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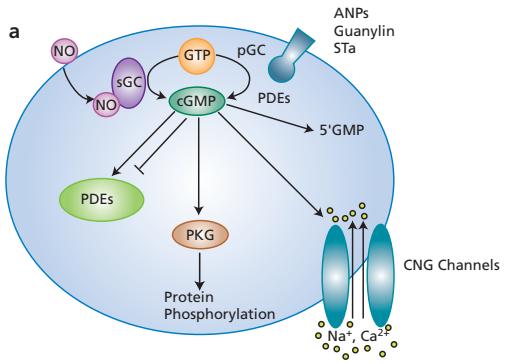
In this Issue...

Functions and Pharmacological Inhibitors of Cyclic Nucleotide Phosphodiesterases

Valeria Vasta and Joe Beavo

The discovery of the cyclic nucleotides **3',5'-cyclic monophosphate** (cAMP, Prod. No. **A 9501, A 4137**) and **guanosine 3',5'-cyclic monophosphate** (cGMP, Prod. No. **G 7504, G 6129**) led to the first formulation of the second messenger concept. These cyclic nucleotides are now known to be ubiquitous intracellular second messengers that mediate the response of cells to a variety of extracellular stimuli through the activation of cyclic nucleotide-dependent protein kinases, ion channels, GTP-exchange factors and their downstream effector systems [1]. The amplitude and duration of cAMP and cGMP signals are controlled by their rates of synthesis by adenylyl and guanylyl cyclases, respectively, and their degradation by 3',5'-cyclic nucleotide phosphodiesterases (PDEs) (Figure 1). PDEs, identified shortly after the discovery of cAMP and cGMP, are



a large superfamily of enzymes that hydrolyze the 3' phosphodiester bond in cAMP or cGMP to form the corresponding 5'-nucleotide

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Application Note:

The New Panorama™ Antibody (Ab) Microarray Cell Signaling Kit: A Unique Tool for Protein Expression Analysis

Eliezer Kopf, Dalia Shnitzer and Dorit Zharhary

Introduction

There is a growing need for technologies that allow global molecular characterization of biological samples. The ability to identify multiple proteins simultaneously has many applications in basic biological research as well as in disease diagnosis and treatment. The use of DNA arrays for profiling mRNA expression in cells has provided valuable information in many biological areas. However, since there is not always a direct correlation between the mRNA level and the expression of the protein, a method that can directly assay proteins is required. Whereas DNA/RNA/oligo arrays give information on the genetic defects that may cause disease, protein microarrays provide

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Functions and Pharmacological Inhibitors of Cyclic Nucleotide Phosphodiesterases

Valeria Vasta and Joe Beavo *(continued from cover)*

monophosphate. This overview focuses on the functions and pharmacological inhibitors of mammalian PDEs; for more extensive reviews see references [2-4].

PDE Superfamily

The PDEs are now recognized to form a superfamily of 11 different, but homologous gene-families that all contain a conserved catalytic domain of around 300 amino acids near their carboxy terminus [3]. The amino acid sequence identity in the catalytic domain is usually 35-40% between different families, while it is generally at least 70% between members of the same gene family. Twenty-one residues, in particular, are absolutely conserved across all PDE families and two PDE signature motifs (H-N-XX-H\H-D-XX-H) in the active site appear to form a single pocket, binding two metal ions involved in catalysis [5]. The crystal structures of several PDE catalytic domains have recently been determined [6-9]. The amino terminal region of the PDEs is distinguished by a domain and motif organization characteristic of each PDE gene family [3]. This region is involved in subunit and protein-protein interactions, localization, and allosteric and covalent modifications affecting catalytic activity. The nomenclature employed to distinguish each PDE isozyme uses the first two letters to designate the species of origin, followed by PDE and the Arabic numeral of the gene family (usually reflecting the order of discovery). The next letter represents the individual gene within the family, followed by an Arabic numeral identifying the transcript variant. For example, HSPDE8A1 refers to the human PDE8 family, gene A, transcript variant 1.

At present, 21 different mammalian PDE genes have been identified with each gene generally being highly conserved between species. In addition, many of the genes are expressed in multiple isoforms due to different transcription initiation sites or alternative splicing. A large number of transcript variants have been reported in the literature or can be deduced from the EST database, but the physiological significance of this phenomenon is only beginning to be elucidated. The PDE families can be differentiated genetically, on the basis of sequence homology, and functionally, on the basis of substrate specificity, kinetic properties and sensitivity to endogenous or pharmacological regulators. The first functional difference identified between the various PDEs was substrate specificity. Thus, some PDEs hydrolyze only cAMP, some hydrolyze only cGMP, while others hydrolyze both. Table 1 depicts the 11 gene families, referred to as PDE1-PDE11, together with their substrate specificity, tissue localization, their basic mode of regulation and some of their selective inhibitors.

