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ASPET Meeting Report

Multiple Cyclic Nucleotide Phosphodiesterases

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Introduction

The last several years have seen a renaissance of interest in PDEs as modulators of second messenger function. Much of the renewed interest has come from the recognition that there are a large number of different cyclic nucleotide-specific PDE isozymes, that these isozymes are differentially expressed in individual cell types, that they are differentially regulated, and that they are specialized to serve specific functions in the cells in which they are expressed. Currently >30 different isozymes are recognized. The realization that individual PDE isozymes are regulated in both positive and negative manners has highlighted the pivotal role played by PDEs in mediating cross-talk between second messenger pathways. It has also provided a conceptual basis for understanding many of the distinctive cell type-specific differences in the amplitude and duration of cAMP and cGMP signals produced in response to stimulation of adenylyl and guanylyl cyclase. The concept of differentially expressed and regulated PDE isozymes also implies that individual PDEs are likely to be good targets for therapeutic intervention in diseases caused or regulated by cyclic nucleotide-modulated transduction mechanisms. Pharmaceutical interest in the area has been further sparked by the promise that different PDE isozymes having distinct sequences at regulatory and catalytic sites should allow the development of selective therapeutic agents that can target a specific cyclic nucleotide pool in a very limited number of cell types. This in turn holds great promise of being able to limit the toxic side effects of many of the first-generation, nonselective inhibitors developed in the 1960s and 1970s.

The recent ASPET Colloquium on Multiple Phosphodiesterases held in Newport Beach, CA, on April 22–23, 1994, and the two companion ASPET Symposia held over the next 2 days at the Experimental Biology Meetings in Anaheim, CA, brought together >150 scientists having interests in this area. The Colloquium was the first in a series to be sponsored by ASPET on rapidly developing topics of interest to both academic and industry pharmacologists and biological scientists. It is the purpose of this report to briefly summarize some of the newer and more interesting information presented at these meetings. This report cannot begin to cover all of the information presented at the meeting. Therefore, it emphasizes data that had not been published before April 22, 1994, has just been published in the last few months, or was judged not to be widely known by many investigators.

Five sessions on different aspects of PDE function and regulation were held. The first three sessions were held at the Colloquium at Newport Beach and included discussions of Cellular Regulation, Pathophysiology, and Organ-Specific Expression and Function. The last two, which were held as part of the Experimental Biology Meeting in Anaheim, included discussions of topics concerning Structure, Function, and Localization and Roads to New Therapies. There was also a contingent of >60 posters presented. Most of these have printed abstracts available in the *FASEB Journal* (Vol. 8, No. 4, 1994). Finally, there was also substantial discussion about the inevitable difficulties in nomenclature caused by a rapidly expanding list of new PDE isozymes. Guidelines for a system tentatively approved by those attending are included in a later section of this article. A list of all PDEs for which sequence information is currently available in GenBank was included as part of the meeting program and is now available for electronic retrieval. The Internet address is < Gopher.hs.washington.edu >. This information can be downloaded via any Gopher retrieval system. It is the intention of the meeting organizers to update it three or four times each year.

Summary of New Data Reported at the Meetings

How many PDEs are there?

The data presented at the meeting suggest that there are at least seven different gene families of PDEs. Most of the families have been shown to contain two or more different genes and, for many, more than one splice variant has been shown to be expressed. In fact, a large portion of the meeting was taken up discussing methods and data relating to which of the putative PDE splice variants are real (i.e., expressed as proteins) and which ones might be artifacts of cloning or incomplete splicing. In several cases, distinct physiological functions related to the properties of the different splice variants can now be determined. These families are summarized in Table 1.

For convenience, the information presented at the meeting has been rearranged by isozyme family rather than strictly

ABBREVIATIONS: PDE, cyclic nucleotide phosphodiesterase; CaM, calmodulin; EHNA, *erythro*-9-(2-hydroxy-3-nonyl)adenine; 8-BDB-TcAMP, 8-[(4-bromo-2,3-dioxobutyl)thio]adenosine monophosphate.

TABLE 1
PDE gene families

Short name	PDE isozyme gene family	Number of gene products identified	Number of splice products	Evidence for additional members
PDE1	CaM-dependent PDEs	3	9+	Yes
PDE2	cGMP-stimulated PDEs	1	2	Yes
PDE3	cGMP-inhibited PDEs	2	2+	Yes
PDE4	cAMP-specific PDEs	4	15+	Yes
PDE5	cGMP-specific PDEs	2	2	Possible
PDE6	Photoreceptor PDEs	3	2	Possible
PDE7	HCP1-PDE[a]	1	1	Possible

[a] Newly reported and likely to be renamed when unique regulatory properties are identified.

according to the session in which it was presented. For the most part only those data that have not been previously published are discussed. In many cases poster session presentations that provided more detail were also made. Many of these poster presentations have abstracts that are published in the *FASEB Journal* (Vol. 8, No. 4, 1994). The nomenclature used in this report follows the recommendations discussed at the meeting (Table 2).

