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Review

Phosphodiesterase: overview of protein structures, potential therapeutic applications and recent progress in drug development

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Abstract. Phosphodiesterases (PDEs) are essential regulators of cyclic nucleotide signaling with diverse physiological functions. Because of their great market potential and therapeutic importance, PDE inhibitors became recognized as important therapeutic agents in the treatment of various diseases. Currently, there are seven PDE inhibitors on the market, and the pharmacological and safety evaluations of many drug candidates are in progress. Three-dimensional (3D) structures of catalytic domains of PDE 1, -3, -4, -5 and -9 in the presence of their inhibitors are now available, and can be utilized for rational drug design. Recent advances in molecular pharmacology of PDE isoenzymes resulted in identification of new potential applications of PDE inhibitors in various therapeutic areas, including dementia, depression and schizophrenia. This review will describe the latest advances in PDE research on 3D structural studies, the potential of therapeutic applications and the development of drug candidates.

Key words: Phosphodiesterase; cAMP; cGMP; 3D structure; drug discovery.

Introduction

Phosphodiesterases (PDEs) are a superfamily of enzymes that degrade cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) [1–3]. There are now 11 PDE families identified, many of which exist as splice variants [4, 5]. The cAMP-specific enzymes include PDE4, -7 and -8. The cGMP-specific PDEs are PDE5, -6 and -9, whereas PDE1, -2, -3, -10 and -11 use both cyclic nucleotides [6]. PDEs influence a vast array of pharmacological processes, including proinflammatory mediator production and action, ion channel function, muscle contraction, learning, differentiation, apoptosis, lipogenesis, glycogenolysis and gluconeogenesis [7]. As essential regulators of cyclic nucleotide signaling with diverse physiological functions, PDEs have become recognized as important drug targets for the treatment of various diseases, such as heart failure, depression, asthma, inflammation and erectile dysfunction [6, 8–10].

cAMP and cGMP are ubiquitous second messengers responsible for transducing effects of various extracellular signals, including hormones, light and neurotransmitters. These cyclic nucleotides are formed from ATP and GTP by the catalytic reactions of adenylyl cyclase and guanylyl cyclase, respectively. Adenylyl cyclase can be activated by forskolin and guanylyl cyclase by nitric oxide (NO). Through cell-surface receptors such as β-adrenoreceptor and prostaglandin E2, these enzymes can also be activated indirectly [10].

As the intracellular concentrations of the cyclic nucleotides rise, they bind to and activate their target enzymes, protein kinase A (PKA) and protein kinase G (PKG). These protein kinases phosphorylate substrates such as ion channels, contractile proteins and transcription factors, which regulate key cellular functions. Phospho-

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rylation alters the activity of these substrates and thus changes cellular activity. Obviously, altering the rate of cyclic nucleotide formation or degradation will change the activation state of these pathways [11].

By the late 1970s and early 1980s it became clear that kinetically distinct PDEs could indeed be inhibited selectively by a variety of small organic molecules [12–15]. As there are big therapeutic markets and unmet medical needs for the diseases related to PDE proteins, research and development on PDE inhibitors is growing rapidly. Three drugs acting on PDE5 and four drugs on PDE3 have been launched. Two drug candidates of PDE4 inhibitors are awaiting approval. Currently, about 20 PDE4 inhibitors are undergoing clinical studies, and hundreds of compounds are reported to be in discovery stages [16]. Recent advances in understanding the 3D structure of PDEs and their inhibitors have led to rational drug discoveries and optimization of lead compounds. The 3D structures of the catalytic domains of PDE1, -3, -4, -5 and -9 are currently available [17–26]. We can access 3D coordinates to investigate binding of inhibitors, substrate discrimination mechanisms of PDEs, inhibitor selectivity and information on further optimization of inhibitors.

The purpose of this review is to describe the latest developments in PDE research from a drug discovery point of view. We present 3D-structural aspects of PDEs involved in regulating each PDE isoenzyme, the rationale and attempts to exploit PDEs as new therapeutic targets, and the chemotherapeutic potential of current PDE inhibitors.

Structural basis of PDE catalysis and inhibition

Various genes encoding human PDEs can be classified by their substrate specificities. One group of PDEs selectively hydrolyzes cyclic AMP (PDE4, -7 and -8), the second group of PDEs are cyclic GMP-specific enzymes (PDE5, -6 and -9), and the rest hydrolyze both cAMP and cGMP (PDE1, -2, -3, -10 and -11) [27–29]. PDEs contain three functional domains, including a conserved catalytic core, a regulatory N-terminus and the C-terminus [30, 31]. Regulatory N-terminal domains of these enzymes that vary widely among the PDE classes are flanked by the catalytic core and include regions that auto-inhibit the catalytic domains, as well as targeting sequences that control subcellular localization [32, 33]. This region contains a calmodulin binding domain in PDE1, cyclic GMP binding sites in PDE2, phosphorylation sites for various protein kinases in PDE1–5, and a transducin binding domain in PDE6. All PDEs contain a conserved catalytic domain of approximately 270 amino acids (18–46% of sequence identity) at the carboxyl terminus. Due to the need to develop selective PDE inhibitors as therapeutic drugs, the structures of the catalytic domains of PDEs, which contain the active pocket that accommodates inhibitors, have been elucidated. The crystal structures of the catalytic domains of PDE4B [34, 35], PDE4D [36–39], PDE5A [40], PDE3B [41], PDE1B [42] and PDE9A [43] have shown that catalytic domains of PDEs have three helical subdomains (fig. 1): an N-terminal cyclin-fold region, a linker region and a C-terminal helical bundle. A deep hydrophobic pocket is formed at the interface of the three subdomains and is composed of four subsites: a metal-binding site (M site), core pocket (Q pocket), hydrophobic pocket (H pocket) and lid region (L region) [23] (fig. 2). The M site is at the bottom of the pocket with several metal atoms, which bind to residues that are completely conserved in all PDE family members. Although the identity of the metal ions cannot be absolutely determined from the crystal structures, the observed geometry of the metal coordinating ligands, anomalous X-ray diffraction behavior and existing biochemical evidence all suggest that at least one of the metals is zinc and the other is likely to be magnesium [44–47]. In the PDE structures, these metal ions have an octahedral coordination geometry. The zinc coordination sphere is made up of three histidines, one aspartate and two water molecules, while the magnesium coordination

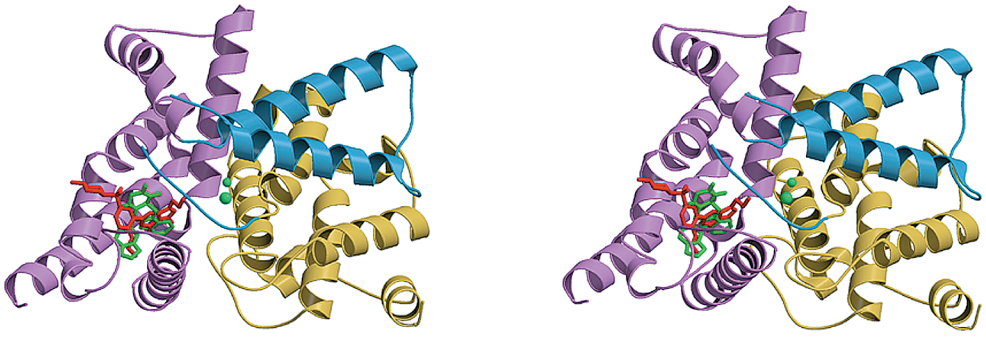

Figure 1. Overview of the PDE5 complex structures. Stereo ribbon diagram of the human PDE5 structure. The catalytic domain of the PDE5 molecule can be divided into three subdomains: an N-terminal cyclin-fold domain (residues 537–678, yellow), a linker helical domain (residues 679–725, blue) and a C-terminal helical bundle domain (residues 726–860, violet). The bound sildenafil and tadalafil molecules are overlapped and shown as stick models (red and green, respectively). Two metal ions are represented as green spheres.

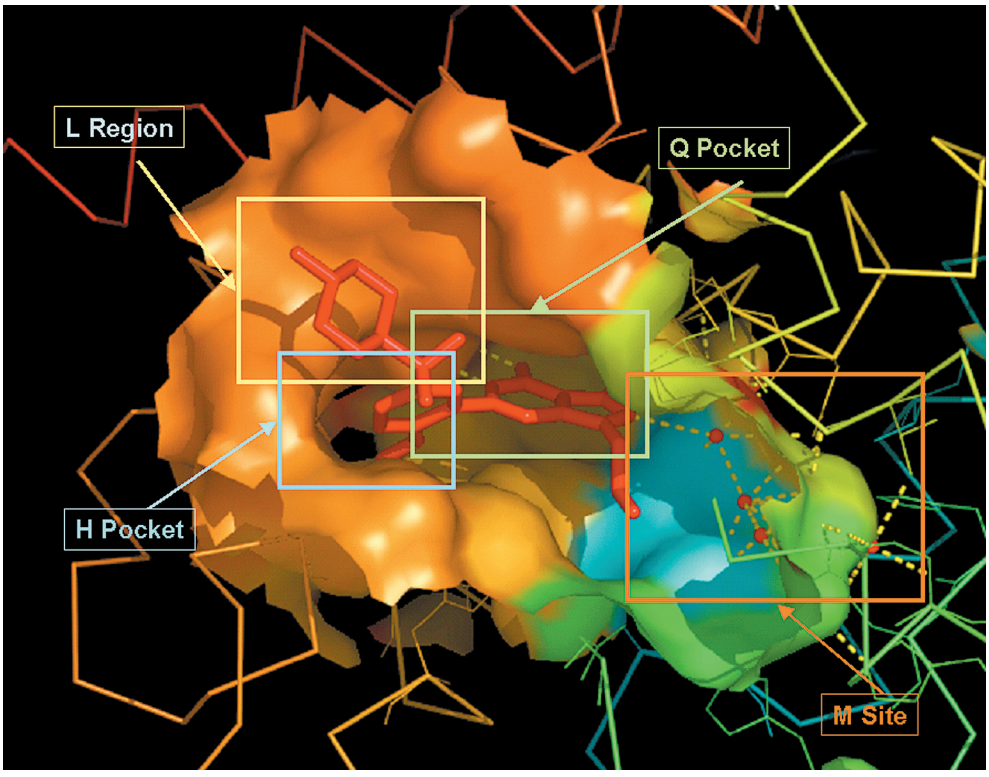

Figure 2. Surface representation of the active site of PDE5A occupied by sildenafil. The active site can be divided into four subsites: a metal-binding site (M site), core pocket (Q pocket), hydrophobic pocket (H pocket) and lid region (L region).

sphere involves the same aspartate and five water molecules, one of which is shared with the zinc molecule. The putative roles of these metal ions include stabilization of the structure and activation of hydroxide to mediate catalysis.

