have not been reported. Hypothetically, an alternative reason for the existence of unique mRNA products is to allow for more finely tuned regulation of expression. To date only the *PDE1B* gene has been disrupted (Reed et al., 2002) (see next section).

4. Localization. PDE1 expression is highly regulated, and individual isoforms and variants are localized to specific tissues and cell types. The localization of PDE1s in the central and peripheral nervous systems exemplifies this principle. All three PDE1 isoforms are expressed in the brain and many peripheral neurons but to greatly differing degrees depending on region. For example, PDE1C2 is highly localized to olfactory epithelium where it is thought to play an important role in rapid regulation of cAMP responses to odorants (Yan et al., 1995). PDE1 expression is differentially localized not only to different regions, but even to individual neurons of the same type within a region. For example, PDE1B is highly expressed in some but not all Purkinje neurons (Shimizu-Albergine et al., 2003). Different PDE1 isoforms are differentially localized in testis and sperm of the reproductive system (Yan et al., 2001), heart and vessels of the cardiovascular system, and macrophages and T lymphocytes of the immune system (Essayan, 2001). Most PDE1 isoforms are reported to be cytosolic. However, there are instances of PDE1s being localized to subcellular regions. For example, in human and mouse sperm, most of the activity is found in the midpiece of the tail and fractionates with the particulate portion of the cell (Vasta et al., 2005). However, little is known about the molecular mechanisms responsible for subcellular localization of PDE1s. It is likely that the unique N-terminal or C-terminal regions of the various isoforms allow the different proteins to be targeted to specific subcellular domains.

5. Pharmacology/Function. Given their in vitro regulation by Ca^{2+}/CaM, PDE1s are presumed to all function at least in part as a mechanism for integrating cell signaling pathways mediated by cAMP and cGMP with pathways that regulate intracellular calcium levels. However, this very reasonable hypothesis has been hard to prove experimentally in most instances. One reason for this is the lack of truly specific cell-permeable inhibitors for this PDE family. Although several molecules that show some selectivity toward the PDE1s in vitro have been described, few if any show enough selectivity or permeability to be useful for distinguishing these PDEs from other families in intact cells. Therefore, few definitive studies on the functional roles of the various PDE1 isoenzymes exist. In addition, mice with gene disruptions are available only for the PDE1B isozyme in this family. Nevertheless, PDE1 has been implicated to play a role in several physiological and pathological processes. PDE1A mRNA is induced in several cell types upon chronic agonist stimulation, suggesting that it performs important feedback regulatory functions in these cells. For instance, PDE1A is up-regulated in rat aorta in response to chronic nitroglycerin treatment (Kim et al., 2001).

PDE1B knockout mice have increased locomotor activity and in some paradigms decreased learning and memory (Reed et al., 2002). Using these mice, a role for PDE1B in dopaminergic signaling also has been suggested. PDE1B may also contribute to other neuronal functions as it is expressed in multiple CNS regions. PDE1B is induced in several types of activated immune cells (Essayan, 2001). It is hoped that exactly how the up-regulation of these PDE1 variants contributes to the function of the cells will be elucidated in the near future.

PDE1C has been demonstrated to be a major regulator of smooth muscle proliferation, at least in human smooth muscle. In humans PDE1C is absent in quiescent smooth muscle but is expressed in proliferating smooth muscle in culture and in smooth muscle cells isolated from atherosclerotic lesions (Rybalkin et al., 2003b). The importance of PDE1C in the proliferative phenotype was underscored by the finding that antisense oligonucleotide treatment against PDE1C halted proliferation (Rybalkin et al., 2003b). It should be noted that this isozyme is not expressed in the smooth muscle of most other animal models, underscoring the species selectivity of some PDE isozymes and functions subserved by them. Another likely role for PDE1C is in olfaction. The very high expression of PDE1C2 in olfactory sensory cilia strongly suggests that this is the isozyme that modulates the amplitude and duration of the cAMP signal in this tissue in response to odorant stimulation (Yan et al., 1995).

B. Phosphodiesterase 2 Family

1. Overview. PDE2 is a dual substrate enzyme that has both a high V_{max} and low K_m for hydrolysis of both cAMP and cGMP. The distinguishing feature of this PDE is that it is allosterically stimulated by cGMP binding to one of its GAF domains (note: GAF domains are small molecule binding motifs present in many regulatory enzymes and discussed in more detail below). PDE2 is expressed in a wide variety of tissues and cell types including brain, heart, platelets, endothelial cells, adrenal glomerulosa cells, and macrophages. Given that PDE2 is expressed in such a diverse number of cell types, has a complex biochemical regulation, and has the ability to hydrolyze both cAMP and cGMP with high activity, it is likely that it will be involved in regulating a variety of different processes and its function(s) therefore are not easily generalized.

2. Biochemistry/Structure. PDE2 hydrolyzes both cAMP and cGMP and displays positive cooperativity for both nucleotides (Martins et al., 1982). This cooperativity that arises from binding of nucleotides to the allosteric GAF-B domain causes a conformational change in the protein and increases enzyme activity. It is well documented that cGMP binding stimulates cAMP hydrolysis. However, there are as yet no known in vivo examples for the reverse, cAMP stimulation of cGMP hydrolysis, although this has not been thoroughly investigated. Given the 30- 100-fold lower affinity for cAMP at the allosteric GAF-B domain site (Wu et al., 2004) and current ideas about levels of cAMP in cells, it is presumed that activation of cGMP hydrolysis by cAMP does not happen in vivo. However, it is possible that localized pools of cAMP and cGMP may be present in cells that allow such activation in some subcellular compartments.

The crystal structure of the PDE2A tandem GAF-A/GAF-B domain has been determined (Martinez et al., 2002) and more recently compared with that of the tandem GAF-A/GAF-B domains of the *Anabaena* adenylyl cyclase, CyaB2 (Martinez et al., 2005). The PDE2 GAF-B domain binds cGMP with high affinity and selectivity whereas in the cyclase both GAF-A and GAF-B bind cyclic nucleotide with a strong preference for cAMP. Many of the important contacts between the nucleotide and the protein are made via the peptide backbone and not side chains. Others contacts are on the α 4 helix that seems to fold over the cGMP, holding it firmly in place. Interestingly, when the GAF domains of PDE2 are used to replace the GAF domains of the cyclase using molecular biological techniques, the chimeric protein is now stimulated by cGMP (Kanacher et al., 2002). This finding strongly suggests that the GAF domains operate as a molecular switch that upon cyclic nucleotide binding can regulate the enzyme activity of adjoining catalytic domains. Moreover, this basic switch has been conserved for >2 billion years of evolution across organisms. Rather unexpectedly, it was also found that the PDE GAF domains exist in a parallel dimeric structure whereas the cyclase GAF domains are antiparallel. Despite the different dimeric configurations, the PDE GAF domains can be joined to the cyclase catalytic domain and still act to regulate cyclase activity. As might be expected, both the PDE2 and cyclase GAF domains have a very similar architecture in the binding region for cyclic nucleotide. As detailed studies on the structure and binding selectivity have been published recently (Wu et al., 2004; Francis et al., 2005), we will not further address these issues in this review. Current work is now focused on obtaining structural information on the PDE2A holoenzyme so that the molecular mechanisms by which activation occurs can be modeled.

3. Genetics/Splicing. PDE2 protein has been found in a wide variety of tissues and cell types. PDE2 activity and protein were originally purified from heart, liver, adrenal gland, and platelets (Martins et al., 1982; Yamamoto et al., 1983) and are also found in brain, endothelial cells, and macrophages (Tenor and Schudt, 1996; Juilfs et al., 1999; Bender et al., 2004). As with other PDEs, the expression of PDE2A can be regulated, and once produced, PDE2 can be localized to discrete regions or specific cell types within tissues. For instance, in endothelial cells PDE2A is expressed under basal conditions only in smaller vessels and capillaries, but not in larger vessels (Sadhu et al., 1999). In addition, although PDE2A is widely expressed in the brain, its highest expression seems to be localized to specific regions and cell types (Juilfs et al., 1999). PDE2A cellular localization will be discussed more in the context of function in a later section. On the subcellular level, PDE2 activity has been purified from both supernatant and particulate fractions as there are both soluble (PDE2A1) and membrane-associated (PDE2A2/3) variants of this PDE. The different localization of PDE2A2/3 is probably mediated by a unique N-terminal sequence that is absent from PDE2A1 (Fig. 5).

4. Localization. Three PDE2A variants have been cloned from several different species and are identical except for the N-terminal-most 17 to 24 amino acids. The mechanism by which the different variants are produced is complex and is not completely understood (Juilfs et

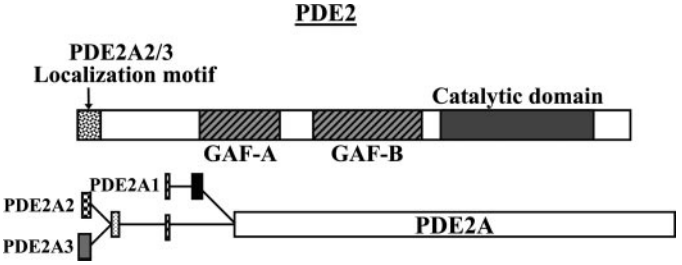

FIG. 5. The cGMP-stimulated PDE. cGMP binds with high affinity to the GAF-B domain causing activation of the PDE2 catalytic domain. PDE2A2/3 is thought to be localized to a particulate fraction due to a N-terminal sequence absent from PDE2A1.

TABLE 4
Pharmacology of PDE family specific inhibitors

PDE	Inhibitors	Source	IC_{50}	Reference	Usage
PDE1	Vinpocetine	W-A	14 μM	Hagiwara et al. (1984)	Only recently have selective PDE1 inhibitors been developed; IC224 may be the most selective in intact cells
	IC224	ICOS	80 nM	Snyder et al. (2005)	
	SCH51866	Schering	13–100 nM	Watkins et al. (1995)	
PDE2	EHNA	W-A	1 μM	Podzuweit et al. (1995)	Inhibitors are being investigated for improving memory and decreasing endothelial permeability under inflammatory conditions
	BAY 60-7550	Bayer	4.7 nM	Boess et al. (2004)	
	PDP	Bayer	0.6 nM	Seybold et al. (2005)	
	IC933	ICOS	4 nM	Snyder et al. (2005)	
PDE3	Cilostamide	W-A	20 nM	Hidaka et al. (1979)	Milrinone is a currently approved treatment for short term congestive heart failure; cilastazol is a treatment for intermittent claudication
	Milrinone	W-A	150 nM	Harrison et al. (1986)	
	Trequinsin	W-A	300 pM	Ruppert and Weithmann (1982)	
	Cilastazol	Otsuka	200 nM	Tanaka et al. (1988)	
	OPC-33540	Otsuka	0.3–1.5 nM	Sudo et al. (2000)	
PDE4	Rolipram	W-A	1 μM	Schwabe et al. (1976)	Multiple compounds have undergone trials for treatment of chronic obstructive pulmonary disease but have experienced limited success because of side effects; compounds are also under investigation for several other inflammatory conditions; interest exists in using PDE4 inhibitors for CNS disorders, including depression and improvement of memory
	Roflumilast	Altana	0.8 nM	Hatzelmann and Schudt (2001)	
	Cilomilat	GlaxoSmithKline	120 nM	Barnette et al. (1998)	
	Ro 20-1724	W-A	2 μM	Sheppard and Tsien (1975)	
	AWD 12-281	Elbion A-G	9.7 nM	Schmidt et al. (2000)	
	SCH351591	Schering	58 nM	Billah et al. (2002)	
	V-11294A	Purdue Frederick	405 nM	Gale et al. (2002)	
PDE5	Zaprinast	W-A	0.13 μM	Lugnier et al. (1986)	Sildenafil, vardenafil, and tadalafil are in usage as erectile dysfunction drugs; these compounds are in trials for other indications such as pulmonary hypertension and benign prostatic hyperplasia
	Sildenafil	Pfizer	10 nM	Boolell et al. (1996)	
	Vardenafil	Bayer	1 nM	Bischoff et al. (2001)	
	Tadalafil	Lilly-ICOS	10 nM	Padma-Nathan et al (2001)	
	DA-8159	Dong-A	6 nM	Oh et al. (2000)	
PDE6					Inhibition of PDE6 may be a source of sildenafil side effects on vision; genetic mutations in PDE6 are the basis for several vision related diseases, but PDE6 has not been investigated as a therapeutic target
PDE7	BRL 50481	GSK	260 nM	Smith et al. (2004)	PDE7-selective inhibitors have been investigated as anti-inflammatory agents in vitro but so far have shown limited utility in vivo
	IC242	ICOS	370 nM	Lee et al. (2002)	
PDE8					No truly selective inhibitors are yet available
PDE9	BAY 73-6691	Bayer	55 nM	Wunder et al. (2005)	BAY 73-6691 is in preclinical development for Alzheimer's disease treatment
PDE10					No truly selective inhibitors are yet available
PDE11					PDE11 has received pharmacological interest because it is also inhibited by tadalafil and thus is a potential source for side effects