PDE1 family (CaM-dependent PDEs). A number of talks presenting new data regarding the PDE1 family (Ca^{2+}/CaM-dependent PDEs) were given. Dr. Bill Sonnenburg (University of Washington) described his experiments suggesting that there is not one but rather are two regions of PDE1A2 (61-kDa CaM-dependent PDE) that are required for CaM binding and activation. The data also suggested that there is an inhibitory region immediately adjacent to the CaM binding sites. He also summarized mass spectroscopic and mutagenesis experiments identifying serine-120 as the enzyme site phosphorylated by cAMP-dependent protein kinase that mediates the decreased affinity for Ca^{2+}/CaM caused by phosphorylation. Many of these latter data were summarized in a poster presentation (Abstract 465). This topic promoted much discussion about whether this phosphorylation mechanism was important physiologically, for example, in the process of long term potentiation in the hippocampus.

Drs. Ken Ferguson (ICOS Corporation) and Joe Beavo (University of Washington) both presented data describing a new member of the CaM-dependent PDE gene family (PDE1C). This gene appears to have at least five different splice variants (see Table 2). Dr. Ferguson presented information about two forms isolated from human brain and heart cDNA libraries and Dr. Beavo presented data suggesting that at least five different splice variants are present in mouse brain. *In situ* hybridization analysis utilizing a conserved region of the mouse sequence showed particularly high levels of PDE1C mRNA in the granule cell layer of the cerebellum and also in the mitral cell layer of the olfactory bulb. Of particular interest were extraordinarily high levels of expression in olfactory neurons. Because expression data from one of these forms indicated a preference for cAMP, compared with cGMP, when measured at low substrate levels, it is likely that this family of CaM-dependent PDEs mediate the rapid decrease in cAMP that occurs in many olfactory neurons in response to odorant stimulation. Finally, *in situ* analysis with the mouse probe also showed very high levels of mRNA in developing spermatocytes in the testis. In this tissue, this isozyme is likely to represent the high affinity, CaM-dependent PDE previously reported by several other investigators in rat and mouse testis. Some of the data presented were discussed in more detail in the poster sessions (Abstracts 469 and 470).

PDE2 family (cGMP-stimulated PDEs). Several interesting and previously unpublished observations about this family of PDEs were presented at the meeting. Of particular interest were the studies reported by Dr. R. Fischmeister (University de Paris-Sud). It is known that the activity of cardiac L-type Ca^{2+} channels is up-regulated by cAMP-dependent phosphorylation. This partly explains the positive inotropic effect of β-adrenergic agonists. By reducing the intracellular concentration of cAMP, PDEs are likely regulators of Ca^{2+} channel activity. Dr. Fischmeister has shown previously that in frog heart PDE2 activity is physiologically coupled to Ca^{2+} channels and its activity regulates the amplitude of cardiac calcium current. Intracellular perfusion of cGMP or external application of micromolar concentrations of n-morpholino-n-nitrosaminoacetonitrile SIN-1, a nitric oxide donor) induces a strong inhibition of cAMP-stimulated calcium current, which was shown to be mediated by PDE2 activation. The new data reported relate to the observation that the well known adenosine deaminase inhibitor EHNA is also a selective and potent inhibitor of PDE2 (cGMP-stimulated PDE). The IC_{50} for PDE2 is approximately 1 μM. This is substantially lower than the concentrations of EHNA commonly used to inhibit adenosine deaminase activity in cardiac tissues. When tested on isolated frog ventricular myocytes, EHNA antagonized the effects of cGMP, as expected from the earlier data presented by this laboratory. Dr. Fischmeister reported that EHNA is the same compound previously described (as MEP-1) by Dr. T. Podzuweit as a selective PDE2 inhibitor. The data are described in more detail in Abstract 472.

Dr. J. Thompson (University of South Alabama) presented evidence suggesting that PDE2 may be a major contributor to vascular endothelial cell function. Dr. P. Epstein (University of Connecticut) reported the cloning of a rat cDNA for PDE2 and its expression in several brain areas as well as in the submandibular salivary gland (Abstract 473). Dr. Beavo described data on a 5′ splice variant of the bovine enzyme, PDE2B. The different amino-terminal sequence appears to direct this isozyme to particulate fractions of the cell.