In the crystal structure of PDE5A in complex with sildenafil (Viagra), the Q pocket accommodates the pyrazolopyrimidinone group of sildenafil. This Q pocket provides the key hydrogen bonding of the conserved glutamine residue with substrates or inhibitors of PDEs, and the hydrophobic interactions, which come from the residues on both sides of the pyrazolopyrimidinone group, forming a 'clamp' like structure.

The ethoxyphenyl group of sildenafil fits into the hydrophobic H pocket. The variation of hydrophobic residues in the H pocket among PDEs can give PDE inhibitors the selectivity to corresponding PDEs.

The L region of PDE5A, composed of residues Tyr 664, Met 816, Ala 823 and Gly 819, surrounds the methylpiperazine group of sildenafil. The conformational change between closed and open forms of this region seems to be involved in inhibitor binding.

Structural features of each PDE have shown how the specificity of the substrate can be achieved. It has been proposed that PDE selectivity toward cyclic nucleotide is controlled by a so-called, 'glutamine switch' mechanism [42]. It has been proposed that an invariant glutamine residue plays an important role in PDE nucleotide selectivity, but the structures reveal an invariant. The γ-amino group of the conserved glutamine residue in the active site of the PDEs can alternatively adopt two different orientations: in one orientation the hydrogen bond network supports guanine binding, resulting in cGMP selectivity, and in the other orientation the network supports adenine binding, leading to selectivity toward cAMP. And in dual-specific PDEs the orientation of the side chain of glutamine can switch between the two orientations, resulting in dual specificity toward both cyclic nucleotides.

As an example, in the structure of PDE4D in complex with AMP, the conserved glutamine Q369 forms a bidentate H-bond with the adenine moiety (fig. 3E): the Nε atom of Q369 donates a H-bond to the N1 atom of the adenine ring, and the Oε atom accepts a H-bond from N6 in the exocyclic amino group of adenine. The orientation of this conformation of Q369 is stabilized by H-bonding of Oε to the phenolic hydroxyl group Oη of Y329. The structure of AMP-bound PDE4B is almost identical to that of AMP-bound PDE4D, implying that the mode of nucleotide recognition discussed above is also applicable to other PDE4 isoforms. By contrast, in the structure of PDE5A co-crystallized with GMP, the orientation of the side chain of conserved glutamine Q817 is switched from that in PDE4D to allow H-bonding specific to the guanine ring of cGMP (fig. 3F): Oε accepts an H-bond from

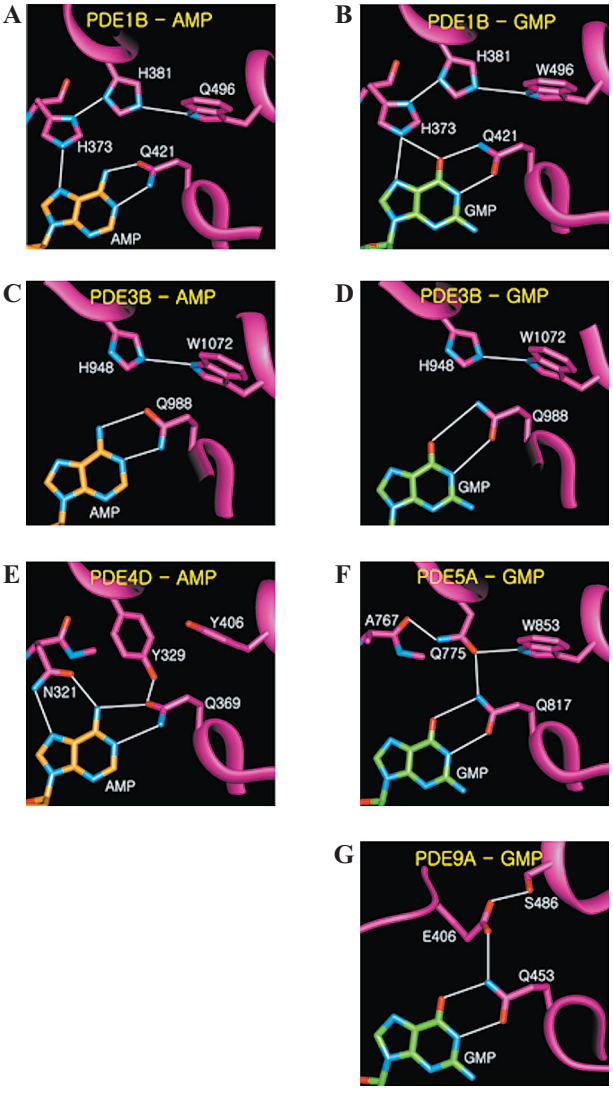

Figure 3. The glutamine switch mechanism for recognizing the purine moiety in cAMP or cGMP. (*A*) Q421 recognizing AMP in the model of AMP bound to PDE1B. (*B*) Q421 recognizing GMP in the model of GMP bound to PDE1B. In (*A*) and (*B*), there are no supporting residues to anchor the orientation of the conserved glutamine residue. (*C*) Q988 recognizing AMP in the model of AMP bound to PDE3B. (*D*) Q988 recognizing GMP in the model of GMP bound to PDE3B. In (*C*) and (*D*), there are no supporting residues to constrain the conformation of the glutamine residue. (*E*) Q369 recognizing AMP in PDE4D. Q369 forms a bidentate H-bond with adenine moiety, and its orientation is stabilized by the H-bond with Y329. In addition, N321 forms a bidentate H-bond with adenine moiety of AMP. (*F*) Q817 recognizing GMP in PDE5. The orientation of γ-amino group of Q817 is well ordered by a H-bond relay involving Q817 to Q775, Q775 to A767 and Q775 to W853. This H-bond relay accounts for the selective recognition of cGMP. (*G*) Q453 recognizing GMP in the model of GMP bound to PDE9A. The orientation of Q453 is anchored by the H-bond relay involving S486 to E406 and E406 to Q453 for selective recognition of GMP.

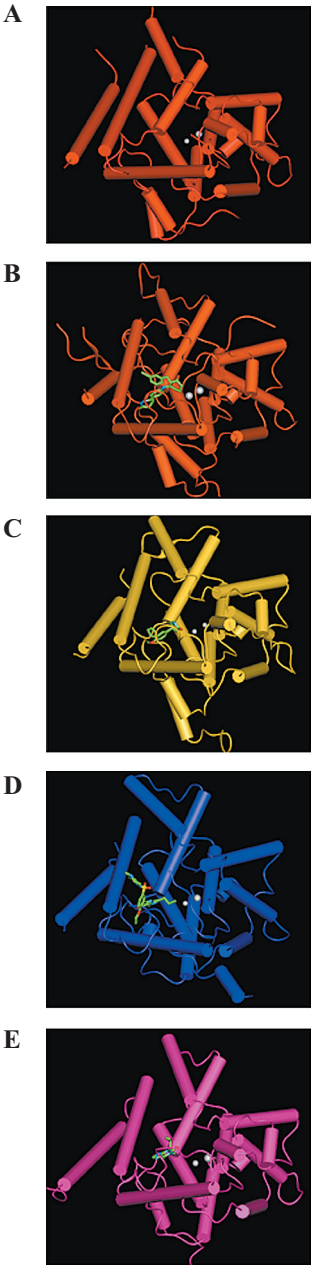

Figure 4. The crystal structures of PDEs in complexed with or without their inhibitors. The overall structures of PDE1B, PDE3B, PDE4D, PDE5A and PDE9A are represented in the same orientation. The bound inhibitors are represented in stick models and the metal ions are shown as white spheres. (*A*) PDE1B apo-structure. (B) PDE3B in complex with MERCK1 compound. (*C*) PDE4D in complex with rolipram. (*D*) PDE5A in complex with sildenafil. (*E*) PDE9A in complex with IBMX.directly involved in metal binding.

N1 in the guanine ring and Nε donates an H-bond to the exocyclic O6 atom of guanine. This orientation of Q817 is constrained by the H-bond relay involving Q817 to Q775, Q775 to A767 and Q775 to W853. This intricate network of H-bonding determines the orientation of the γ-amide group of Q817 that is favorable for cGMP but unfavorable for cAMP binding in PDE5.

PDE1B is a dual-specific enzyme that can hydrolyze either cAMP or cGMP. From the structure of PDE1B with a model of bound AMP or GMP (fig. 3A, B), the conserved glutamine Q421 may be able to adopt both of the orientations observed in the PDE4 and -5 structures, since there is no H-bonding network to constrain the orientation of its γ-amide group of Q421 in this enzyme. The binding of either cAMP or cGMP can be accommodated by the H-bonding flexibility by two histidine residues, H373 and H381.

PDE3B is also dually specific to both cyclic nucleotides. When a model of AMP or GMP is overlaid on the nucleotide binding site of PDE3B (fig. 3C, D), the dual specificity of PDE3B can be easily explained. The conserved glutamine Q988 can adopt any conformation, which can recognize both substrates owing to the lack of H-bonding constraints. Because the orientation of H948 is fixed by a H-bond with W1072, the conformation of Q988 is not affected by the neighboring residue, H948.