W-A, widely available; SCH51866, (+)-*cis*-5,6*a*,7,8,9,9*a*-hexahydro-2-[4-[trifluro-methyl] phenylmethyl]-5-methyl-cylopent[4,5]imidazo[2,1-*b*]purin-4(3*H*)one; EHNA, *erythro*-9-(2-hydroxy-3-nonyl)adenine; PDP, 9-(6-phenyl-2-oxohex-3-yl)-2-(3,4-dimethoxybenzyl)-purin-6-one; Ro 20-1724, 4-[(3-butoxy-4-methoxyphenyl)-methyl]-2-imidazolidinone; AWD 12-281; *N*-(3,5-dichloro-pyrid-4-yl)-[1-(4-fluorobenzyl)-5-hydroxy-indole-3-yl]-glyoxylic acid amide; SCH351591, 3,5-dichloro-4-[8-methoxy-2-(trifluoromethyl)-quinoline-5-ylcarboxamido]pyridine-1-oxide; V-11294A, 3-[3-(cyclopentyloxy)-4-methoxybenzyl]-6-(ethylamino)-8-isopropyl-3*H*-purine hydrochloride; DA-8159, 5-[2-propyloxy-5-(1-methyl-2-pyrollidinylethylamidosulfonyl)phenyl]-1-methyl-3-propyl-1,6-dihydro-7*H*-pyrazolo(4,3-*d*)pyrimidine-7-one.

al., 1999). PDE2A1 and PDE2A2 seem to be splice variants. PDE2A1 has a 62-nucleotide insert that encodes a frame shift. This allows initiation of translation at a methionine downstream of the PDE2A2 start site. PDE2A2 has so far only been found in rat. PDE2A3 lacks the insert of PDE2A1 and shares 19 amino acids in common with PDE2A2 that are not present in PDE2A1. PDE2A3 also has 25 amino acids at the N terminus that are unique. There are no known differences in kinetic behavior between the PDE2A variants. However, as

mentioned before the different N-terminal sequences likely mediate different localizations of the proteins.

5. Pharmacology/Function. PDE2 is involved in a variety of physiological processes. The availability of PDE2 selective inhibitors has greatly aided in the elucidation of PDE2 functions (Table 4). However, to date, PDE2 inhibitors have served primarily as research tools and have not entered clinical usage. Interestingly, the enzyme has been found to regulate either cAMP or cGMP, depending on the cell type in which it is expressed. It is thought that the cooperative kinetic behavior seen for cAMP and cGMP hydrolysis in vitro is important physiologically for the ability of cGMP to stimulate cAMP hydrolysis. Thus, a unique property of PDE2 is its ability to mediate negative "cross talk" between the cGMP and cAMP pathways. This is perhaps best exemplified in adrenal glomerulosa cells of several species in which PDE2 is highly expressed and is thought to mediate the inhibitory effects of atrial natriuretic peptide (ANP) on aldosterone secretion (MacFarland et al., 1991; Nikolaev et al., 2005). In these cells, elevation of cGMP by ANP activates PDE2 that in turn lowers cAMP. Functionally, the PDE2 activation by ANP decreases cAMP that has been stimulated by adrenocorticotropin and results in decreased aldosterone secretion. This cross-talk between the cGMP and cAMP pathways probably occurs in other cellular settings as well. For example, in platelets it is thought that high levels of NO elicit high cGMP accumulation that activates PDE2 and reduces cAMP (Dickinson et al., 1997; Dunkern and Hatzelmann, 2005). However, at lower cGMP levels PDE2 inhibitors alone have little effect on platelet aggregation in comparison to PDE3 inhibitors as PDE3 seems to play a more prominent role in cAMP regulation under these conditions. These results probably reflect a complicated interplay between cGMP-mediated inhibition of PDE3 and stimulation of PDE2. Together the two PDEs mediate opposing regulation of cAMP hydrolysis.

Cardiac myocytes are another cell type in which PDE2 is stimulated by cGMP and interplay between PDE2 and PDE3 may occur. The role of PDE2 in regulating cardiac function was well reviewed in a recent report (Fischmeister et al., 2005). In human cardiac myocytes, PDE2 has been shown to be a regulator of cardiac L-type Ca^{+2} current. L-type Ca^{+2} channels are classically recognized as targets for activation by β-adrenergic receptor-stimulated cAMP and PKA, and alterations in channel activity have both chronotropic and ionotropic effects. Several studies have demonstrated that cGMP can oppose cAMP in myocytes by activating PDE2, thus reducing cAMP and affecting cardiac function (Mery et al., 1995; Vandecasteele et al., 2001; Fischmeister et al., 2005). Using real-time imaging PDE2 in cardiac cells was found to be compartmentalized and shaped the cAMP response to catecholamine stimulation (Mongillo et al., 2006). PDE2 not only regulates cAMP and Ca^{+2} current but also seems to mediate the ability of NO and cGMP to affect cAMP. As in platelets, the activation of PDE2 by cGMP is opposed by the inhibition of PDE3, and the balance of the two effects can be different depending on the tissue and species (Fischmeister et al., 2005). In addition, cGMP may directly inactivate the channel via cGMP-dependent protein kinase (PKG) phosphorylation.

As PDE2 also has a high activity toward cGMP, in some cells it may serve simply as a regulator of cGMP. This seems to be especially true in the brain in which PDE2 is highly expressed in several discrete regions and also in olfactory neurons (Juilfs et al., 1997). For instance, in cultured neurons and hippocampal slices PDE2 serves to regulate cGMP, and functionally PDE2 inhibitor treatment of rats enhanced long-term potentiation and memory without affecting basal synaptic transmission (Boess et al., 2004). PDE2 has also been proposed to regulate cGMP elevated by *N*-methyl-D-aspartate in rat neurons from cortex and hippocampus (Suvarna and O'Donnell, 2002), NO elevated cGMP in rat striatal cells (Wykes et al., 2002), and cGMP in rat olfactory sensory neurons (Meyer et al., 2000).

PDE2 has been found to be up-regulated upon monocyte to macrophage differentiation (Bender, 2003) and is highly expressed in rat (Witwicka et al., 2002) and mouse glycolate-elicited peritoneal macrophages (Tenor and Schudt, 1996). In these cells PDE2 activity represents much of the cGMP PDE activity and may serve as the primary regulator of cGMP. However, these cells also respond to ANP, raising the possibility that PDE2 may also serve to regulate cAMP in response to cGMP in macrophages. It is hoped that future experiments will identify what role is played by PDE2 in macrophages.

PDE2 may play a role in regulation of fluid and cell extravasation during inflammatory conditions as PDE2 is localized to microvessels, especially venous capillary and endothelial cells, but apparently not to larger vessels (Sadhu et al., 1999). One recent observation that is likely to be of considerable importance is the finding that in endothelial cells, PDE2A mRNA and activity are highly induced in response to tumor necrosis factor-α stimulation in vitro (Seybold et al., 2005). Moreover, blockade of PDE2 activity with the PDE2 selective inhibitor 9-(6-phenyl-2-oxohex-3-yl)-2-(3,4-dimethoxybenzyl)-purin-6-one (PDP) seems to greatly alter the barrier function of endothelial cells. This suggests that PDE2 is likely to play an important role in regulating fluid and protein integrity of the circulatory system under pathological conditions. Therefore, PDE2 may be a good pharmacological target for pathological states such as sepsis or in more localized inflammatory responses.

The availability of PDE2 selective inhibitors has greatly facilitated the elucidation of PDE2 function in a variety of tissues as evidenced in the previous paragraphs. One of the first PDE2 selective inhibitors, *erythro*-9-(2-hydroxy-3-nonyl)adenine, was found to have an IC_{50} for PDE2 in the high nanomolar to low micromolar range and an at least 50-fold selectivity over

other PDEs (Podzuweit et al., 1995). Although this compound also potently inhibits adenosine deaminase, with the proper controls it has been successfully used as a tool to probe PDE2 function. Recently, Bayer has developed several newer inhibitors with increased potency and improved selectivity (Boess et al., 2004; Seybold et al., 2005). For example, one of these compounds, PDP, has an IC_{50} of 0.6 nM and a >1000-fold selectivity (Seybold et al., 2005). This compound was found to inhibit thrombin-induced edema formation in mouse lung (Seybold et al., 2005). Another PDE2-selective inhibitor, BAY 60-7750, has been reported to improve memory in animal models. Although studies of these compounds in humans are lacking, they may hold promise in treating disorders of endothelial permeability or learning and memory.

C. Phosphodiesterase 3 Family

1. Overview. The PDE3 family isoforms have been extensively studied, especially in regard to their physiological functions and their usefulness as drug targets. One distinguishing feature of the PDE3 family is their biochemical property of being able to hydrolyze both cAMP and cGMP, but in a manner suggesting that in vivo the hydrolysis of cAMP is inhibited by cGMP. Thus, they have earned the title "the cGMP-inhibited PDE". They also are distinguished by their ability to be activated by several phosphorylation pathways including the PKA and PI3K/PKB pathways. Two PDE3 genes, *PDE3A* and *PDE3B,* have been identified, but splice/start variants have been conclusively demonstrated only for the PDE3A isoform.

2. Biochemistry / Structure. PDE3s were initially purified and described as enzymes that hydrolyze both cAMP and cGMP with relatively high affinities (K_{mcAMP} <0.4 μM; K_{mcGMP} <0.3 μM). However, the V_{max} for hydrolysis of cAMP is nearly 10-fold higher than the V_{max} for cGMP. Therefore, in vitro cGMP can act as an inhibitor of cAMP hydrolysis with an apparent K_i of 0.6 μM. This inhibition also occurs in intact cells as was first demonstrated in platelets (Maurice and Haslam, 1990) and is now thought to occur in most other cells containing PDE3 (see section II.C.5.). It may also be that under some conditions, e.g., low cGMP levels, that this family of PDEs also is important for controlling the levels of cGMP in the cell since it does have a very high affinity for this nucleotide.

The PDE3A and PDE3B isoforms have a high degree of amino acid identity (>80% for much of the catalytic region) and very similar kinetic properties. Both PDE3 isoforms contain an insert in the catalytic domain that is not present in other PDEs. Currently the function of the insert is unknown. Recently a crystal structure of the PDE3B catalytic domain in complex with the inhibitor, cilostamide, was published and should enhance our understanding of the molecular nature of PDE3 catalysis and aid in inhibitor design as well (Scapin et al., 2004).

Both PDE3A and PDE3B activity are regulated by phosphorylation in response to hormonal stimulation in several cell types. In platelets and perhaps also in heart, prostaglandins and epinephrine feedback through PKA to activate PDE3A (Shakur et al., 2001). PDE3B is also a substrate for PKA and is activated by it (Shakur et al., 2001). Similarly, first-messenger signals such as insulin, IGF1, and leptin acting through the PI3K/PKB pathway can induce a phosphorylation of PDE3B and probably also PDE3A to stimulate activity (Shakur et al., 2001). It is thought that these phosphorylation events are important to many of the physiological processes controlled by these hormones. It is likely that in cell types expressing large amounts of PDE3, phosphorylation by PKA and activation of PDE3 can mediate part or all of the tachyphylaxis seen in response to continued agonist stimulation (over the minute to hour time scale). Several of the phosphorylation sites have been identified (Fig. 6).

3. Genetics / Splicing. Three variants of the PDE3A isoform (PDE3A1/2/3) have been identified (Choi et al., 2001; Wechsler et al., 2002). The PDE3A2 and PDE3A3 variants are alternate start site truncations of PDE3A1. PDE3A2 is a shortened version of PDE3A1 due to a separate downstream transcriptional start site. PDE3A3 is a truncated version of PDE3A2 that is thought to be a product of the PDE3A2 mRNA in which translation begins at a downstream ATG (Wechsler et al., 2002). Although PDE3B proteins of multiple sizes have been reported, no PDE3B splice or alternative start variants have been identified. It is thought that the multiple protein sizes reported for PDE3B isolated from tissues are likely to be due to proteolysis. However, the possibility that isoforms of different lengths might be generated by an as yet unidentified alternative start sites in some tissues has not yet been fully ruled out. The genetic regulation of PDE3 expression is relevant as PDE3 expression is altered with adipocyte differentiation (Rahn Landstrom et al., 2000) and heart failure (Ding et al., 2005) and has been noted to change in

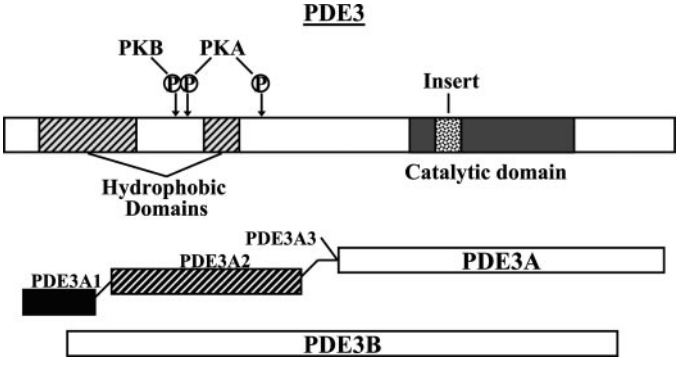

FIG. 6. The cGMP-inhibited PDE. Several shorter variants of the PDE3A gene exist, but only one variant of PDE3B has been identified. PDE3 activity can be regulated by both PKA and PKB phosphorylation. The hydrophobic domains are thought to be involved in protein localization.

vascular smooth muscle (Maurice et al., 2003). In addition, down-regulation of PDE3A expression is observed in human failing hearts or mouse hearts subjected to chronic pressure overload (Ding et al., 2005). Decreased PDE3A activity is associated with increased apoptosis of myocytes. This was demonstrated in isolated cardiomyocytes subjected to pharmacological inhibition of PDE3 or adenovirus-delivered antisense PDE3A (Ding et al., 2005).

