Cyclic Nucleotide Phosphodiesterases: Functional Implications of Multiple Isoforms

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Beavo, Joseph A. Cyclic Nucleotide Phosphodiesterases: Functional Implications of Multiple Isoforms. Physiol. Rev. 75: 725-748, 1995.—In the last few years there has been a veritable explosion of knowledge about cyclic nucleotide phosphodiesterases. In particular, the accumulating data showing that there are a large number of different phosphodiesterase isozymes have triggered an equally large increase in interest about these enzymes. At least seven different gene families of cyclic nucleotide phosphodiesterase are currently known to exist in mammalian tissues. Most families contain several distinct genes, and many of these genes are expressed in different tissues as functionally unique alternative splice variants. This article reviews many of the more important aspects about the structure, cellular localization, and regulation of each family of phosphodiesterases. Particular emphasis is placed on new information obtained in the last few years about how differential expression and regulation of individual phosphodiesterase isozymes relate to their function(s) in the body. A substantial discussion of the currently accepted nomenclature is also included. Finally, a brief discussion is included about how the differences among distinct phosphodiesterase isozymes are beginning to be used as the basis for developing therapeutic agents.

I. INTRODUCTION

A. Historical Perspective

Cyclic nucleotide phosphodiesterase (PDE) activity was first described shortly after the discovery of adenosine 3',5'-cyclic monophosphate (cAMP) by Dr. Earl Sutherland and co-workers. In fact, the existence of an enzyme activity able to catalyze the degradation of this molecule was used as one of the initial pieces of supporting evi
idence that cAMP is a physiologically relevant molecule. Similarly, the discovery that most preparations of PDE would also hydrolyze guanosine 3',5'-cyclic monophosphate (cGMP) was one of the more important early criteria for the physiological relevance of this second messenger molecule.

The initial purification and characterization of PDE activity was reported by Butcher and Sutherland in 1962 (26). These and subsequent early studies documented the inhibitory effect of the methylxanthines, caffeine, and theophylline on PDE activity and also demonstrated the specificity of the reaction for the hydrolysis of the 3'-phosphoester bond of the 3',5'-purine ribose cyclic monophosphate cAMP and cGMP. It was soon discovered that PDE activity from some tissues could be activated by calcium and a small heat-stable molecule now known as calmodulin (CaM). In fact, many of the initial studies of CaM were made in an effort to determine the mechanism by which Ca²⁺ activated PDE1 (32).

With the development of PDE assays based on radio-labeled cyclic nucleotides (41,77), investigators were able to measure PDE activity at substrate levels nearer to their physiological concentrations. It soon became clear that several PDE activities, each having different substrate specificity and kinetic properties, could be separated from most tissues (9, 158). Since that time, many of these PDE activities have been isolated, purified, and characterized (reviewed in Ref. 6). More recently, additional isozymes have been identified using techniques of molecular biology. A major purpose of this review is to summarize much of the more recent data relating to the number, localization, regulation, and physiological roles for these expanding families of PDEs. It also introduces a very brief review of isozyme-selective PDE inhibitors and their clinical applications.

B. Current Concepts for Roles and Functions of PDE Activity

1. Modulation of amplitude and duration of signal

The very high catalytic activity of PDE in tissue extracts coupled with the very low efficacy of AMP and GMP on the known targets of cAMP and cGMP have led to the assumption that the major function for PDEs in the cell is to terminate the cyclic nucleotide second messenger signal. Phosphodiesterases have been most commonly thought of as regulators of the steady-state levels of cAMP and cGMP and, therefore, the functions of these nucleotide second messengers. More recently, the realization that in many tissues cyclic nucleotide levels oscillate rapidly has emphasized the likely importance of the PDEs for modulating not only the amplitude of a cyclic nucleotide signal but also its duration. The best example of this is the role played by the photoreceptor PDEs in the modulation of cGMP in response to changes in light intensity. These changes in cGMP in turn modulate the activity of a cGMP-gated cation channel in the photoreceptor outer segment membranes on a millisecond time scale. However, it is likely that similar functions for other PDEs in the modulation of synaptic transmission, cardiac contractility, platelet aggregation, and odorant transduction are also operative. Importantly, differences in specific isozyme expression are now thought to be controlling factors in the "cyclic nucleotide phenotype" of a cell, i.e., the duration and size of the cyclic nucleotide signal that is elicited in response to a given agonist. Finally, it is now becoming clear that regulation of PDE activity is a major mechanism by which many cells modulate their response to prolonged agonist stimulation.

2. Regulation by different pathways

Phosphodiesterases also are now thought of as major mediators of "cross talk" between different second messenger signaling pathways. For example, many of the effects of Ca²⁺ on the amplitude and duration of cAMP and cGMP signals are mediated through activation of CaM-dependent PDE activity and probably also by Ca²⁺/CaM-dependent phosphorylation of PDEs. This type of control is often in addition to effects of Ca²⁺ on cAMP or cGMP synthesis. Similar scenarios for both positive and negative effects of cGMP effects on cAMP levels have also been extensively discussed for the cGMP-inhibited and cGMP-stimulated PDE families (ii).

II. PHOSPHODIESTERASE GENE FAMILIES

A. Basis for Classification Into Gene Families

Once primary protein sequence and initial cDNA isolation and sequencing had been accomplished for several of the different PDEs, it became clear that a number of different cyclic nucleotide PDEs gene families exist (7). Currently, seven different but homologous families are recognized. A classification based on both primary sequence and regulation is discussed in more detail in section III. All PDEs contain a core of ~270 amino acids that are highly conserved (29) and are likely to make up the catalytic domain of the enzyme. In each case where there has been substantial time for analysis, each family has been shown to contain more than one gene product. Overall, the PDEs within each family are 65% or more homologous with each other when compared at the amino acid level. However, when compared between different families, the similarity drops to <40%, with most of this occurring in the catalytic domains.

B. Nomenclature

Initially, PDEs were classified solely on the basis of their known regulatory properties and to a certain extent
on their elution patterns from DEAE-cellulose column chromatography. There was a tendency to name PDEs based on their order of discovery within a laboratory and/or by homology with other known PDEs. As might be expected, this approach had major drawbacks as the field developed, particularly as new and often closer homologies were discovered. It is clear that as in any developing field, nomenclature will have to evolve as new information is reported. An attempt has been made recently to organize and reorganize the existing nomenclature in a manner that takes into account all of the existing published data (8). The most current general nomenclature method is summarized in Figure 1. In the top panel of Figure 1, the general designation for an as yet undiscovered PDE8 isolated from a human source is given. The bottom panel of Figure 1 gives an abbreviated version, as is used in the Human Genome Project nomenclature.

More specific nomenclature data, including both old and new names as well as GenBank accession numbers for all of the known PDEs of which I am aware, are listed in Table 1. These names have been adopted recently in an American Society for Pharmacology and Experimental Therapeutics (ASPET) sponsored symposia on multiple PDEs that was held last year in Newport Beach, California, and most have been published previously in a report summarizing that meeting (8).

C. Alternative Splice Variants of PDE

The application of cloning, polymerase chain reaction, and other techniques of molecular biology to the problems of PDE number, function, and regulation has resulted in an explosion of information. One of the more interesting consequences has been the realization that most PDE genes have more than one alternatively spliced mRNA transcribed from them. Moreover, in many cases, the alternative splicing appears to be highly tissue specific. This provides a mechanism for selective expression of different isoforms in individual tissues and cell types. Cell type-specific expression in turn strongly suggests that the different isoforms are likely to have different cell type-specific properties. It also provides a probable molecular explanation for many of the different kinetic forms of PDEs described in the past 30 years. Clearly one of the major tasks of the next few years is for investigators to sort out which isozyme variant is expressed where and perhaps more importantly what functional consequences result from the differential expression of unique gene products and the individual splice variants of them.