PDE9A is a cGMP-specific enzyme whose cGMP specificity originates from its unique H-bonding network, which determines the orientation of the conserved residue Q453 (fig. 3G). The orientation of Q453 is fixed by H-bonding with the side chain of E406, whose orientation is also determined by H-bonding with the hydroxyl group of S486.

The structural understanding of ligand interaction aids in the design of specific PDE inhibitors. The crystal structures of the catalytic domains of PDEs in complex with several inhibitors are available now. The overall folding patterns of the catalytic domains of PDEs are very similar, with compact α-helical structures (fig. 4). However, the comparison of ligand binding sites to different PDE family members can aid in understanding what is common to ligand binding and what regions of inhibitors or drugs are important for selectivity for individual PDE family members. Common features in ligand binding of PDEs are as follows (fig. 5): The central rings of inhibitors on the position of the purine rings of cAMP or cGMP interact with the conserved glutamine by a bidentate or single H-bond. In the structure of PDE3B in complex with MERCK1 compound, the dihydropiridazinone nitrogens form a bidentate H-bond with the conserved glutamine Q988. In the structure of PDE4D in complex with rolipram, the methoxy and cyclopentoxy oxygen atoms individually make H-bonds with the side chain NH2 of the conserved glutamine Q369. In the structure of PDE5A in complex with sildenafil, the pyrazolopyrimidinone group of sildenafil mimics that of guanine in cGMP and has the same H-bond donor and acceptor features to form a bidentate H-bond with Q817 through its amide orientation evolved to bind cGMP. Another common character is that the central rings of inhibitors are tightly held by a 'hydrophobic clamp' composed of side chains of hydrophobic residues. For example, the guanine moiety of cGMP or the pyrazolopyrimidinone group of sildenafil is sandwiched between the side chains of hydrophobic residues, F820 and V782. Finally, in contrast to the substrate binding, PDE inhibitors are not involved in interaction with metal ions. Effective interaction with metals directly or indirectly via water molecules may improve the potency of inhibitors.

Some side effects of sildenafil are known, and the main reason of the side effects is thought to be interaction with PDEs other than PDE5. To overcome the side effects of PDE inhibitors, the selectivity of inhibitors should be improved. Tadalfil is known to have fewer side effects

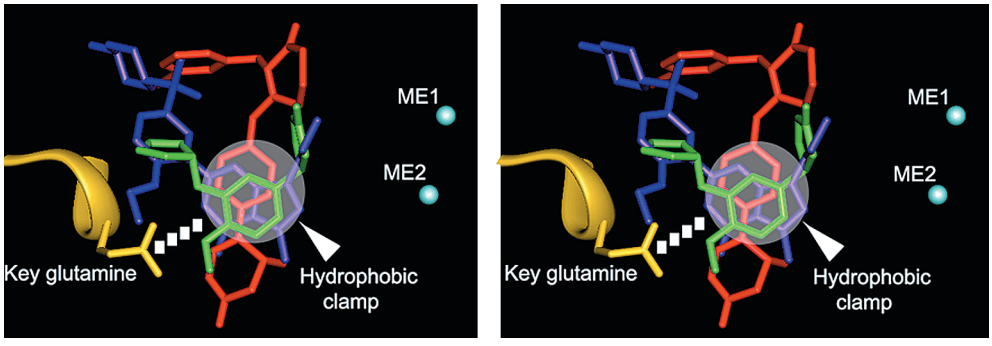

Figure 5. Stereo view of the common features of the inhibitor binding in PDEs. MERCK1 compound bound in PDE3D is colored as red, rolipram in PDE4D as green, and sildenafil in PDE5A as blue. The conserved glutamine residue is colored as yellow and the bound metal ions in cyan spheres. The central ring moieties of inhibitors on the position of the purine bases of substrate nucleotides interact with the conserved glutamine by a bidentate or single H-bond (dash line of squares), and are held tightly in the active site by the hydrophobic clamp represented as a whitened circle. No inhibitor is directly involved in metal binding.

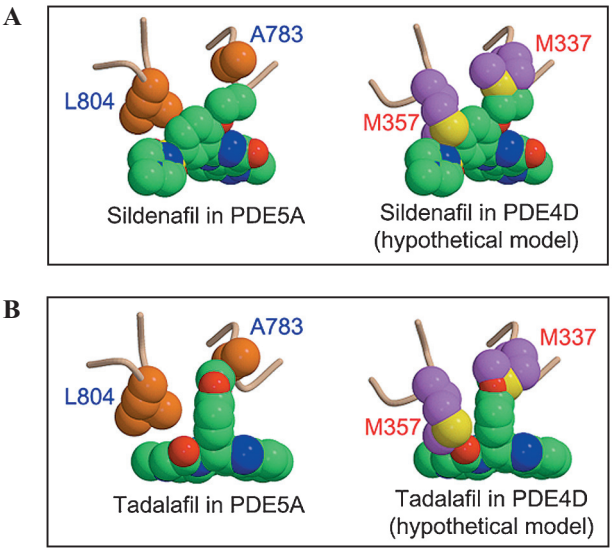

Figure 6. Effective hydrophobic interaction of PDE5 inhibitors is crucial for the selective inhibition. The key hydrophobic residues and PDE5A inhibitors are represented in space-filling models. (*A*) The hydrophobic residues, A783 and L804 of PDE5A, provide important interactions with sildenafil. In the hypothetical model of sildenafil bound to PDE4D, the corresponding residues, M337 and M357, could collide with sildenafil, hindering its binding. The side chain of M337 collides with the ethyloxy group of sildenafil. (*B*) In PDE5A complexed with tadalafil, the hydrophobic interactions of A783 and L804 with tadalafil are critical for the inhibitor binding to PDE5A. In the hypothetical model of tadalafil bound to PDE4D, the structural rigidity of methylenedioxyphenyl group of tadalafil inevitably causes a severe bumping with M337 of PDE4D.

than sildenafil. Sildenafil interacts hydrophobically with A783 and L804 of PDE5A (fig. 6A). In the hypothetical model of sildenafil bound to PDE4D, the corresponding residues, M337 and M357, would bump into sildenafil and greatly reduce the binding affinity. Nonetheless, the severe steric hindrance of M337 of PDE4D can be reduced slightly by the high flexibility of the ethyloxy group of sildenafil. In contrast to sildenafil, tadalafil has a very rigid molecular structure. In the hypothetical model of tadalafil bound to PDE4D (fig. 6B), the collision of M337 with the methylenedioxyphenyl group of tadalafil is inevitable due to the rigidity of this moiety. In addition to the severe steric barrier, this structural rigidity of tadalafil has an advantage in that tadalafil does not need to undergo entropic loss in order to bind at the active site.

These structural insights can further facilitate the discovery processes for more potent and selective drugs for the treatment of a wide array of diseases related to PDEs. And studies of other PDEs, whose structures have not yet been eluciated, are needed to obtain more information for designing PDE inhibitors specific to target PDEs of interest.

PDEs as therapeutic targets

Recent advances in the molecular pharmacology of PDE isoenzymes support new applications of PDE inhibitors for various diseases. Genomics and proteomics research may provide new rationales or possibilities for PDEs to be exploited for new drug applications. Many new pathways are being elucidated that involve specific PDE isoenzymes. Validation studies of PDEs as drug targets that use model animals and specific inhibitors are also in progress. The potential adverse effects of PDE inhibitors must be considered.

Pulmonary diseases (asthma, COPD, pulmonary hypertension)

The majority of PDE4 inhibitor patent claims address use of compounds as treatment for inflammatory airway diseases such as asthma and chronic obstructive pulmonary disease (COPD). Pretreatment with PDE4 inhibitors reduces antigen-induced bronchoconstriction in guinea pigs [48–50], rabbits [51] and cynomolgus monkeys [52], primarily due to inhibition of mast cell degranulation. PDE4 inhibitors also abolish antigen-driven eosinophil infiltration in models of pulmonary inflammation, including guinea pig [48], rat [53], rabbit [51] and monkey [52]. Additional beneficial effects of PDE4 inhibitors in vivo include their ability to reduce airway hyperreactivity [48, 49, 52] and pulmonary microvascular leakage [49], induced by a number of challenges and in a number of species. These studies indicate that PDE4 inhibitors are active in a wide spectrum of pulmonary inflammation models.

Excessive airway constriction is a severe problem in asthma. Accordingly, bronchodilators are a mainstay of current asthma therapy. However, even though current bronchodilators are effective, there is still a need for improved anti-asthma drugs [54], mainly because bronchodilators do not help against the chronic airway inflammation that drives the asthma process. Therefore, PDE4 inhibitors which have both airway-relaxing and anti-inflammatory properties might be ideal as anti-asthma drugs [55, 56].

Clinical trials suggest that PDE4 inhibitors such as cilomilast [212] and roflumilast [219] do have a real FEV1 effect, largely by reducing inflammation, but whether these relatively small physiologic responses translate into truly improved outcomes is still open to question. Moreover, although preliminary adverse effect profiles of the newer PDE4 inhibitors appear to be improved, older prototype PDE4 inhibitors had substantial adverse effects, the most notable being headache, nausea and emesis. Trials comparing PDE4 inhibitors to current strategies (not just placebo) using meaningful clinical outcomes (not just FEV1) will be needed to determine

whether these drugs are an important addition or replacement medication in asthma or COPD treatment [57].

Several reports suggest that PDEs play important roles in the development and maintenance of pulmonary hypertension. PDE activity is increased in pulmonary arteries of rats with chronic hypoxia-induced pulmonary hypertension [58], and this is correlated with a decrease in intracellular cAMP and cGMP levels [59]. Recently, combined inhibition of PDE3 and -4 was found to be effective in pulmonary hypertension. It was shown that PDE3 and -4 inhibitors promote acute pulmonary vasodilation in experimental models of pulmonary hypertension [60–63].