4. Localization. PDE3A is relatively highly expressed in platelets, as well as in vascular smooth muscle, cardiac myocytes, and oocytes (Shakur et al., 2001). PDE3B is a major PDE in adipose tissue, liver, and pancreas, as well as in several cardiovascular tissues (Shakur et al., 2001). Both PDE3A and PDE3B contain a large and a small hydrophobic domain at their N termini (Fig. 6). The large domain is 195 amino acids and is predicted to form six transmembrane helices that allow association of PDE3 with membranes (Shakur et al., 2001). However, it is not yet well established how many, or if any, of these helices actually transect the membrane. The second smaller domain of roughly 50 amino acids is thought to be involved in targeting of the enzyme and forms weaker interactions (Shakur et al., 2001). Full-length PDE3A and PDE3B expressed in Sf9 cells are both predominantly particulate. However, truncations eliminating the first hydrophobic region resulted in ~50% of the enzyme becoming cytosolic, whereas truncating both of the domains results in 100% of the enzyme becoming cytosolic (Kenan et al., 2000). In tissues, PDE3B is almost always found to be particulate, whereas PDE3A has been found to be both cytosolic and particulate. PDE3A and PDE3B are expressed in a variety of tissues and have distinct but overlapping localizations (Table 2). For instance, both PDE3A and PDE3B are found in vascular smooth muscle cells (Palmer and Maurice, 2000). In contrast, PDE3A is distinctly expressed in platelets and oocytes, whereas PDE3B is unique to T lymphocytes, macrophages, β cells, and adipocytes (Shakur et al., 2001).

5. Pharmacology/Function. There are a relatively large number of PDE3 selective inhibitors (Table 4). Many of them are commercially available and pharmaceutical firms have also developed a significant number of proprietary compounds. PDE3 selective inhibitors include amrinone, milrinone, cilostamide, and cilostazol. The most potent is trequinsin. Amrinone was the first recognized but possesses only modest affinity and selectivity. To date there have been no inhibitors described that clearly distinguish between PDE3A and PDE3B, although at least one, OPC-33450, has been reported to show some selectivity (Sudo et al., 2000). The plethora of available PDE3 specific inhibitors and the use of genetic techniques have allowed elucidation of PDE3 functions in multiple physiological processes. PDE3 inhibitors antagonize platelet aggregation, block oocyte maturation, increase myocardial contractility, and enhance vascular and airway smooth muscle relaxation.

In platelets, aggregation is highly regulated by cyclic nucleotides. PDE3A is a regulator of this process and PDE3 inhibitors effectively prevent aggregation (Shakur et al., 2001). In fact one drug, cilastazol (Pletal), is approved for treatment of intermittent claudication. Its mechanism of action is thought to involve inhibition of platelet aggregation along with inhibition of smooth muscle proliferation and vasodilation. There is also substantial evidence that cGMP, acting as a competitive inhibitor of PDE3A, exerts most of its antiplatelet effects by increasing cAMP via inhibition of PDE3A (Maurice and Haslam, 1990).

PDE3A has been found to be important not only for platelet function, but also for oocyte maturation. It has been demonstrated that inhibition of PDE3A prevents oocyte maturation in vitro and in vivo (Conti et al., 2002). In more recent gene disruption studies it was found that PDE3A$^{-/-}$ mice are viable and ovulate a normal number of oocytes but are completely infertile as their oocytes contain higher levels of cAMP and fail to undergo spontaneous maturation (Masciarelli et al., 2004). Further studies showed that this occurred because ovulated oocytes were arrested at the germinal vesicle stage. Male PDE3A$^{-/-}$ mice are fertile and to date no obvious phenotypes as a result of the disruption have been reported.

PDE3 enzymes are also involved in regulation of cardiac contractility and vascular smooth muscle (Maurice et al., 2003). PDE3 inhibitors were initially investigated for the treatment of heart failure, but their use for this indication has fallen out of favor because of untoward arrhythmic side effects. Nonetheless, in intravenous form the PDE3 inhibitor milrinone (Primacor) is approved for use in heart failure. Recently it has been reported that PI3Kγ can be associated with PDE3B, and this interaction controls PDE3B activity (Patrucco et al., 2004). The PDE3B interaction with PI3Kγ is thought to be important for the regulation of cardiac contractility and was found to affect cardiac hypertrophy in a mouse model of chronic pressure overload (Patrucco et al., 2004). Interestingly, both PDE3A and PDE3B are expressed in vascular smooth muscle cells and are likely to modulate contraction. Their expression in vascular smooth muscle cells is altered under several conditions such as elevated cAMP, the switch from contractile to proliferative phenotype, and hypoxia (Dunkerley et al., 2002; Murray et al., 2002; Maurice et al., 2003).

The most intensely studied roles for PDE3B have been in the areas of insulin, IGF1, and leptin signaling. Activation of PDE3B is thought to be important for the antilipolytic and antiglycogenolytic actions of insulin (Shakur et al., 2001), as well as for IGF1 and leptin inhibition of glucagon-like peptide-1-stimulated insulin release from pancreatic islets (Zhao et al., 1997, 1998). This idea has now been expanded to include at least part

of the effects of leptin on food intake and body weight (Zhao et al., 2002). At the molecular level it is thought that leptin, IGF1, and insulin activation of PI3K in turn stimulates PKB phosphorylation of PDE3B triggering activation of the enzyme (Zhao et al., 1998; Rondinone et al., 2000; Shakur et al., 2001) and its possible association with 14-3-3 proteins (Onuma et al., 2002). Opposing the phosphorylation by PKB is thought to be protein phosphatase 2A (Shakur et al., 2001). The involvement of PDE3B in regulation of these important metabolic pathways has encouraged researchers to begin exploring the possible roles of this enzyme in disorders such as obesity and diabetes.

D. Phosphodiesterase 4 Family

1. Overview. Almost immediately after assays able to detect PDE activity at low substrate levels were developed, what we now recognize as PDE4 was described. That is, a "low K_m, cAMP-specific PDE activity." This activity was initially characterized by the fact that it could be selectively inhibited by the drug rolipram and the enzymes were once named RoI-PDEs (rolipram-inhibited PDEs) based on this property. One consequence of the early discovery of PDE4 is that it has been one of the most studied PDEs, and a great deal is known about its biochemistry, genetics, and physiological function. It is now recognized that there are four genes (*PDE4A–PDE4D*) that make up this family. Moreover, each gene has multiple variants. Currently, >20 have been described (Fig. 7). PDE4 is expressed in a plethora of tissues and cell types and plays a role in a large number of physiological processes.

2. Biochemistry/Structure. The four PDE4 genes generate >20 different variants by means of alternative start sites and alternative splicing. In general each gene has a so-called long form as well as one or more short forms. As shown in Fig. 7, all of the long forms have an N-terminal domain, two regions termed "upstream conserved regions" (UCR1 and UCR2) that are thought to be regulatory in nature, and a rather highly conserved catalytic domain. The catalytic domains of each PDE4 gene exhibit ~75% sequence identity to any other PDE4 family member. The PDE4 enzymes are universally selective for cAMP with K_m values typically between 1 and 10 μM. V_{max} values of the PDE4 enzymes for cAMP, although lower than those for PDE1s and PDE2s, are comparable with those for most other PDEs (Table 1).

3. Genetics/Splicing. The original cloning of the PDE4 gene arose from the isolation of the "dunce" gene in *Drosophila,* which turned out to be a cyclic nucleotide phosphodiesterase of the PDE4 subtype (Davis et al., 1989). Since then, a great deal of study has been focused on the PDE4 family. The genetics of the PDE4s are exceedingly complex as a number of variants exist for each of the four isoforms. PDE4 gene products are distributed very widely, and one or more can be found in most tissues and cell types. Many of the isoforms and

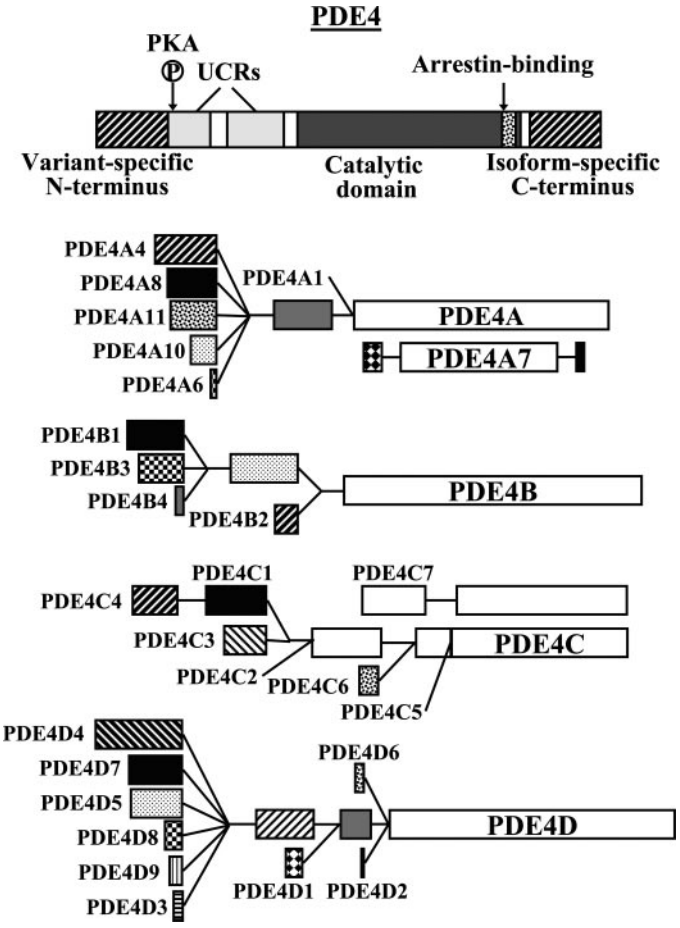

FIG. 7. The high-affinity, rolipram-sensitive, cAMP-specific PDE. PDE4 long forms contain two UCRs, whereas short forms produced by alternative start sites are missing the UCRs. The UCRs contain sites for regulation of activity by phosphorylation and protein-protein interactions. In addition, all PDE4s have a binding site for arrestin in their catalytic domains. Only the human variants of PDE4A are shown.

variants show tissue and cell type-specific expression. For example, nine PDE4D variants have been identified and have varied mRNA tissue expression, with each variant typically expressed in several tissues but frequently overlapping with multiple variants (Richter et al., 2005). The PDE4 variants arise due to differences in their N termini. As the N termini of PDE4s encode regulatory domains and phosphorylation sites, it is not difficult to imagine how different variants having unique N termini will be subject to different regulatory mechanisms. Several of the variants are known to have distinct cellular localizations as well. Several PDE4 promoters have been characterized, and one of the common findings for many of them is regulation by cAMP via CRE/CRE-binding protein.

Several recent studies have shown links between PDE4 expression and disease conditions. For example, one region of the *PDE4D* gene was found to confer increased risk of ischemic stroke (Gretarsdottir et al., 2003; Saleheen et al., 2005). Scientists at deCODE Genetics Inc. identified three haplotypes of individuals that could be grouped by their stroke risk as "wild-type",

"at-risk", or "protected". Interestingly, there was variation in the expression of multiple PDE4D variants in these individuals. The link between PDE4D and ischemic stroke was proposed to be through atherosclerosis. In another recent study a link between the *PDE4D* gene and bone density was found (Reneland et al., 2005). The implication of the study was that *PDE4D* genetic variation could be a factor in osteoporosis. This is supported by the finding that treatment of rodents with PDE4 inhibitors increases bone density (Miyamoto et al., 1997; Kinoshita et al., 2000).