PDE3 family (cGMP-inhibited PDEs). It has been shown by several investigators that many of the metabolic effects of insulin on carbohydrate and lipid metabolism are mediated at least in part by activation of a low-K_m, cGMP-inhibitable, PDE activity. Dr. V. Manganiello (National Institutes of Health) summarized the present knowledge, largely based on data from his own laboratory, on the structure and mechanisms of regulation of these groups of isozymes. Insulin antilipolytic effects are dependent on a decrease in intracellular cAMP, which is caused by activation of a PDE (PDE3B). Recently, Dr. Manganiello and colleagues reported the cDNA cloning and characterization of PDE3B from adipose tissue. PDE3B is rapidly phosphorylated after insulin stimulation of the cell. Because the enzyme is phosphorylated mostly on serine and threonine and not on tyrosine, activation of intermediate kinases and not direct phosphorylation by the insulin receptor tyrosine kinase is responsible for PDE activation. New data were presented at this meeting on the characterization of the insulin-dependent kinase activity responsible for activation. The kinase does not bind to phosphotyrosine antibodies and is not one of the S-6 kinases. It does require vanadate to maintain activity and also

TABLE 2

PDE isozymes

Included are most of the human, bovine, and rodent cDNA references listed in GenBank or in recent literature references. Only those thought to be full length products or known to be catalytically active are listed.

Human Genome Project name	New GenBank name	Old locus	GenBank accession number	Alternate name	Comments
PDE1 gene family					
	BTPDE1A1			59-kDa CaM-PDE	
	BTPDE1A2	BOVCNPA	M90358	61-kDa CaM-PDE	
	BTPDE1B1A	BOVCALPHOS	M94867	63-kDa CaM-PDE	
	RNPDE1B1B	RATCAMPDE	M94537	63-kDa CaM-PDE	
	MMPDE1B1C	MUSPDE1B1	L01695	63-kDa CaM-PDE	
PDE1C	HSPDE1C			70-kDa CaM-PDE	5+ splice variants
	MMPDE1C			70-kDa CaM PDE	
PDE2 gene family					
	BTPDE2A1	BOVCGMPCN	M73512	cGS-PDE	
PDE3 gene family					
PDE3A	HSPDE3A	HUMGMPIPDI	M91667	cGI-PDE	
	RNPDE3B	RNCAMPPHA	Z22867	cGI-PDE	
PDE4 gene family[a]					
	RNPDE4A1A	RATDUNCEA	M26715	RD1	
	RNPDE4A1B	RATPHOSF	L27062	RPDE8	
	RNPDE4A2	RATDUNCEC	M26717	RD2	Contains a deletion
	RNPDE4A3	RATDUNCEB	M26716	RD3	
	RNPDE4A?.	RATPHOCNA	M25348	Rat PDE2	Small early clone
PDE4A	HSPDE4A4	HUMPDEAA	M37744	h-PDE1	
PDE4A	HSPDE4A5	HUMPDEA	L20965	PDE46	
PDE4A	HSPDE4A5?	HUMPDEC	L20967	DPDE2, TM3	Introns?
	RNPDE4A5	RATPHOSA	L27057	RPDE6	
PDE4B	HSPDE4B1	HUMPDEB	L20966	TM72	
	RNPDE4B1	RATDPD	J04563	DPD	
PDE4B	HSPDE4B2A	HSPDE2A	M97515		
PDE4B	HSPDE4B2B	HUMPDEG	L20971	PDE32	
PDE4B	HSPDE4B2C	HUMCAMPB	L12686		
	RNPDE4B2A	RATPHOCAMB	M25350	Rat PDE4	
	RNPDE4B2B	RATPHOSB	L27058	RPDE18	
PDE4C	HSPDE4C1	HUMPDED	L20968	DPDE1, PDE21	
	RNPDE4C1A	RATPHOCN	M25347	Rat PDE1	
	RNPDE4C1B	RATPHOSE	L27061	RPDE36	
PDE4D	HSPDE4D1	HUMPDEE	L20969	DPDE3, PDE39	
	RNPDE4D2A	RATPHOCAMA	M25349	Rat PDE3.1	
	RNPDE4D2B	RATPHOSD	L27060	RPDE13	
	RNPDE4D3	RATPDE32	U09456	Rat PDE3.2	
PDE4D	HSPDE4D4	HUMPDEF	L20970	DPDE3, PDE43	
	RNPDE4D4A	RATPHOSC	L27059	RPDE3	
	RNPDE4D4B	RATPDE33	U09457	Rat PDE3.3	
PDE5 gene family					
	BTPDE5A1	BOVPCGB8X	L16545	cGB-PDE	Not full length
PDE6 gene family					
PDE6A	HSPDE6A1	HUMCGPRA	M26061	ROS-PDEa	
	BTPDE6A1A	BOVCGMPO1	M27541	ROS-PDEa	
	BTPDE6A1B	BOVCGPDAG	M26043	ROS-PDEa	
	MMPDE6A1	MMPA	X60664	ROS-PDEa	
PDE6B	HSPDE6B1	HSCGMPPM	X66142	ROS-PDEb	
	BTPDE6B1	BOVCGMP	J05553	ROS-PDEb	
	MMPDE6B1A	MUSPDE	X55968	ROS-PDEb	Conflict with X60133
	MMPDE6B1B	MMPB	X60133	ROS-PDEb	
	BTPDE6C1	BOVPDE	M37838	COS-PDEa′	
PDE7 gene family					
PDE7A	HSPDE7A	HUMCAMPHOS	L12052	HCP1	Not full length