Different PDE isozymes can have specific tissue, cellular and subcellular distributions and more than one type of PDE is usually present in any given cell. However, the pattern of PDE expression can exhibit some degree of variability between different species. The types of PDEs expressed in a cell, together with their relative proportions and subcellular localization, clearly control the cyclic nucleotide phenotype of that cell. In addition to literature data on the tissue/cell specific expression of the various PDEs, which is not entirely catalogued as yet, information on the expression can be obtained from mRNA, ESTs and microarray analysis databases. For example, the sequences (mRNA and ESTs) belonging to each PDE gene family and their tissue of derivation are listed in a cluster with the name of that PDE in the UniGene database (http://www.ncbi.nlm.nih.gov/entrez/-query.fcgi? db=unigene) and in the Geo Microarray analysis database (http://www.ncbi.nlm.nih.gov:80/entrez/query.fcgi?CMD= search&DB=geo).

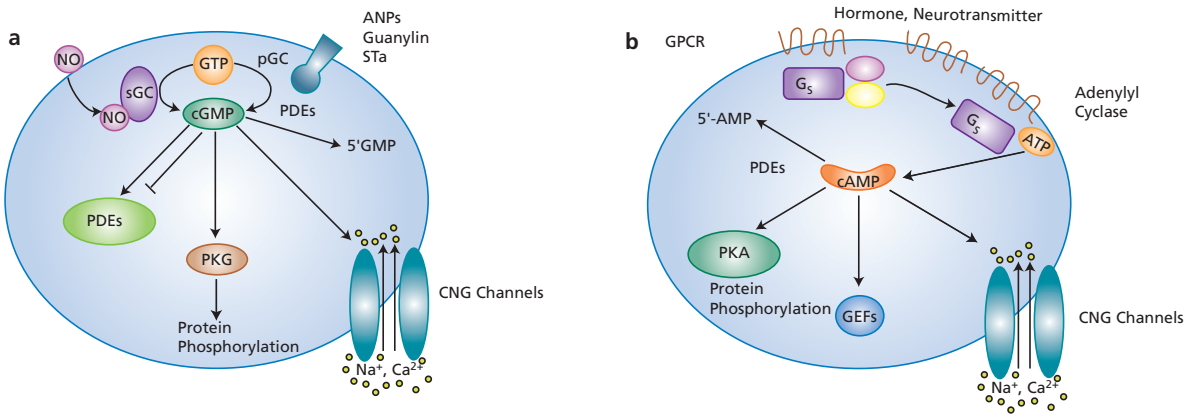

Figure 1. Cellular pathways of cyclic nucleotide function and regulation.
The above schematics show the basic synthetic and regulatory pathways for a) cGMP and b) cAMP metabolism. Various agonists can activate guanylyl cyclases (Panel a) and adenylyl cyclase (Panel b) in different cell types and increase the intracellular levels of cGMP or cAMP, respectively. This in turn activates downstream effector systems, such as PKA (protein kinase A), PKG (protein kinase G), cAMP-GEFs (guanine nucleotide exchange factors) and CNG (cyclic nucleotide-gated) channels. By degrading cyclic nucleotides, PDEs control the amplitude, duration and compartmentalization of the cyclic nucleotide signal. Panel a) also illustrates the feedback control of PDE activity by cGMP, ANPs (atrial natriuertic peptides), NO (nitric oxide), pGC (particulate guanylyl cyclase), sGC (soluble guanylyl cyclase) and STa (heat-stable enterotoxin). Figure adapted from reference [1].

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Inhibitors of Cyclic Nucleotide Phosphodiesterases...(continued)

Table 1 Biochemical and pharmacological characteristics of cyclic nucleotide phosphodiesterase (PDE) isozymes

Gene Family	Genes	Major Tissue Expression	Substrate	Regulators	Inhibitors (Sigma Prod. No.)	IC_{50} in µM* [Reference]
PDE1	PDE1A PDE1B PDE1C	Brain, heart, smooth muscle, olfactory cilia, testis	cAMP/cGMP	Ca^{2+}-CaM PKA CaMKII	8-Methoxymethyl-IBMX (M 2547) Vinpocetine (V 6383)	4 [2] 20 [2]
PDE2	PDE2A	Adrenal cortex, brain, heart	cGMP/cAMP	cGMP	EHNA (E-114) Trequinsin (T 2057)	1 [2] 0.64-2 [48]
PDE3	PDE3A PDE3B	Heart, adipose tissue, pancreas, platelets	cAMP>cGMP	cGMP PKA PKB	Cilostamide (C 7971) Cilostazol (C 0737) Enoximone (E 1279) Imazodan (I 0782) Milrinone (M 4659) Trequinsin (T 2057)	0.005 [2] 0.12 [2] 1 [2] 6 [2] 0.30 [2] 0.0003 [2]
PDE4	PDE4A PDE4B PDE4C PDE4D	Many tissues	cAMP	PKA PKC	Rolipram (R 6520) YM976 (Y 4877)	2 [2] 0.002 [49]
PDE5	PDE5A	Lung, platelets, smooth muscle, corpus cavernosum	cGMP	PKA PKG cGMP	Dipyridamole (D 9766) DMPPO Sildenafil (Viagra) T-0156 (T 8067) T-1032 (T 7692) Tadalafil (Cialis) Vardenafil (Levitra) Zaprinast (Z 0878)	0.90 [2] 0.003 [42] 0.004 [50] 0.0002 [43] 0.001 [44] 0.005 [51] 0.0007 [52] 0.76 [2]
PDE6	PDE6A (α) PDE6B (β) PDE6C (α)	Rod and cone photoreceptor outer segments	cGMP	Transducin PDE6 γ and δ subunits	Dipyridamole (D 9766) Sildenafil Tadalafil Vardenafil Zaprinast (Z 0878)	0.38 [2] 0.074 [51] 5.1 [51] 0.011 [52] 0.15 [2]
PDE7	PDE7A PDE7B	Skeletal muscle, T-cells, B-cells	cAMP	Unknown	Dipyridamole (D 9766)	9-42 [53]
PDE8	PDE8A PDE8B	Testis, liver, thyroid	cAMP	Unknown	Dipyridamole (D 9766)	4.5 [54]
PDE9	PDE9A	Kidney	cGMP	Unknown	Sildenafil Zaprinast (Z 0878)	7 [55] 29 [55]
PDE10	PDE10A	Testis, brain	cAMP/cGMP	PKA	Dipyridamole (D 9766)	1.10 [56]
PDE11	PDE11A	Skeletal muscle, prostate	cAMP/cGMP	Unknown	Dipyridamole (D 9766) Zaprinast (Z 0878)	0.82-1.8 [57] 5-28 [57]