4. Localization. This family of PDEs has been the most widely studied regarding its subcellular distribution and how this distribution is important to cellular function. Multiple different targeting molecular interactions have been identified. These include interactions with arrestins, myomegalin (Verde et al., 2001; Perry et al., 2002; Terry et al., 2003), receptor for activated C-kinase 1 (RACK) (Yarwood et al., 1999), several AKAPS (Dodge et al., 2001; Tasken et al., 2001; Wong and Scott, 2004), and several tyrosine kinases (Ekholm et al., 1997; Beard et al., 1999; McPhee et al., 1999). Two very good reviews describing what is known about the subcellular localizations of the various PDE4 family members have been published relatively recently (Houslay and Adams, 2003; Houslay, 2005). They include discussions not only on where and how the localizations occur but also on why it is likely to be important for shaping the amplitude and duration of cAMP in different regions of the cell. Brief descriptions of some of these interactions are given below.

All of the PDE4 gene products can be recruited to β-arrestin1/2 by binding sites on the catalytic site of the PDE4 that are conserved in each isoform. This interaction is thought to be important for recruitment of PDE4 activity to the subcellular compartment(s) enriched in β-receptor/G-protein-mediated signaling, thereby providing a mechanism for the cell to modulate and attenuate the amplitude and duration of the β-receptor signal (Perry et al., 2002).

The RACK1 protein contains seven WD repeats (tryptophan/aspartic acid) that form a classic β-propeller structure. The PDE4D long form, PDE4D5, has been shown to bind tightly to RACK1. Because RACK1 also recruits a number of other proteins including PKC (for which it was named), Src, integrins, and GABA receptors, an appealing proposal is that recruitment of PDE4D5 to this complex modulates the local cAMP signal in this region of the cell. This in turn presumably then can regulate local effector molecules, some of which are also recruited to this cellular locale. However, concrete direct experimental proof of this hypothesis is currently not available and will probably await good measurements of local cAMP levels within the cell (Adams et al., 1991; Evellin et al., 2004; Nikolaev et al., 2005).

Several forms of PDE4 have also been shown to bind to and be colocalized with a number of different AKAPS. For example, a long form of PDE4D (4D3) can bind to both AKAP450 and the muscle-specific mAKAP (Houslay and Adams, 2003). This interaction is likely to be through the N terminus region of the PDE. It is postulated that this interaction may be important not only in normal functioning of the muscle but also perhaps in certain disease processes. For example mAKAP is induced in hypertrophic cardiocytes, causing part of the PDE4D3 isozyme to be redistributed from the cytosol to a perinuclear compartment (Houslay and Adams, 2003). This could have pathological consequences as cAMP is well known to be a regulator of cardiac contractility and perhaps more importantly in this case of cardiocyte apoptosis.

PDE4D3 was further demonstrated to be associated with the cardiac ryanodine receptor complex (Lehnart et al., 2005). PDE4D knockout mice were found to experience several cardiac problems such as progressive cardiomyopathy and accelerated heart failure. On a molecular level a reduction in PDE4D3 activity resulted in increased PKA-mediated phosphorylation of the ryanodine receptor, rendering the channels "leaky" and contributing to cardiac dysfunction (Lehnart et al., 2005). These findings have important pharmacological implications as patients treated with PDE inhibitors have experienced heart failure and arrhythmias. Thus, cardiac side effects should be considered when PDE4 inhibitors are used clinically.

An example suggesting the physiological importance of PDE4 localization is the recent report of the association of PDE4B and the protein for the disrupted in schizophrenia 1 (*DISC1*) gene (Millar et al., 2005). The *DISC1* gene is one candidate thought to increase susceptibility to schizophrenia, and PDE4B levels seem to be reduced in psychiatric patients. The DISC1 protein was shown to interact with PDE4B in resting cells. However, upon increased cAMP levels the interaction was disrupted and the activity of PDE4B increased (Millar et al., 2005).

Not all of the targeting of PDE4 isozymes is thought to be via protein-protein interactions. For example, several of the short forms of PDE4, including PDE4A1, can bind directly to membranes as a consequence of variant-specific N-terminal sequences. In the case of PDE4A1, it is thought that a hydrophobic sequence in the N terminus can be inserted into the lipid bilayer in a calcium- and phosphatidic acid-specific manner (Houslay and Adams, 2003). Again, the physiological consequences of this localization remain to be established.

5. Regulation by Phosphorylation. The long forms of each PDE4 subtype can be phosphorylated by PKA (Sette et al., 1994; Sette and Conti, 1996). Phosphorylation of a site on the UCR1 module causes a conformation change and an ~60 to 250% increase in activity of the catalytic domain. Functionally, phosphorylation also leads to an increased sensitivity to Mg^{2+}, increased affinity for rolipram, and an altered effect of ERK phos-

phorylation. The PDE4B, C, and D catalytic domains all contain consensus motifs for ERK phosphorylation (Houslay and Adams, 2003). In contrast to PKA phosphorylation, phosphorylation by ERK leads to an inhibition of activity. However, this inhibition can be overcome by PKA phosphorylation of the UCR1. Therefore, physiologically, it is thought that activation of the mitogen-activated protein kinase pathway will initially lead to local increases in cAMP. This increase in turn will activate PDE4 phosphorylation by PKA that will cause a return of cAMP to a lower level. Therefore, these two phosphorylation steps probably form a timing loop for controlling the duration of the cAMP signal (Houslay and Adams, 2003).

6. Pharmacology/Function. A substantial amount is known about the functions of various cell types that are influenced by PDE4s. Primarily this is due to the fact that a number of inhibitors selective for PDE4 are available, although knockout mice have also provided valuable insight. There has been more study of PDE4s using knockout mice than for any other PDE family as three of the four *PDE4* genes have been disrupted. PDE4A, 4B, and 4D have been knocked out in mice in the laboratory of Marco Conti (Jin et al., 2005b). However, detailed information on the resulting phenotypes are at present limited largely to the PDE4B$^{-/-}$ and PDE4D$^{-/-}$ mice (Jin et al., 1999; Jin and Conti, 2002; Mehats et al., 2003; Richter et al., 2005). Probably the most important generalization to be made from these studies is the fact that each of the individual genes can play a nonredundant regulatory role that is not compensated for by the fact that there are three other very similar *PDE4* genes encoding proteins with almost identical kinetic characteristics. This is true even though many tissues and even individual cell types can express two, three or even all four of the PDE4 genes. These observations are at present the strongest evidence for specific functions of individual PDE genes within a family.

At the time of this writing, only preliminary characterization of the PDE4A knockout mice has been published. However, a number of studies on the PDE4B and 4D mice have been published. The first publications were on PDE4D. In these studies it was found that the airways of PDE4D-deficient mice are refractory to cholinergic stimulation, and therefore the pool of cAMP modulated by PDE4D plays a major role in modulating the cholinergic response (Mehats et al., 2003). More recently, it has been shown using the same knockout model that PDE4D is intimately involved in the control of β_2-adrenergic receptor-regulated responses in cardiac cells but has little or no effect on β_1-receptor-mediated responses (Xiang et al., 2005). Furthermore, PDE4D deficiency promotes heart failure progression as demonstrated in PDE4D$^{-/-}$ mice (Lehnart et al., 2005). It was found that PDE4D3 is associated with the cardiac RyR2 channel, and an absence of the PDE results in hyperphosphorylation of the channel, resulting in altered Ca^{+2} control and cardiac dysfunction. So, clearly, individual PDEs are specifically coupled to different functional pools of cAMP in cells. It is not yet known whether the same regulatory mechanisms are used in humans.

Using PDE4B knockout mice, it has been demonstrated that this isoform plays an important role in immune cells. PDE4B was found to be essential for mounting an inflammatory response to lipopolysaccharide in monocytes (Jin and Conti, 2002) and macrophages (Jin et al., 2005a). In particular, PDE4B is required for tumor necrosis factor-α production. PDE4B along with PDE4D was also found to be required for neutrophil recruitment in a model of lung injury induced by endotoxin inhalation (Ariga et al., 2004).

A significant number of PDE4 inhibitors have been developed, and a great deal of investigation is ongoing to explore their use as therapeutic agents. The prototypical PDE4 inhibitor is rolipram, which was originally developed by Schering AG as a possible antidepressant agent (Wachtel, 1982). This compound and now many others like it (Table 4) can have >100-fold selectivity for inhibition of PDE4 versus other PDE family members. In general most PDE4 inhibitors are thought to be relatively selective for PDE4 as opposed to other enzymes in the cell. For example, most investigators feel that any cellular response that is modulated by 10 μM rolipram is likely to involve PDE4 regulation of cAMP. One problem with PDE4 inhibitors as therapeutic agents has been their propensity to promote emesis. It is thought that most of these effects are mediated, at least in part, via actions in the CNS, and so far it has been difficult to separate the effects on emesis from more desirable effects. Nearly all of the PDE4 isozymes show both low (micromolar)- and high (nanomolar)-affinity rolipram-binding sites. Most investigators now feel that these two different types of sites represent two or more different "states" of the catalytic domain of the PDEs (Souness and Rao, 1997). At least part of the high-affinity state seems to involve the bivalent metal ion sites in the catalytic domain.

Several of the newer generation PDE4 inhibitors are thought to have decreased emetic side effects. These include roflumilast (Daxas) and cilomilast (Airflo) that are currently in phase III clinical trials for treatment of chronic obstructive pulmonary disease. Also, recently it has been suggested that inhibition of PDE4D in the brain is responsible for the emetic side effects of PDE4 inhibitors (Lipworth, 2005). Thus, development of PDE4B specific inhibitors is being undertaken as a possible means of maintaining anti-inflammatory activity without causing the emetic side effect of nonspecific PDE4 inhibition.

In addition to chronic obstructive pulmonary disease, other inflammatory diseases for which PDE4 inhibitors are currently being developed include asthma, arthritis, and psoriasis. Most of these uses stem from the effect of decreased PDE4 activity to reduce inflammatory re-

sponses of multiple cell types, as described in the knockout mouse studies detailed above. It is well established that PDE4 inhibitors can effectively suppress release of inflammatory mediators and immune cell infiltration (Essayan, 2001; Castro et al., 2005). In particular, major dampening effects have been seen on neutrophil, monocyte, and T-lymphocyte function (Abrahamsen et al., 2004). However, PDE4 is a major cAMP hydrolyzing activity in a large number of proinflammatory and inflammatory cells, and PDE4 inhibitors probably target multiple cell types.

Finally, in animal models, treatment with rolipram or other PDE4 inhibitors seems to enhance several models of learning and memory (Zhang et al., 2004a, 2005). In addition, initial studies on CNS function with the PDE4D and PDE4B knockout animals are beginning to be reported. As a result, several companies are developing PDE4 inhibitors as memory-enhancing agents and even as antidepressants again. Whether or not these second- and third-generation agents will be able to overcome the emetic side effects of earlier compounds or whether they will be useful for decreasing the memory loss that occurs in various types of neurodegenerative disease remains to be seen.

E. Phosphodiesterase 5 Family

1. Overview. PDE5 was originally identified, isolated, and characterized from platelets (Coquil et al., 1980) and later lung (Francis et al., 1980). However, this PDE received little notoriety until it was discovered to be a regulator of vascular smooth muscle contraction and more importantly the target for the drug, sildenafil. PDE5 is now best known as the molecular target for several well-advertised drugs used to treat erectile dysfunction and more recently pulmonary hypertension. PDE5 is characterized by a relative specificity for cGMP hydrolysis at low substrate levels and by the presence of high affinity-binding sites for cGMP. These binding sites are now known to be on the N-terminal regulatory GAF domains of the enzyme. Only one PDE5 gene has been discovered to date, *PDE5A,* although several variants under the control of differentially regulated promoters have been identified (Fig. 8). Very recent data suggest that PDE5 may modulate pressure-induced cardiac hypertrophy and fibrosis (Takimoto et al., 2005).

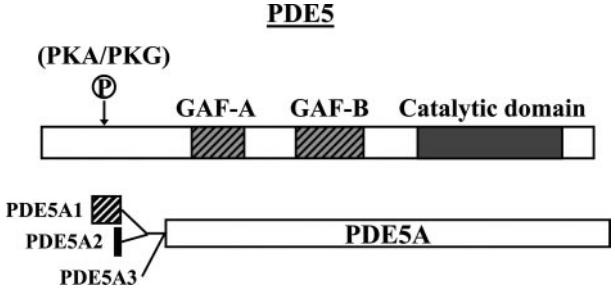

FIG. 8. The cGMP-specific PDE. Binding of cGMP to the high-affinity GAF-A domain activates the enzyme. Binding affinity is modulated by phosphorylation of a nearby site.

