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Review Article

PHOSPHODIESTERASE: *WHAT IS IN AN ENZYME?*

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ABSTRACT

Phosphodiesterases (PDEs) are hydrolytic enzymes that degrade intracellular cyclic nucleotides. By altering the concentration of these second messengers, PDEs tend to control cellular and subcellular functioning. PDEs are expressed ubiquitously, yet in a compartmentalized fashion, throughout the human body. PDEs aid for optimal co-ordination of cellular transduction by cross-linking diverse molecular pathways. Hence, PDEs act as *'homeostasis regulators*' of numerous biological processes in health and disease. Erectile dysfunction, pulmonary arterial hypertension, congestive heart failure, intermittent claudication and chronic obstructive pulmonary disease are some of the disease states in which inhibitors of PDEs are utilized. This review focuses on the pathophysiological effects and therapeutic potentials of different PDE isozymes.

Key words: Phosphodiesterases, phosphodiesterase types, phosphodiesterase inhibitors

INTRODUCTION

Phosphodiesterases (PDEs), more specifically, cyclic nucleotide PDEs are enzymes (hydrolases) which are involved in the hydrolysis of the 3',5'-cyclic phosphodiester bond foundin the cyclic nucleotides, cAMP (3',5'-cyclic adenosine monophosphate) and cGMP (3',5'-cyclic guanosine monophosphate) (**Figure 1**). Phosphodiester bonds are covalent bonds present between the sugar group and phosphate moiety of these nucleotides. Breakage of phosphodiester bonds leads to conversion of cAMP and cGMP to physiologically inactive elements 5'-AMP and 5'-GMP, respectively. Hence, by destroying the backbone of these regulatory molecules, PDEs play a pivotal role in cellular signal transduction in both health and disease.

Both cAMP and cGMP are ubiquitous second messengers synthesized intracellularly from ATP (adenosine triphosphate) and GTP (guanosine triphosphate) with the catalytic function of adenylyl cyclase (AC) and guanylyl cyclase (GC), respectively. When adequate concentrations of cAMP and cGMP are achieved within the cell, activation of cyclic nucleotide-dependent protein kinases, protein kinase A (PKA) and protein kinase G (PKG), respectively, occurs. These kinases phosphorylate serine and/or threonine residues of downstream proteins leading to altered function of the internal milieu of a cell. cAMP and cGMP also activate other cellular effectors or 'signalosomes' such as cyclic nucleotide-gated ion channels, exchange proteins activated by cAMP(Epacs), cAMP-regulated guanine nucleotide exchange factors (cAMP-GEFS) and phosphoprotein phosphatases.[1–3]

Expression of PDEs is conserved along the phylogenetic spectrum of organisms.They are found abundantly both in the lower organisms such as *Drosophila melanogaster* (fruit fly) and *Caenhorhabiditselegans* (a free-living nematode) and also in mammals including humans.

- **Classification**

PDEs constitute a superfamily of around105 different PDE enzymes categorized into 11 families (classes) based on the amino acid sequences, substrate specificity, catalytic properties, pharmacological features and regulatory features. There are 21 distinct genes coding for these PDEs. Nomenclature of a PDE is as depicted below,

- ❖ HsPDE4D3➔ where,
  - o 'Hs' stands for *Homo sapiens*
  - o '4' (Arabic numeral) signifies the PDE family,
  - o 'D' (capital letter) denotes the coding gene (sub-family/ sub-class)and

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- '3' (Arabic numeral) indicates the specific variant or isoform

Substrate specificity of PDEsis as follows,

- cAMP-specific PDEs: PDEs 4, 7 and 8
- cGMP-specific PDEs: PDEs 5, 6 and 9
- dual-specific PDEs: PDEs 1, 2, 3, 10 and 11

PDEs are present universally throughout the human body. However, different isoforms of PDEs predominate in a particular cell or organ (**Table 1**). Each isoform of a PDE class vary in its three-dimensional structure, subcellular localization and expression, substrate specificities, regulatory and kinetic properties.[4–6]