E-4010, a selective PDE5 inhibitor, attenuates hypoxic pulmonary hypertension in rats [64]. Long-term treatment with a PDE type 5 inhibitor improves pulmonary hypertension by enhancing the natriuretic peptide-cGMP pathway [65], downregulating the Ca2+ signaling pathway and altering vascular tone in pulmonary arteries in chronic hypoxia-induced pulmonary hypertensive rat models [66]. PDE5 inhibition attenuates the rise in pulmonary artery pressure and vascular remodeling when given before chronic exposure to hypoxia and when administered as a treatment during ongoing hypoxia-induced pulmonary hypertension [67].

Sexual dysfunction

A variety of physiological processes in the cardiovascular, nervous and immune systems are controlled by the NO/cGMP signaling pathway. In smooth muscle, NO and natriuretic peptides regulate vascular tone by inducing relaxation through cGMP [68]. Degradation of cGMP is controlled by cyclic nucleotide PDEs, and PDE5 is the most highly expressed PDE that hydrolyzes cGMP in these cells. The physiological importance of PDE5 in regulation of smooth muscle tone has been demonstrated most clearly by clinical use of its specific inhibitors, sildenafil (Viagra), vardenafil (Levitra) and tadalafil (Cialis) in the treatment of erectile dysfunction [69]. When a man is sexually stimulated, either physically or psychologically, NO is released from noncholinergic, nonadrenergic neurons in the penis, as well as from endothelial cells. NO diffuses into cells, where it activates soluble guanylyl cyclase, the enzyme that converts GTP to cGMP. cGMP then stimulates PKG, which initiates a protein phosphorylation cascade. This results in a decrease in intracellular levels of calcium ions, leading ultimately to dilation of the arteries that bring blood to the penis and compression of the spongy corpus-cavernosum tissue. This compression contracts veins, which reduces the outflow of blood and increases intracavernosal pressure, resulting in an erection [70]. A PDE5 inhibitor will retard enzymatic hydrolysis of cGMP in the human corpus cavernosum, leading to the same outcome.

Although the male excitation process has been widely investigated, the physiology of the female sexual response is still poorly understood. It has been found only recently that the clitoris consists of an erectile tissue complex surrounding the urethra and embedded in the anterior vaginal wall [71]. Recent data from Burnett and colleagues [72] showed the presence of NO synthase (NOS) isoforms in the human clitoris, suggesting that NO may be involved in the erectile physiology of the clitoris as a modulator of smooth muscle activity. This is consistent with the presence of PDE5 activity in the clitoral corpus cavernosum [73], suggesting that the mechanisms underlying sexual excitation in both sexes share common local neurovascular pathways. Sildenafil has recently been demonstrated to improve sexual performance in women affected by arousal disorders in a double-blind, crossover and placebo-controlled study [74]. However, there are some controversies for the evidence of efficacy of the PDE5 inhibitor for the treatment of female sexual dysfunction (FSD) [75]. In the phase I trial of tadalafil, an orally active PDE5 inhibitor for the treatment of erectile dysfunction (ED), reported in June 2001, the results showed no conclusive treatment effect relative to placebo in women with FSD (IC351 shows no benefit over placebo in an exploratory female sexual arousal disorder [FSAD] trial, Lilly ICOS LLC press release posted on 18 June 2001). By December 2003, it was still in phase II development for the potential treatment of FSD [16].

Neurodegenerative diseases

PDE1A2 is predominantly expressed in brain [76, 77], and its inhibition by deprenyl (selegeline hydrochloride) and amantadine can lead to enhanced intracellular levels of cAMP. There is considerable evidence that cAMP is involved in the regulation of metabolism and function in the nervous system and is important in neuronal survival [78–82]. It has been reported that in patients having Parkinson's disease with dementia, there is a significant decrease in cAMP [83]. It was demonstrated that PDE1A2 is inhibited by antiparkinsonian agents, suggesting a potential role of PDE1 in Parkinson's disease [84, 85].

On the other hand, isoenzyme PDE1A2 has a PEST motif and acts as a substrate for m-calpain. In brain, calpains are implicated in synaptic modification, neurite pruning, receptor characteristics, neurofilament turnover and neural differentiation [86–88]. Several reports indicate that calpains are involved in axonal neurofilament degradation, motorneuronal degradation, neuronal ischemia and other neurodegenerative diseases, including Alzheimer's and epilepsy [86–90]. The proteolysis of PDE1A2 by m-calpain results in a CaM-independent form which in turn could decrease the intracellular levels of cAMP [91]. These studies suggest that PDE1 isoenzymes may be useful targets for therapeutic interven-

tion with respect to disorders of the central nervous system.

Besides Alzheimer disease, there are many severe learning and memory disorders that involve heredity, disease, injury or age. Neurobiological research has begun to identify the molecular biology of memory formation and has shown that the ability to form memories is fundamentally based on neuronal 'plasticity'. This is to say that a particular experience is registered in the brain as a circuit-specific pattern of neural activity and that, as a result of plasticity, the structure of this circuit is modified so as to form a memory. This knowledge is generating new gene targets, drug screens, chemical compounds and preclinical data to suggest drug classes capable of directly enhancing the memory process, such as PDE4 and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors.

Neurogenetic studies have shown cAMP response-element-binding protein (CREB) to be a key control point for long-term memory (LTM) formation [92]. Loss-of-function manipulations of CREB leave learning and short-term memory (STM) intact, but impair LTM [93–98]. Gain-of-function manipulations also leave learning and STM intact, but enhance LTM formation specifically by reducing the amount of training required to produce maximal LTM [99, 100]. Similar manipulations of CREB, moreover, also produce concomitant changes in the underlying synaptic structure and function in several animal models and in various regions of the mammalian brain [101–107]. The observation that opposing genetic manipulations produce opposing effects on LTM indicates that CREB functions as a rate-limiting 'molecular switch' in biochemical pathways. Following genetic modulation of CREB in *Drosophila*, transgenic flies overexpressing CREB do not exhibit more memory, but rather demonstrate induction of long-term memory after less training. PDE4 inhibitors such as rolipram induce behavior analogous to CREB-dependent memory enhancement (the so-called 'CREB signature'), which suggests that PDE4 inhibitors can be used as memory enhancers. PDE4 inhibitors enabled memory to form following less than half the normal amount of training [108].

It is also reported that selective PDE2 inhibitors can be used to produce pharmaceuticals for improving perception, concentration, learning and/or memory, though it is cGMP-specific PDE. In the object recognition test, which measures the ability of rats to distinguish between familiar and unfamiliar objects, administration of PDE2-specific inhibitors leads to improve recognition of the familiar object. Rats treated with PDE2 inhibitors investigated the new, unfamiliar object in more detail than familiar ones. Memory capacity was improved in the second run after treatment with 0.3 and 1.0 mg/kg of PDE2 inhibitors, compared with controls [109].

Alterations of PDE7 and -8 isoenzyme messenger RNA (mRNA) expression in Alzheimer's disease brains indicate that the expression of specific cAMP PDE isoforms may be selectively regulated in Alzheimer's disease and associated with different stages of the disease [110]. Their differential regulation in AD brains suggests that the isoenzymes of these two families could be implicated in neurodegenerative and inflammatory diseases.

Vascular disease

Atherosclerotic lesions occur in the context of endothelial cell dysfunction and involve activation, migration and proliferation of smooth muscle cells (SMCs). Endothelial derived relaxing factors, such as NO or prostacyclin (PGI2), relax blood vessels and inhibit the proliferation and migration of SMCs by increasing synthesis of the cyclic nucleotides cAMP or cGMP. In fact, cAMP and cGMP inhibit the proliferation of arterial SMCs [111], and elevation of cyclic nucleotides reduces neointimal formation after angioplasty in animal models.

Oral administration for 3–21 days of milrinone (0.3–3.0 mg/kg), a bipyridine derivative that specifically inhibits PDE3, suppressed intimal thickening by up to 56% in a dose- and time-dependent manner in a mouse model of photochemically induced vascular injury [112]. In this model, oral administration of milrinone decreased the number of activated SMC and consequently suppressed intimal thickening by preventing SMC proliferation within the media.

PDE1C is expressed in proliferating human SMCs, but is absent from the quiescent human aorta. Inhibition of PDE1C in SMCs isolated from normal aorta or from atherosclerotic lesions, using antisense oligonucleotides or a PDE1 inhibitor, results in suppression of SMC proliferation. Because PDE1C is absent from quiescent SMCs, PDE1C inhibitors may target proliferating SMCs in atherosclerotic lesions or during restenosis [113].

Atherosclerosis and other cardiovascular diseases are much more prevalent in diabetics than in the human population at large, and they represent a significant cause of morbidity and early mortality in diabetes [114–116]. It has been reported that alterations in PDEs occur in diabetes-associated cardiovascular disease [117, 118]. In clinical studies, flow-mediated dilation (FMD), induced by occlusion of the brachial artery, is an index of NO-dependent endothelial function, and this is impaired in patients with type 2 diabetes. Desouza et al. assessed the acute and prolonged effects of a low dose of sildenafil (25 mg), an inhibitor of PDE5, on FMD in patients with type 2 diabetes [119].

Sildenafil increases brain levels of cGMP, evokes neurogenesis and reduces neurological deficits when given to rats 2 or 24 h after stroke [120]. These data suggest that sildenafil may have a role in promoting recovery from stroke.

Gretarsdottir et al. present association analyses (single-marker and haplotype analyses) that support the idea that

PDE4D confers risk of ischemic stroke. They observed significant disregulation of multiple PDE4D isoforms in affected individuals. It is proposed that this gene is involved in the pathogenesis of stroke through atherosclerosis, and inhibition of PDE4D might decrease the risk of stroke in those who are predisposed by genotype at PDE4D [57].

