

## Phosphodiesterase Enzymes – Target Overview

a report by

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Phosphodiesterase enzymes (PDEs) hydrolyse the important intracellular second-messenger cyclic nucleotides cyclic guanosine monophosphate (cGMP) and cyclic adenosine monophosphate (cAMP) to produce the corresponding nucleoside phosphates. The substrate cyclic nucleotides are formed by their cognate nucleotide cyclase enzymes, which in turn are associated with cell surface receptors. Receptor activation can either stimulate or inhibit the cyclase reaction, leading to fluctuations in cyclic nucleotide levels and initiation or inhibition of signal transduction pathways. By virtue of its ability to control cyclic nucleotide levels, the phosphodiesterase reaction is an important mechanism by which biological processes and pathways can be controlled.

### Class I Phosphodiesterase Enzymes Gene Family

In the most interesting class I PDE gene family, there is a total of 11 different families of mammalian enzymes (see *Table 1*). The cAMP-specific enzymes include PDE4, PDE7 and PDE8, and the cGMP-specific enzymes are PDE5, PDE6 and PDE9. The remaining family members can hydrolyse either cyclic nucleotide; some have a preference for one over the other. The various isozymes within a family are designated by a letter, and in many cases there are distinct splice variants designated by a Roman numeral. For example, PDE5A1 refers to one of the three known isozymes of PDE5A. These isozymes are often associated with specific regions of the cell, such as membranes or Golgi apparatus, as well as the cytosol, and it has been hypothesised that these N-terminal modifications help to direct and/or anchor the enzymes in their respective location within the cell.

### Phosphodiesterase Enzymes and Regulatory Terminal Domains

Structurally, PDEs share a common motif, with an N-terminal regulatory domain, a C-terminal catalytic domain and a carboxy terminal domain. In some cases the regulatory domain regions incorporate peptide sequences

that share common functional purposes, such as a cGMP-regulated adenylyl cyclase and Fh1A (GAF)-binding domain or a targeting domain. Each phosphodiesterase has a unique affinity ( $K_m$  value) and catalytic efficiency ( $V_{max}$ ) for its cyclic nucleotide substrate(s). The multiplicity of enzymes, their specific subcellular location(s) and even their affinity for substrate are thought to contribute to the role these enzymes play in modulating signal transduction pathways. For example, it is not uncommon for one cell type, e.g. cortical cells in the brain, to contain more than one PDE, each of which is responsible for distinct functions in

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cell biology and signalling. From a lead optimisation and clinical development perspective, it is important to pay attention to PDE selectivity in order to minimise potential side effects associated with inhibition of other enzymes in the family. For example, it is well-known that visual disturbances are associated with inhibition of PDE6 and nausea is associated with PDE4, although this may be associated with inhibition of a specific isoform, and inhibition of PDE3A can result in cardiac arrhythmia.

### Therapeutic Applications of Phosphodiesterase Enzymes

As the role(s) these enzymes play in second messenger pathways have been uncovered, they have been investigated for a wide variety of potential therapeutic applications. Perhaps the best known of these are PDE5 inhibitors and their use in the treatment of erectile dysfunction (ED). The first PDE5 inhibitor approved for use was sildenafil (Viagra<sup>®</sup>, Pfizer) in 1998, and it remains the most widely used PDE5 inhibitor today. This was followed in 2001 by vardenafil (Levitra<sup>®</sup>, Bayer/GSK/Schering Plough), and in 2003 by tadalafil (Cialis<sup>®</sup>, ICOS/Lilly). All three of these compounds are potent inhibitors ( $IC_{50}$  values less than 10nM) of PDE5. Sildenafil and vardenafil are chemically similar, differing primarily in their core heterocyclic structures. Sildenafil has a pyrazolopyrimidine core and vardenafil has an isomeric imidazotriazene template, while tadalafil is a tetrahydrocarboline derivative. These compounds can be differentiated primarily based on their comparative *in vitro* PDE selectivity profile and/or duration of action. There are other PDE5 inhibitors undergoing clinical development, and this remains an active area of research. PDE5 is effective in the treatment of ED because it is the primary cGMP-metabolising enzyme present in the corpus cavernosum, the spongy tissue in the penis that helps to control blood flow. Enzyme inhibition



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asthma. In addition to participation in phosphodiesterase lead optimisation programmes, Dr Rotella has also been involved in metabolic and CNS disease drug-discovery projects. He is National Programme Chair of the Division of Medicinal Chemistry of the American Chemical Society. He has published over 25 papers, including invited reviews on phosphodiesterase enzymes, and has given a number of invited lectures on the same subject. Dr Rotella carried out post-doctoral research with Professor Ken Feldman at Penn State University after earning his PhD in medicinal chemistry under Professor Donald T Witiak at the Ohio State University.

elevates levels of cGMP, leading to smooth muscle relaxation that restricts blood flow from the tissue. More recently, sildenafil was approved for use in the treatment of pulmonary hypertension. PDE5 inhibition is also being clinically investigated as a potential treatment for benign prostatic hypertrophy, based on the high level of enzyme expression in this tissue.