*IC_{50} values (µM) were obtained from *in vitro* determination of PDE activity at the substrate concentration reported in the specific references (shown in brackets). Differences in inhibitor sensitivity may occur between different members of each PDE family and between transcript variants of each gene. All inhibitors listed, except dipyrimadole and zaprinast, exhibit substantial selectivity for the PDE family listed; in most cases at least 20-fold as compared to the next nearest family member. However, these inhibitors are not absolutely selective and the caveats mentioned in the text should be noted, especially when using these inhibitors in intact cells. Note that IBMX inhibits all PDEs with the exception of PDE8 and PDE9, although the IC_{50} values can vary from 1-50 µM depending on the isozyme and species examined.

Function and Regulation of PDEs

It is assumed that the major function of PDEs in cells is to control the resting level of cyclic nucleotides and to restore these steady-state levels following stimulatory events that utilize cAMP or cGMP as second messengers. The realization that, in many cells, cyclic nucleotide levels oscillate rapidly and locally has emphasized the importance of PDEs for modulating not only the amplitude of a cyclic nucleotide signal, but also its duration and compartmentalization. Perhaps the best example of this is the photoreceptor PDEs that modulate rapid oscillations in light-induced cGMP and thus control cGMP-gated cation channel opening that transmit the visual signal via changes in membrane potential [2,10]. It is likely that similar functions are exerted by PDEs in the modulation of synaptic transmission, cardiac contractility, platelet aggregation, sensorial transduction and many other cyclic nucleotide-modulated cell processes.

It is becoming increasingly evident that compartmentalization is an important aspect of cyclic nucleotide signaling. The combined actions of the cyclases and PDEs control the intensity and spatial progression of cyclic nucleotide transients [11,12]. Evidence for the existence of cAMP microdomains has been obtained in cardiac myocytes [12] as well

Inhibitors of Cyclic Nucleotide Phosphodiesterases...(continued)

as other cell types [13,14]. The recruitment of PDEs to activated receptors, as seen in cardiac myocytes and T cells [15-17], would be responsible for degradation of cAMP near the site of synthesis, thus delimiting the spatio-temporal wave of cAMP propagation in combination with intracellular molecular and physical barriers to diffusion. This role for PDEs has been confirmed by the use of new indicators that allow real-time measurements of cyclic nucleotides [18-20]. The existence of soluble guanylyl cyclase and adenylyl cyclase, distributed in various subcellular compartments, point to new sites of cyclic nucleotide synthesis where PDEs can be activated to reset basal levels following a stimulatory event [21,22]. This scheme also fits well with what is known about the regulation of PDE activity. Some PDEs are known to be activated by phosphorylation, either by cAMP- or cGMP-dependent protein kinases (PKA, PKG) or by other kinases following an increase in cyclic nucleotides [23-26]. In some instances, PKA and PDE have been shown to be part of the same scaffold signaling complex where the negative feedback loop can be rapidly activated [27,28]. Elevation of cGMP also provides a negative feedback control by causing allosteric activation of PDE2 and PDE5 through cGMP binding to the cGMP-binding GAF domains of these proteins [26,29]. Cyclic GMP will also inhibit PDE3 thus establishing a crosstalk between cGMP and cAMP pathways [30]. Other established mechanisms of regulation of PDE activity include activation of PDE1 by Ca^{2+}/calmodulin (CaM) binding [31] and activation of PDE6 by transducin [10].

Another mechanism of PDE regulation occurs at the level of transcription and translation. The basis of the developmental and cell-specific transcription regulation is still largely unknown. Up-regulation of PDEs following an increase in cAMP levels seems to be a common compensatory feedback mechanism that augments the ability of a cell to catabolize cAMP and adapt to chronic activation. This type of regulation has been observed for PDE3, PDE4 and PDE7 [32-34] and likely involves PKA activation of transcription factors acting on the cAMP-response elements found in the promoters of these genes [33,35-37].