Diabetes

Type 2 diabetes mellitus is characterized by impaired insulin secretion and peripheral insensitivity to the hormone [121]. Treatment of type 2 diabetes is currently unsatisfactory, and new agents are needed. One approach is to develop non-sulfonylurea drugs that will augment insulin secretion through mechanisms other than blocking K_{ATP} channels. Agents increasing islet beta-cell cAMP have potential as therapeutic agents, and GLP-1 and its derivatives have been shown to normalize insulin responses to glucose and nearly normalize overnight and daytime glucose concentrations [122–123]. However, GLP-1 has the disadvantages associated with peptides, namely rapid degradation and inactivity by the oral route. Selective inhibition of PDE3 in the islet beta cell might augment meal-related insulin secretion, due to amplification of the effect of incretin factors, particularly GLP-1. Thus, PDE3 offers a potential target for developing drugs for the treatment of type 2 diabetes mellitus. Development of PDE3 inhibitors for this purpose will require their selectivity for islet beta-cell PDE3, as PDE3 also seems to be an important isoenzyme in the liver and adipose tissue [124], where its activation mediates some of the effects of insulin.

The mammalian PDE3 family consists of two members, PDE3A and PDE3B, which have strikingly similar pharmacological and kinetic properties but distinct expression profiles [125, 126]. PDE3A is mainly expressed in the cardiovascular system and platelets [127]. PDE3B has been recognized for its importance in mediating the antilipolytic and antiglycogenolytic action of insulin in adipose and liver tissues [127–129]. Upon insulin binding to its receptor in adipose tissue, a Ser/Thr kinase is activated through a wortmannin-sensitive phosphorylation cascade [129]. This insulin-sensitive kinase in turn activates PDE3B [127, 129]. The activated PDE3B decreases cAMP and protein kinase A activity, thereby inactivating a hormone-sensitive lipase and thus inhibiting lipolysis.

Another intriguing possibility lies in the potential of PDE inhibitors to prevent beta-cell loss in both type 1 and type 2 diabetes. The non-selective PDE inhibitor pentoxifylline and the PDE4-selective agent rolipram were shown to reduce insulitis and prevent diabetes in non-obese diabetic (NOD) mice [130]. These results suggest the importance of PDEs as therapeutic targets for treatment of diabetes.

Osteoporosis

Osteoporosis is a disease characterized by an imbalance between bone resorption and formation. Excessive bone resorption causes changes in the microstructure of the bone matrix, which make bones prone to fracture. Current therapies are mostly directed to decrease the rate of bone resorption. Antiresorptive therapies and compounds include estrogen replacement therapy, selective estrogen receptor modulators, calcitonin, vitamin D, calcium supplements, PTH and PTH analogues, and bisphosphonates [131]. All therapies show efficacy but reveal various problems, such as increased cancer rates in the estrogen-replacement therapy [132] or upper gastrointestinal symptoms and problems in patient compliance in the case of bisphosphonates [133]. Thus, other nonhormonal and more specific therapies are needed. Several cytokines, including tumor necrosis factor α (TNF-α) are thought to promote bone resoroption by osteoclasts, which implies that agents which are able to suppress the production of these cytokines could have a role in reducing bone loss. In addition, cAMP and cGMP act as second messengers in the functional responses of various cells to hormones, neurotransmitters and other agents. In osteoblasts, for example, cAMP produced in response to parathyroid hormone (PTH) or prostaglandins (PGs) regulates osteoblastic differentiation [134–137]. There are corresponding data that show that administration of PTH or PGs also leads to increases in cancellous bone volume in animal models [138–143]. The elevation of cAMP in osteoporosis has been shown to enhance bone formation, and therefore suggests that agents which elevate cAMP levels could have the potential to increase bone mass. Since PDE4 inhibitors are able to inhibit the production of TNF-α and are also able to elevate cAMP, a therapeutic effect in osteoporosis would be predicted [144–147]. Several recent studies provide evidence to support this hypothesis. The effectiveness of XT-44 in three osteopenia models has been described [144]. Oral administration of XT-44 inhibited the decrease in bone mineral density in Walker 256/s tumor-bearing mice, in the sciatic neurectomized rat model and in ovariectomized rats. The mechanism by which XT-44 exerts these effects has been discussed but is not entirely clear [145]. Recently the effects of rolipram and pentoxifylline in normal mice were investigated. Both compounds were able to increase significantly both cortical and cancellous bone mass, predominantly by the acceleration of bone formation [146]. It has also been suggested that the disease-modifying effects of PDE4 inhibitors in animal models of rheumatoid arthritis are related to their ability to suppress osteoporosis [148].

Cancer

The possible utility of PDE inhibitors as anti-cancer drugs has been prosposed [149]. Potent inhibitors of PDE could elevate intracellular levels of cAMP; increased cAMP in a variety of cancer cells may suppress RAS activity [150] and, as a consequence, reduce the constitutive activity of MAPK, which could be relatively increased in cancer cells. On the other hand, cAMP could attenuate Bcl2 intracellular levels [151] and MDM2 [152–153]. Reduction in Bcl2 expression, which is considered as a survival factor, and MDM2, which blocks the proapoptotic activity of P53, could lead to enhanced apoptosis. Attenuation of cell migration by PDE inhibitors may also be exerted by cAMP, which can lead to cytoskeleton reorganization via phosphorylation of specific components of the microtubular network [154–156].

There are some indications that high intracellular levels of cAMP could arrest growth, induce apoptosis and attenuate cancer cell migration [157–161]. It has been known that elevation of cAMP levels functions as another stimulus that can induce growth arrest or cell death (or both) in many cultured lymphoid cells, including resting B cells, germinal center B cells, T lymphocytes and thymocytes [162–166]. cAMP also induces cell death in cells derived from lymphoid malignancies, including murine lymphoma cell line S49.1, B-CLL cells and multiple myeloma cells [167–169]. These results suggest that screening of PDE inhibitors could open a possibility to improved chemotherapeutic cancer treatments with reduced undesired side effects.

Rheumatoid arthritis

Rheumatoid arthritis (RA) is a highly inflammatory joint disorder that affects 1–2% of the U.S. population. Direct and indirect costs are estimated to be over $14 billion annually. Similarly to osteoporosis, three times more women than men are affected by the disease. Besides its inflammatory component, RA is characterized by a progressive articular cartilage and subchondral bone destruction that eventually leads to loss of joint function [170].

Of the PDE isoenzymes, PDE4 has been of particular interest because PD4 is highly expressed in most immune and inflammatory cells [55]. It is also evident that PDE4 inhibitors as promising anti-inflammatory drugs potently regulate the specific function of immune cells through enhancement of cAMP [171–172]. In vitro and in vivo evidence suggesting that PDE4 inhibitors would be expected to be beneficial in the treatment of RA has recently been summarized [173]. PDE4 inhibitors have been found to be efficacious in several animal models of arthritis. Rolipram has been shown to exhibit anti-inflammatory effects in carrageenan-induced paw edema and the adjuvant arthritis model. It has also been shown to ameliorate collagen II-induced arthritis (CIA) in mice. Recent studies in the adjuvant arthritis model demonstrated that rolipram abrogated edema formation and significantly inhibited hyperalgesia. Inhibition of cellular influx and inhibition of bone and cartilage destruction were also achieved [174]. The efficacy of rolipram in the streptococcal cell wall (SCW)-arthritis model also has been documented [175]. Several PDE4 inhibitors have also been evaluated in animal models of arthritis, particularly in models which involve LPS-induced TNF release [176–178].

Depression

Impairment of signal transduction that regulates neuroplasticity and cell survival is thought to be an important mechanism contributing to major depressive disorders [179]. In particular, cAMP-mediated signaling appears to have a key role in the pathophysiology and pharmacotherapy of depression [180]. Elevating intracellular cAMP, either via inhibition of PDE4, which specifically catalyzes the hydrolysis of cAMP, or stimulation of adrenergic receptors, produces antidepressant-like effects in animal models [181–184]. PDE4 is particularly important for controlling intracellular cAMP concentrations and is considered to be a prime target for therapeutic intervention in a range of disorders such as depression and impaired cognition [185–187]. Notably, PDE4 is the predominant mediator of hydrolysis of cAMP formed by stimulation of β-adrenergic receptors, which are involved in the mediation of the effects of antidepressant drugs [188–189]. Consistent with this, inhibition of PDE4 by rolipram produces antidepressant-like and memory-enhancing effects in animals [180, 182, 185, 186].

The distribution of PDE4A, PDE4B and PDE4D varies among regions of the brain [190]. This differential distribution suggests that PDE4 subtypes may subserve distinct roles; these roles in the central nervous system have only recently begun to be examined [189, 191]. Using a gene knockout technique, mice lacking a single PDE4 subtype, PDE4D, exhibit delayed growth, decreased fertility and reduced responsiveness to the respiratory effect of a muscarinic agonist [192–193]. Given the potent antidepressant-like effect of rolipram [181, 183] and the important role of PDE4D in the control of cAMP concentrations [192, 194], it was thought that this subtype might be involved in the mediation of depressive symptomatology and antidepressant responsiveness. Recently, the behavioral phenotype and pharmacological sensitivity of PDE4D knockout mice were investigated in models sensitive to antidepressant drugs [195]. Immunoblot analysis showed the loss of PDE4D expression in the cerebral cortex and hippocampus of PDE4D knockout (PDE4D–/–) mice, but unchanged PDE4A and

PDE4B expression, relative to the wild-type (PDE4D–/–) and heterozygous knockout (PDE4D–/–) mice. PDE4D–/– mice exhibited decreased immobility in tail-suspension and forced-swim tests, which is indicative of an antidepressant-like effect on behavior. PDE4D-regulated cAMP signaling may play a role in the pathophysiology and pharmacotherapy of depression.