2. Biochemistry/Structure. Historically, what is now known as PDE5 was first described as an enzyme activity that was termed the cGMP-specific or cGMP-binding PDE. Originally this activity was described and characterized in platelets by Hamet and colleagues (Hamet and Coquil, 1978; Coquil et al., 1980). Later, studies on cGMP-dependent protein kinase and other cGMP-binding proteins in lung tissue also identified PDE5A as one of the major cGMP binding proteins in this tissue (Francis et al., 1980). Because this PDE had kinetic inhibitor characteristics that were very similar to the previously described rod and cone photoreceptor PDEs, it was initially thought that PDE5 might be related to the already well-known light-sensitive, cGMP-selective PDEs found in the eye (now known as PDE6s). Both enzyme families selectively used cGMP compared with cAMP as substrate [at low substrate levels (Table 1)], both bound cGMP with high affinity, and both had a very similar size. However, differences in V_{max} activities and the lack of regulation of PDE5 by light or G-proteins suggested that they might be different PDEs. Thus, PDE5 was originally labeled as the "cGMP-binding, cGMP-specific PDE". The issue was finally resolved when the bovine lung cGMP-binding PDE was finally cloned, expressed, and sequenced (McAllister et al., 1993) and could be compared with the PDE6 sequences (Ovchinnikov et al., 1986).

The structural basis for the high-affinity cGMP binding to PDE5A was solved when it was found to have two highly homologous GAF domains (GAF-A and GAF-B). In contrast with PDE2, high-affinity cGMP binding occurs only to the GAF-A domain (K_D <40 nM) of PDE5 (Zoraghi et al., 2005). Cyclic nucleotide binding to this domain is >100-fold selective for cGMP over cAMP (Zoraghi et al., 2005). Mutational analysis based on homology models to the crystal structure of the PDE2A GAF-B domain with cGMP bound has revealed that the determinants for cGMP binding are likely to be highly similar to those found in the structure of PDE2 GAF-B (Martinez et al., 2002; Sopory et al., 2003). As with cGMP binding to PDE2 GAF-B domain, it has been shown that the binding of cGMP to the PDE5 GAF-A domain stimulates the enzyme activity 9- to 11-fold and that blockade of this binding inhibits activity. This would suggest that the enzyme may be inactive in the absence of GAF-A domain ligand binding (Rybalkin et al., 2003a). However, under many physiological conditions, it is thought that the GAF-A domain is likely to be occupied by cGMP and therefore fully active. cGMP binding is stabilized by phosphorylation of a nearby serine (Francis et al., 2002). The major kinase responsible for this phosphorylation would seem to be PKG (Corbin et al., 2000). However, when cGMP levels are high and GAF-A is already occupied by cGMP, PKA can also phosphorylate this site. This phosphorylation then stabilizes the in-

creased catalytic activity by enhancing the affinity of cGMP binding to the GAF-A domain (Corbin et al., 2000). It is postulated that this mechanism provides the cell with a method for prolonging the activation of PDE5 in a feedback loop initiated by cGMP synthesis. Phosphorylation of PDE5 by PKG has been demonstrated in vivo (Shimizu-Albergine et al., 2003). By both of these mechanisms, the product of guanylyl cyclases and the substrate for PDE5, cGMP, acts as a feed-forward activator of the enzyme.

3. Genetics/Splicing. Only one gene for PDE5 has been discovered. However, three variants of the PDE5A mRNA and protein have been identified (Loughney et al., 1998; Kotera et al., 1999a; Lin et al., 2000). The three variants (PDE5A1–PDE5A3) differ at their N termini, and all three have unique first exons followed by a common sequence of 823 amino acids. The order of the first exons from the 5′ end in the gene is PDE5A1, PDE5A3, and PDE5A2. A promoter has been characterized upstream of the PDE5A1 first exon and an alternative intronic promoter was also found upstream of the PDE5A2 first exon (Lin et al., 2002). PDE5A3 seems to be regulated by the same promoter as PDE5A1, although it has a separate first exon. Transcription from the PDE5A2 promoter was found to be positively regulated by both cAMP and cGMP (Kotera et al., 1999a; Lin et al., 2001a). Mutation analysis suggests that AP2 and Sp1 elements may be most responsible for the cyclic nucleotide responsiveness (Lin et al., 2001b), although CREs are also present (Kotera et al., 1999a). It is assumed, but has not yet been clearly shown, that the different promoters for the PDE5 isoforms allow physiologically relevant differential control of PDE5 gene expression.

4. Localization. PDE5A is generally considered to be a cytosolic protein. In rodents, relatively high levels of PDE5A mRNA have been localized to vascular smooth muscle, heart, placenta, skeletal muscle, pancreas, brain, liver, several gastrointestinal tissues and lung (Loughney et al., 1998; Stacey et al., 1998; Yanaka et al., 1998). However, the highest levels of PDE5A mRNA are found in the cerebellum, kidney, and pancreas (Kotera et al., 2000), and prominent expression is also seen in lung and heart (Giordano et al., 2001). PDE5A protein is also expressed in platelets, where it was originally discovered. Of course, it is prominently expressed in smooth muscle including the vascular tissues of the penis. PDE5 protein is also found in the brain and is particularly abundant in Purkinje neurons (Shimizu-Albergine et al., 2003). The expression of different PDE5A variants is apparently regulated as PDE5A1 and PDE5A2 variant mRNAs have been detected at varying levels in a wide variety of tissues (Kotera et al., 1999a; Lin et al., 2002), whereas PDE5A3 expression seems to be restricted to vascular smooth muscle (Lin et al., 2002).

5. Pharmacology/Function. To date, pharmaceutical companies have found more commercial success with PDE5 inhibitors than with inhibitors of any other PDE family. This success is attributable to the three widely prescribed drugs sildenafil (Viagra), vardenafil (Levitra), and tadalafil (Cialis). These drugs have proven very effective for the treatment of erectile dysfunction and are being tested for other pathological conditions as well. These highly selective inhibitors have also been useful as research tools to study the physiological role of PDE5 in various tissues. The most established function of PDE5A is as a regulator of vascular smooth muscle contraction through regulation of cGMP. Two tissues in which this role is evident are the lung and the penis. In the cavernosal smooth muscle of the penis, PDE5 inhibition enhances relaxation of smooth muscle by nitric oxide and cGMP and thereby stimulates penile erection (Rosen and Kostis, 2003; Corbin, 2004). Of both clinical and physiological interest is the fact that, in general, large effects of PDE5 inhibitors are seen only in the presence of nitric oxide release or ANP stimulation. In the case of many types of erectile dysfunction, this potentiation effect allows near-normal control of erectile function to be reestablished without occurrence of an unwanted response. That is, efficacy of the drug still requires release of NO by the penile nerves. However, these drugs are contraindicated in patients also taking nitrovasodilators as the PDE5 inhibitors can then elevate cGMP levels in all smooth muscle beds. The regulation of PDE5 in smooth muscle and the use of PDE5 inhibitors in treatment of erectile dysfunction have been the subject of a number of recent reviews and readers are referred to these articles for more detailed information (Bischoff, 2004; Corbin, 2004; Setter et al., 2005).

In the lung, inhibition of PDE5 opposes smooth muscle vasoconstriction, and PDE5 inhibitors are in clinical trials for treatment of pulmonary hypertension (Lewis and Semigran, 2004; Steiner et al., 2005) and more recently for treatment of pulmonary hypertension of neonates (Hoeper, 2005; Humpl et al., 2005). It is not clear whether efficacy in this case also requires a high stimulatory tone for guanylyl cyclase. Similarly, PDE5 is also thought to be important in the regulation of platelet aggregation. Inhibition of PDE5 in the presence of NO increases platelet cGMP and enhances the inhibitory effect of NO on platelet aggregation and secretory function (Ito et al., 1996; Dunkern and Hatzelmann, 2005). At least part of the effect of PDE5 inhibitors on platelet function is thought to be mediated through cGMP inhibition of PDE3 and subsequent increases in cAMP (see discussion of PDE3 in previous sections). PDE5 has also been implicated to play a role in learning and memory (Prickaerts et al., 2004) and as discussed below in cardiac remodeling. It should be noted that although the PDE5 inhibitors are generally well tolerated, they are reported to have some side effects that are likely to be related to their primary mechanism of action. Among the most common are headaches, nasal congestion, and gastric acidosis. These effects suggest additional roles

for cGMP (and PDE5) in these tissues. To date, however, the molecular mechanisms for these side effects are not well understood.

Several studies have suggested roles for cGMP (Wollert et al., 2002; Zahabi et al., 2003) and PDE5 (Das et al., 2002, 2005; Hassan and Ketat, 2005) in cardiac function. One of the most graphic was the recent observation by Takimoto et al. (2005) on the effects of sildenafil on pressure-induced cardiac hypertrophy. These authors have shown that in several models of cardiac hypertrophy and heart failure due to pressure overload, the PDE5 selective inhibitor sildenafil will completely prevent the hypertrophic and fibrotic response caused by the increased pressure. Although the levels of PDE5 in the cardiomyocyte are rather low, the authors further suggest that the effects of sildenafil may be due to expression of a small amount of PDE5 that is localized to regions of the cardiocyte near the Z-line. However, because these studies were conducted in whole animals, the possibility that part or all of the reduction in hypertrophy may be due to effects on other cell types, perhaps not even in the heart, has not been ruled out. Regardless of the physiological mechanism, if these observations in rodents transfer to human hypertension and heart failure, the possible implications for treatment of cardiac disease with PDE5 inhibitors are enormous.

F. Phosphodiesterase 6 Family

1. Overview. The PDE6 family members are better known as the photoreceptor phosphodiesterases. There are three genes in the family, *PDE6A, PDE6B,* and *PDE6C*. In addition, there are also PDE6 γ and PDE6 δ "subunits" that modulate the activity and localization of these enzymes (Fig. 9). The PDE6s are highly expressed in the photoreceptor outer segments of the mammalian retina, in which they mediate the conversion of a light signal into a photoresponse. PDE6A and 6B are expressed in the rods, and PDE6C is expressed in cones. In response to a photon of light absorbed by the rod or cone photopigment, a G-protein called transducin is activated by GDP-GTP exchange. The activated transducin then interacts with the PDE6 holoenzyme, causing its activation by removal of the inhibitory γ subunit. The activated PDE then rapidly hydrolyzes cGMP, causing deactivation of a cGMP-gated cation channel in the nearby plasma membrane. The ensuing hyperpolarization initiates the photoresponse of the photoreceptor neuron. A number of relatively recent reviews of photoreceptor function and photoreceptor PDEs have been published (Ridge et al., 2003; Cote, 2004). In addition to their role in retinal phototransduction, substantial evidence has been presented demonstrating that in avian species at least a functioning photoreceptor cascade including activation of a PDE6 is present in pineal gland, which is known to be able to sense light.

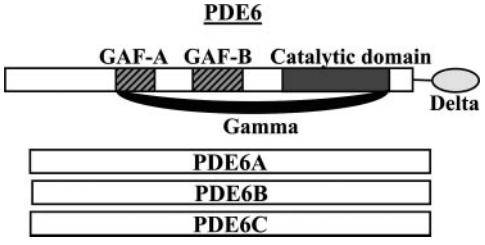

FIG. 9. The photoreceptor cGMP-specific PDE. There are three different PDE6 genes that encode a catalytic domain, PDE6A, B, and C. Depending on the cell and species, the enzymes exists as an AB or CC dimer plus two inhibitory γ subunits. Some of the molecules also bind to a δ subunit via a C-terminal prenyl group.

2. Biochemistry/Structure. In the rod cells of most species, the catalytically active form of the enzyme is a dimer composed of an α subunit (PDE6A) and a β subunit (PDE6B). Each of these subunits has two N-terminal GAF domains (GAF-A and GAF-B) and a C-terminal catalytic domain. The GAF-A domain contains a high-affinity binding site for cGMP and also probably much of the dimerization interface. The catalytic domain shows very high specificity for cGMP at low substrate levels but will hydrolyze cAMP efficiently at high, presumably unphysiological, levels. The catalytic efficiency of this family of enzymes is the highest of any known PDE, with catalytic rates for the fully activated enzyme of >2000 μmol/min/mg or >3000 molecules of cGMP hydrolyzed per molecule of PDE per second (Gillespie and Beavo, 1988). It has been estimated from ^{18}O turnover studies that under full light illumination, the total pool of cGMP in the retina can turnover in <15 ms (Goldberg et al., 1983). This allows the animal to detect and respond very rapidly to changes in light intensity, obviously an advantage for vision-dominated species.

In contrast to the subunit structure of the rod PDE6 enzymes from most species studied to date, in birds there is as yet no evidence for a PDE6 α subunit from studies on both the pineal gland (Morin et al., 2001) and the chicken retina (Huang et al., 2004). In both cases only a PDE6 β subunit has been found. This suggests that a heterodimer is not essential for activity although a heterodimer does seem to be the major form of the enzyme in mammalian rod photoreceptors.