[a] Note some of the groupings of cDNAs listed in several of the families and particularly in this family are at present tentative because there is still some controversy among investigators about such issues as introns within sequences, possible cloning artifacts, and interpretations of cross species alignments. Updated information may be obtained by e-mail from the PDE Information Gopher Site listed earlier in this article.

phosphorylates PDE3A from platelets. The adipose PDE3B is also phosphorylated and activated by cAMP-dependent protein kinase. Phosphorylation by this kinase is on serine-427.

In platelets, cAMP is generally considered to be a primary regulator of function. Dr. R. Colman (Temple University) reported the isolation and characterization of cDNA clones for PDE3A (cGMP-inhibited PDE) from a human erythroleukemia cell line (HEL cells). PDE3A is an abundant, high affinity, cAMP-specific PDE that is probably a principal homeostatic regulator of basal cAMP levels in platelets. This pluripotent stem cell line can give rise to platelets, which are a rich source of what was thought to be a PDE3A based on earlier kinetic and enzymological studies. The data from Dr. Colman supported this earlier speculation, because the sequence was essentially identical to the human heart PDE3A isolated by Manganiello and colleagues. Ongoing studies have sought to further

define the kinetic characteristics of the PDE3s, by comparing the kinetic characteristics of the recombinant full length PDE3 with those of the proteolyzed PDE3 found in aged platelets. Dr. Colman also reported the use of a selective affinity label, 8-BDB-TcAMP, for PDEs. 8-BDB-TcAMP inactivates PDE3 in a rapid time-dependent manner, with a second-order rate constant of 0.031 min^{-1} mM^{-1}, presumably by interacting with a cysteine or tyrosine near the PDE3 catalytic site. Isolation of peptide fragments labeled with 8-BDB-TcAMP should facilitate mapping of the PDE3 catalytic site and comparison of this site with those of other cAMP PDEs.

Dr. K. Tang (McMaster University) gave a short talk and presented a poster describing the use of cGMP as a specific photolabel for PDE3. Interestingly, although selective inhibitors of PDE3 inhibited photolabeling in a manner expected from their affinities as inhibitors of activity *in vitro*, several did not have as great an effect in intact platelets as expected, indicating that substantial differences in membrane permeability may exist. More data can be found in Abstract 479.

Studies investigating the role of PDE isozymes as regulators of cardiac muscle contractility have demonstrated that the mechanisms for regulation of cAMP-dependent cardiac contractility by individual PDE isotype families have evolved differently in several animal species. Dr. M. Endoh (Yamagata University School of Medicine) reported that, in rabbit cardiac muscle, PDE3 localized at the sarcoplasmic reticulum has been identified as the primary modulator of cAMP levels and cAMP-dependent positive inotropic responses; milrinone (a selective PDE3 inhibitor) increased cardiac contractility and enhanced the inotropic effects of isoproterenol in rabbit cardiac muscle, whereas rolipram and Ro 20–1724 (selective PDE4 inhibitors) did not. In contrast, PDE4 apparently is an important regulator of rat cardiac muscle, where rolipram and Ro 20–1724 were demonstrated to increase cardiac inotropy in the presence (but not in the absence) of isoproterenol. Milrinone produced only modest positive inotropic effects and neither elevated cAMP levels nor enhanced the effects of isoproterenol in rat cardiac muscle. Interestingly, the data from rats suggest that PDE isozyme subtypes may differentially regulate basal and isoproterenol-stimulated cardiac contractility. Newly developed quinolinone PDE inhibitors, like OPC-18790, exhibit unique cardiac activity profiles, apparently by inhibiting both the PDE3 and PDE4 isozymes in cardiac muscle and perhaps by affecting myofibrillar responsiveness to intracellular calcium. OPC-18790, as well as several other related PDE inhibitors, caused a greater increase in force than did isoproterenol at doses that gave essentially equal effects on calcium levels. Ongoing studies will determine whether these quinolinone PDE inhibitors, by virtue of their distinct pharmacological profiles, possess clinically significant advantages over more traditional cardiotonic agents. Some of these data are reported in Abstract 481.