Brain-derived neurotrophic factor (BDNF) is reported to play an important role in the survival of mature neurons as well as damaged neurons in the central nervous system [196–197]. Recent studies revealed that activation of the cAMP system, as well as β-adrenergic receptors that couple to this system, is closely involved in the regulation of BDNF mRNA expression [198–201]. Studies were undertaken to examine the influence of acute or chronic administration of PDE4 inhibitors with an antidepressant on the expression of BDNF mRNA [202–203]. These findings demonstrated that administration of PDE4 inhibitors shortens the time required for the upregulation of BDNF mRNA, supporting the possibility that this treatment may provide an effective therapy for major depression.

Chemotherapeutic potential of PDE inhibitors

Given the multitude of cellular responses that cAMP and cGMP can elicit, it is clear that to achieve specificity of signal transduction, cells must be able to tightly regulate the magnitude and duration of cAMP/cGMP elevation, and also in specific cellular locations. Mammalian cells have evolved a complex and highly conserved complement of enzymes in order to generate, recognize and inactivate cyclic nucleotides. Inactivation of cAMP/cGMP is achived by hydrolysis of the 3′-ester bond catalyzed by the PDEs, of which more than 50 have been identified [204]. If cells did not possess PDEs, intracellular cAMP levels should rapidly become uniform. These enzymes therefore provide a key ability for the cell to generate nonuniform intracellular distribution of cAMP/cGMP, and hence differentially activate distinct compartmentalized protein kinase species.

PDE inhibitors reduce the hydrolysis of cAMP/cGMP, and hence elevate the intracellular level of cAMP/cGMP. Thus, PDE inhibitors will change the activation state of cyclic nucleotide signaling pathways, resulting in the regulation of various physiological functions. An important issue in the development of one PDE inhibitor is specificity for the other PDEs. The molecular diversity of the PDE inhibitors and structure-based design of PDE inhibitors may provide opportunities for development of newer, more selective drugs. This section will update the recent progress of the development of PDE inhibitors.

PDE4 inhibitors

The PDE4 family is highly specific for cAMP as substrate, having a low Km for cAMP (1–3 μM), being insensitive to cGMP and Ca2+/calmodulin, and being potently and specifically inhibited by rolipram [205]. Rolipram (fig. 7) is the most extensively studied inhibitor of PDE4 [206–208]. It binds to two sites with different affinities [208], of which the high-affinity binding site (HPDE4) is 50–1000 times higher than inhibitory Ki at the catalytic site (LPDE4) on the PDE4 [209]. This differential activity of rolipram may explain the variable potency of rolipram relative to inhibitors such as RP73401 (piclamilast; fig. 7)

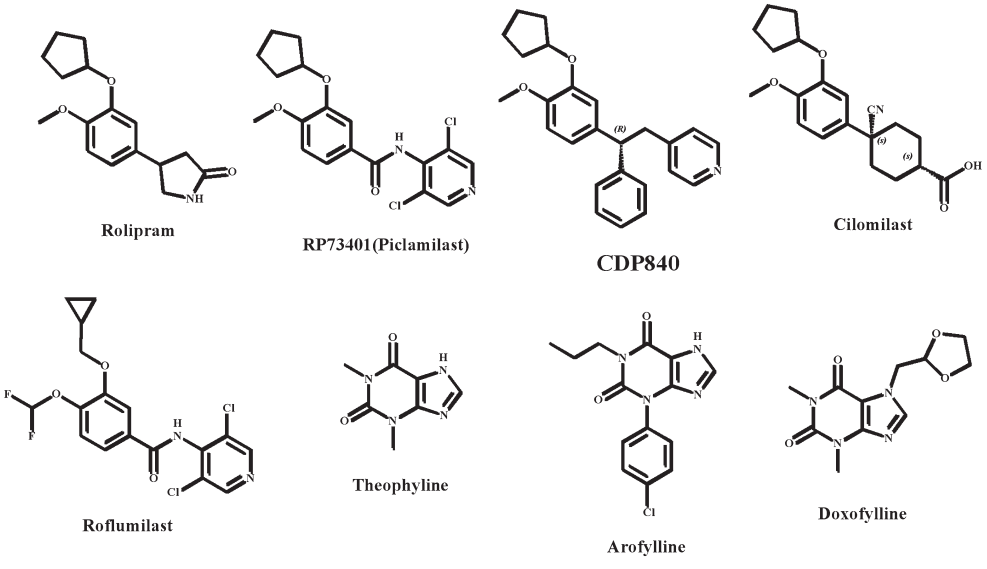

Figure 7. PDE4 inhibitors discussed in this review.

and CDP840 (fig. 7), which exhibit similar HPDE4 and LPDE4 affinity in in vitro and in vivo functional assays [210]. This has led to speculation that activity at the HPDE4 site is responsible for the nausea and emetic profile of PDE4 inhibitors [211], but overall the mechanisms responsible for the side effects of PDE4 inhibitors are still not well understood. Rolipram has been investigated for its anti-inflammatory effect in asthma, but central nervous system and cardiovascular side effects have precluded its development for this condition. In an attempt to limit these side effects, many pharmaceutical companies have tried to synthesize different compounds.

The most clinically advanced selective PDE4 inhibitors (cilomilast and roflumilast; fig. 7) have a superior side-effect profile compared with theophylline (fig. 7) and first-generation compounds [212]. These compounds were designed with the knowledge that PDE4 exists in two distinct conformations, high-affinity rolipram-binding PDE4 (HPDE4, which predominates in the central nervous system and parietal glands) and low-affinity rolipram-binding PDE4 (LPDE4, which predominates in immunocompetent cells) [212]. Unlike rolipram, which targets HPDE4, second-generation compounds (such as cilomilast) primarily target LPDE4, resulting in an improved therapeutic index [212]. Cilomilast and roflumilast inhibit the activity of cells that have been implicated in the pathogenesis of asthma and COPD, e.g. neutrophils, monocytes, macrophages, CD4T cells, epithelial cells and fibroblasts [213–217]. Additionally, cilomilast has recently been shown to decrease levels of CD8+ T cells and CD68+ macrophages, thus demonstrating its potent anti-inflammatory effects [218]. Other effects of cilomilast and roflumilast include reduced chemotaxis, activation, degranulation and adherence of inflammatory cells, impact on key mechanisms involved in airway remodeling and modulation of the release of inflammatory mediators, such as TNF-α, interleukin (IL)-8, and granulocyte-macrophage colony-stimulating factor (GM-CSF). Significantly, cilomilast retains the anti-inflammatory actions of rolipram, but is substantially less likely to stimulate gastric acid secretion [215–218].

According to published data, the most advanced PDE4 inhibitor in clinical development is roflumilast. In April 2004, clinical data were presented at the SMi Asthma Therapeutics meeting in London, UK. In a double-blind, three-period crossover study, 28 patients received 250 or 500 µg/day of roflumilast, or placebo. Following allergen challenge, a dose-dependent reduction in the late asthmatic response was observed, compared to placebo. It was well tolerated, and most adverse events were mild to moderate, with headache being the most common. In addition, there were no changes in vital signs, electrocardiograms or other clinical laboratory parameters. In a dose-range finding study, 500 µg was selected as the most effective dose for a 40-week, open-label extension trial, which enrolled 456 patients. This study showed that roflumilast had a constant efficacy, as assessed by FEV1 measurement, over 1 year. It also showed that the adverse events observed in short-term trials were transient and there was no incidence of vomiting [219]. Roflumilast has also shown encouraging efficacy in patients with COPD. In September 2003, Altana completed a phase III study (RECORD) with roflumilast in 1400 patients with COPD. An initial review of the data showed positive results [220]. Data from this trial were presented in September 2003 at the 13th Annual Congress of the European Respiratory Society in Vienna, Austria. More than 1400 patients received placebo, 250 or 500 µg of roflumilast for 24 weeks. Forced expiratory volume in 1 s (FEV1) was significantly improved in patients with both 250 and 500 µg doses. After 24 weeks the difference in FEV1 between 500 µg and placebo equaled approximately 100 ml. Quality of life (QOL) measures showed a dose-related improvement compared to placebo. Roflumilast was well tolerated; the most frequent drug-related adverse event was diarrhea [221]. Similar data were presented in October 2003, at the SMi Anti-Inflammatory Therapeutics meeting in London, UK [222], and at the SRI's Phosphodiesterases in Disease meeting in Princeton, NJ. Similar data were also presented at the American Thoracic Society meeting in Durham, NC, in May 2004; patients treated with 500 µg roflumilast had 34% fewer exacerbations than those treated with placebo. There was some interaction with erythromycin. Similar data were also presented at the 14th Annual European Respiratory Society Congress in Glasgow, UK. The rank order of potency was as follows: roflumilast = piclamilast > roflumilast N-oxide > rolipram > cilomilast.

Doxofylline (fig. 7), theophylline (fig. 7) and arofylline (LAS 31205; fig. 7) are xanthine PDE4 inhibitors which shows no cardiovascular or central nervous system side effects on animal models. Doxofylline has been launched in Italy as a treatment for asthma; it has been reported to exhibit similar efficacy to theophylline, with reduced incidents of adverse events [223]. Theophylline, in once-daily and twice-daily formulations, has been put forth as the PDE inhibitor for treatment of asthma and other respiratory disorders by Elan. Once-daily theophylline utilizes Elan's proprietary Spheroidal Oral Drug Absorption System (SODAS) technology, which is a multiparticulate system for controlled release and absorption of drugs. It is marketed as Theolan in Ireland by Elan, and in Southeast Asia by Elan Pharma. Theophylline is the most frequently prescribed oral bronchodilator for the chronic maintenance treatment of chronic obstructive airway disorders [224]. However, there is no evidence that theophylline has any selectivity for a particular isoenzyme, such as PDE4 [225]. Non-selective PDE inhibition can lead to elevation of cGMP, as well as cAMP, levels, resulting in the activation of both cAMP- and cGMP-dependent kinases

(PKA and PKG); this could be linked with an increase in adverse events [225, 226]. Arofylline (LAS-31025) is under development by Almirall Prodesfarma as a potential treatment for chronic obstructive airway disease; as of October 2001, phase II trials in Europe were ongoing for this indication. In April 2004, Almirall listed arofylline as being in phase II/III trials for bronchitis.