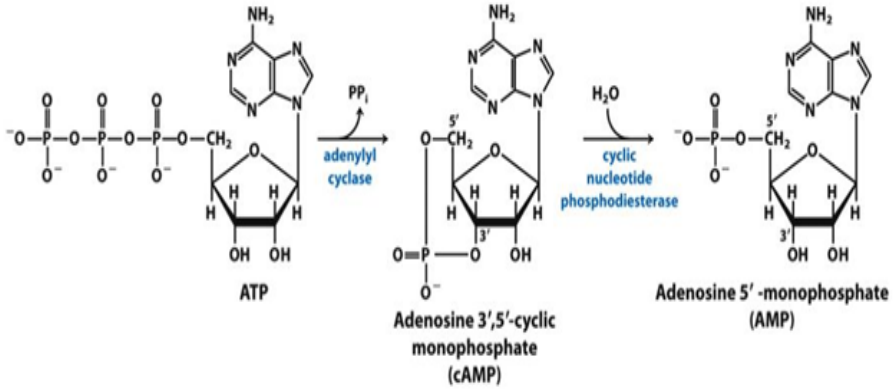

Figure 1: Synthesis and breadown of cAMP

Table 1: Types and tissue localization of PDEs[3,7,8]

Family	Subfamily (variant)	Location
1	A (nine) B (two) C (five)	Olfactory mucosa, kidney, heart, vascular muscle cell, brain, lung, sperm, testis
2	A (four)	Brain, adrenal cortex, kidney, cardiac muscle, platelets, endothelial cells, lung, liver
3	A (three) B (one)	Cardiac myocytes, kidney, blood vessels, bronchi, hypothalamus, platelets, adipocytes, macrophages, pancreatic β-cells, T lymphocytes, oocytes
4	A (seven) B (four) C (seven) D (nine)	Hippocampus, cerebral cortex, olfactory bulb, hypothalamus, amygdala, midbrain, cerebellum, cardiac myocytes, lymphocytes, kidney, pulmonary nerves, airway smooth muscle, sertoli cells, endothelial cells
5	A (four)	Vascular & airway smooth muscle, corpus cavernosum, platelets, cerebellar Purkinje cells, gastrointestinal epithelial cells, endothelial cells, kidney, heart, platelets
6	A (one) B (one) C (one)	Retinal photoreceptors, pineal gland, melanoma cells
7	A (three) B (four)	Cells of the immune system, skeletal muscle, heart, spleen, pancreas, brain, lung
8	A (five) B (six)	Cardiac myocytes, Leydig cells (PDE8A), adrenals (PDE8B), thyroid (PDE8B), embryo, ovary, brain
9	A (twenty)	Small intestinal smooth muscle, skeletal muscle, lung, liver, kidney, heart, testis, spleen
10	A (six)	Neuronal cells, testis
11	B (four)	Ventral hippocampus, prostate, testis, salivary gland, pituitary gland, corpus cavernosum

- **Structure & regulation**

X-ray crystallography has helped in delineating the structure of PDEs. A regulatory NH_2-terminus (R domain) flanked by a catalytic core (C domain) along with the COOH-terminus (COOH domain) constitute the three functional domains of any PDE. Generally PDEs are made up of 250 amino acids. The catalytic domain of all the PDEs is highly conserved, i.e., with 25% to 51% of amino acid sequence identity. However, the regulatory N-terminal domains are highly variable across the various isoforms. The need to understand these structural details of various PDE isoforms is to aid in the design and development of more potent and selective drugs modulating PDE function.