Another interesting general talk was the summary by Dr. W. Colucci (Harvard University) of the clinical data concerning the use of PDE3 inhibitors as therapeutic agents for congestive heart failure. He reviewed the results of the PROMISE Trial for Milrinone (Stirling Winthrop), as well as several other smaller studies. Most of the clinical data currently available are from studies using milrinone, although results of more recent studies with enoximone (Merrill Dow) and vesnarinone (OPC-8212; Otsuka) are now becoming available. The discussion centered around the fact that many investigators now think that most of the increased incidence of cardiac arrhythmias and other serious side effects that caused the initial clinical trials to be prematurely stopped may well have been due to the use of an excessively high dosage schedule. It is also now clear that the patients at highest risk for adverse reactions were those who were the most seriously ill (i.e., class IV). More recent analyses with less severely ill patients (and unfortunately with fewer patients) suggest that at lower doses there may be substantial improvement in patient status with no apparent increase in toxic side effects. The recent reports on vesnarinone, which is a very potent PDE3 inhibitor (as well as perhaps having additional sites of action) have shown not only an increase in cardiac performance but also a decrease in deaths. It seems quite possible that, as more confirmatory information becomes available, experimental use of selective inhibitors of this PDE isozyme family as therapeutic agents for cardiac disease may enjoy renewed attention. Interest is now focused not only on the use of lower doses, which would be likely to have effects only when sympathetic tone is increased, but also on the use of PDE3 inhibitors with different combinations of drugs other than those used in the PROMISE Trial.

Dr. John Colicelli (University of California, Los Angeles) reported a method for using the heat-shock resistance of yeast expressing plasmid coding for a cAMP-specific PDE to screen for mutants defective in drug binding. The method should have general utility for analyzing which parts of a PDE molecule are required for high affinity drug-binding interactions. Essentially any PDE that hydrolyzes cAMP should be able to be tested using any inhibitor that is able to easily enter the yeast in this assay. Dr. Colicelli also described the results of some initial deletion analyses of PDE3B using the yeast expression system.

Although T lymphocyte proliferation is regulated by intracellular cAMP, the roles of individual cAMP-metabolizing PDE isozymes in regulating this proliferative response are less well understood. Dr. R. Hurwitz (Baylor College of Medicine) reported the use of a synchronized cell proliferation assay using HT-2 lymphocytes. HT-2 cell proliferation, like primary T cell proliferation, appears to be under cAMP control; dibutyryl-cGMP was without effect on HT-2 cell proliferation, whereas dibutyryl-cAMP, 8-bromo-cAMP, and the nonselective PDE inhibitor isobutylmethylxanthine significantly inhibited proliferation. cAMP-metabolizing PDE isoforms from HT-2 cells could be separated into a particulate PDE that was characterized as PDE3 because it was inhibited by CI-930 (a selective PDE3 inhibitor) and a soluble PDE fraction that was activated by trypsin, calcium, or dilution. This latter PDE does not appear to fall neatly into an existing PDE isozyme family and may be a new form.

PDE4 family (cAMP-specific PDEs). There were a very large number of presentations concerning both the basic properties of the PDE4 genes and gene products and the identification of this family of PDEs as targets for therapeutic intervention in various inflammatory diseases. Several investigators, including Drs. M. Conti (Stanford University), K. Ferguson (ICOS Corp.), and G. Bolger (University of Utah) reported on the organization of the human and rat PDE4 genes (Abstracts 2129–2139). Dr. Ferguson's talk was particularly memorable, in that it was accompanied by a rather loud dance band in the adjoining meeting room. Dr. Conti and Dr. M. Houslay (University of Glasgow) both reported the development of antibodies and nucleotide probes able to distinguish between various

gene products within the PDE4 family, a few of which were able to distinguish between splice variants from a single gene (Abstracts 2132, 2134, and 2136). Immunological analyses of the cAMP-specific PDE forms expressed in different rat tissues have provided an initial indication that proteins of different molecular weights are derived from the same gene. Dr. Houslay also reported on a novel isoform that appears to be enriched in olfactory tissue (Abstract 2134) and on the probable role of the amino-terminal domain for conferring membrane binding to the PDE4 gene products.