It has been clear for many years now that each PDE6 catalytic subunit is associated with a γ subunit and that this subunit inhibits activity in the absence of activated transducin. Thus, the mechanism by which transducin activates the enzyme is by relieving an inhibition, although the exact molecular mechanism by which the PDE6 γ subunit inhibits activity is still a bit unclear. It is known that the rod and cone γ subunits are different gene products and are likely to have different affinities for their respective catalytic domains; however, the functional significance of this difference is not known. Similarly, the number and location of the binding site(s) for the γ subunits on the holoenzyme are not entirely clear as a crystal structure for the complex is not yet available. To date no crystal structure has been obtained for either the catalytic domains or the GAF domains of

PDE6, in part because of the great difficulty in expressing any appreciable quantity of any form of PDE6 from cDNA constructs. So far only chimeric PDEs made up of PDE5 and PDE6 have been successfully expressed in any quantity (Granovsky et al., 1998). Several reports of very low yield expression have appeared, however. The structure of native enzyme with and without proteolytic removal of the γ subunits has been investigated by cryo-electron microscopy (Tcheudji et al., 2001; Kajimura et al., 2002). Although the resolution is limited (~29 Å), it seems that both the GAF-A and GAF-B domains form a dimerization contact. There is little difference between the structures with and without the γ subunits. This three-dimensional electron microscopic structure does not have high enough resolution to determine the details of the interaction. It is known from various binding and cross-linking studies that the γ subunit binds to or at least is very close to both the catalytic and the GAF-A domains and it is presumed that both interactions are important for function.

Similar to the γ subunit, neither the details of the binding site nor the physiological role(s) for the δ subunit are entirely clear.[4] This protein can bind tightly to the C-terminal region of the catalytic subunit, and it is known that this binding requires an isoprenyl farnesyl or geranylgeranyl lipid modification for the binding to occur (Cook et al., 2000). It is also known that binding of the δ subunit is associated with localization of the enzyme to the soluble fraction of the retina extract. Under normal fractionation conditions, nearly all the cone PDE6C is associated with the δ subunit and found in the soluble fraction. Under the same conditions, ~50% of the rod PDE6A/B is soluble and bound to the δ subunit. However, given the widespread nature of the δ subunit, and the fact that it probably is not expressed at the same levels as the PDE6A and B subunits, it is not yet clear what physiological role PDE6 δ has for photoreceptor function. It has been hypothesized that the fraction of PDE6 that is soluble would be less able to be activated by transducin binding, thereby extending the light sensitivity of the rod to higher intensities (Cook et al., 2001). Recent localization studies suggest a possible role in synthesis and transport of the PDE6 to the disc or plasma membrane (Norton et al., 2005).

3. Genetics/Splicing. PDE6s were the first PDEs in which genetic mutations were found to cause disease. In particular, several forms of stationary night blindness (Gal et al., 1994) and retinitis pigmentosa (McLaughlin et al., 1993) have been mapped to different locations on the PDE6 α and β subunits. There are also diseases associated with mutations in the γ subunit. A number of genetic causes for various forms of stationary night blindness and retinal degeneration in various animal models are known, and only a fraction of them map to the PDE, although most map to somewhere in the phototransduction cascade. Most of the PDE6 mutations are thought to result in protein misfolding and thereby lead to progressive dysfunction and cell death. Because in mammals it is likely that heterodimerization between the α and β subunits is required for activity, a folding problem in either subunit may be sufficient to explain the progressive nature of most of these conditions. It has been postulated that any mutation that chronically increases cGMP in the photoreceptor cell can lead to death of photoreceptor cells (Farber and Tsang, 2003). One interesting mutation studied recently leads to progressive night blindness and seems to alter binding sensitivity for the γ subunits to PDE6 (Muradov et al., 2003).

4. Localization. PDE6 has found little pharmacological interest as a drug target. However, it is inhibited to some extent by the erectile dysfunction drug sildenafil, and this inhibition has been suggested to be the source for some of the visual side effects reported with sildenafil usage. PDE6 is, of course, best known for its role in regulating photoreceptor signal transduction. However, several recent studies hint that PDE6 may have additional roles. For example, Malbon and colleagues have reported in mouse embryonal cells that the Wnt/Frizzled pathway is coupled to a decrease in cGMP (Wang et al., 2004). They further provide evidence that this is caused by activation of a PDE that is likely to be a PDE6. A number of investigators have reported the presence of PDE6 α and/or β mRNA in nonretinal tissues, particularly in many recent gene chip studies. These data are consistent with the idea that PDE6s are involved in embryonic development and perhaps also in transformation. In *Drosophila,* a PDE isozyme most homologous to PDE6 (DmPDE6, *Drosophila* PDE6-like enzyme) has been observed in several tissues including the Malpighian gland, suggesting that this PDE may have some role in kidney function (Day et al., 2005). In *Drosophila* Malpighian tubules the enzyme has been posited to play a role in regulating cGMP transport (Day et al., 2006). As it is known that the related PDE5 is highly expressed in mammalian kidney, it remains to be established what the real homolog of this Malpighian gland PDE is in higher species.

G. Phosphodiesterase 7 Family

1. Overview. The PDE7 family, like PDE4 and PDE8, is highly selective for cAMP as substrate, especially at low substrate levels. It consists of two genes, *PDE7A* and *PDE7B.* There are no known regulatory domains on the N terminus as established for most of the other PDE families, although consensus PKA phosphorylation sites exist in this region (Fig. 10). The functions of the enzyme remain largely to be determined. However, PDE7 mRNA and protein are expressed in a wide variety of immune cells (Smith et al., 2003), and evidence suggests that PDE7 may play a role in T-

[4] The δ subunit is found in a number of cell types and tissues in addition to the photoreceptors and probably should not be called a photoreceptor PDE subunit because it can also bind to other proteins.

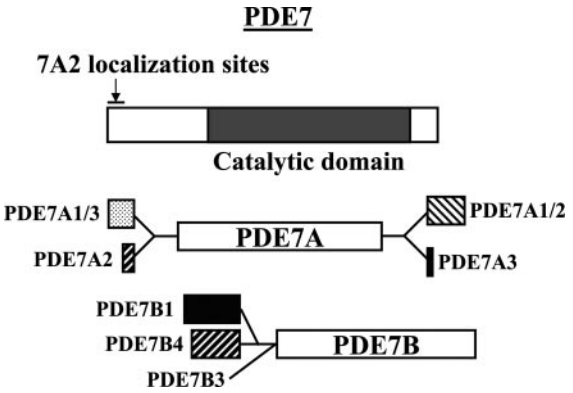

FIG. 10. The high-affinity, rolipram-insensitive cAMP-specific PDE. The two PDE7 isoforms both contain an N-terminal sequence of unknown function that does not contain any of the regulatory domains identified in other PDE isoforms.

lymphocyte activation (Li et al., 1999; Glavas et al., 2001; Nakata et al., 2002; Smith et al., 2004). Given the high value of pharmacological targets that are involved in inflammation, a great deal of effort has been expended in developing PDE7 selective inhibitors. Despite the discovery of PDE7 more than a decade ago, until recently very few PDE7 selective inhibitors had been reported. Now, several compounds have been described, including BRL 50481 (Smith et al., 2004) and BMS-586353 (Yang et al., 2003), that have been used for in vitro pharmacological testing. Unfortunately, none are commercially available yet.

2. Biochemistry/Structure. PDE7A was originally found in a genetic screen for mutations that suppressed Ras function in yeast (Michaeli et al., 1993). The K_m for cAMP hydrolysis is ~0.1 μM for PDE7A and is slightly lower for PDE7B. The V_{max} values for PDE7A (Michaeli et al., 1993; Han et al., 1997) and PDE7B (Sasaki et al., 2000) have been reported to be very low (<1 nmol/min/mg) in comparison with those for most other PDEs. Thus, it is thought that either these enzymes may function largely to regulate "basal" levels of cAMP or perhaps have activators yet to be identified. Both isoforms have little activity against cGMP.

3. Genetics/Splicing. In humans there are three known PDE7A splice variants, PDE7A1, PDE7A2, and PDE7A3, that differ in their N- and C-terminal regions (Han et al., 1997; Glavas et al., 2001). The PDE7A2 splice variant contains a unique N terminus that localizes it to membrane fractions of muscle and heart (Han et al., 1997). Three splice variants for PDE7B have been identified in rat and have unique N-terminal sequences (Sasaki et al., 2002). These variants are probably transcriptionally regulated in a differential manner as activation of only the PDE7B1 variant promoter was observed in striatal neurons by cAMP activation of PKA (Sasaki et al., 2004). The PDE7A1 promoter has been characterized and is also thought to contain several cAMP-responsive elements that contribute to its activity (Torras-Llort and Azorin, 2003), and activation of PKA in B lymphocytes up-regulates PDE7A (Lee et al., 2002b).

4. Localization. PDE7A1 mRNA is expressed in multiple tissues including muscle, spleen, brain, lung, thymus, and various lymphocyte populations (Bloom and Beavo, 1996; Han et al., 1997; Lugnier, 2005). PDE7A1 protein has been found in a wide variety of immune cells and has thus engendered a great deal of interest as a target for treating inflammation (Smith et al., 2003). In T lymphocytes PDE7A protein can be highly localized in the cell, probably to the Golgi apparatus, through its association with the AKAP protein MTG (Asirvatham et al., 2004). PDE7A1 protein is primarily cytosolic, whereas PDE7A2 is mostly associated with a particulate fraction and its expression is not widespread but is high in heart (Han et al., 1997). Like the PDE7A variants, the PDE7B variants are also expressed in a tissue-specific manner. mRNA for PDE7B1 was observed in multiple tissues such as heart, brain, lung, kidney, liver, and muscle (Gardner et al., 2000; Hetman et al., 2000b), whereas PDE7B2 mRNA is restricted to testis and PDE7B3 mRNA is found only in heart (Sasaki et al., 2002).

5. Pharmacology/Function. A great deal of effort from the pharmaceutical industry has been invested in developing PDE7 selective inhibitors (Yang et al., 2003; Smith et al., 2004; Castro et al., 2005; Lugnier, 2005). These inhibitors and some genetic knockout technologies have been used to probe the function of PDE7 in cells and whole animals. To date only two major functions have been ascribed to PDE7. Initial studies suggested that PDE7A could be induced in T lymphocytes in response to activation of the T-cell receptor (Li et al., 1999). PDE7 antisense oligonucleotides inhibited proliferation and IL-2 production in a PKA-dependent manner (Li et al., 1999). With PDE4/PDE7 dual inhibitors, it was also reported that PDE7 may play a role in T-lymphocyte activation (Nakata et al., 2002). However, in another study the PDE7 selective inhibitor BRL 50481 had only a small effect on $CD8^+$ lymphocyte cell proliferation and only in the context of concomitant PDE4 inhibition (Smith et al., 2004). As a result a substantial effort is now being made in testing compounds that are combination PDE4/PDE7 inhibitors for anti-inflammatory activity. It should be noted that initial data from PDE7A gene disruption studies in mice have not shown that PDE7 is required for T-lymphocyte activation (Yang et al., 2003). However, these mice may have a decrease in insulin-stimulated glucose secretion in skeletal muscle. It is not yet clear if all variants of PDE7 were deleted by the disruption. Thus, the role PDE7 plays in T-lymphocyte activation and function is unclear at this time. However, the development and availability of PDE7 selective inhibitors should facilitate future studies of PDE7 in other immune cells and in tissues such as brain or skeletal muscle in which it is also expressed.

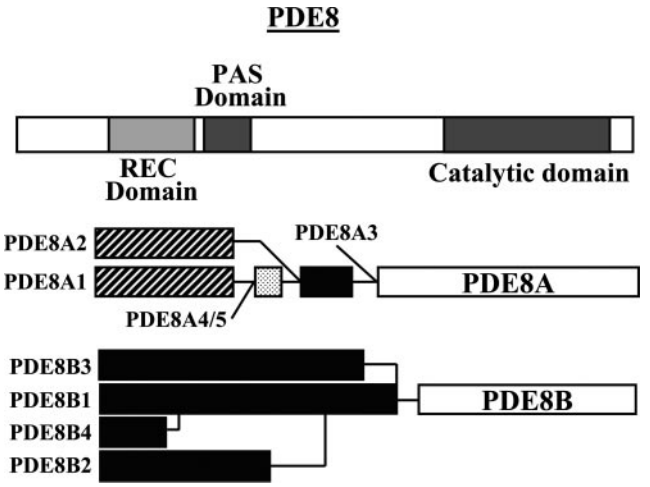

FIG. 11. The high-affinity and IBMX-insensitive cAMP-specific PDE. Both PDE8 isoforms contain N-terminal REC and PAS domains. However, the functions of the REC and PAS domains are unknown at the present time.

H. Phosphodiesterase 8 Family

1. Overview. The PDE8 family was the first of four families identified initially with the help of bioinformatic analysis of the EST (Expressed Sequence TAG) cDNA databases. Subsequent analysis has shown it to contain two genes, *PDE8A* and *PDE8B*. Both isoforms are specific for cAMP and have a very high affinity for the substrate. Each of these two gene products contains two putative regulatory domains of unknown function in their N-terminal region (Fig. 11). The first is a "REC domain" homologous to the "receiver" domains of bacterial two-component signaling systems (Galperin et al., 2001). This is followed closely by a PAS domain (an acronym for *P*eriod, *A*rnt, and *S*im) first described as a regulatory domain present in several proteins involved in the control of circadian rhythms (Dunlap et al., 1999; Gilles-Gonzalez and Gonzalez, 2004). In other systems, PAS domains have been shown to be sites of ligand binding and protein interactions. However, little is known about the physiological function of either the REC or the PAS domain in PDE8 or for that matter of the PDE8s themselves.