PDEs are regulated by dephosphorylation/ phosphorylation, self-inhibitory mechanisms, several protein-protein interactions with scaffolding or anchoring proteins and binding of calcium-calmodulin complex. And even binding of cAMP and cGMP, themselves, at a different allosteric sitecould modulate the expression of PDEs. In addition to these endogenous structural and biochemical modulations, various patho-physiological states also impact the regulation of these PDEs. Hence, there exists a cross-talk between these cellular signaling pathways which provide for integration of complex regulatory processes.

Though the functions of PDEs are yet to be deciphered completely, convincing evidences are available for diseases states wherein PDEs are dysfunctional. Bronchial asthma, chronic obstructive pulmonary disease (COPD), heart failure, hypertension, stroke, intermittent claudication, depression and erectile dysfunction (ED) are some of the disease conditions associated with malfunctioning PDEs.[7]

PDEsas therapeutic targets

PDEs are one of the major determining factors of intracellular cyclic nucleotide levels. Compartmentalized expression of cyclic nucleotides and so the subsequent cellular signaling pathways show the therapeutic significance of selective PDE inhibition. However it should be noted that a particular cell type can express several different isoforms of PDE. Presence of multitude of PDEs on cell-type specific basis with specific functioning characteristics has led to the development of selective and specific inhibitors of these enzymes, viz., phosphodie-sterase inhibitors (PDE-Is).

Once the PDE activity was discovered (late 1950s), many non-selective PDE-Is such as caffeine and theophylline were used in clinical practice. However, they causedmany untoward side effects with a very narrow therapeutic index as they inhibited almost all PDEs present in every cell and tissue.

The exploration of PDEs as drug targets was pursuedrigorously and successfullydue to the following reasons,

- Measurement of PDE activity was easier than that of cyclic nucleotides themselves and also that of adenylyl/ guanylyl cyclase, the enzymes involved in the synthesis.
- Regulation of degradation of any second messenger, including these cyclic nucleotides, will cause a faster and higher percentage alteration in the concentration rather than modifying the synthetic pathway.
- Greater activity (higher V_{max}) of PDE than the cyclase enzymes.
- Abundantnumbers of isoforms of different PDE classes act as potential targets altering specific physiological systems or pathological states.
- Lower concentrations of the substrates (cAMP and cGMP levels are usually less than 10 micromoles) lead to optimal inhibition even with minimal concentration of inhibitor.

Mutations and polymorphisms of the genes encoding PDE families have been associated with many disorders (**Table 2**).

Table 2: Diseases associated with genetic mutations and polymorphisms in PDEs[3]

Gene (mutated or polymorphic)	Diseases
PDE4D	Ischemic stroke
PDE4B	Schizophrenia
PDE6A, PDE6B	Retinitis pigmentosa
PDE6A	Leber congenital amaurosis type 4
PDE7	Chronic lymphocytic leukemia
PDE8B	Micronodular adrenal hyperplasia, adrenal adenomas
PDE11	Prostate, adrenal, testicular tumours
PDE11A	Cushing syndrome, bilateral micronodular adrenal hyperplasia

Furthermore, with the help of real-time imaging modalities estimation of intracellular cyclic nucleotides in the vicinity of PDEs was possible. Herget*et al*[9]reported one such technique based on the fluorescence resonance energy transfer (FRET) technique wherein PDE activity in intact living cells was measured. This and other such biosensors of intracellular signaling confirmed the compartmentalized expression of PDEs and also supported in thedevelopment of selective PDE-Is.

PDE5

PDE5 is a cGMP specific PDE; there is only one subtype (PDE5A) reported in humans with four isoforms (PDE5A1, PDE5A2, PDE5A3 and PDE5A4). The gene for PDE5A is located in chromosome 4q27. Both PDE5A1 and PDE5A2 are extensively distributed in tissues such as heart, lung, liver, kidney, brain, bladder, urethra, prostate, skeletal muscles, uterus and penis. Smooth muscle tissues are the major areas where PDE5A3 is seen; heart, bladder, urethra, prostate, uterus and penis also express PDE5A3.