Finally, Dr. Conti described a direct phosphorylation mechanism for activation of the PDE4 gene products (see also Abstracts 2140 and 2141). This is in addition to the previously described transcriptional activation for some of the PDE4 genes. Using polymerase chain reaction and cloning methods, it was demonstrated that the PDE4D gene contains multiple promoters that generate mRNA with different 5′ regions. These mRNAs encode three distinct proteins of different molecular masses (93, 72, and 68 kDa). Studies on the thyroid-stimulating hormone-dependent activation of cAMP-specific PDEs present in thyroid cells have demonstrated that only the 93-kDa protein is a target for this regulation. Studies using recombinant variants derived from the PDE4D gene have reached the same conclusion, that only PDE4D1 can be activated by cAMP-dependent phosphorylation. These studies therefore provide a functional explanation for the presence of more than one PDE protein derived from the PDE4 gene. One variant is activated by phosphorylation, whereas the others are probably constitutively active proteins. It seems likely that both of these mechanisms are important contributors to the tachyphylaxis many tissues exhibit with continued stimulation of adenylyl cyclase.

Therapeutic implications for selective PDE4 inhibitors, as well as the physiological roles for the PDE4 gene products, were investigated by several groups. Dr. J. Souness (Rhône-Poulenc Rorer Ltd.) used selective inhibitors developed by his company to show that it is possible to distinguish between the PDE4 isozymes expressed in guinea pig eosinophils and pig aorta. Drs. T. Torphy and M. Barnette (SmithKline Beecham) also presented data on the effects of various PDE inhibitors on airway reactivity and tracheal smooth muscle function. In response to questions from the audience, Dr. Torphy indicated that his company (SmithKline Beecham) has obtained some success in developing compounds that would distinguish between PDE4 isozymes. Dr. J. Hanifin (Oregon Health Sciences University) reviewed the relationships between cAMP levels, interleukin release, PDE activity, and selective PDE4 inhibitors in monocytes from patients with various atopic disease. Taken together, all of these results point to the potential importance of the PDE4 isozyme family as a target for the treatment of asthma and other inflammatory diseases.

Although selective PDE4 inhibitors possess admirable efficacy as both anti-inflammatory and bronchodilatory agents in animal models, it is not clear whether their other activities in nonpulmonary tissues will limit their use. It was noted by Dr. R. Heaslip (Wyeth-Ayerst Research) that, at intravenous doses 10–100 times greater than those that induce bronchodilation, most PDE4 inhibitors have caused emesis, stepping behaviors, sedation, anxiety, and tremors in dogs (Abstract 2146). Moreover, bronchodilatory doses of rolipram (a selective PDE4 inhibitor) also induced cardiovascular changes (tachycardia, increased blood pressure, and positive cardiac inotropy) in dogs that were associated with reversal of pentobarbital anesthesia in this species, activities that are clearly undesirable in an antiasthma drug. The challenge that remains is to determine the relevance of these observations to the pharmacological profile of candidate antiasthmatic PDE4 inhibitors in humans. Species-specific differences in the functions of PDE isozymes often make this difficult; whereas even bronchodilatory doses of PDE4 inhibitors produce significant behavioral effects in rats, these doses are associated with minimal cardiovascular system effects in this species. Clinical studies available to date indicate that in humans (as in dogs) the dose-limiting side effect of the PDE4 inhibitors is usually nausea, presumably resulting from the inhibition of neuronal PDE4. It remains to be determined whether a molecular basis for selectively modulating inflammatory cell and respiratory smooth muscle PDE4 versus neuronal PDE4 can be identified. The general feeling was that this should be possible. Finally, several of the speakers discussed new high affinity inhibitors of the PDE4 family. Probably the highest affinity described was for one of the Syntex compounds (RS14203) described by Dr. R. Alvarez (Syntex). It had an apparent IC_{50} of 0.023 nM.

A few studies on structure-function relationships among the PDE4 isozymes were discussed. Dr. T. Torphy (SmithKline Beecham) reported studies by his group utilizing site-directed mutagenesis to expand on the earlier studies from the laboratories of Drs. M. Conti and H. S. Ahn (Schering Plough) that indicated the importance but not absolute necessity of several of the conserved histidines for activity (Abstract 2143). Dr. J. Souness (Rhône-Poulenc Rorer Ltd.) and Dr. Torphy presented their evidence supporting the idea there may be a rolipram binding site on the PDE4 isozymes that is not identical to the catalytic site (Abstract 2145). Finally, Dr. R. Heaslip (Wyeth-Ayerst) reported on the studies of his group showing that phosphatidic acid activates some of the PDE4 isozymes and that rolipram shifts the activation curve to the right. Neither the site(s) on the enzyme for the phosphatidic acid effect nor the mechanism was elucidated in these initial studies.