The new approaches and resultant explosion of information have also brought about a whole new set of problems, not the least of which is the question of how many of the new cDNA clones for putative PDEs are "real," and how many might be due to artifacts of cDNA library construction or incomplete intron excision. Actually, in only a few cases have the properties of the expressed protein been firmly linked to the properties and function of well-characterized PDEs isolated by more conventional means. In many cases, the "new" PDEs are known only by their nucleotide structure, and little kinetic or regulatory information is available.

III. PHYSIOLOGICAL REGULATION AND ROLES FOR INDIVIDUAL PHOSPHODIESTERASE ISOZYMES

A. PDE1 Family

1. Number of family members

At present, there are three different genes described within the CaM-stimulated PDE family (PDE1). Each of these appears to have at least two different alternative splice variants. The isoforms within this family were first distinguished on the basis of their apparent molecular mass on sodium dodecyl sulfate-polyacrylamide gel electrophoresis and called the 58- to 59-kDa (lung, heart), the 61 kDa (brain), the 63 kDa (brain), and the 75 kDa isoforms (brain). We now know that this is a greatly over-simplified scheme, both in terms of the number and localization of the different PDEs that these terms were used to represent.

More specifically, the 58-kDa lung isoform (142), the 59-kDa heart isoform (68, 73, 91), and the 61-kDa brain isoform (140) are now known to be the products of one gene currently called PDE1A. These conclusions are based largely on protein sequence and cDNA cloning studies carried out in the last few years (31, 124, 148;
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<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSPDE7A1</td>
<td>HUMCAMPHOS</td>
<td>L12052</td>
<td>HCP1</td>
<td>cAMP specific PDE</td>
<td>003-1499</td>
<td>5'-Truncated, not full length</td>
</tr>
</tbody>
</table>

Most of the human, bovine, and rodent cDNA references listed in GenBank or in recent literature references are included. ORF, open reading frame; PDE, phosphodiesterase; CaM, calmodulin; Kₘ, Michaelis constant; CA kinase, cAMP-dependent protein kinase. Routinely updated information containing much of the data shown here is available for electronic retrieval via the Internet gopher system at the following address (gopher://hs.washington.edu). On the World Wide Web, the address is (gopher://www.hs.washington.edu).
perhaps due to a contaminant in the preparation. Therefore, it is not yet clear whether or not this peptide is actually a part of a unique heart isozyme or if it was due to a contaminant in the preparation.

It is now clear that the 63-kDa isozyyme, originally described in bovine brain (140), is the product of a different gene (13). This enzyme does contain a few short sequences in common with the 59/61-kDa isozyyme, which probably accounts for early reports of antibody cross-reactivity, but is otherwise entirely distinct. This CaM-stimulated PDE family is now called PDE1B.

One of the first indications that there might be a third gene family was the report of a 74-kDa PDE having a substrate preference for cGMP (143). This difference in substrate specificity suggested that it might have a different structure from the other CaM-PDEs known at that time. There were also several reports of very-high-affinity CaM-dependent PDE activities, which also suggested additional family members. The recent discovery of several cDNAs for a group of PDEs having predicted molecular masses of ~70–80 kDa may tie these early observations together (99, 174). This PDE family is now called PDE1C. There appear to be at least five different 3'-splice variants of this gene (99, 174). It is not yet clear, however, whether or not these PDEs actually are responsible for the early brain 74-kDa form. At least two of the PDE1C variants do have a high affinity for cAMP. One of these is highly expressed in olfactory receptor neurons and is likely to mediate some aspects of odorant-induced signal transduction (176).

2. Substrate specificity

The substrate specificity of the different CaM-dependent PDEs is still a matter of some confusion and controversy. In general, two different types of CaM PDE activity have been reported. A so-called low-affinity form, which we now know contains several different CaM-PDE isozymes, has been widely studied (reviewed in Ref. 167). A relatively uncharacterized high-affinity form has also been reported in several tissues (18, 57, 147). As noted above, it is likely that a number of different isozymes of high-affinity CaM-dependent PDE are also present in different tissues.

The "classical" PDE1A isozymes (59- and 61-kDa CaM-PDEs) have been reported to have Michaelis constant ($K_m$) values for cAMP in the range of 34–40 $\mu$M and $K_m$ values for cGMP in the range of 2–3 $\mu$M (167). The maximum velocity ($V_{max}$) ratio for cAMP/cGMP is ~2 for this isozyyme. The PDE1B isozymes (63-kDa CaM-PDEs) have reported $K_m$ values for cAMP and cGMP of ~11 and 1 $\mu$M, respectively, but a $V_{max}$ ratio of 0.3. In general, a wide range of kinetic constants for CaM-PDE activity has been reported (167). Part of the reason for the wide variation between values reported in the literature was the lack of appreciation in most early studies for the large number of different CaM-PDE isozymes present in many tissues. We now know that many of the supposedly pure isozymes used in these second generation studies were in fact not kinetically pure. For example, in my laboratory, we have recently shown that the "heart 59-kDa CaM-dependent PDE" isolated by ACC-1 monoclonal antibody affinity chromatography is likely to contain at least two different kinetic variants (12). In this case, the second form appears to be one of the high-affinity PDE1C isozymes recently discovered to be present in heart and other tissues (99, 174). A similar situation is now thought to occur in several other tissues including brain. The problem of interpretation of kinetic data obtained from most early CaM-PDE preparations is magnified by the fact that even a relatively small contamination of an isozyyme having a much higher affinity for one nucleotide can greatly change the ratio of cAMP to cGMP hydrolysis at low substrate levels. This effect also greatly complicates interpretation of drug inhibi-

<table>
<thead>
<tr>
<th>PDE</th>
<th>Family</th>
<th>$K_m$, $\mu$M</th>
<th>$V_{max}$ (cAMP/cGMP)</th>
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</thead>
<tbody>
<tr>
<td>PDE1A2</td>
<td>cAMP: 113 ± 8</td>
<td>cGMP: 5.0 ± 0.6</td>
<td>2.9 ± 0.1</td>
</tr>
<tr>
<td>PDE1B1</td>
<td>cAMP: 24 ± 2.9</td>
<td>cGMP: 2.7 ± 0.2</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>PDE1C2</td>
<td>cAMP: 1.2 ± 0.1</td>
<td>cGMP: 1.1 ± 0.2</td>
<td>1.2 ± 0.1</td>
</tr>
</tbody>
</table>

$K_m$, Michaelis constant; $V_{max}$, maximum velocity ratio.
tion for CaM-PDEs in several brain areas (101). More recently, in situ hybridization analysis using probes to three different CaM-PDE isozymes shows very distinct localization to different areas of the brain and peripheral nervous system (87, 128, 175). An example of this type of localization from the work of Yan et al. (175) is shown in Figure 2.

It is of interest to note that a strong hybridization signal for PDE1A is seen in the cortex and also the CA1 and CA2 regions of the hippocampus. On the other hand, PDE1B is particularly high in the striatal region and also in the granule layer of the dentate gyrus. An obvious implication here would be that the kinetic and regulatory properties of PDE1A may be important toward the normal functioning of the hippocampal CA1 and CA2 regions. As discussed in section 4.5, this might have to do with the fact that PDE1A is a substrate for cAMP-dependent protein kinase, and phosphorylation inhibits
its activity. Similarly, the properties of the PDE1B would be predicted to be compatible with the cyclic nucleotide regulation of dentate granule cell function. Because this enzyme is likely to be regulated by CaM kinase II, a functional regulation including phosphorylation by this kinase is likely. Similar arguments regarding dopamine function and PDE activity in the striatal area can be envisioned.

4. Regulation by Ca\textsuperscript{2+}/CaM

The PDE1 gene products are called Ca\textsuperscript{2+}/CaM-dependent PDEs because of their requirement for Ca\textsuperscript{2+} and CaM for activity. It has been known for some time that the Ca\textsuperscript{2+}-CaM complex will increase the activity of any of the isozymes at least 10-fold. The kinetic mechanism seems to be largely due to an increase in the V\textsubscript{max} of the enzyme, although small changes in K\textsubscript{m} have also been noted. Initial data suggested that CaM bound to a single NH\textsubscript{2}-terminal domain on the enzyme and a high-affinity CaM binding peptide from this region have been identified (31). More recent studies from my laboratory strongly suggest that there is an additional CaM binding site immediately adjacent to the initially identified NH\textsubscript{2}-terminal region. The data would suggest that there is also an inhibitory domain present in this area. The mechanism of activation by CaM is to remove the influence of the inhibitory domain.