- **Genitourinary system**

The first of the PDE5-Is to be synthesized and developed was sildenafil. While it was initially investigated for the management of coronary heart disease many patients reported a distinctive side effect of penile erection. Later it was worked over and the side effect potential of sildenafil was transformed to a therapy for ED. Pfizer got the U.S. Food and Drug Administration (FDA) approval for Viagra (sildenafil) in 1998. Thereby, sildenafil became the first oral remedy for the management of ED.

Sexual arousal with stimulation of nonadrenergic, noncholinergic system along with parasymphathetic discharge leads to release of nitric oxide (NO) in the endothelial cells of penile corpora cavernosa. NO released diffuses into the smooth muscle cells where it activates the GC resulting in production of cGMP. cGMPin turn activates PKG which phosphorylates many downstream proteins inhibiting entry of calcium into the cells finally leading on to relaxation of smooth muscles of the corpora cavernosa and penile arteries. Hence, engorgement of the sinusoids of corpora cavernosa results in penile erection. Sildenafil by inhibiting these cGMP-specific PDE5 enzymes present in the smooth muscle cells of corpora cavernosa causes accumulation of cGMP and the consequent penile erection.[4,10]

Sildenafil is usually prescribed at an oral dose of 50 mg taken approximately one hour before sexual intercourse. For males aged more than 65 years and males with severe renal or hepatic impairment the starting dose is reduced to 25 mg.

Tadalafil (Cialis; Eli Lilly) andvardenafil (Levitra; Bayer), the congeners of sildenafil, got FDA approval for the management of ED in 2003.Tadalafil is the most potent PDE5-I with a longer duration of action (36 h) and its selectivity for the enzyme is 9000 times more than the other PDE5-Is. Owing to its poor solubility and consequent variable bioavailability (slowest absorption)the time to peak concentration is delayed (Median: 2 h). A transdermal drug delivery system is in the making for tadalafil to overcome these pharmacokinetic shortcomings.Similarly an orodispersibletablet formulation of vardenafil was tried successfully in previous studies.[11]

Avanafil (Stendra; Vivus), udenafil, lodenafil and mirodenafil are some of the other PDE5-Is available for the management of ED.[12]

Co-administration of any nitric oxide donors (organic nitrates or organic nitrites) is highly contraindicated in patients on PDE5-Is as it may result in catastrophic fall in blood pressure. A time gap of 24 hours is advised between the intakes of these types of drugs. Sildenafil and other PDE5-Is also cause difficulty in blue-green colour discrimination due to their inhibitory potential on PDE6 isoenzyme present in the retina. However, very rarely sudden loss of vision can occur which could be a sign of nonarteritic anterior ischemic optic neuropathy (NAION). However, occurrence of these visual abnormalities is lesser in tadalafil (< 0.1%) and vardenafil. Sudden decrease in hearing capacity or loss of hearing along with tinnitus and dizziness are also associated with the usage of these drugs owing to increased exposure of cochlear hair cells to cGMP.

Sildenafil was indeed a blockbuster "lifestyle drug" which was reflected in its annual sales and usage since its approval. However, owing to its rampant use, regulatory bodies had to face more problems when sildenafil was incorporated into the so-called "energy food" supplements. Certain analogs of sildenafil, not approved by FDA, such as hydroxythiohomosildenafil and sulfoidenafil are marketed as "male enhancement" recreational agents. These drugs may also cause deleterious interactions with nitrates.[13]

Tadalafil was also later approved for the treatment of signs and symptoms of benign prostatic hyperplasia (BPH) or ED with BPH. A recent meta-analysis of 12 RCTs reaffirmed that a PDE5-I alone or in combination with an α–adrenergic blocker was both effective and safe for the treatment of lower urinary tract symptoms (LUTS) due to BPH.[14]

The effect of PDE5-Is in the management of men with premature ejaculation (PE) was conclusively proved in the meta-analysis by Sun *et al*[15] in 10 RCTs comprising 775 patients. It was noted that PDE5-Is were more effective than selective serotonin reuptake inhibitors or placebos. The possible mechanisms are reduction in the central sympathetic outflow, regulation of the contractile responses from the prostate, vas deferens, seminal vesicles and urethra.