### Anti-inflammatory and Central Nervous System Applications

PDE4 has been widely investigated for anti-inflammatory and central nervous system (CNS) applications, including cognition and depression/anxiety. However, unlike PDE5 no compounds have been approved for marketing that function primarily as PDE4 inhibitors. Their anti-inflammatory activity – as it relates to pulmonary function for the potential treatment of asthma and chronic obstructive pulmonary disease – has been the focus of much of the clinical investigation into this phosphodiesterase. Several compounds are undergoing clinical evaluation for pulmonary applications, and others are being studied for conditions such as psoriasis, where the anti-inflammatory action of the compound may be useful. Anti-inflammatory activity is associated with a reduction in biosynthesis of tumour necrosis factor (TNF)- $\alpha$ , an important cytokine involved in tissue response to insult or inflammatory stimuli. Questions have arisen about side effect profile, efficacy and safety with many PDE4 inhibitors in the clinic.

More recently, CNS indications have come to the forefront and are being actively studied. Many investigators have demonstrated that prototypical PDE4 inhibitors such as rolipram are active in animal models of memory and learning, cognition and depression. Detailed studies in animals suggested that the antidepressant effects of PDE4 inhibitors are exerted in part by upregulation of brain-derived neurotrophic factor biosynthesis. The precise mechanism is not well understood, but is related to the cellular effects associated with increased cAMP levels in target cells. However, questions such as which PDE4 isozyme to target and the degree of selectivity needed to reduce the risk of mechanism-based adverse events have eluded clear definition because it is difficult to identify PDE4 inhibitors with sufficient isozyme selectivity to use as tools for such work. The two isozymes of primary interest in this regard are PDE4B and 4D. These enzymes share a high degree of sequence homology in and near their active sites, where most inhibitors bind.

### Immunological Applications

There has been some interest in the identification of PDE7 inhibitors for immunological applications based on preliminary work using small interfering RNA (siRNA) that indicated PDE7 inhibition could regulate T-cell proliferation. Interestingly, a series of small-molecule PDE7 inhibitors showed similar effects on T-cell proliferation in both wild-type and knockout mice. The disconnection between these two independent experiments remains to be resolved. PDE7B messenger RNA (mRNA) has been identified in the brain; however, no specific physiological function(s) associated with this isozyme have been elucidated. More recently, attention has been focused on three other PDEs for their potential as targets for CNS disorders. PDE2, PDE9 and PDE10 are being studied as possible targets for treatment of cognitive disorders, depression/anxiety and schizophrenia. For example, papaverine is a moderately potent PDE10 inhibitor that has demonstrated activity in animal models predictive of antischizophrenic activity (conditioned avoidance responding). When papaverine was administered to PDE10A knockout mice, no positive effect

**Table 1: The Type I Phosphodiesterase Family**

PDE	Number of Isozymes	Cyclic Nucleotide Substrates
PDE1	3 (A–C)	1A-cGMP>cAMP; 1B, 1C, no preference
PDE2	1	cGMP>cAMP
PDE3	2 (A, B)	No preference
PDE4	4 (A–D)	cAMP
PDE5	1	cGMP
PDE6	3 (A–C)	cGMP
PDE7	2	cAMP
PDE8	2	cAMP
PDE9	1	cGMP
PDE10	1	cAMP>cGMP
PDE11	1	No preference

was noted, suggesting a mechanistic link between activity in the animal model and the enzyme. Another small molecule, BAY 73-6691 – manufactured by Bayer – is a PDE9 inhibitor that was shown to elevate cGMP levels in rat cortical brain sections. cGMP has been shown to play a role in cognition, suggesting that PDE9 inhibitors may be of interest in the treatment of Alzheimer's disease or other cognitive disorders. Another Bayer compound, BAY 60-7550, a potent PDE2 inhibitor, has shown similar activity in tissue culture, and has demonstrated activity in animal models of learning and memory. These investigations are closely linked to ongoing work to identify the cell types and brain regions where the enzymes are expressed, and the mechanism(s) by which modulation of cyclic nucleotide levels influences responses in the pharmacological assays.

### Challenges for the Future

One of the features that makes these studies challenging is the tight and multiple means by which cells regulate cyclic nucleotide levels. As noted above, a particular cell type may express a given PDE in one region, and others in separate portions of the cell. The signal that is initiated by a cyclic nucleotide in the former may be ultimately expressed in the latter, where

Phosphodiesterase enzymes that hydrolyse both substrates can do so based on the local concentration of a given cyclic nucleotide and/or the precise intracellular location of the enzyme.

distinct regulatory processes are operative. For example, it has been hypothesised that PDE4, a cAMP-selective enzyme with comparatively low affinity for its substrate, functions to modulate cyclic nucleotide levels at relatively high concentrations, while high-affinity cAMP-selective enzymes such as PDE7 or 8 function at the lower end of the cAMP concentration spectrum. PDEs that hydrolyse both substrates can do so based on the local concentration of a given cyclic nucleotide and/or the precise intracellular location of the enzyme. Consequently, there is a great deal of attention paid to the identification of PDE inhibitors with a sufficient degree of selectivity to attempt to address these questions with a minimum of ambiguity. The challenges associated with this objective have been, and continue to be, the basis of ongoing medicinal chemistry studies of this therapeutically promising enzyme family. ■