2. Biochemistry/Structure. The primary structure of both PDE8A and PDE8B enzymes includes N-terminal REC and PAS domains, although in some variants these domains are incomplete or deleted (Soderling et al., 1998a; Hayashi et al., 2002). PAS motifs are found in proteins existing in organisms ranging from bacteria to man. These domains often act as environmental sensors for signal transduction (Gilles-Gonzalez and Gonzalez, 2004). For example, the *Escherichia coli* protein EcDOS, which exhibits some phosphodiesterase activity, contains a PAS domain that binds heme and acts as a redox sensor as the oxidation state of the heme group affects the catalytic activity of the enzyme (Gilles-Gonzalez and Gonzalez, 2004). However, a ligand for the PDE8 PAS domain has not yet been identified. REC, or receiver domains, are best known for their role in prokaryotes as response regulators for sensor kinases in two component systems (Galperin et al., 2001). A relevant PDE example has been found for a *Dictyostelium* cAMP PDE that has an N-terminal REC domain and serves as the second protein in a two-component system (Thomason et al., 1999).

It will be interesting to determine whether and how the PAS and REC domains of the PDE8s serve to regulate enzyme activity in mammals. The catalytic properties for PDE8A and PDE8B have been characterized in vitro. Both enzymes are cAMP-specific and have a very high affinity for the substrate (40–60 nM or >40-fold higher affinity than PDE4) (Fisher et al., 1998a; Gamanuma et al., 2003). In fact, their cAMP affinity is the highest of any PDE. The V_{max} values for none of the PDE8s are known for certain, but may be as much as 10- to 100-fold lower than the V_{max} values for the PDE4s (Fisher et al., 1998a). It is not yet known whether there are ligands or other regulators that interact with the PAS and REC domains in the cell that significantly alter these catalytic properties.

3. Genetics/Splicing. Two separate but highly homologous genes for PDE8 (PDE8A and PDE8B) exist. The PDE8A isoform has several variants that seem to be produced by alternative splicing and alternative start sites. PDE8A2 is a spliced variant of PDE8A1 that lacks the PAS domain, whereas PDE8A3 is a truncated protein missing both the PAS and REC domains (Wang et al., 2001). PDE8A4 and PDE8A5 are identical truncated proteins, with different untranslated sequences, that are longer than PDE8A3 but are still missing both the PAS and REC domains (Wang et al., 2001). The various PDE8B variants arise from alternative splicing. It should be noted that for the most part the different splice variants are predicted from mRNA and cDNA data but have not yet been verified to yield different protein products.

4. Localization. PDE8 enzymes seem to be primarily cytosolic, although recombinant expressed PDE8 has been found in both cytosolic and particulate fractions (Gamanuma et al., 2003). PDE8A mRNA expression is widespread and has been detected in a variety of tissues but is highest in testis, spleen, small intestine, ovary, colon and kidney (Fisher et al., 1998a; Soderling et al., 1998a; Wang et al., 2001). In addition, PDE8A1 protein has been detected in primary T lymphocytes and T cell lines (Glavas et al., 2001). Of all the variants, the PDE8A1 variant seems to be most abundant. PDE8B expression is more restricted and its mRNA seems to be primarily confined to brain and thyroid although there is also a substantial expression in testis. PDE8B variants also show differential expression as PDE8B1 is expressed only in brain, whereas PDE8B3 is expressed equally in brain and thyroid.

5. Pharmacology/Function. Selective inhibitors of PDE8 have not been reported, and no known specific

functions for the PDE8s have been identified. However, the finding that PDE8A1 is highly up-regulated upon CD3/CD28 T lymphocyte stimulation suggests that it may be involved in T cell activation. The existence of the PAS and REC domains and the comparison of function of these domains in other proteins suggest that the PDE8s may serve as environmental sensors for regulation of cAMP in the cell. However, to date no firm examples of this putative regulation have been shown.

I. Phosphodiesterase 9 Family

1. Overview. PDE9 is one of the more recently discovered PDE families. It is perhaps most notable as the PDE family having the highest affinity for cGMP. PDE9A is the only isoform identified to date, but its mRNA processing seems to be extremely complex as 19 variants have been reported (Fig. 12) and multiple mRNAs are present in many tissues. Little is known about the functional role of PDE9A, although some data suggest it may be a regulator of cGMP signaling in the brain (Van Staveren et al., 2002). PDE9A is possibly involved in pathological settings as its genomic location maps to a region that contains genes involved in several neurological diseases including bipolar disorder (Guipponi et al., 1998).

2. Biochemistry/Structure. The primary structure of PDE9A is relatively simple as it does not appear to contain any GAF domains or other N-terminal regulatory sequences found in other PDEs. However, unknown regulatory regions may be present. PDE9A is a cGMP specific PDE as it has a K_m for cAMP that is greater than 1000 fold its K_m for cGMP. In fact, PDE9A has the lowest K_m of any PDE for cGMP (70–170 nM) (Fisher et al., 1998b; Soderling et al., 1998b) of all the PDE families. No good estimate for the V_{max} of PDE9A has been reported for a purified enzyme. In crude expression systems, the enzyme had a V_{max} for cGMP (4.9 nmol/min/mg in Sf9 cell extracts) that was approximately twice the V_{max} of PDE4A for cAMP expressed under similar conditions (Fisher et al., 1998b), suggesting that it may be substantial. This isozyme is relatively insensitive to most common PDE inhibitors, including the generally nonselective ones (Table 4). The amino acid sequence of the PDE9A catalytic domain has a low degree of homology to other mammalian PDE catalytic domains and may explain the insensitivity of the enzyme to most PDE inhibitors (Fisher et al., 1998b; Soderling et al., 1998b). A crystal structure of the PDE9A2 catalytic domain with the nonselective inhibitor IBMX has given insight to the structure and conformation of the PDE9A active site (Huai et al., 2004b).

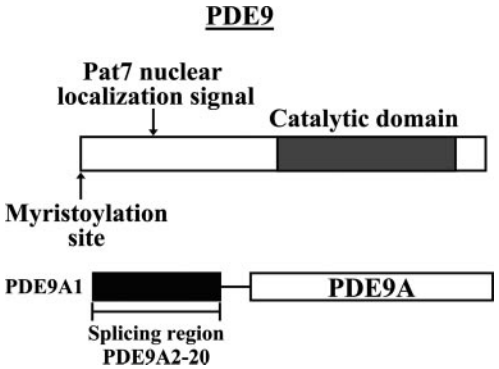

FIG. 12. The high-affinity cGMP-specific PDE. There is a multitude of PDE9 alternatively spliced mRNAs reported with variations in the N-terminal region. PDE9A has N-terminal sites that can regulate its localization.

3. Genetics/Splicing. The regulation of PDE9A mRNA processing seems to be quite complex as many variant mRNAs have been discovered. By using reverse transcription-polymerase chain reaction, rapid amplification of cDNA ends, and alignment of EST sequences, 19 N-terminal mRNA variants have been identified (Guipponi et al., 1998; Rentero et al., 2003). However, other than PDE9A1, the protein for only PDE9A5 has been expressed and characterized (Wang et al., 2003). The variants all use the same transcriptional start site but are alternatively spliced to produce unique N-terminal mRNAs. What the functional consequence(s) of these amino acid sequence alterations would be in the cell is unclear as the variations are not in the catalytic domain or any other recognized regulatory domain. Furthermore, it is dubious whether all these mRNAs are translated into protein in vivo. There is likely to be post-transcriptional regulation of PDE9A mRNA as the 5′-UTR sequence has been found to have a GC-rich region that is capable of folding back on itself and forming a hairpin that could inhibit translation (Fisher et al., 1998b).

4. Localization. A few studies have been conducted concerning PDE9A protein localization. PDE9A5 has been reported to be cytosolic, whereas PDE9A1 has been observed to be nuclear (Wang et al., 2003). PDE9A1 is thought to be localized to the nucleus by a pat7 nuclear localization motif that is not present in PDE9A5 (Wang et al., 2003). PDE9A1 was found to be nuclear despite the presence of an N-terminal myristoylation site in its primary sequence. So far no reports of the cellular localization of other PDE9A variant proteins have appeared. However, several studies have reported PDE9A mRNA tissue localization. In general, PDE9A mRNA is widely expressed and has been found in nearly every tissue tested, with especially high levels detected in kidney, brain, spleen, various gastrointestinal tissues, and prostate (Fisher et al., 1998b; Soderling et al., 1998b; Rentero et al., 2003; Wang et al., 2003). Although PDE9A mRNA is widely expressed in mouse and rat brain, it has a distribution pattern of varying intensity. Two studies have found PDE9A mRNA expression to vary by region but agree that the highest levels are in the olfactory bulb, dentate gyrus, Purkinje cells, and olfactory tubercle (Andreeva et al., 2001; Van Staveren et al., 2002). Expression was considered to be predominantly neuro-

nal, although some astrocyte expression was detected (Van Staveren et al., 2002).

5. Pharmacology/Function. No studies clearly elucidating a specific function for PDE9A have been published to date. However, the finding that PDE9A localizes to rat brain areas also expressing soluble guanylyl cyclase and neuronal nitric oxide synthase suggests that it may regulate cGMP signaling in these regions (Andreeva et al., 2001). Recently, the compound BAY 73-6691 has been reported as a potent (IC_{50} 55 nM) cell permeable PDE9 selective inhibitor (Wunder et al., 2005). This compound was identified using a novel cell-based assay that used a cell line stably expressing PDE9 and guanylyl cyclase. By indirectly measuring cellular cGMP levels using fluorescence indicators, this assay provides a measure not only of how test agents interact with PDE9 but also of how well they enter cells. This type of assay may serve as a tool and model for identification of future cell-permeable PDE inhibitors. BAY 73-6691 is currently under preclinical investigation as a possible treatment for Alzheimer's disease. Other PDE9 inhibitors have been reported to improve long-term potentiation and cognition in several animal models (Hendrix, 2005).

J. Phosphodiesterase 10 Family

1. Overview. The PDE10 family was initially reported essentially at the same time by three different groups (Fujishige et al., 1999a; Loughney et al., 1999; Soderling et al., 1999). To date there is only one gene in this family, *PDE10A*. At present, four variants, PDE10A1-4 (Fig. 13), have been described. Little is known about the function of PDE10, although inhibitors are reportedly under development for schizophrenia and Parkinson's disease. Recently, it was reported that the GAF domains of PDE10 have specificity for cAMP rather than cGMP (Gross-Langenhoff et al., 2006).

2. Biochemistry/Structure. PDE10A will hydrolyze both cAMP and cGMP in vitro, but it is not yet clear whether or not both cyclic nucleotides are substrates in vivo. Kinetically, the enzyme has a higher affinity for cAMP than for cGMP, but a roughly 2- to 5-fold lower V_{max} for cAMP compared with cGMP (Fujishige et al., 1999a; Loughney et al., 1999; Soderling et al., 1999). This is just the opposite from the PDE3 family enzymes.

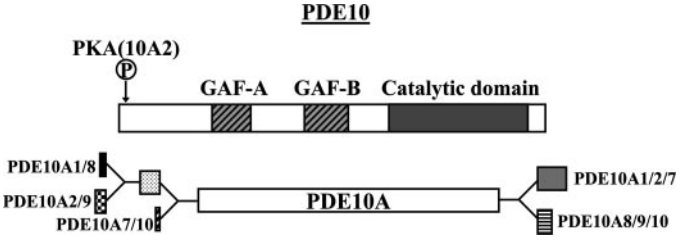

FIG. 13. The cAMP-inhibited PDE. Recent reports indicate the PDE10 GAF domain binding of cAMP. PKA phosphorylation of PDE10A2 seems to alter its subcellular localization. Only the human PDE10A variants are depicted in this diagram.

Therefore, there is, by analogy to the PDE3s, the possibility that it may function in vivo as a cAMP-inhibited cGMP phosphodiesterase. Nevertheless, little is known about how this enzyme's activity is regulated, either in vitro or in vivo. Recently, it was reported that cAMP will bind to one or more of the GAF domains of PDE10 (Gross-Langenhoff et al., 2006). This at least occurs for a chimeric protein of the PDE10 GAF domains with a bacterial adenylyl cyclase catalytic domain as the binding of cAMP stimulated cyclase catalytic activity. It remains to be seen whether there is a functional allosteric effect of cAMP on PDE10 in vivo.

3. Localization. The original reports showed relatively high levels of PDE10A in several tissues. The level of mRNA transcript is particularly high in the brain with the highest signal in the striatal area but substantial levels in the cerebellum, thalamus, hippocampus, and spinal cord (Fujishige et al., 1999a; Loughney et al., 1999; Soderling et al., 1999). Very high levels are also seen in the testis, much of which occurs in developing spermatocytes. PDE10 transcript levels are also easily seen in the thyroid and pituitary gland as well as in striated and cardiac muscle. Several rather detailed descriptions of PDE10 localization in the brain have been published (Fujishige et al., 1999b; Seeger et al., 2003).