However, in comparison with roflumilast, these xanthine PDE4 inhibitors have significantly lower potency as PDE4 inhibitors than roflumilast, which exhibits an IC_{50} (inhibitory concentration 50%) value of 20 nM for the PDE4D isoform found in inflammatory cells such as eosinophils, and failed to demonstrate significant benefit in a 4-week study in asthmatics at 15 mg twice a day (bid). Although apparently lacking the emetic effects seen with PDE4 inhibitors, such as rolipram, it seems unlikely that these xanthine inhibitors will prove sufficiently effective to become a major product in the treatment of asthma.

PDE5 inhibitors

In contrast to PDE4, PDE5 catalyzes the hydrolysis of cGMP with absolute specificity. The enzyme is active as a homodimer, which has a molecular mass of approximately 200 kDa. Either PKA or PKG can phosphorylate PDE5, and this results in a significant increase in PDE5 activity [227]. The protein is widely distributed throughout the smooth muscle in the body, and is also found in platelets [228]. However, PDE5 exhibits a more limited tissue distribution than PDE1 and -2; it is particularly prevalent in vascular smooth muscle [229]. PDE5 is the primary cGMP-hydrolyzing activity in human corpus-cavernosum tissue. Erection is largely a hemodynamic event which is regulated by vascular tone and blood-flow balance in the penis. Because cGMP levels modulate vascular tone, PDE5 is an obvious target for therapeutic intervention in the process. Oral PDE5 inhibitors can increase the cGMP, smooth muscle relaxation in the penis and, thus, penis election. Similar mechanisms appear to be involved in genical vasodilatation in the human female [230]. This, coupled with its specificity for cGMP, has identified PDE5 as a target of considerable interest for the pharmaceutical industry.

Sildenafil (Viagra; fig. 8) is an orally active, potent and selective inhibitor of cGMP-specific PDE5 [231, 232]. Following oral administration, sildenafil is rapidly absorbed, with an absolute bioavailability of 40%. The time to peak plasma concentration (T_{max}) after oral absorption in the fasting state has a range of 30–120 min, but a high-fat meal increases the T_{max} by 60 min and reduces the peak plasma concentration by 29% (there is no effect on area under the curve [AUC]). From a clinical point of view, the onset of efficacy is optimal if sildenafil is taken on an empty stomach. The terminal half-life of sildenafil is 3–5 h [233]. Sildenafil was approved for use in the United States in March 1998, and still accounts for more than 50% of all pharmaceutical sales for the treatment of erectile dysfunction (ED). Worldwide sales in 2003 exceeded US$ 2.1 billion. Because of its mechanism of action, sildenafil is contraindicated in patients taking NO donors or organic nitrates. The patient population with the greatest risk of developing ED comprises men over the age of 40. Many men in this age group also have other chronic diseases, such as depression, diabetes, atherosclerosis, hypertension or ischemic heart disease. All of these conditions increase the risk of developing ED, and in some cases, the pharmacological treatment for the disorder can also induce ED. Consequently, the safety and efficacy of sildenafil and other PDE5 inhibitors in this group of patients needed to be established. Several studies have been done with sildenafil in men with cardiovascular disease. The data indicate that, with the exception of patients taking organic nitrates, sildenafil does not have a synergistic effect on blood pressure with antihypertensive agents, such as ACE inhibitors, α-adrenoceptor or β-adrenoceptor blockers, calcium channel blockers or diuretics [234]. There was no increase in the incidence of drug-related adverse events, and the overall safety profile indicated that there was no significant difference in the incidence of stroke, myocardial infarction or other serious cardiovascular events in patients taking sildenafil. The drug improved erectile function in up to 70% of men with ischemic heart disease [235], and gave similar results in trials with other groups of men with cardiovascular disease [236].

Tadalafil (Cialis; fig. 8) is another novel PDE5 inhibitor recently approved both in Europe and in the United States. It has a maximum T_{max} of 2 h and a half-life of 17.5 h. The latter values clearly distinguish tadalafil from the other PDE5 inhibitors. When the selectivity profile of tadalafil was evaluated against 14 human recombinant PDEs, tadalafil was found to be highly selective for PDE5, with 700-fold greater affinity for PDE5 than for the related retinal PDE6 [236]. Furthermore, tadalafil has shown 14-fold greater affinity for PDE5 compared with PDE11, which closely resembles PDE5 (71% amino acid similarity). Tadalafil also has a more rapid onset of action than sildenafil, often showing effects in 20 min or less [237]. It is likely to be contraindicated in patients taking organic nitrates, in spite of a substantial increase in PDE5 selectivity compared with other PDE enzymes [238]. In healthy subjects who received a single 20-mg dose, there was no significant change in heart rate, standing systolic or diastolic blood pressure [238]. Analysis of the data from phase III clinical trials showed that the incidence of adverse events in patients taking tadalafil, including those with various cardiovascular diseases, was no different from that in placebo-treated patients [239]. In double-blind, placebo-controlled phase III trials that included

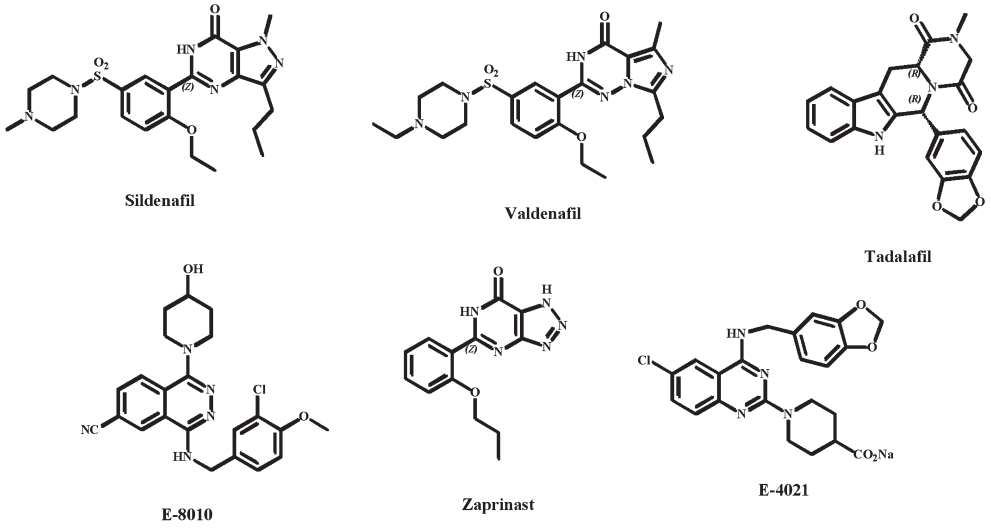

Figure 8. PDE5 inhibitors discussed in this review.

over 1100 men, tadalafil doses of 2.5–20 mg once daily, as needed, significantly improved erections in up to 81% of men. The mean percentage of successful intercourse attempts was 75%, and efficacy was maintained in both hypertensive and nonhypertensive patient groups [240].

Vardenafil (Levitra; fig. 8) is a novel PDE5 inhibitor recently approved for marketing in Europe and US. Vardenafil is characterized by a very high potency in vitro (IC_{50}=0.6 nM, compared to sildenafil, 3.0 nM). Pharmacokinetic data for vardenafil were obtained in two randomized, double-blind, placebo-controlled studies with a single oral dose of 10, 20 and 40 mg. The T_{max} of vardenafil was 0.7–0.9 h. As with sildenafil, the absorption of vardenafil is delayed if taken after a meal containing >30% fat. Thus, practically, patients should be advised to use vardenafil on an empty stomach to maximize its efficacy [241, 242]. The efficacy was evaluated by using real-time technique with the RigiScan Plus device [243]. Phase II trials showed that vardenafil was effective in men with severe ED after nerve-sparing radical prostatectomy. After 3 months using 10- or 20-mg doses, patients recorded successful penetration and maintenance of erection significantly more often than placebo-treated men (47% compared with 22%, and 36% compared with 10%, respectively, for each end point) [243]. Data from two phase III studies were pooled to evaluate the safety and efficacy in hypertensive men with mild-to-moderate ED. The drug was dosed at 5, 10 or 20 mg, and all three groups reported results far superior to placebo. Side effects were generally mild, as noted above, and did not occur more frequently in the hypertensive patient population. A smaller study showed that a single 10-mg dose of vardenafil did not increase the risk of exercise-induced cardiac ischemia in patients with stable coronary artery disease [243].

There are other PDE5 inhibitors in earlier stages of clinical development, and – on the basis of an evaluation of patent publications – it seems that several companies have preclinical discovery programs. Pfizer has reported that a 'second-generation' PDE5 inhibitor, UK357903, is now in phase II trials for ED. Tanabe is investigating avanafil in phase II trials for ED and FSD. Dong-A Pharmaceutical entered DA-8159 into phase II clinical trials for ED. DA-8159 is a pyrazolopyrimidinone that has shown erectogenic activity after oral administration of 0.3–1.0 mg kg^{-1} to rats. In anesthetized dogs, intravenous administration of 1–300 μg kg^{-1} potentiated an increase in intracavernosal pressure in a dose-related manner. Eisai Pharmaceutical entered E-8010 (fig. 8) into phase I clinical trials for ED [16].

PDE3 inhibitors

Due to their inotropic and vasodilatory actions, several PDE3 inhibitors – including pimobendan, anagrelide, milrinone, cilostazol, amrinone, vesnarinone and enoximone (see fig. 9) – were developed as therapeutic agents for the treatment of ischemic and idiopathic dilated cardiomyopathy, a syndrome characterized by impaired myocardial contractibility and inappropriate systemic and pulmonary vasoconstriction. In numerous studies, the use of these agents was shown to have beneficial effects on parameters of cardiovascular function in treated patients, and expectations for sustained benefits were high [244]. However, this optimism was largely quashed by the results of subsequent clinical trials.