PDE5-Is are also triedin overactive bladder and in ureteral colic due to stone in the urinary tract owing to their smooth muscle relaxing properties in concerned tissues.

- **Lungs**

PDE5 is overexpressed in the vascular smooth muscle cells of the pulmonary vasculature and also in the cardiomyocytes of the right ventricle. PDE5 causes vasoconstriction and cellular proliferation by decreasing cGMP levels. By acting as PDE5-Is, both sildenafil (Revatio; Pfizer) and tadalafil (Adcirca; Eli Lilly) caused marked vasodilatation of the pulmonary blood vessels leading to profound fall in pulmonary arterial pressure. Hence, they were also approved for the treatment of pulmonary arterial hypertension (PAH). Patients falling under WHOfunctional classes of II and III exhibited better response, such as improved exercising ability, when treated with Revatio or Adcirca. Concomitant intake of riociguat (a guanylyl cyclase stimulator) or nitrates is contraindicated with these drugs. Revatio is available as oral tablets, oral suspensions and intravenous injections.[16]

- **Cardiovascular system**

The therapeutic potential of PDE5-Is in the management of various cardiovascular diseases has been explored through preclinical and clinical studies. Several animal trials and few nascent human studies have put forth the strong protective effect of PDE5 inhibition against ischemia reperfusion injury, ischaemic cardiomyopathy, improvement of stem cell efficacy for myocardial repair, cardiac hypertrophy, doxorubicin-induced cardiotoxicity. Similar effects were also seen in diabetic mice models where tadalafil was tested for its potential to activate SIRT1 (Silent Information Regulator 1). It was observed that PDE5-Is could protect against endothelial dysfunction, ischemia reperfusion injury and promote anti-oxidant and anti-inflammatory effects in diabetic hearts. Augmented expression of NO synthases, stimulation of PKG and its dependent pathways and phosphorylation of glycogen synthase kinase-3β are the possible mechanistic actions of PDE5-Is leading to cardioprotection.[3,17]Despite equivocal results in various clinical trials on systolic and diastolic heart failure and cardiomyopathies, a

latest meta-analysis by Giannetta*et al*[18] demonstrated convincingly that PDE5-Is exerted anti-remodeling properties with enhanced cardiac inotropism. Their meta-analysis reviewed 24 RCTs evaluating the safety and efficacy of PDE5-Is on cardiac morphology and function involving 1,622 subjects. PDE5-Is were also found to relieve vasoconstriction in peripheral artery diseases (PADs), Raynaud's phenomenon and hypoxic brain states.

- **Neoplasms**

The proposed role of PDE5-Is as chemotherapeutic agents for the management of various neoplastic conditions is being studied extensively. Increased expression of PDE5 was seen in several human carcinomas such as the breast, colon, bladder, prostate, lung and pancreatic cancers. PDE5-Is activate PKG resulting in activation of β-catenin pathway which causes downregulation of survival proteins and also activation of pro-apoptotic proteins. PDE5-Is also enhance sensitivity of multiple chemotherapeutic agents by inhibiting the efflux ATP-binding cassette(ABC) drug transporters such as ABCB1 and ABCG2. PDE5-I-induced increased reactive oxygen species generation leads to activation of pro-apoptotic factors and inhibition of anti-apoptotic factors culminating in cancer cell death.[3,17]