PDE5 family (cGMP-specific PDEs). Dr. P. Silver presented data from his group at Stirling Winthrop on the use of PDE5-selective inhibitors as potentiators of guanylyl cyclase. Particularly impressive were the large effects of low doses of PDE5 inhibitors to potentiate the effects of nitric oxide-generating drugs on smooth muscle relaxation.

Dr. J. D. Corbin (Vanderbilt University) presented data on the cloning, expression, initial mutagenesis, and intact cell phosphorylation of the bovine PDE5 (Abstracts 2149 and 2150). The differences in sequence between this form and the photoreceptor PDEs provide the basis for splitting the cGMP-binding and photoreceptor PDEs into two different families (PDE5 and PDE6, respectively). The mutagenesis data suggest that at least two different types of cGMP binding sites are present on this PDE. Drs. Corbin and S. Francis also reported on several other aspects of PDE5 structure and function. Of particular interest was the hypothesis that the conserved Hx_3Hx–20E motif observed previously to be present in all PDEs may serve the function of a zinc binding site. These investigators provided evidence that zinc binds with high affinity to PDE5 and appears to be necessary for catalytic activity. They speculated that it may be required as part of the catalytic mechanism for all PDEs, because it serves a similar function in various other "zinc hydrolases" and because the sequence is conserved in all

TABLE 3
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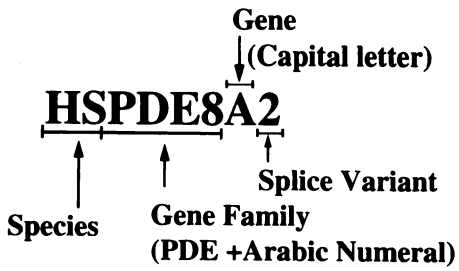

Fig. 1. GenBank/literature nomenclature.

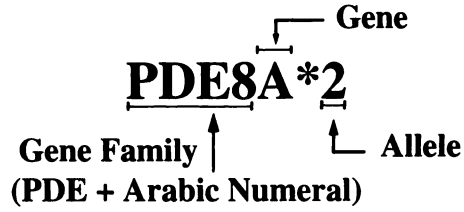

Fig. 2. Human Genome Project nomenclature.

PDEs (Abstract 2148). Dr. N. Pyne also presented some of the data from his laboratory on the activation of PDE5 by cAMP-dependent protein kinase. He also reported the interesting observation that the selective PDE5 inhibitor zaprinast is a chelator of Zn^{2+}, suggesting that this may provide part of the selectivity of this inhibitor for PDE5s.

PDE6 family (photoreceptor PDEs). It is well documented that cGMP-specific PDE (PDE6) is an effector enzyme in photoreceptor signal transduction. Its light-dependent activation by the retinal G protein transducin leads to a decrease in intracellular cGMP concentrations and closure of the cGMP-gated cationic channels of the photoreceptor plasma membrane. Dr. V. Arshavsky (University of Wisconsin) and Dr. T. Wensel (Baylor University) both presented data regarding possible mechanisms by which the GTPase activity of transducin can be altered by the inhibitory γ subunit of PDE6 or by other effectors in retinal extracts. Drs. Arshavsky and Bownds reported previously that in amphibians the GTPase activity of transducin is altered depending on whether it is bound to the PDE γ subunit or to the PDE $\alpha\beta\gamma$ complex. Moreover, cGMP binding to the noncatalytic sites on PDE inhibits the off rate of transducin from the PDE $\alpha\beta\gamma$-transducin complex. Thus, cGMP in the photoreceptor cell can feed back on the primary excitatory pathway by regulating the lifetime of activated PDE. In amphibians, cGMP dissociation from PDE noncatalytic sites occurs on the time scale of tens of seconds and may be a part of the mechanism of photoreceptor light adaptation.

Dr. T. Wensel described recent findings from his laboratory on the regulation and structure of the mammalian retinal cGMP PDE. The active form of the enzyme from rod cells was reported to be a tightly membrane-bound complex containing the inhibitory γ subunit of the PDE and the activated α subunit of the G protein transducin, as well as the catalytic α and β subunits. Inactivation of this complex by GTP hydrolysis was reported to be accelerated by a novel GTPase-activating protein that appears to be distinct from PDE and its subunits. In the absence of the membrane-bound GTPase-activating protein, the PDE γ subunit was found to have no effect on GTPase. During discussions of these two ideas it was pointed out that they may not be mutually exclusive, in that one pertained

largely to the dark-adapted state of mammals and the other to a substantially bleached state in amphibians.