The PDE10A2 variant is found largely in the particulate fraction of the cell. This variant can be phosphorylated on T16 by cAMP-dependent protein kinase (Kotera et al., 1999b). This phosphorylation seems to promote the translocation of the enzyme from its normal residence in the Golgi apparatus to the cytosol (Kotera et al., 1999b).

4. Pharmacology/Function. Little is definitively known about the functions of PDE10A. PDE10A localization to the caudate region of the brain suggests a role(s) in modulating striatonigral and striatopallidal pathways (Seeger et al., 2003). Thus, it has been suggested that PDE10 might be a good therapeutic target for treatment of psychiatric disorders of frontostriatal dysfunction (Rodefer et al., 2005). Supporting this idea, it was found that the semiselective PDE10A inhibitor, papavarine, reverses deficits in attentional set-shifting in rats caused by PCP (Rodefer et al., 2005). This is used as a model for the cognitive deficits suffered by schizophrenics. In addition, there is some indirect evidence that PDE10A may be involved in long-term potentiation in the hippocampus as the level of its mRNA has been reported to be elevated after long-term potentiation (O'Connor et al., 2004). PDE10A mRNA and protein levels are decreased in the striatal region of brains from Huntington's diseased transgenic mice and the decrease precedes the onset of symptoms (Hebb et al., 2004; Hu et al., 2004). Thus, it is postulated that loss of PDE10 may cause an alteration in cyclic nucleotide signaling that contributes to the loss of neuronal function produced by the disease.

Many of the compounds that inhibit other PDEs are poor inhibitors of PDE10 and only inhibit the enzyme at concentrations much higher than those for their specific families (Fujishige et al., 1999a; Loughney et al., 1999; Soderling et al., 1999). However, both of the nonselective PDE inhibitors IBMX and dipyridamole will inhibit PDE10 and papaverine has also been shown to be an inhibitor as well (Rodefer et al., 2005). Given the suggested involvement of PDE10 in processes affected in several neural disorders, this PDE will probably receive increasing attention from many investigators in the coming years.

K. Phosphodiesterase 11 Family

1. Overview. PDE11 is the most recently discovered PDE enzyme family and was first reported in 2000 (Fawcett et al., 2000). As the family was only recently identified, little is known about the PDE11 enzymes beyond their biochemical characteristics and basic genetics. Only one gene product, PDE11A, has been discovered. However, four variants of PDE11A have been identified (PDE11A1-4) (Fig. 14). The longest variant, PDE11A4, has two N-terminal GAF domains, whereas the other variants are truncations of this variant of varying lengths. Recently a controversy has developed about whether or not this PDE might be an additional target of the drug tadalafil.

2. Biochemistry/Structure. PDE11A enzymes hydrolyze both cAMP and cGMP, and little preference for either nucleotide is seen. For PDE11A both the K_m and V_{max} values for the two cyclic nucleotides are nearly equal, ranging from 1.0 to 6.0 μM for cAMP and 0.5 to 4.0 μM for cGMP, depending on the tissue and splice variant studied (Fawcett et al., 2000; Hetman et al., 2000a; Yuasa et al., 2000, 2001b). The K_m and relative cGMP/cAMP V_{max} values do not vary significantly among the variants. However, major differences in the absolute V_{max} values between the variants are reported to exist. From truncation mutagenesis studies it has been reported that the V_{max} generally increases with length for the human variants as the PDE11A2 and PDE11A3 have roughly 30- and 15-fold greater V_{max} values compared with PDE11A1, whereas PDE11A4 has a nearly 100-fold greater V_{max} than PDE11A1 (Yuasa et al., 2001b). However, the reported V_{max} for even the most active variant, PDE11A4, is only 270 nmol/min/mg (Yuasa et al., 2000) and is lower than many of the other PDEs. It is not yet clear if the reported kinetic constants are reflective of the native stable enzyme or if endogenous activators or inhibitors may be present in tissues. Although stimulation of PDE11A activity through binding of a cyclic nucleotide to their GAF domains has not been directly demonstrated, the observation that the variants with complete GAF domains have the highest activity suggests that activation may be taking place. Very recently it has been reported that the PDE11 GAF domains can confer cGMP stimulation on an *Anabaena* adenylyl cyclase (Gross-Langenhoff et al., 2006).

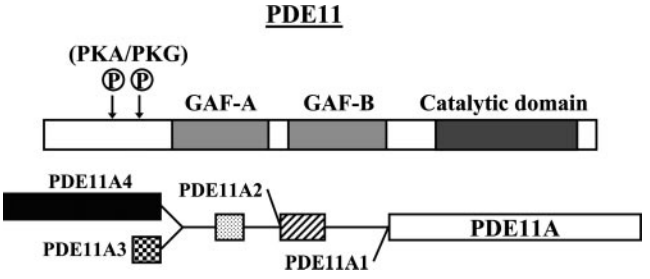

FIG. 14. The dual specificity cGMP-binding PDE. The four PDE11A variants differ largely in how many and how complete their GAF domains are. The significance of the PDE11 phosphorylation sites is unknown.

3. Genetics/Splicing. The *PDE11A* gene occupies >300 kb of genomic DNA and contains 23 exons of generally small size. The four PDE11A variants have different amino termini due to separate transcriptional start sites (Hetman et al., 2000a; Yuasa et al., 2000). Only PDE11A4 contains two complete GAF domains. Oddly enough, the separate transcriptional start sites for PDE11A1-3 result in production of proteins with incomplete GAF domains (Hetman et al., 2000). PDE11A1 completely lacks the N-terminal-most GAF domain, but contains the complete second GAF domain. PDE11A3 and PDE11A2 have one complete GAF domain and one partial GAF domain. The functional consequence(s) of these truncations is unknown, but the truncations do seem to affect catalytic activity.

4. Localization. Most of the localization studies for PDE11 have been for PDE11A mRNA products. The data for protein are less certain as few highly specific antisera have been described. There also is likely to be substantial differences in expression between species. Nevertheless, it is clear that the PDE11A variants demonstrate differential tissue expression. In humans, PDE11A1 mRNA is most prominent in skeletal muscle and prostate (Fawcett et al., 2000; Yuasa et al., 2001a). PDE11A3 mRNA is found specifically in testis and PDE11A4 mRNA is highly expressed in prostate (Yuasa et al., 2000). PDE11A protein localization studies have been somewhat contradictory in their findings, probably because of differences in the specificity of the antibodies used. PDE11A1 protein was originally detected in prostate and skeletal muscle (Fawcett et al., 2000), although a later study did not detect PDE11A1 protein in any tissues (Loughney et al., 2005). In fact, only PDE11A4 protein has been verified and is found in prostate, pituitary, heart, and liver. Another study suggested that PDE11A is widely expressed, and immunohistochemistry using an antibody reported to recognize all PDE11A variants localized it to the epithelial, endothelial, and smooth muscle cells of many tissues, but at highest levels in the prostate, testis, kidney, adrenal gland, colon, and skin (D'Andrea et al., 2005). However, a separate study did not find any PDE11 protein expression in human testis (Loughney et al., 2005). As with many

PDEs, it is still not clear if the same tissue, cellular, and subcellular localization is found among species. Future work will hopefully rectify the discrepancies regarding the tissue expression of PDE11A.

5. Pharmacology/Function. Little is known about the function of PDE11A. Furthermore, selective inhibitors of PDE11 have not been reported. However, recent studies with a PDE11 knockout mouse model have been interpreted to suggest that PDE11 may be important for sperm development and function. Ejaculated sperm from knockout mice displayed slightly lower sperm concentration and decreased viability compared with controls, and the sperm had a lower rate of forward progression (Wayman et al., 2005). However, the animals were fertile. Moreover, it is still not clear whether PDE11 is expressed in reproductive tissue of the mouse.

The expression pattern of PDE11 has become the source of some controversy recently because of the drug tadalafil. Of the three major PDE5 inhibitors commonly used to treat erectile dysfunction (tadalafil, sildenafil, and vardenafil), only tadalafil has been found to have a relatively high affinity for PDE11 with a reported IC_{50} of ~73 nM (Tables 4 and 5) (Card et al., 2004; Weeks et al., 2005). Therefore, some scientists speculate that tadalafil will be more likely to have nonspecific side effects than sildenafil or vardenafil because of inhibitory effects on PDE11. Given that tadalafil inhibits PDE5 with substantially higher affinity (IC_{50} ~10 nM), our lack of information about physiological roles for PDE11, and the very similar safety profiles for all three drugs, it will probably take long-term trials to determine the validity of these speculations.

TABLE 5

Multi-isoform inhibitors and secondary targets of selective inhibitors

Although many inhibitors are called "selective", this is really a relative term. The compounds are termed selective based on the fact that other isoforms are only inhibited at higher concentrations. Some compounds such as dipyridamole and zardaverine are selective for a limited number of isoforms. However, some of the inhibitors that target multiple isoforms may be useful because of this property. For example, dipyridamole is a component of the stroke treatment Aggrenox (aspirin/extended-release dipyridamole).

Inhibitor	PDE	IC_{50}
Sildenafil	PDE1	350 nM
	PDE6	50 nM
Tadalafil	PDE6	2 μM
	PDE11	73 nM
Vardenafil	PDE6	11 nM
Zaprinast	PDE1	6 μM
	PDE5	0.13 μM
	PDE10	22 μM
	PDE11	12 μM
Zardaverine	PDE3	0.5–2 μM
	PDE4	0.8–4 μM
Dipyridamole	PDE5	0.9 μM
	PDE6	125 nM
	PDE7	0.60 μM
	PDE8	9 μM
	PDE10	1 μM
	PDE11	0.4 μM
Trequinsin	PDE4	0.2–0.8 μM
	PDE2	~1 μM
IBMX	Multiple	2–50 μM

III. Concluding Remarks: The Future of "Phosphodiesterase Pharmacology"?

Research into the structure, function, and regulation of PDEs has progressed a long way from the initial identification of PDE activity nearly 50 years ago. The PDE enzymes are now well recognized to be important regulators of many different cellular functions. Research in the field has evolved from early studies primarily concerning identification of PDE enzymes and characterization of their kinetic and regulatory properties to more recent work on their structure and how their activities are regulated in the cellular context. This accumulation of basic knowledge should further foster the targeting of these enzymes for drug development and clinical treatment.

The entire field of PDE investigation has received enormous benefits from recent technological innovations. New tools have helped to overcome some problems that have plagued the field. For example, the high degree of identity among the active sites of many of the PDEs has made them challenging targets for identification of selective inhibitors. This problem is being addressed by the use of high throughput screening and X-ray crystallography. In particular the solution of several PDE catalytic domain crystal structures in the presence of drugs has yielded crucial information on the active sites of these enzymes and allowed rational design to improve inhibitor potency and selectivity. Both high throughput screening and crystallization are now able to be performed efficiently with robotics, further decreasing the cost and increasing the speed with which PDE selective inhibitors can be discovered. Unfortunately, much less effort has gone into identification of activators of PDEs although much of the same technology could be applied.

At the functional level the identification of new PDE enzymes has been greatly aided by developments in molecular biology. In particular the publication of the human and mouse genomes has led to the discovery of new PDE isoforms and the identification of variants of previously known ones. These advances have implicated the involvement of PDEs in many pathological conditions. Newer genetic techniques for probing PDE function such as RNA interference and tissue-specific conditional mouse gene disruptions and conditional RNA interference expression should allow for the validation of newly discovered enzymes as targets in specific diseases and forewarn of potential inhibitor side effects.

Several key problems still need to be addressed. For instance, it would be of great benefit to determine the structure of several PDE holoenzymes to fully understand their mechanisms of regulation. Therefore, substantial effort is being expended in this area. A concept gaining great support is the idea that different PDEs subserve different pools of cAMP and cGMP in the cell. We are only just beginning to be able to measure PDE

activity in subcellular compartments of the cell in real time. This is an area that clearly needs to be developed, including identification of molecular mechanisms that provide and control PDE subcellular localization. We also need to identify good animal models that accurately reflect the regulation and roles of PDEs in human tissues as many examples now are available for different modes of regulation between mice and men. At a minimum, putative regulatory schemes identified from studies in mouse, *Drosophila*, or zebrafish need to be tested quickly in human tissues.

Finally, despite the long history of PDE research and the potential of PDEs as high-quality drug targets, to date only a few PDE inhibitors have been approved for clinical usage. This is likely to change in the near future as the number of PDE inhibitors in clinical trials and in the drug company development pipelines has increased greatly in the last few years. The enormous clinical and financial success of the erectile dysfunction drugs has validated the concept that PDE inhibitors can be clinically successful and profitable and has attracted much commercial interest to the PDE superfamily. The future for PDE research seems bright as increased interest from pharmaceutical companies and academic researchers should accelerate the pace of discovery.

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