Mutations in PDEs have been found to cause certain inherited conditions in humans. For example, mutations in subunits of PDE6 in the retina are the basis of some forms of hereditary retinitis pigmentosa and stationary night blindness [38,39]. Recently, the PDE4D gene has been identified as the most likely candidate gene for susceptibility to cardiogenic and carotid ischemic stroke [40]. In particular, reduced expression of some PDE4D variants has been detected in affected individuals. The precise pathogenic mechanism of this disregulation of PDE4D expression for stroke is still not clear, but it is known that PDE4D is expressed in many cells that are important for the pathogenesis of atherosclerosis, an important risk factor in stroke.

PDE Inhibitors

As discussed earlier, the complexity associated with the cellular distribution patterns of PDEs contributes to the specificity and compartmentalization of cyclic nucleotide signaling. From the point of view of pharmacological intervention, the differential tissue distribution of PDEs makes them desirable molecular targets for the development of cell-specific drugs. For example, the relative smooth muscle-specific expression of PDE5 has been exploited for the treatment of erectile dysfunction. It is well known that upon sexual stimulation, penile nerve excitation causes the release of nitric oxide that in turn increases intracellular cGMP levels in vascular smooth muscle cells. This causes relaxation and allows blood flow into the corpus cavernosum leading to penile erection (Figure 2). The cGMP pathway can be triggered pharmacologically by selective PDE5 inhibitors, including sildenafil (Viagra®), tadalafil (Cialis®) and vardanafil (Levitra®), that prevent the breakdown of cGMP [41]. Other potent selective PDE5 inhibitors have been developed and are available as research tools, specifically DMPPO [42], **T-0156** (Prod. No. **T 8067**) [43] and **T-1032** (Prod. No. **T 7692**) [44]. It is now recognized that PDE5 is also expressed in various other cell types such that new effects of PDE5 inhibitors are being described that could lead to other clinical applications for the above drugs. However, the presence of PDE5 in other cell types, including lung platelets and smooth muscle, cautions their use [45-47].

Phosphodiesterase Inhibitors Available from Sigma-RBI

A 1755	**Aminophylline hydrate** (nonselective)
B 8279	**4-(3-Butoxy-4-methoxybenzyl)imidazolidin-2-one** (Ro 20-1724) (PDE4)
C 0750	**Caffeine** (nonselective)
C 7971	**Cilostamide** (PDE3)
C 0737	**Cilostazol** (PDE3)
D 5385	**1,7-Dimethylxanthine** (Paraxanthine) (nonselective)
D-108	**1,3-Dipropyl-7-methylxanthine** (nonselective)
D 9766	**Dipyridamole** (nonselective)
E-114	**EHNA** (PDE2)
E 1279	**Enoximone** (PDE3)
E 1896	**Etazolate hydrochloride** (SQ20,009) (PDE4)
F-124	**Furafylline** (nonselective)
H 9006	**7-(β-Hydroxyethyl)theophylline**
I 0157	**Ibudilast** (PDE3)
I 0782	**Imazodan** (PDE3)
I 5879	**3-Isobutyl-1-methylxanthine** (IBMX) (nonselective)
M 2547	**8-Methoxymethyl-3-isobutyl-1-methylxanthine** (8-Methoxymethyl-IBMX) (PDE1)
M 4659	**Milrinone** (PDE3)
P 3510	**Papaverine hydrochloride** (nonselective)
P 1784	**Pentoxifylline** (nonselective)
P 9689	**Propentofylline** (nonselective)
Q 3504	**Quazinone** (PDE3)
R 6520	**Rolipram** (PDE4)
T 8067	**T-0156** (PDE5)
T 7692	**T-1032** (PDE5)
T 4500	**Theobromine** (PDE5)
T 1633	**Theophylline** (PDE5)
T 2057	**Trequinsin hydrochloride** (PDE3)
V 6383	**Vinpocetine** (PDE1)
Y 4877	**YM976** (PDE4)
Z 0878	**Zaprinast** (nonselective)
Z 3003	**Zardaverine** (PDE 3/4)

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Inhibitors of Cyclic Nucleotide Phosphodiesterases...*(continued)*

Research on the function of specific PDEs has so far relied heavily on a limited number of commercially available compounds that display only partial selectivity for specific PDE isoforms. Truly selective compounds are still not available for many PDE families and, in particular, for the more recently discovered PDE families 7 to 11. Table 1 lists a variety of PDE inhibitors, some of which are non-selective (i.e. they inhibit more than one family) while others are relatively selective (i.e. they exhibit some degree of specificity when tested in *in vitro* PDE activity assays). Most of the available inhibitors are methylxanthines or isoquinoline derivatives and are substrate competitors, a characteristic that to some extent compromises their selectivity. For example, sildenafil possesses a high affinity for PDE5A (IC_{50} 10 nM). However, at higher concentrations, it can also inhibit PDE6 (IC_{50} 50 nM), likely explaining the visual disturbances experienced by some patients when taking this compound. Moreover, differences in inhibitor sensitivity can be observed between different members of each PDE family [53,58,59] and between transcript variants [53,60]. Specific drugs for different genes and transcript variants within a PDE family have yet to be developed.