Because the total amounts of CaM do not change rapidly in the cells, most investigators think that regulation of the enzyme in vivo is most likely due to changes in Ca\textsuperscript{2+}, which in turn determines the amount of active Ca\textsuperscript{2+}-CaM complex available. The situation in the cell is complex because of competition for the active Ca\textsuperscript{2+}-CaM complex between the PDEs and other CaM binding proteins. Additional regulation is introduced by Ca\textsuperscript{2+}/CaM activation of other enzymes that modulate cyclic nucleotide levels. Examples include Ca\textsuperscript{2+}/CaM activation of some of the isozymes of adenylyl cyclase, Ca\textsuperscript{2+}/CaM-dependent protein phosphatases, CaM kinase II, CaM kinase IV, and Ca\textsuperscript{2+}/CaM-dependent nitric oxide synthase. Therefore, differences in the affinity for CaM of these enzymes as well as the relative affinities for CaM of the PDEs versus the cyclases will be determining factors in the overall cyclic nucleotide phenotype of the cell. In other words, the amplitude and duration of the cAMP or cGMP signal will be directly determined by which specific isozymes of CaM-dependent cyclase and PDE are expressed. They will also be indirectly influenced by other CaM-dependent enzymes that impinge on the pathway.

5. Regulation by phosphorylation

At least three of the CaM-dependent PDEs are regulated in vitro by phosphorylation/dephosphorylation. Both the 59-kDa heart isozyme and the 61-kDa brain isozyme (PDE1A1 and PDE1A2) are phosphorylated by cAMP-dependent protein kinase (70, 141). The 63-kDa isozyme (PDE1B) is phosphorylated by CaM kinase II (70). They can be dephosphorylated by CaM-dependent phosphatase. The effect of phosphorylation is to decrease the affinity of the enzyme for CaM. In the cell, this should be the equivalent to decreasing the affinity for Ca\textsuperscript{2+}. Two serines, serine-120 and serine-138, have been identified as the sites of phosphorylation of PDE1A in vitro (53). Site-directed mutagenesis suggests that it is serine-120 that is responsible for the CaM affinity change.

6. Possible role in plasticity

The physiological role(s) for phosphorylation of these enzymes is not yet clear. However, in vitro studies would suggest that the effect of phosphorylation should be to decrease PDE activity. This in turn would be expected to further increase the steady-state level of cAMP in the cell. This type of control loop in a cell is unusual in that it is “feedforward” and therefore potentially irreversible. However, in this case, any signal pathway that causes an increase in Ca\textsuperscript{2+} in the cell would allow reversal to occur. One possible role suggested by the postsynaptic localization data mentioned above would be for PDE1A to mediate some of the effects of cAMP on synaptic transmission. In this case, initial small changes in cAMP and cAMP-dependent phosphorylation would lead to an augmentation of the amplitude and duration of the cAMP signal. This in turn would allow a small signal initiated at a nerve ending to reach the cell body and nucleus. Once there, longer term changes mediated by phosphorylation of transcription factors like cAMP response element binding protein could promote more lasting changes in the synapse. An increase in Ca\textsuperscript{2+} could then reset the system. Similar scenarios for effects of phosphorylation of CaM-PDEs in smooth and cardiac muscle could be envisioned. A general theoretical model for such a feedforward interaction is shown in Figure 3.
7. Possible role in olfaction

The localization of a CaM-dependent PDE1C in the olfactory neuronal epithelia suggests that this isozyme may play a major role in olfactory signal transduction. It has been known for some time that a high-affinity Ca2+/CaM-dependent PDE is present in olfactory neuronal cilia (18). Recently, PDE1C has been implicated as being at least one of the PDEs that makes up this activity, and it seems likely that it will be intimately involved in regulation of the amplitude and duration of the odorant second messenger signal(s), as illustrated in the model shown in Figure 4.

For the case illustrated in Figure 4, it is known that many odorants will cause a rapid but transient increase in cAMP (22). This second messenger is then thought to bind to a CAMP/cGMP-dependent cation channel in the membrane of the cilia, causing a depolarization and initiation of the neuronal signal. It is also known that the duration of the signal can be very brief, <100 ms. Because both Ca2+ and Na+ are permeable to this channel, it seems likely that a major reason the cyclic nucleotide signal is rapid is due to the fact that the type III adenylyl cyclase is synergistically stimulated by the initial Ca2+ entry. One reason that it is brief is because as more Ca2+ enters, the PDE1C is stimulated. Because most PDEs have greater catalytic capacity than cyclases, the levels of cyclic nucleotide drop quickly. There is also evidence for phosphorylation of odorant receptors and inhibition of adenylyl cyclase by higher (Ca2+) as additional methods of attenuating the cAMP signal (22). Therefore, the system is tuned to be able to make rapid distinctions in amounts of odorant as would be necessary in many situations for the animal. It also seems quite possible that the inositol 1,4,5-trisphosphate (IP3)/Ca2+, a second messenger system activated in response to other odorants, also could elicit at least part of its effects via this pathway.

8. Role in cross talk

The general role that CaM-dependent PDEs play in the coordination of signaling pathways that work through increases in Ca2+ and those that work through cAMP or cGMP is clear. For example, in some tissues, muscarinic agonists increase Ca2+ and also decrease cAMP and/or cGMP. In many of these cases, part or all of the effects on cyclic nucleotide levels are mediated via stimulation of a CaM-dependent PDE. Early examples of this include the effect of muscarinic agonists to activate CaM-PDE activity in 1821N1 astrocytoma cells (154), thyroid cells (45), and WI 38 fibroblasts (120). These early studies have not, however, identified which isozyme(s) of CaM-PDE was present in these cells nor did they address possible effects on cGMP levels. It is likely the coordination of calcium effects on cAMP and cGMP levels will be very complex in many cells, since both the PDEs and cyclases can be stimulated by Ca2+ (guanylyl cyclase indirectly via nitric oxide synthase). Several studies suggest that at least some isozymes of adenylyl cyclase are activated at lower concentrations of Ca2+ than the CaM-PDE (33, 34, 167). However, the dose-response curve is biphasic, and Ca2+ becomes inhibitory at higher concentrations. It has been known for many years that in most tissues the maximal catalytic activity of fully stimulated phosphodiesterase is much greater than that of adenylyl or guanylyl cyclase. Therefore, one would expect that at the initial low concentrations elicited, for example, in response to opening of an IP3 or voltage-gated channel, Ca2+ would be expected to first increase cAMP via stimulation of cyclase, then later decrease it again as the higher calcium levels stimulated the more active PDE. This is of course just what happens in many cell types. Therefore, a cell can regulate the amplitude and duration of either cAMP or cGMP depending on the relative affinities for Ca2+/CaM of the
adenylyl cyclase and PDE that it expresses. The kinetics of the response depend on which isozymc(s) is expressed and also on the phosphorylation state of the enzyme(s).

B. PDE2 Family

1. Number of family members

At present, only one gene has been identified for this PDE family. However, at least two different 5'-splice variants are known (177). Indirect evidence would suggest that a third form may be present in liver and perhaps other cells (75).

2. Substrate specificity: regulation by cGMP

Both cAMP and cGMP are hydrolyzed by this enzyme, and in fact, the \( V_{\text{max}} \) values for both are very similar (107). Both substrates show positively cooperative kinetic effects of substrate with Hill coefficients of 1.9 and 1.3 for cAMP and cGMP, respectively. The cGMP-stimulated PDEs are unique in that they contain a noncatalytic binding site having high specificity for cGMP. When cGMP binds to this site, the affinity of the catalytic site is increased by allosteric interactions such that a large increase in activity occurs under normal substrate conditions. This results in a marked stimulation of activity when cAMP is the substrate. The activity increase is transient unless cGMP synthesis is constantly elevated, since the enzyme will hydrolyze both cAMP and cGMP.