Anagrelide is a quinazoline derivative PDE3 inhibitor that was developed by Bristol-Myers Squibb (BMS) and has been launched, under an exclusive license, by Shire (formerly Roberts) and its collaborators for the treatment

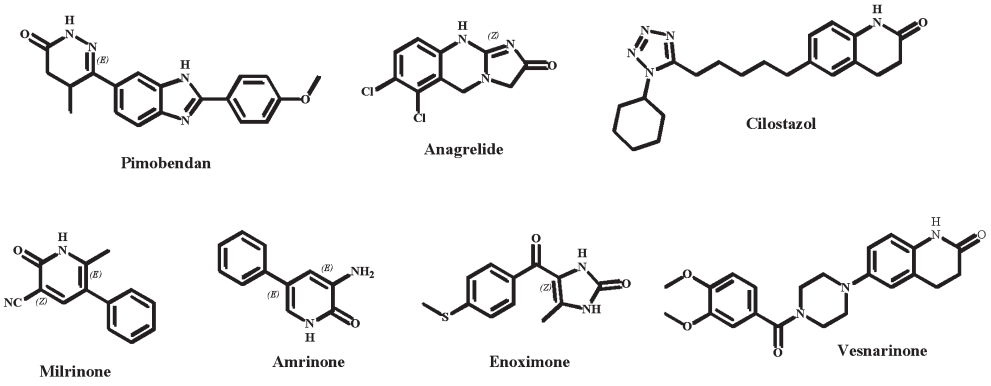

Figure 9. PDE3 inhibitors discussed in this review.

of thrombocytosis in patients with chronic myeloproliferative disease. Its use, for the management of thrombocythemia in polycythemia vera (PV) and other myeloproliferative disorders, seems reasonably well established and was recently approved by the Food and Drug Adminisstration (FDA). Although anagrelide was originally assayed as an inhibitor of platelet aggregation [245], clinical trials of the use of anagrelide as an antithrombotic drug were discontinued because of thrombocytopenic effects [16].

Pimobendan is an orally active PDE3 inhibitor developed by Boehringer Ingelheim for the treatment of acute and mild-to-moderate chronic heart failure. It was initially launched in Japan, in September 1994. The development in the United States was put on hold following results of the PICO (pimobendan in congestive heart failure) study, which showed that it may be associated with an increased risk of death. In the study, 317 patients were randomized to one of two doses of pimobendan (2.5 or 5 mg daily) or placebo. After 24 weeks, patients in the treatment groups had significantly increased exercise duration, but this was offset by a nonsignificant increase in mortality. The development in Japan was unaffected, as a mortality study was not required [16].

Milrinone is a PDE3 inhibitor developed by Sterling Winthrop. It was first launched in Europe in 1989 and has since been launched in the United States. It is in use for the treatment of acute heart failure [16]. However, the long-term treatment of the milinone showed increased risk of mortality. The prospective randomized milrinone survival evaluation study, which randomized patients with class III/IV heart failure to oral milrinone versus placebo, showed a 28% increase in all-cause mortality in the treated group [246].

A meta-analysis of 13 randomized, placebo-controlled trials of PDE3 inhibitors in patients with dilated cardiomyopathy concluded that the risk of death was increased by as much as 40% in treated patients [247]. The Vesnarinone Trial (VEST), which randomized patients with left ventricular ejection fractions (LVEF) of <30% to vesnarinone versus placebo, was terminated early because of an 11% increase in the relative risk of death in the treated group [248, 249]. The Enoximone Multicenter Trial, which randomized patients to enoximone versus placebo, showed a two- to threefold increase in mortality in the treated group [250].

Taken together with the studies described earlier, these observations are consistent with the conclusion that PDE3 inhibition may be beneficial in the short term in patients with dilated cardiomyopathy whose contractility is so impaired as to cause cardiogenic shock or hypotension, but the long-term administration of PDE3 inhibitors is not only ineffective in reversing the pathological features of dilated cardiomyopathy, but also increases the risk of death in treated patients. The specific cause of increased mortality in patients treated with PDE3 inhibitors has not been established [244].

PDE1 inhibitors

A normal artery consists of quiescent arterial smooth muscle cells (SMCs) covered by a monolayer of endothelial cells lining the interior of the blood vessel. If the artery is injured by an excess amount of atherogenic lipid, by oxidative stress, diabetes, smoking, viruses or by mechanical means, the SMCs respond by proliferating and forming a neointimal lesion [251]. Atherosclerotic lesions occur in the context of endothelial cell dysfunction and involve activation, migration and proliferation of SMCs. Therefore, considerable effort has been devoted to the identification of factors that regulate SMC proliferation [252]. Inhibition of PDE1C in SMCs isolated from normal aorta or from atherosclerotic lesions, using antisense oligonucleotides or a PDE1 inhibitor, results in suppression of SMC proliferation. Because PDE1C is absent from quiescent SMCs, PDE1C inhibitors may target proliferating SMCs in atherosclerotic lesions or during restenosis [113]. Unfortunately,

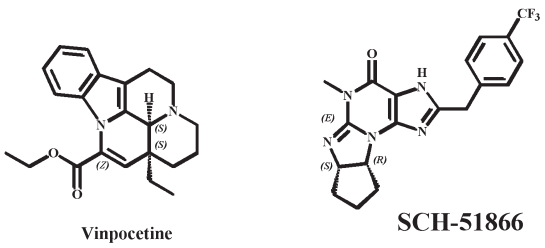

Figure 10. PDE1 inhibitors discussed in this review.

the lack of specific PDE1 inhibitors means that the functional role of PDEl isoenzymes remains speculative.

Richter Gedeon has developed and launched vinpocetine (fig. 10), a sodium channel blocker and PDE1 inhibitor which has potent vasodilatory actions, for the potential treatment of vascular diseases including cerebrovascular and opthalmological diseases. Vinpocetine was launched, as Cavinton, worldwide during the 1980s. Takeda launched vinpocetine, as Calan, in Japan; however, it was later withdrawn due to the poor efficacy observed in a double-blind, placebo-controlled study, where the primary side effect was dizziness of chronic cerebrovascular origin [16]. Vinpocetine directly inhibits PDEl, whereas other common PDEl inhibitors, the phenothiazines, act indirectly via their binding to CaM. Both vinpocetine and the phenothiazines lack specificity of action to explore the functional role of PDE1. Initially it was claimed that PDEl inhibitors were effective vascular relaxants. With the availability of purified cloned enzymes, however, it is now known that such inhibitors are in fact equally active against PDE5. Indeed, it remains very much the case today that potent inhibitors of PDEl are also active against PDE5; however, selective PDE5 inhibitors have been used to try and separate the roles of the two isoenzymes. Thus it has been suggested that PDE1 inhibitors may prevent intimal hyperplasia following angioplasty on the basis that SCH 51866 (a PDEl and -5 inhibitor; see fig. 10) but not E4021 (a PDE5 inhibitor; fig. 8) is effective in the rat carotid artery injury model [253]. However, we cannot conclude about the functional role of PDE1 until the development of selective PDE inhibitors [254].

Other PDE inhibitors

As mentioned earlier there exist seven other PDEs, PDE2, -6, -7, -8, -9, -10 and -11. Much is known about the structure and function of these enzymes, their complex subcellular distribution and regulation. Their potential as targets for therapeutic intervention in a broad range of biological abnormality is being investigated. Central nervous system applications of PDE inhibitors have begum to emerge. Bayer is studying PDE2 and PDE9 inhibitors as therapeutics for cognition deficits, and Neuro3d is investigating in phase I trials an orally active PDE4 inhibitor for the treatment of depression [16]. Memory Pharmaceuticals and Helicon Therapeutics are investigating PDE4 inhibitors as potential cognitive enhancers [16].

The huge commercial success of the PDE5 inhibitor Viagra has indicated their optimism in the concept of PDE-based therapy. We expect that burgeoning information on the regulation, molecular nature and newly developing inhibitors of these PDEs will open additional avenues toward the production of novel therapeutics for many life-threatening diseases.

Conclusion

Advances in our understanding of the molecular pharmacology of cyclic nucleotide PDE isoenzymes have led to the development of selective inhibitors for many of the PDE families. These inhibitors are being evaluated as potential therapeutic agents for a variety of clinical indications. Substantial study data from preclinical and clinical studies of PDE inhibitors support the concept that PDE inhibitors could be very useful drugs for treatment of pulmonary, vascular, CNS and many inflammatory diseases, as well as for the improvement of quality of life, e.g. sexual function.

PDE inhibitors are now being investigated because of their market potential as well as their therapeutic importance. For example, since Viagra (sildenafil), Pfizer, was approved for use in the United States in March 1998, worldwide sales of drugs to treat ED have grown continuously from 0.78 billion at 1998 to 2.2 billion at 2003. As of 2003 Viagra had been taken by an estimated 23 million men worldwide. Cialis (tadalafil), Eli Lilly and ICOS, is growing to become a second market leader following Viagra in the ED industry. The therapeutic market for inflammatory diseases including asthma and COPD is also substantial. There are about 150 million asthma patients in the world. The number of patients, especially younger patients, is increasing, and about half need chronic treatment. As many as 10 million Americans have COPD and, as a consequence, experience disabling symptoms, high cost of care and substantial mortality. In addition, recent clinical trials of PDE inhibitors in central nervous system diseases such as depression and dementia are increasing their therapeutic potential even more.

Recent achievements in the determination of 3D structures of PDEs provide new opportunities for rational, rapid and productive drug discovery. Crystal structures of PDEs will help to understand mechanisms of action of PDE inhibitors at the atomic level, and are essentially ‘blueprints’ for the chemical and physical space that can be filled by small molecules as inhibitors. Thus, by exploiting the structures of PDE proteins, we may be able to design

more potent and safer drugs. In this way, it would be possible to reduce overall R&D time and expenses, and generate novel developmental candidates faster and more precisely.

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