The evaluation of PDE inhibitors *in vivo* or *ex vivo* has to take into consideration a number of factors including the issue of differential cell permeability, the uncertainty of the actual intracellular concentration of inhibitor and the profile and subcellular localization of the PDEs in the specific cell type being studied. The concentration dependence of an inhibitor effect *in vivo* might also differ from the *in vitro* profile as a small inhibition of PDE activity may have a significant effect on the physiological proccess being studied.

Moreover, a disparity between the concentration dependence for an inhibitor *in vitro* and *in vivo* can result from the complexity of the *in vivo* situation versus a broken cell extract or a purified enzyme assay. For example, **trequinsin** (Prod. No. **T 2057**) has been described as a PDE2 inhibitor and, when tested *in vivo* in some tissues, has been shown to act as such [48]. However, *in vitro* and in many other tissues, it is actually much more potent at inhibiting PDE3 [61,62]. Therefore, trequinsin can be used to obtain information on the function of either PDE2 or PDE3, depending on the differential expression of PDEs by a specific tissue or cell type. Potent and partially selective PDE1 inhibitors have been described [58] including **vinpocetine** (Prod. No. **V 6383**) and **8-methoxymethyl-IBMX** (Prod. No. **M 2547**), but their use to access the function of these enzymes in cells has been limited by the requirement to use high doses, thus compromising their specificity. Conversely, various PDE3 and PDE4 inhibitors, such as **milrinone** (Prod. No. **M 4659**) and **rolipram** (Prod. No. **R 6520**) have been more amenable to *in vivo* studies allowing the role of these enzymes in numerous cell processes to be more effectively investigated [63,64].

The development of drugs that target PDEs will clearly take advantage of the recent clarification of the structure of their catalytic domain, their mechanism of catalysis and the structure of their regulatory domains. The definition of the crystal structures of PDE4D and PDE5A in complex with the non-selective inhibitor **3-isobutyl-1-methylxanthine** (IBMX; Prod. No. **I 5879**) shows that the majority of the IBMX binding residues are conserved between PDE4 and PDE5, underscoring the lack of selectivity of this inhibitor [7]. More

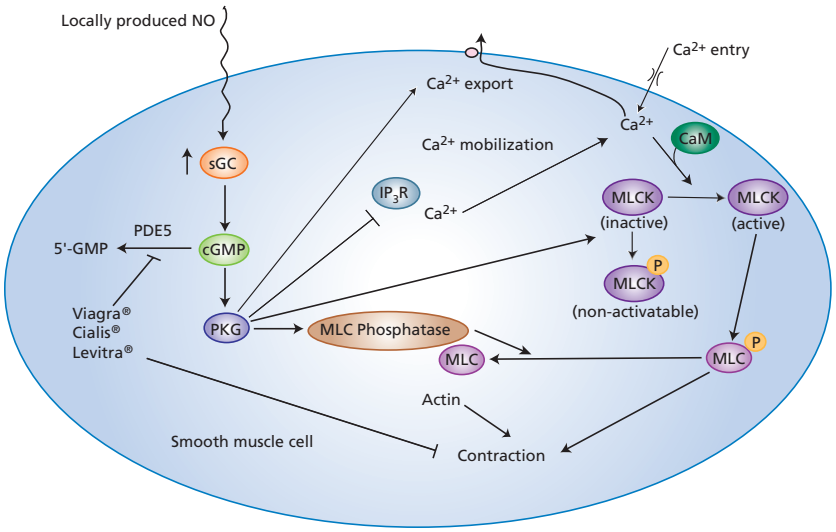


Figure 2. Mechanism of action of the drugs sildenafil (Viagra®), tadalafil (Cialis®) and vardenafil (Levitra®).
Penile erection occurs when blood swells the corpus cavernosum, an effect facilitated by relaxation of regional smooth muscle. Smooth muscle tone is regulated by cellular Ca^{2+}, which activates the Ca^{2+}/calmodulin (CaM)-dependent enzyme myosin light chain kinase (MLCK), which leads to MLC phosphorylation and contraction. The nitric oxide (NO) pathway leads to relaxation of smooth muscle by stimulating the soluble guanylyl cyclase (sGC), which results in the production of cyclic GMP (cGMP) and the activation of cGMP-dependent protein kinase (PKG). PKG causes smooth-muscle relaxation by mechanisms that are still being defined, but which include a reduction in cytosolic Ca^{2+} (by enhanced Ca^{2+} export and/or by reduced inositol trisphosphate ($InsP_3$; **inositol 1,4,5-trisphosphate**; Prod. No. **I 7012**) receptor-mediated Ca^{2+} mobilization) and dephosphorylation of myosin light chains (by activation of MLC phosphatase and/or by sequestration of MLCK in a phosphorylated form that is not readily activated by Ca^{2+}/CaM). Sildenafil (Viagra®), tadalafil (Cialis®) and vardenafil (Levitra®) specifically inhibit the breakdown of cellular cGMP by PDE5 that is localized in erectile tissue, thereby prolonging and enhancing the effects of NO and cGMP on blood flow.