3. Localization

In the adrenal cortex and also in several areas of the brain, PDE2 is found in very high concentrations. Recent studies in my laboratory also suggest that it is highly expressed in a subset of goblet cells and also in a subset of olfactory neurons (D. Jullis and J. Beavo, unpublished data). The physiological function of this isozyme in most tissues is still not entirely clear. Presumably, it mediates much of the effect of cGMP on cAMP metabolism in the tissues where it is expressed. In fact, the tissues in which it is expressed are in general those in which the effects of cGMP are opposite to those of cAMP. Several examples are given below.

4. Role in atrial natriuretic peptide regulation of aldosterone

It has been known for several years that one of the major effects of atrial natriuretic peptide (ANP) on natriuresis is mediated via its inhibitory effects on aldosterone synthesis. The demonstration by MacFarland et al. (102) that the cells in the adrenal cortex expressing the highest levels of this enzyme were the glomerulosa cells suggested that this enzyme might play a central role in ANP action in this tissue. He was able to show that cGMP made in response to ANP stimulated isolated glomerulosa cell PDE2 activity, that cAMP levels were decreased in response to this stimulation, and that as a result cAMP-dependent aldosterone production was decreased. The central role of PDE2 in regulation of adrenal steroidogenesis is depicted in Figure 5.

5. Role in catecholamine secretion

A similar role for PDE2 in the control of cAMP levels in PC 12 cells was proposed by Whalin et al. (171). Because these cells are derived from adrenal medullary chromaffin cells that normally secrete epinephrine, the implication is that a normal function of this isozyme in chromaffin cells is to mediate the effects of cAMP on epinephrine release. Similar studies on effects of ANP on cAMP levels in fibroblasts have also been reported (93).

6. Role in cardiac Ca\(^{2+}\) channel control

It was shown several years ago that the activity of cardiac L-type Ca\(^{2+}\) channels is increased by cAMP dependent phosphorylation. This partly explains the positive inotropic effect of \(\beta\)-adrenergic agonists. In frog cardiac myocytes, it has been shown that CAMP and PDE2 activity are physiologically coupled to decreased Ca\(^{2+}\) channel activity (50). For example, intracellular perfusion of cGMP or an external application of micromolar concentrations of SIN-1 (a NO donor) inhibits CAMP-stimulated Ca\(^{2+}\) current (114). Recent studies suggest that the well-known adenosine deaminase inhibitor erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) is also a selective and potent inhibitor of PDE2. Moreover, when tested on isolated...
frog ventricular myocytes, EHNA antagonized the effects of cGMP on Ca^{2+} channel activity (112). The effect on mammalian heart is much more complex, since multiple mechanisms for the effects of cGMP on Ca^{2+} channel activity appear to operate (113, 114). It seems likely that different mechanisms may have quantitatively different levels of importance among species and even that different regions of the heart may show different regulatory schemes.

7. Role in olfaction

At present, a unique role for PDE2 in olfaction has not been demonstrated. The fact that it is very highly expressed only in a subset of olfactory sensory neurons (Julifs and Beavo, unpublished data) strongly suggests that cGMP activation of PDE2 activity will be important to the function of these cells. Because the cyclic nucleotide-gated channel in most (and perhaps all) olfactory neurons will respond to both cAMP and cGMP, the PDE2 may be responsible for rapid quenching of both cAMP and cGMP signals. This isozyme may also play a role in cGMP modulation of cAMP in these cells. It has been recently reported that a unique form of membrane-associated guanylyl cyclase is expressed in a subset of olfactory neurons. It will be interesting to learn if PDE2 is also expressed in these same neurons. Much more needs to be learned about olfactory signal transduction pathways and their kinetic and spatial relationships before the role of cGMP-stimulated PDE (cGS-PDE) in these neurons can be clarified.

8. Role in central nervous system function

The role of PDE2 in brain neuronal function is also not clear. Likely candidates include a role in nitric oxide effects on synaptic plasticity. The high expression of the enzyme in selected neurons of the hippocampus suggests this as a likely location for such effects (132).

C. PDE3 Family

1. Number of family members and localization

There are currently two different gene products identified as being part of the PDE3 family (111, 153). The first, PDE3A, has been identified in smooth muscle, platelets, and cardiac tissues. The second, PDE3B, is most abundant in adipocytes and liver. Both forms are found in lower amounts in other tissues. Several other PDE activities that have not yet been fully characterized are likely to also belong to the PDE3 family. In particular, a PDE overexpressed in a mutant cell line of mouse S49 lymphocytes called K30a is likely to be a PDE3 (65).

2. Substrate specificity: regulation by cGMP

Members of the cGMP inhibited PDE family share the property of having a relatively high specificity for cAMP as substrate. They are unique compared with other PDEs in that they also have a high affinity for cGMP; however, the V_max for cGMP is rather low, thereby conveying specificity for cAMP. Because cGMP binds tightly to the enzyme but is hydrolyzed poorly, many investigators now think that an important physiological role for cGMP is to act as an inhibitor of cAMP hydrolytic activity by this PDE.

3. Role in platelet aggregation

One of the first tissues in which the cGMP inhibited PDE was extensively studied was platelets (64,103). More recent studies suggest that many of the antiaggregatory effects of drugs and naturally occurring agents that act via cGMP are mediated at least in part by the inhibitory effect of cGMP on this enzyme (109). Because both cAMP and cGMP inhibit platelet aggregation, and because cGMP inhibits the hydrolysis of cAMP by this enzyme, one might expect agents that work via cGMP should act synergistically with agents that raise cAMP. This in fact usually turns out to be the case.

4. Role in regulation of blood pressure

Because PDE3 is one of the major PDE isozymes present in vascular smooth muscle, as one might expect, PDE3 inhibitors are potent smooth muscle relaxants. This may also contribute substantially to their effect on cardiac performance in clinical settings (see sect. vii). It also implies that at least part of the effects of agents like endothelial-derived relaxation factor or nitric oxide, which relax smooth muscles by increasing cGMP, also employ this same pathway. In general, tissues in which cAMP and cGMP have similar physiological effects express a cGMP inhibited PDE3. This allows cAMP and cGMP to work synergistically in the tissue. Often, the other major target of cGMP in these tissues is cGMP-dependent protein kinase. Both platelets and vascular smooth muscle are similar in this regard.

5. Role in cardiac function

A large number of selective inhibitors of PDE3 are currently undergoing clinical assessment as cardiac inotropic agents for treatment of cardiac disease (see sect. vii). Most studies indicate that inhibition of this enzyme causes a substantial increase in cardiac contractility, especially in conditions where sympathetic tone is elevated. The major mechanism for this increase appears to be an increase in calcium current, presumably due largely to cAMP-dependent phosphorylation and activation of the voltage-sensitive calcium channels in the plasma mem-
brane of the cardiocytes (reviewed in Ref. 131). Increases in cAMP-dependent protein kinase activity and also in phosphorylation of cardiac regulatory proteins in response to PDE3 inhibitors have been reported (reviewed in Ref. 131). Not only cardiotonic drugs but also cGMP and agents that increase cGMP appear to have at least part of their cardiac effects via this mechanism, at least in some regions of the heart (88, 114).

6. Regulation by phosphorylation/dephosphorylation

Both the PDE3A and PDE3B isozymes are phosphorylated and activated by serine/threonine phosphorylation mechanisms. Early studies with the platelet enzyme showed that both in vitro and in intact platelets, PDE3A could be phosphorylated and activated by cAMP-dependent protein kinase (104, 105). Physiologically, this is likely to be a very important part of the “normal” tachyphylaxis that occurs in response to continued adenylyl cyclase stimulation in platelets and probably also in other tissues expressing this PDE isoform. In this instance, the phosphorylation can be thought of as one mechanism for attenuating the amplitude of the cAMP signal in the cell. For many cell types, this mechanism works in conjunction with downregulation of cAMP synthesis. A model for effects of phosphorylation and cGMP on PDE3 activity is shown in Figure 6.