Dr. Wensel also showed some of the first generation of high-resolution electron cryomicrographic images of photoreceptor PDE subunit complexes. Analysis of the rod PDE has revealed a compact structure with at least four globular domains and approximate dimensions of 40 × 80 × 120 Å. Thus, the initial data seemed to be biased towards images viewed down the long axis. This may have been due to the PDE molecule lining up nonrandomly at the interface. Nevertheless, with refinement it seems likely that the technique will be useful for determining the spatial relationships of the various subunits and perhaps, when used with antibodies or other domain-specific tags, it may be useful for even finer resolution.

In a poster session, Dr. S. Florio (University of Washington) presented the first data on cloning and characterization of the 15-kDa subunit of the soluble photoreceptor PDE6. Unexpectedly, this subunit did not appear to be highly enriched in cone outer segments, as might be expected from the fact that a 15-kDa band has been reported to be associated with the cone enzyme.

PDE7 family (HCP1 PDE). The only new data reported about the PDE7 family were in a poster by Dr. T. Bloom (University of Washington), who reported that, in addition to earlier reports of relatively high levels of mRNA for this PDE in skeletal muscle, PDE7 mRNA levels were also high in T cells (Abstract 2154). This PDE may be responsible for the high affinity activity reported recently by Dr. M. Ichimura (Kyowa Hakko Kogyo) in several T cell lines.

PDEs and disease. There is now a growing list of pathological states in which a defect in PDE activity has been postulated or shown to be an important contributing cause. Dr. T. Dousa (Mayo Medical School) presented new data regarding the involvement of PDE4 in diabetes insipidus. Also discussed at this meeting was the involvement of PDEs in the genesis of retinitis pigmentosa (Dr. D. Farber, University of California, Los Angeles) and atopic dermatitis (Dr. J. Hanifin, Oregon Health Sciences University). All of these disease states appear to be either caused by or associated with altered PDE activity.

Nomenclature

A request was made at the meeting for volunteers to form a committee whose purpose will be to help organize and, as necessary, revise a standardized system of nomenclature for existing and newly discovered PDE isozymes. Investigators who have volunteered to help edit this list include all of the meeting organizers, as well as several other scientists. Their names and addresses are listed in Table 3.

It was decided to utilize the electronic "mailbox" mentioned above < gopher.hs.washington.edu > for this purpose. The major function of this mailbox is to provide an easily accessible source for the most current data concerning the names and descriptions of all PDEs that are already known. In addition, authors wanting to list any new PDEs that they discover will be able to contact one of the committee members and be assigned an appropriate name based on the set of criteria described below. Suggestions for changes also can be made directly to any of committee members. The criteria for naming are summarized in Fig. 1 for a theoretical new entry into the LOCUS field of GenBank. The first two letters represent the species, for example HS for *Homo sapiens*. The next three letters plus an Arabic numeral designate the PDE gene family. The next letter represents the individual gene product within the family. The final Arabic numeral represents the splice variant, and the final letter allows GenBank to assign a unique locus field designation based on when the entry was submitted and also to give different locus names to conflicting or incomplete sequences. It is hoped that most investigators will change their current GenBank LOCUS entries to this system and will in the future use this nomenclature in the titles and abstracts of their manuscripts and in their GenBank submissions, so that a common order for nomenclature may be instituted. This system should allow for future expansion (at least until the number of gene families or splice variants exceeds 99 or the number of genes within any one gene family exceeds 26). It was also the consensus from discussion that more descriptive names based on regulation and function continue to serve a useful role in the text of most manuscripts. For examples, a paper on a PDE6 family member would be likely to include the terms "light-activated," "ROS," or "photoreceptor PDE"; similarly, one discussing the PDE2 family would be likely to include the terms "cGMP-stimulated PDE" or "cGS-PDE."

A similar nomenclature system has been approved for adoption by the Human Genome Project Nomenclature Committee, with the following differences. First, they omit the species, because all are human sequences. Second, they use an asterisk followed by a second Arabic numeral to represent alleles, rather than splice variants. The theoretical example shown in Fig. 1 for a GenBank entry would be as shown in Fig. 2 for a Human Genome Project entry. It is expected that the few current names in the Human Genome Project databank will be changed to reflect this new nomenclature.

Concluding Remarks

A particularly encouraging aspect of these meetings was the large number of new young investigators in attendance. These scientists came not only from academic institutions but also from pharmaceutical and biotechnology firms. The renewed interest in this area, particularly by young investigators new to the field, promises an increasing output of information in the coming years. The fact that many different firms are now starting to bring isozyme-selective PDE inhibitors to clinical trials also is an encouraging sign. It seems likely that this important area of signal transduction will continue to develop in the next few years.

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