interestingly, these models show that loop regions are also involved in the formation of the active site and in the binding of the inhibitor suggesting that the conformational flexibility of the loops, as well as the chemical nature of the residues making contact, may play a role in inhibitor selectivity. Similarly, comparison of PDE5A complexed with different inhibitors has suggested modifications to improve binding affinity and selectivity [8]. While more selective drugs will clearly need to be developed in order to fully explore the pharmacological potential of PDE inhibition, several compounds are currently approved or been evaluated in clinical trials for the treatment of various conditions including **dipyridamole** (Aggrenox; Prod. No. **D 9766**) for the prevention of stroke, and the PDE3 inhibitors **enoximone** (Prod. No. **E 1279**) and **milrinone** (Prod. No. **M 4659**) in heart failure. The PDE4 inhibitors rolipram, roflumilast and cilomilast are also being evaluated as anti-inflammatory drugs to treat multiple sclerosis, asthma and chronic obstructive pulmonary disease (see http://clinicaltrials.gov).

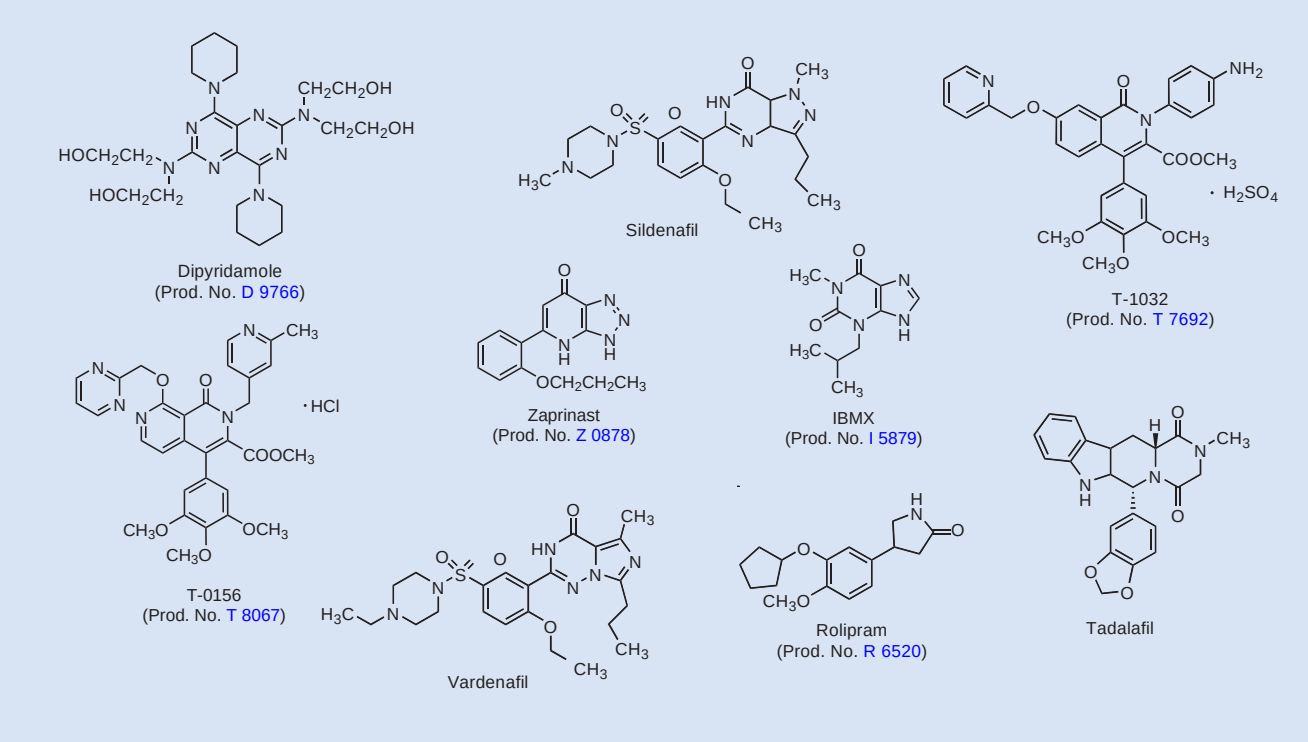
PDE Knockout Models

It is likely that the study of specific PDE knockout models, which are just beginning to become available, will be crucial for understanding the roles of individual PDEs in physiological and pathophysiological processes. Such studies should also provide the rationale for developing new therapies. This potential is illustrated by the first PDE4 knockout studies. When the phenotype of the PDE4D null mouse was tested in an asthma model (by measuring airway resistance), the bronchoconstriction response to muscarinic cholinergic stimulation was abolished [65]. This phenotype reinforces the pharmacological potential of PDE4D-specific inhibitors in the treatment of airway diseases associated with smooth muscle contraction, such as asthma and chronic obstructive pulmonary disease.