7. Role in insulin action

An additional level of regulation by phosphorylation occurs with the PDE3B isozyme. In addition to regulation by cAMP-dependent protein kinase, the PDE3B isozyme is also regulated by one or more insulin-dependent kinases (or kinase cascades) (96, 97, 106, 130, 146, 164). At present, it is not known which of the several serine/threonine kinases known to be in the insulin-dependent pathway is responsible for the phosphorylation. It is likely that the site of phosphorylation due to insulin treatment is different from that for CAMP-dependent phosphorylation (130). Although the stimulation of PDE activity by insulin has been known for many years (98), the physiological role for insulin-dependent activation of this enzyme is only partially clear. It seems likely that many of the metabolic effects of insulin, which often are most apparent when cAMP levels in a cell are first elevated by stimulation of adenylyl cyclase, are mediated at least in part by this mechanism. For example, Corbin and co-workers (11, 58, 59) have shown that most of the effects of insulin action on lipolysis in isolated fat cells can be eliminated by selective inhibitors of PDE3 activity and that lipolysis stimulated by analogues of cAMP activate cAMP-dependent protein kinase is only antagonized by insulin if the analogue is also a reasonably good substrate for PDE3. As illustrated in Figure 6, the overall effect of insulin will be
to antagonize the effects of an agonist of adenyl cyclase such as epinephrine. It will be important to determine the relative rates of phosphorylation by cAMP-dependent and insulin-dependent protein kinase systems to evaluate the quantitative importance of these pathways in the regulation of PDE3 activity. Because inhibition of cAMP-stimulated lipolysis is one of the most important metabolic effects of insulin, it could be argued that lack of PDE3B phosphorylation in diabetic patients is the major precipitating cause of the excess lipolysis and ketoacidosis that occurs in these patients.

D. PDE4 Family

1. Number of family members

This family of PDEs is currently the largest. At least four different gene products are known. Moreover, most have two or more alternative splice variants that are differentially expressed in different tissues. The complexity of this family illustrates a problem likely to occur in several of the other PDE families, namely, that as slightly different isoforms are found in different species, it is difficult to know if the sequence differences are due to isoform variant differences or just to species or even allelic variation. The regulation and control of this PDE isozyme family has been reviewed recently (38).

2. Localization

This group of isozymes is widely expressed in many tissues. However, very little information has been published to show exactly which gene is expressed in which tissue, not to mention which splice variant is present where. A number of tissues and cell types have received particular attention recently. These include the brain, where a PDE4 is likely to be involved in such processes as control of mood (166), emesis, and olfactory sensory transduction (17). In lung lymphocytes, a PDE4 is likely to be involved in regulation of inflammatory processes (160). In Sertoli cells, two or more PDE4s are thought to attenuate the CAMP response to follicle-stimulating hormone (38).

3. Regulation of transcription

No endogenous allosteric or small molecule regulators of PDE4 have been identified. However, some of the PDE4 isozymes are regulated at the level of transcription. For example, Conti and co-workers (151) have shown that in response to prolonged stimulation by cAMP, a >100-fold increase in PDE4D2 (rat PDE3.1) mRNA can be demonstrated in Sertoli cells. Smaller changes in PDE4B transcription have also been noted (152). Physiologically, this mechanism of regulation is thought to be important in setting the sensitivity of Sertoli cells to stimulation by follicle-stimulating hormone. It is likely that similar mechanisms operate in other cell types that express this isozyme of PDE4. More work needs to be done to determine how many of the different PDE4 genes are transcriptionally activated and if all of the alternative splice variants are subject to this type of regulation. It is likely that there will be substantial variation between tissues and cell types in this regard. A model of this type of regulation is shown in Figure 7.

4. Regulation by phosphorylation

More recently, it has been reported that some of the PDE4 gene products can also be activated by cAMP-dependent phosphorylation (139). Presumably, the physiological role for this type of covalent regulation is to provide a more rapid decrease in CAMP than would occur with the regulation of transcription alone. It is not yet clear whether or not both types of regulation always occur in the same cells or even if it is always the same PDE4 isozyme that is being regulated. In Sertoli cells, it appears that both types of regulation do occur. In vitro phosphorylation studies with recombinant enzyme suggest that the PDE4D4 (rat PDE3.3) but not the PDE4D2 (rat PDE3.1) or PDE4D3 (rat PDE3.2) alternative splice variants are substrates for cAMP-dependent protein kinase (139). Presumably, the direct phosphorylation provides a short-term response, and the regulation of transcription provides a longer term response. Both are likely to be important mechanisms of downregulation or tachyphylaxis to long-
term hormone and neurotransmitter stimulation of cAMP synthesis. The model shown in Figure 7 also illustrates this type of regulation.

E. PDE5 Family

1. Number of family members

This PDE isozyme has traditionally been termed the cGMP-specific PDE or the cGMP binding PDE. These names are based on the substrate specificity of the enzyme and on the fact that it contains a high-affinity noncatalytic cGMP binding site. To date, only one gene product has been reported for this family. However, ribonuclease protection analysis and preliminary cloning data from my laboratory suggest that at least two alternative splice variants are present in bovine lung. Until the initial cloning of a cDNA for this isozyme (110), it was thought that it would probably be very similar in sequence to one of the photoreceptor PDEs (PDE6), since it had very similar kinetic, cGMP-binding, and size characteristics. However, sequence comparison revealed <60% sequence identity even within the catalytic domain. Therefore, it has been placed in a separate family.

2. Localization

Little is known about the cellular and subcellular localization of this PDE. It is selectively inhibited by the drug zaprinast (62) and perhaps somewhat less selectively by dipyridamole (169). This feature, along with its high selectivity for cGMP as substrate, has been used to determine the tissue distribution. Relatively high levels are found in most smooth muscle preparations including vascular and tracheal smooth muscle (55). It has also been studied in platelets. Zaprinast effects on cation flux in the kidney suggest that it is also expressed in this tissue (172).

3. Role in physiological processes

The physiological role(s) played by this isozyme is not well understood. Its substrate specificity and kinetic characteristics suggest that it is most directly involved in regulation of cGMP levels. However, in tissues expressing both PDE5 and PDE3, it may also indirectly affect cAMP levels. In general, however, it has traditionally been thought of mostly as a regulator of cGMP function.

4. ANP and kidney function

The most suggestive information for a specific function of this enzyme in the kidney has come from studies with the selective inhibitor of PDE5, zaprinast (M&B 22,948). For example, in the presence of zaprinast, ANP will cause an extensive loss of Na⁺ and diuresis even in the face of the ensuing loss of blood volume and blood pressure (172, 173). This strongly suggests a role for this PDE in modulating the effect of ANP (and probably other agents that alter cGMP synthesis) on renal handling of Na⁺. It further suggests that a major part of the effects of cGMP and PDE5 is tubular.

5. Pulmonary vascular tone

This enzyme also has been implicated in the control of pulmonary vascular resistance. For example, whole animal and perfused organ studies with dipyridamole, another PDE5 inhibitor, suggest that it is particularly effective in decreasing pulmonary vascular resistance (35).

6. Function of cGMP binding and regulation by phosphorylation

Although it has been known for some time that PDE5s contain a high-affinity noncatalytic cGMP binding site, the function(s) of this site is still not clear. It is known that when this site is occupied by cGMP, the enzyme becomes a very good substrate for cGMP-dependent protein kinase (156). However, no major effect of phosphorylation on activity or function has been described for the purified enzyme. Some investigators have reported that treatment of partially purified preparations of PDE5 from lung with the catalytic subunit of cAMP-dependent protein kinase (24, 25). Because both cAMP- and cGMP-dependent protein kinases appear to phosphorylate the same serine residue (157), it suggests that there is some factor present in the partially pure enzyme that allows the activation to be seen. Conversely, this factor must be absent in the pure preparations. Phosphorylation may also decrease the affinity of the enzyme for inhibitors such as zaprinast (25).