The PDE4-family inhibitor, rolipram, is known to be an effective antidepressant, although the emetogenic side-effects of this agent have limited its use to date. However, in this situation, PDE inhibition is considered to be one of the more promising new targets in the treatment of depression [66]. Behavioral and pharmacological tests performed on PDE4D knockout mice have revealed that this specific variant most likely mediates the antidepressant-like effects of rolipram [67]. It seems possible, therefore, that PDE4D-specific drugs may possess therapeutic potential that is devoid of the side effects associated with rolipram, provided that PDE4D inhibition is not also responsible for the side effects of this drug [68]. Similarly, targeting the PDE4B gene has revealed an essential role for this isoform in the innate immune response mediated by **tumor necrosis factor** α (TNF-α; Prod. Nos. **T 7539, T 5944, T 6674**) [69]. Therefore, selective inhibitors of PDE4B may represent a favorable pharmacological strategy for the treatment of TNF-α-mediated diseases such as rheumatoid arthritis, Crohn's disease and septic shock.

While these studies confirm previous pharmacological data on the involvement of PDE4 in various disease states, they also point to specific variants of PDE4 as drug targets for different conditions, perhaps opening a new era for PDE-isoform tailored drug development. It is expected that a

Figure 3. Structures of selected phosphodiesterase inhibitors. See Table 1 for isozyme selectivities.



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similar approach will provide clues to the role of PDEs that have yet to be functionally characterized. Other PDE knockout models investigated to date have generated more subtle phenotypes, possibly due to compensation by other PDEs or simply due to the intrinsic specific role of that PDE. Also, as is the case in many knockout models, determining the experimental approach that will reveal an altered phenotype can be the limiting step. For example, only sophisticated metabolic tests showed that PDE7A and PDE3B knockout mice possess defects in glucose homeostasis [70,71]. These observations suggested a new physiological role for PDE7A in the regulation of glucose metabolism and confirm the role of PDE3B in modulating insulin action. Finally, PDE1B knockout mice show a defect in spatial learning while also exhibiting locomotor hyperactivity [72].

Conclusions

Several new PDEs have been discovered in the past 10 years, although their specific functions are often still unclear due in part to the lack of availability of selective inhibitors. It seems likely, therefore, that functional testing of specific PDE knockout mice will lead to a new understanding of the role played by different PDE isozymes, particularly when tissue-specific conditional knockout animals become available. These transgenic model systems, and possibly RNA interference, will provide tools with which to dissect the function of individual PDEs, especially for those isoforms for which no PDE inhibitor is currently commercially available. In addition, these new approaches will help guide the development of targeted drugs. While most of the drugs developed so far are based on the cyclic nucleotide structure and are substrate competitive, better structural knowledge of the PDE catalytic and allosteric sites should permit the development of more potent and selective drugs to target the major PDEs of specific cell types believed to be involved in pathophysiological processes.