7. Regulation by Zn²⁺

Recently, it has been suggested that PDE5s (and perhaps all PDEs) may contain two or more tightly bound molecules of Zn²⁺ that are involved in catalysis. This was postulated based on the observations that several of the amino acids conserved in all PDEs (29) form a "consensus" sequence motif previously identified in zinc hydrolases (54). The direct data for this are that stoichiometric amounts of Zn²⁺ can be detected in the pure enzyme and that radioactive Zn²⁺ can be shown to bind to the purified enzyme. Indirect evidence includes the observations that the enzyme is purified by binding to a Zn²⁺ chelate affinity column and that cyclic nucleotide PDEs isolated from baker's yeast had previously been shown to bind Zn²⁺. N. J. Pyne reported recently (ASPET Multiple PDE Colloquium, Newport Beach, CA) studies suggesting that zaprinast may act in part by binding the zinc of the enzyme.
F. PDE6 Family

1. Number of family members

There are several different gene products represented in this family of PDEs. The most abundant photoreceptor isozyme is the rod membrane-associated PDE (PDE6A/B). This enzyme appears to be a tetramer containing one α-subunit, one β-subunit, and two γ-subunits (42). Both the α- and β-subunits of the rod membrane-associated photoreceptor PDE contain consensus sequences present in the catalytic domain of all PDEs and therefore are presumed to be active. The polypeptides encoded by the two different genes for these subunits are highly similar but not identical over most of their sequence. In addition to this rod isozyme, a slightly different isozyme is found in cone outer segments (61). The subunit stoichiometry of this enzyme is not as well defined but is thought to consist of two identical α'-subunits, one or two 13-kDa inhibitory γ-subunits, and one or two 15-kDa δ-subunits. A cDNA for the cone photoreceptor α'-subunit has been isolated (95). It is homologous to both the α- and β-rod subunits. Neither the rod nor cone enzyme has been expressed in high yield; therefore, many questions about subunit number and function still remain to be answered.

2. Localization

As suggested by the names, all of the photoreceptor enzymes are highly enriched in the outer segments of the retinal photoreceptor neurons. Most of the enzyme in rod outer segments is loosely bound to the disk membranes. Rod photoreceptors also contain a soluble form of enzyme that appears to consist of one α-subunit, one β-subunit, two 11-kDa γ-subunits, and one or two 15-kDa δ-subunits (63). It has not yet been determined whether or not the α- and β-subunits are identical to the ones found in the membrane-associated rod isozyme or if the 15 kDa subunit is the same as its homologue in the cone isozyme. Preliminary data from my laboratory would suggest that the rod and cone 15 kDa subunits may be antigenically different (52). The cone enzyme also is found predominantly in the soluble fraction after fractionation with mildly hypotonic buffers, at least in bovine cones (61). The physiological significance, if any, of this apparent partitioning between a soluble versus membrane-associated localization remains to be determined. One might expect that the soluble enzyme would be less effectively activated by the membrane-associated transducin in the outer segments. Some evidence exists that the COOH-terminal of both the rod α and β subunits is isoprenylated and that this modification alters the membrane-associated properties of the enzyme (129).

3. Physiological role in vision: regulation by light, transducin, and cGMP

The physiological function for the photoreceptor PDEs is probably the best understood of any of the PDE families. This has been reviewed extensively (90). Briefly, light is detected by the isomerization of photopigments in individual rod or cone membranes. This isomerization alters the conformation of the opsin, allowing it to interact with a heterotrimERIC GTP-binding protein called transducin (T). This interaction allows GDP to dissociate from the transducin and for GTP to bind. Guanosine 5'-triphosphate causes the heterotrimer to dissociate into a Ta-GTP complex and a Tβγ complex. The Ta-GTP complex then can associate with the PDEα, β, γ complex, causing it to be catalytically active. The active PDE lowers the local concentration of cGMP in its vicinity, which in turn decreases the occupancy of cGMP on the binding sites of cGMP-gated cation channel present in the membrane. Because the channel is open when cGMP is bound, the decrease in cGMP levels leads to a hyperpolarization of the cell and ultimately a decrease in neurotransmitter release. This scheme is summarized in Figure 8.

4. Role of cGMP binding sites

Also shown in the model depicted in Figure 8 is a role cGMP is likely to play in regulation of PDE6 activity in addition to its role of regulating the cation channel. All photoreceptor PDEs contain a high-affinity noncatalytic binding site for cGMP. Under conditions of low cGMP, this site is less likely to be occupied at least with some of the PDE6s. When cGMP is not bound to the PDE, the large PDEα, β, γ-Ta complex dissociates rapidly into a catalytically active PDEα, β and an inactive PDEγ-γ-Ta complex. At higher cGMP levels, this dissociation does not take place or takes place more slowly. Because the guanosine triphosphatase (GTPase) activity of PDE-γ-Ta is faster than that of PDEα, β, γ-Ta, a physiological consequence of this is that the duration of the activation of PDE is dependent on the local concentration of cGMP in the vicinity (40). In Figure 8, this is illustrated as having only the PDE not containing bound cGMP dissociate. Actually, there is no evidence whether or not partially associated/dissociated species exist. There may also be additional mechanisms by which Ta-GTPase activity is regulated (4, 5). It is also not clear whether or not all species and isoforms are subject to this type of control. Because the apparent affinity for cGMP varies substantially between species, at least the quantitative aspects of the model are likely to differ between species.

5. Role of γ- and δ-subunits

The physiological role for the 11 kDa PDE γ-subunit is relatively clear. It serves to inhibit PDE activity in the absence of light stimulation. A similar role is played by the 13 kDa PDE γ-subunit of the cone isozyme. The physiological role for the 15 kDa PDE δ-subunits is less clear. Because it is found only on those PDE6 isoforms that appear in the soluble fraction, it seems possible that this...
subunit serves to modulate binding of the PDE to the membrane.

**G. PDE7 Family**

1. **Number of family members**

   At present there has been only one member of this family described (116). This PDE was originally found by a genetic screening procedure developed in yeast to identify functional high-affinity cAMP PDE cDNA clones. At the primary sequence level, this PDE family is most closely related to the PDE4 family, however, this similarity is <60% even in the most conserved regions. Because a full-length cDNA has not yet been isolated, only a truncated recombinant form has been studied in any detail. This truncated form has a high affinity and specificity for cAMP and is not selectively inhibited by the usual inhibitors of PDE4 such as rolipram and Ro-20-1724. No substantial amounts of protein have been isolated either from recombinant or natural sources.

   2. **Localization: physiological role**

   For the reasons described in section IIIG, localization studies have not been carried out, since there are as yet no isozyme-specific antibodies or isozyme-specific inhibitors available. Northern analysis of PDE7 mRNA indicates that relatively high concentrations may be present in skeletal muscle. Recent data from several laboratories suggest that some but not all lymphocytes may contain a high-affinity cAMP-specific PDE that is a PDE7 gene product or perhaps closely related to one (14, 78). The physiological role(s) of this enzyme is completely unknown at this time.

**IV. GENERAL PHOSPHODIESTERASE STRUCTURE-FUNCTION RELATIONSHIPS**

**A. Oligomeric Structure**

All mammalian PDEs studied to date appear to exist as dimers of two catalytic subunits. In addition, the photoreceptor PDEs (PDE6s) also contain tightly associated 11- or 13-kDa inhibitory subunits. Each monomer of the Ca2+/CaM-dependent PDEs (PDE1s) contains a tightly bound molecule of CaM if isolated in the presence of micromolar levels of Ca2+.

**B. Conserved Catalytic Domain: Motifs**

All mammalian PDEs contain a highly conserved region of ~270 amino acids in the COOH-terminal half of the protein. Limited proteolysis (30, 31), deletion mutagenesis, and conserved amino acid point mutation analysis all suggest that this region is required for catalytic
activity. It is not known whether or not dimerization is required for catalytic activity. A conserved "signature motif" of the sequence HDX2HX4N has been identified in the catalytic domain of all PDEs isolated to date (30). This sequence motif is not found in enzymes that do not have PDE activity. It is presumed that these amino acids are important to catalytic function. In fact, initial mutagenesis studies with some of the histidine residues show loss of activity (80, 81), and chemical modification of the histidines also decreases activity (1). However, the exact roles of these residues in catalysis and/or nucleotide or metal binding are not yet clear. In the PDE5s, some evidence for a role in metal binding has been reported (54). The zinc binding consensus sequence (HX3HX24–26E) overlaps the PDE consensus sequence, perhaps implying a role in metal binding for this sequence in all PDEs.

V. PHOSPHODIESTERASES AND DISEASE

A. PDE4: Diabetes Insipidus

One form of diabetes insipidus in the mouse (74) has been attributed to increased PDE4 activity. Neither the mechanism by which the activity is increased nor the PDE isoform(s) responsible has been identified. Similarly, an increase in a low-K_m cAMP PDE activity (possibly a PDE4) has been reported in leukocytes of atopic patients (28). Again, it is not clear whether the increased PDE activity is a cause or result of the disease.

B. PDE6: Retinal Diseases

Several different genetic diseases likely to be caused by defects in PDE6 function or expression have been characterized. Retinal degeneration in the rd mouse (19) has been attributed to mutations in the PDE6B gene. In the mouse, this appears to be due either to the insertion of a viral element into the gene (20) or a stop-codon mutation in the gene (127). In humans, a series of other diseases of vision including autosomal dominant congenital stationary night blindness and autosomal recessive retinitis pigmentosa have been attributed to PDE6B mutations (56). In addition, rod/cone dysplasia 1 in Irish Setter dogs (47, 150) is also associated with mutations in the PDE6B locus. How the loss of the PDE β-subunit leads to loss of cell function is not yet well understood.

VI. PHOSPHODIESTERASES AS TARGETS FOR THERAPEUTIC INTERVENTION: ROLE OF MULTIPLE ISOZYMES

The realization that the PDE activity of any one cell is due to the action of only a small subset of the known PDE isoforms has triggered a massive reevaluation of the possibilities for therapeutic intervention based on modulation of individual PDE activities. Nearly all of the first generation inhibitors of PDE turned out not to be isozyme specific. Even so, agents like theophylline are still used in the treatment of resistant forms of asthma and are used as an adjunct in several other types of therapy. In addition, the more recent widespread clinical applications of pentoxifylline for a variety of disease states (27, 46, 135) have generated a resurgence of interest in the potential usefulness of all methylxanthines and their metabolites. The usefulness of the more potent nonspecific agents like theophylline and papaverine has suffered from a large number of toxic side effects at doses that are very close to therapeutic ranges. As a result, their usefulness is limited. In retrospect, such side effects are to be expected if their major mechanism of action is to inhibit all PDEs in all tissues and thereby potentiate the effect of a very large number of hormones, neurotransmitters, and paracrine secretions. The reason that pentoxifylline appears to have much lower toxicity is not clear but may reflect differences in pharmacokinetics or additional target enzymes.

Our more recent understanding that more than 25 different PDEs representing 7 or more families exist in humans greatly increases the therapeutic potential for PDE inhibitors. In principle, one should be able to inhibit one family of PDEs (or better yet a small subset of the family) and achieve much more selective results and therefore fewer side effects. In practice, several of the second generation drugs that are specific for individual PDE families do appear to be much better both in terms of therapeutic efficacy and also decreased number of side effects. A list of many of the more commonly used PDE inhibitors that are selective for individual PDE families is given in the first part of Table 3. In the latter part of Table 3, several older nonselective or partially selective inhibitors are listed. It should be noted that for modulation of some physiological (or pathological) conditions, an inhibitor that affects more than one PDE may be preferable. Also included in Table 3 is an estimate of the half-maximal inhibitory concentration (IC_{50}) for each PDE obtained under conditions where substrate was low relative to the K_m for the substrate. In this case, it is likely that the IC_{50} will be close to the inhibitory constant for the inhibitor. The degree of selectivity varies among the different drugs. In general, they show ~100-fold or greater selectivity, that is, the IC_{50} determined for the enzyme listed is 100-fold lower than the IC_{50} for any of the other PDEs when measured under comparable conditions. Preclinical or clinical trials for several of the selective inhibitors of the PDE1, PDE3, PDE4, and PDE5 families have been carried out or are currently in progress. These compounds are marked with an asterisk.

It should be noted that the in vivo efficacy for all of these inhibitors may not necessarily match their in vitro potency due to issues of permeability, distribution, and metabolism. For similar reasons, the selectivity for any given inhibitor in vivo may not be the same as in vitro. Nevertheless, for most cases where it has been carefully studied, a reasonably good correlation has been found. It should also be emphasized that for many of the inhibitors, additional mechanisms of action have not been ruled out.
<table>
<thead>
<tr>
<th>PDE Family</th>
<th>Inhibitor</th>
<th>IC₅₀, µM</th>
<th>Reference Number; Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDE₁</td>
<td>KS-505</td>
<td>0.06</td>
<td>119; may differentiate among CaM-PDEs</td>
</tr>
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<td></td>
<td>8-Methoxymethyl-IBMX</td>
<td>4.0</td>
<td>170</td>
</tr>
<tr>
<td></td>
<td>Vinpocetine (TCV-3B)</td>
<td>20.0</td>
<td>66</td>
</tr>
<tr>
<td>PDE₂</td>
<td>EHNA*</td>
<td>1.0</td>
<td>112; also adenosine deaminase inhibitor</td>
</tr>
<tr>
<td>PDE₃</td>
<td>Trequinsin (HL-725)</td>
<td>0.0003</td>
<td>134</td>
</tr>
<tr>
<td></td>
<td>8-Methoxymethyl-IBMX</td>
<td>0.0005</td>
<td>3; also contains aegrelide moiety</td>
</tr>
<tr>
<td></td>
<td>(LY-186126)</td>
<td>0.004</td>
<td>86; available tritiated</td>
</tr>
<tr>
<td></td>
<td>Cilostamide (OPC38690)</td>
<td>0.005</td>
<td>72</td>
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<td></td>
<td>Bemoradan (RWJ-22967)</td>
<td>0.02</td>
<td>117</td>
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<tr>
<td></td>
<td>Anergrelide (BLA167A)*</td>
<td>0.05</td>
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</tr>
<tr>
<td></td>
<td>Indolidan (LY195115)*</td>
<td>0.08</td>
<td>85</td>
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<tr>
<td></td>
<td>Cilostazol (OPC-13013)*</td>
<td>0.12</td>
<td>178, 179</td>
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<td></td>
<td>Milrinone (WIN47203)*</td>
<td>0.30</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Sigazodan (SKF-94836)*</td>
<td>0.40</td>
<td>159</td>
</tr>
<tr>
<td></td>
<td>S-Methyl-imazodan (CI 930)</td>
<td>0.60</td>
<td>145</td>
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<td></td>
<td>SKF-95554</td>
<td>0.70</td>
<td>118</td>
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<tr>
<td></td>
<td>Pinobendan (UD-CG 116 BS)*</td>
<td>1.0</td>
<td>18; may also be a Ca⁺⁺ sensitizer</td>
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<tr>
<td></td>
<td>Enoximone (MDL17043)*</td>
<td>1.0</td>
<td>84</td>
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<td></td>
<td>Imazodan (CL 914)*</td>
<td>6.0</td>
<td>168</td>
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<tr>
<td></td>
<td>SKF-94120</td>
<td>8.0</td>
<td>159</td>
</tr>
<tr>
<td></td>
<td>Vesnarinone (OPC 8212)*</td>
<td>8.5</td>
<td>108; may have multiple targets and may differentiate between PDE3 isozymes</td>
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<tr>
<td>PDE₄</td>
<td>Rolipram (RO-20-1724)</td>
<td>2.0</td>
<td>144</td>
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<tr>
<td></td>
<td>(ZK-62711)*</td>
<td>1.0</td>
<td>136</td>
</tr>
<tr>
<td></td>
<td>Denbufylline*</td>
<td>1.0</td>
<td>122</td>
</tr>
<tr>
<td>PDE₅</td>
<td>Zaprinast (M&amp;B 22,948)</td>
<td>0.76</td>
<td>62</td>
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<tr>
<td></td>
<td>Dipyridamole*</td>
<td>0.90</td>
<td>120</td>
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<tr>
<td>PDE₆</td>
<td>Zaprinast (M&amp;B-22, 948)</td>
<td>0.15</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>Dipyridamole*</td>
<td>0.38</td>
<td>62</td>
</tr>
<tr>
<td>PDE₇</td>
<td>None reported</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed PDE3/PDE1</td>
<td>Zardaverine</td>
<td>PDE3</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>PDE4</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AIH21-132</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PUE3</td>
<td>0.55</td>
</tr>
<tr>
<td>Mixed PDE3/PDE5</td>
<td>Sulmazol (AR-L 115 BS)*</td>
<td>50.0</td>
<td>2, 36; Ca⁺⁺ sensitizer; 103, adenosine receptor antagonists, 126</td>
</tr>
<tr>
<td>Nonselective</td>
<td>Theophylline*</td>
<td>50-300</td>
<td>26; probably has additional targets</td>
</tr>
<tr>
<td></td>
<td>IBMX (MIX)</td>
<td>2-50†</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Papaverine*</td>
<td>5-25†</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>Pentoxifylline (Trental)*</td>
<td>45-150</td>
<td>39</td>
</tr>
</tbody>
</table>