References

1. Beavo, J.A. and Brunton, L.L., *Nat. Rev. Mol. Cell Biol.*, **3**, 710-718 (2002).
2. Beavo, J.A., *Physiol. Rev.,* **75**, 725-748 (1995).
3. Soderling, S.H. and Beavo, J.A., *Curr. Opin. Cell Biol.,* **12**, 174-179 (2000).
4. Mehats, C., et al., *Trends Endocrinol. Metab.*, **13**, 29-35 (2002).
5. Huai, Q., et al., *Biochemistry*, **42**, 13220-13226 (2003).
6. Xu, R.X., et al., *Science* **288**, 1822-1825 (2000).
7. Huai, Q., et al., *J. Biol. Chem.,* in press (2004).
8. Sung, B.J., et al., *Nature,* **425**, 98-102 (2003).
9. Patel, S.B., et al., *Acta Crystallogr. D. Biol. Crystallogr.*, **60**, 169-171 (2004).
10. Baylor, D., *Proc. Natl. Acad. Sci. USA,* **93**, 560-565 (1996).
11. Tasken, K. and Aandahl, E.M., *Physiol. Rev.,* **84**, 137-167 (2004).
12. Brunton, L.L., *Sci STKE* 2003, PE44 (2003).
13. Cooper, D.M., *Biochem. J.*, **375**, 517-529 (2003).
14. Karpen, J.W. and Rich, T.C., *Science,* **293**, 2204-2205 (2001).
15. Bolger, G.B., et al., *J. Biol. Chem.*, **278**, 49230-49238 (2003).
16. Arp, J., et al., *Mol. Cell Biol.*, **23**, 8042-8057 (2003).
17. Baillie, G.S., et al., *Proc. Natl. Acad. Sci. USA,* **100**, 940-945 (2003).
18. Rich, T.C., et al., *Proc. Natl. Acad. Sci. USA*, **98**, 13049-13054 (2001).
19. Honda, A., et al., *Proc. Natl. Acad. Sci. USA*, **98**, 2437-2442 (2001).
20. Zaccolo, M., et al., *Curr. Opin. Cell Biol.,* **14**, 160-166 (2002).
21. Xie, F. and Conti, M., *Dev. Biol.*, **265**, 196-206 (2004).
22. Zippin, J.H., et al., *FASEB J.,* **17**, 82-84 (2003).
23. Smith, C.J., et al., *J. Biol. Chem.*, **266**, 13385-13390 (1991).
24. Houslay, M.D. and Adams, D.R., *Biochem. J.*, **370**, 1-18 (2003).
25. Murthy, K.S., *Biochem. J.*, **360**, 199-208 (2001).
26. Rybalkin, S.D., et al., *EMBO J.*, **22**, 469-478 (2003).
27. Tasken, K.A., et al., *J. Biol. Chem.*, **276**, 21999-22002 (2001).
28. Dodge, K.L., et al., *EMBO J.*, **20**, 1921-1930 (2001).
29. Martinez, S.E., et al., *Proc. Natl. Acad. Sci. USA*, **99**, 13260-13265 (2002).
30. Maurice, D.H., et al., *Mol. Pharmacol.*, **64**, 533-546 (2003).
31. Sonnenburg, W.K., et al., *J. Biol. Chem.*, **270**, 30989-31000 (1995).
32. Seybold, J., et al., *J. Biol. Chem.*, **273**, 20575-20588 (1998).
33. D'Sa, C., et al., *J. Neurochem.*, **81**, 745-757 (2002).
34. Lee, R., et al., *Cell Signal,* **14**, 277-284 (2002).
35. Niiya, T., et al., *FEBS Lett.,* **505**, 136-140 (2001).
36. Le Jeune, I.R., et al., *J. Biol. Chem.*, **277**, 35980-35989 (2002).
37. Torras-Llort, M. and Azorin, F., *Biochem. J.*, **373**, 835-843 (2003).
38. Dryja, T.P., et al., *Invest. Ophthalmol. Vis. Sci.*, **40**, 1859-1865 (1999).
39. Gal, A., et al., *Nat. Genet.*, **7**, 551 (1994).
40. Gretarsdottir, S., et al., *Nat. Genet.*, **35**, 131-138 (2003).
41. Francis, S.H. and Corbin, J.D., *Curr. Urol Rep.*, **4**, 457-465 (2003).
42. Coste, H. and Grondin, P., *Biochem. Pharmacol.*, **50**, 1577-1585 (1995).
43. Ukita, T., et al., *Bioorg. Med. Chem. Lett.*, **13**, 2341-2345 (2003).
44. Ukita, T., et al., *J. Med. Chem.*, **44**, 2204-2218 (2001).
45. Raffelmann, T. and Kloner, R.A., *Circulation*, **108**, 239-244 (2003).
46. Manganiello, V., *Mol. Pharmacol.,* **63**, 1209-12311 (2003).
47. Kruse, C., et al., *Brain*, **126**, 241-247 (2003).
48. Whalin, M.E., et al., *Mol. Pharmacol.*, **39**, 711-717 (1991).
49. Aoki, M., et al., *J. Pharmacol. Exp. Ther.*, **295**, 255-260 (2000).
50. Boolell, M., et al., *Int. J. Impot. Res.*, **8**, 47-52 (1996).
51. Daugan, A., et al., *J. Med. Chem.*, **46**, 4533-4542 (2003).
52. Saenz de Tejada, I., et al., *Int. J. Impot. Res.*, **13**, 282-290 (2001).
53. Hetman, J. M., et al., *Proc. Natl. Acad. Sci. USA*, **97**, 472-476 (2000).
54. Soderling, S.H., et al., *Proc. Natl. Acad. Sci. USA,* **95**, 8991-8996 (1998).
55. Soderling, S.H., et al., *J. Biol. Chem.*, **273**, 15553-15558 (1998).
56. Soderling, S.H., et al., *Proc. Natl. Acad. Sci. USA*, **96**, 7071-7076 (1999).
57. Hetman, J. M., et al., *Proc. Natl. Acad. Sci. USA*, **97**, 472-476 (2000).
58. Yan, C., et al., *J. Biol. Chem*, **271**, 25699-25706 (1996).
59. Wang, P., et al., *Biochem. Biophys. Res. Commun.*, **234**, 320-324 (1997).
60. Bolger, G.E., et al., *Biochem. J.*, **328**, 539-48 (1997).
61. Ruppert, D. and Weithmann, K.U., *Life Sci.*, **31**, 2037-2043 (1982).
62. Haynes, J. Jr., et al., *Am. J. Physiol.,* **261**, H487-492 (1991).
63. Manganiello, V.C. and Degerman, E., *Thomb. Haemost.*, *82*, 407-411 (1999).
64. Conti, M., et al., *J. Biol. Chem.*, **278**, 5492-5496 (2003).
65. Mehats, C., et al., *FASEB J.,* **17**, 1831-1841 (2003).
66. Holden, C., *Science,* **302**, 810-813 (2003)
67. Zhang, H.T., et al., *Neuropsychopharmacology*, **27**, 587-595 (2002).
68. Robichaud, A., et al., *J. Clin. Invest.*, **110**, 1045-1052 (2002).
69. Jin, S.L. and Conti, M., *Proc. Natl. Acad. Sci. USA*, **9**, 7628-7633 (2003).
70. Michaeli, T, et al., *Diabetologia* **43**, 138 Suppl. 1 (2000).
71. Choi, Y.H., et. al., *FASEB J.,* **17**, A593 Part 1 Suppl (2003).
72 Reed, T.M., et al., *J. Neurosci.*, **22**, 5188-5197 (2002).

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