EHNA, erythro-9-(2-hydroxy-9-nonyl) adenine; IBMX, 3-isobutyl-1-methylxanthine; IC₅₀, half-maximal inhibitory concentration. * Clinical data in humans available. † Refers to range of IC₅₀ values seen with different isozymes.

VII. CLINICAL STUDIES

A. PDE₁ Inhibitors

Inhibitors selective for several different PDE families have undergone clinical evaluation. For example, there have been a number of clinical studies with vinpocetine (TCV-3B), which is also called Cavinton (apovincamic acid ethyl ester or RGH-4405). Most recent studies have been directed toward its use as a nootropic agent (memory enhancing). In general, most studies suggest a small but consistent effect on some components of increased cognitive function. These would be consistent with earlier studies in rodents suggesting increased long term potentiation after vinpocetine treatment. It should be emphasized that it is not yet clear if these effects are due to selective inhibition of PDE₁ activity or to other effects of the drug.

B. PDE₃ Inhibitors

The greatest number of studies and the greatest number of compounds are those selective for the PDE₃ family. This reflects both the wide range of different structures that can yield selectivity and the wide range of potential applications. For example, PDE₃ inhibitors are being developed as antithrombotic agents [by virtue of their inhibitory effect on cGMP-inhibited PDE (cGIPDE) activity in platelets], as antihypertensive agents (by virtue of their effects on vascular smooth muscle cGIPDE activity), and as cardiotonic agents potentially useful as inotropes for
congestive heart failure (by virtue of their effects on cGMP production). Most of the clinical studies with this class of compounds are for various forms of cardiac disease. For example, enoximone is reported to show substantial promise as an agent to treat patients waiting for heart replacement (165) and also for various types of low cardiac output after cardiac surgery (49, 71, 79). It should be noted that the largest trial, the PROMISE trial using milrinone in conjunction with digoxin, diuretics, and a converting enzyme inhibitor, was directed toward patients having severe congestive heart failure. This trial was stopped prematurely due to an increase in deaths in the drug-treated patients (37, 125). It is now felt by many investigators that the serious side effects that caused these initial trials to be stopped may well have been due to use of an excessively high dosage schedule and perhaps the concurrent administration of the other agents. It seems likely that patients at highest risk for adverse reactions were those who were the most seriously ill (i.e., class IV). More recent studies with less severely ill patients suggest that at lower doses there may be substantial improvement in patient status and no apparent increase in toxic side effects (43). The recent reports on vesnarinone, which is a very potent PDE3 inhibitor (as well perhaps as having additional sites of action), have shown not only an increase in cardiac performance but also a decrease in deaths (48, 108, 123). It seems quite possible that as more confirmatory information becomes available, that experimental use PDE3 inhibitors as therapeutic agents for cardiac disease may increase. Interest is now focused not only on use of lower doses that would be likely to have effects only when sympathetic tone was increased, but also on the use of PDE3 inhibitors with different combinations of other drugs than were used in the PROMISE trials. Cilostazol has been used extensively in Japan as an antithrombotic agent (122a, 178, 179).

C. PDE4 Inhibitors

The clinical literature on PDE4 inhibitors has been limited largely to studies of rolipram as an antidepressant (15, 51, 138). In general, it has been found to be an effective antidepressant in some patients but not greatly more efficacious than many more traditional agents. In many patients it has the unwanted side effect of causing nausea, although its anticholinergic effects may be less than, for example, amitriptyline (138) or desipramine (15). Many of the current studies are related to the possible use of PDE4 inhibitors as an anti-inflammatory agent (121, 155, 161).

D. Other Clinical Uses

Little clinical data on PDE5 selective inhibitors are available. Evidence from animal models implies that regulation of PDE5 activity is likely to have direct relevance to pathophysiology and clinical therapy of pulmonary hypertension. From a clinical perspective, abnormalities of the pulmonary vascular resistance leading to pulmonary hypertension are an important cause of cardiovascular disease in all age groups and especially in neonates, where persistent pulmonary hypertension of the neonate is a major source of mortality and morbidity. Recent data suggest that selective inhibitors of the PDE5 family will differentially decrease pulmonary as compared with systemic blood pressure in newborn lambs (21, 35). When taken together with recent clinical observations that agents that increase cGMP via the nitric oxide/nitric oxide synthase pathway can be quite efficacious in treatment of pulmonary hypertension (70, 82, 83), the data suggest that a cGMP-specific PDE plays a critical role in regulating pulmonary vascular tone. Early clinical experience with dipyridamole, a PDE5 inhibitor, is promising in this regard. Whether the mechanism(s) by which these drugs are effective is via direct relaxation of pulmonary vessels or indirect via alteration in the release or synthesis of secondary mediators remains to be determined. Dipyridamole, of course, has an extensive clinical background as an antithrombotic agent (100) and more recently as an aid to imaging of diseased blood vessels (94). This latter effect may be largely due to its effects as an adenosine uptake inhibitor.

E. Nonselective PDE Inhibitors

Theophylline has been used for years as a bronchodilator in the treatment of asthma. Its usefulness is limited largely by its toxicity. At doses often only twice what is minimally effective as a bronchodilator, theophylline can cause anorexia, nausea, and headache. At slightly higher doses it can cause seizures or arrhythmias. It is still not entirely clear how much of the effects and side effects of this drug arc due to inhibition of PDEs and how much may be due to other causes such as inhibition of adenosine action.

Pentoxifylline, another nonspecific PDE inhibitor, currently has a number of clinical applications (135), including treatment of intermittent claudication (46) and of diabetes-induced peripheral vascular disease (27), inhibition of granulocyte and platelet function (67), improvement of mobility and viability of spermatozoa (162), and reduction of organ damage due to tumor necrosis factor-α production (92, 133), among others. Many of the early studies suggested that its mechanism of action was to increase red cell deformability. However, its site(s) of action is in most cases not well documented. It is possible that at least some of its effects are due to PDE inhibition by it or its metabolites. However, neither which effects nor which PDE isozyme(s) is involved have been elucidated.

The original data shown in this review were obtained with the support of National Institute of Health Grants DK-21723, HL-43948, and FY-08197.
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