- **Kidneys**

PDE5 inhibition is also protective to the kidneys, especially in the long term. PDE5 is expressed in the glomeruli, cortical tubules, mesangial cells and other areas of kidney. The nephroprotective action of PDE5 inhibition is due to alteration of the renal hemodynamics and excretory function. Decreased apoptosis and necrosis; improvement in endothelial function; decreased DNA damage, renal inflammation and fibrosis; increase in free radical scavenging enzymes (catalase & superoxide dismutase) are some of the pathophysiological mechanisms which contribute to the renal beneficial and protective effects of PDE5 inhibition. Essential hypertension, diabetes, ischemia reperfusion injury, nephrotoxic nephropathy and renovascular hypertension are some of the clinical conditions wherein PDE5-Is can be utilized to protect from chronic kidney disease. Along with renin-angiotensin system blockers PF-0048791, a long-acting PDE5-I, has shown to reduce albuminuria in overt diabetic nephropathy patients in phase 2 clinical trials.[19]

PDE5-Is are also being tried for primary oesophgeal motility disorders as they relieve spasm and promote smooth muscle relaxation. Sildenafil is also used for management of sexual dysfunction in females due to type 1 diabetes mellitus, multiple sclerosis, spinal cord injury and antidepressant drugs. PDE5-Is are also used as alternatives to calcium channel blockers for the management of high-altitude pulmonary oedema.[20]

As of now there are 641 completed or ongoing clinical trials related to PDE5-Is focusing on its various putative therapeutic and other beneficial effects (http://www.clinicaltrials.gov).

PDE4

The PDE4 family consists of four sub-families, viz., PDE4A (seven variants), PDE4B (four variants), PDE4C (seven variants) and PDE4D (nine variants). Hence, in total there 27 splicing variants or isoforms of PDE4 making it as the largest of the PDE families.

- **Lungs – inflammatory conditions**

Inflammatory cells including neutrophils, macrophages, monocytes, eosinophils, mast cells and T lymphocytes express PDE4. PDE4 inhibition resulted in decreased release of inflammatory mediators such as cytokines and chemokines causing reduced immune cell migration and activation in the lung tissue. This anti-inflammatory effect was utilized in the management of chronic obstructive pulmonary disease (COPD). PDE4 inhibition also resulted in relaxation of airway smooth muscle and modulation of pulmonary nerve activity. All these effects were due to upregulation of cAMP which controls functioning of the inflammatory cells, mucociliary clearance and pulmonary vascular remodeling.Roflumilast (Daliresp; Takeda) was the first selective PDE4-I to be approved by FDA (2011) for reducing the risk of COPD exacerbations in patients with severe COPD characterized by chronic bronchitis and history of frequent exacerbations.[13,21]

PDE4B inhibition is involved in mediating the anti-inflammatory effects whereas inhibition of PDE4D results in gastrointestinal side effects such as

nausea, vomiting and diarrhoea.[4]Rolipram, the prototypical PDE4-I, was a failure owing to its higher affinity towards the PDE4D isoforms.

A systematic review published by the Cochrane Collaboration divulged that PDE4-Is improved pulmonary function [mean difference of 45.6 mL in forced expiratory volume in one second (FEV_1)] and decreased the frequency of COPD exacerbation (OR: 0.77; 95 CI%: 0.71 to 0.83) compared to placebos. The analysis comprised of 15 roflumilast-based RCTs (12,654 patients) and 14 cilomilast-based RCTs (6,457 patients).[22] However, cilomilast (GSK), another PDE4-I, was stopped from further development due to inconclusive results of some of the phase III trials.

Based on earlier evidence, Takeda Pharmaceutical Company is currently working on the therapeutic prospective of roflumilast as an anti-asthmatic medication. They conducted a multi-centric trial in Europe, North America and South Africa and found out that roflumilast decreased allergen-induced bronchoconstriction and airway inflammation along with reduction in eosinophils and neutrophils in asthmatic patients.[23]Inhalational drugs CHF6001 (a PDE4-I) and RPL554 (a dual PDE3/4-I)are currently in clinical trials for asthma.[24,25]

- **Psoriasis**

In 2014, FDA gave approval for a novel small-molecule PDE4-I viz., apremilast (Otezla; Celgene) for the treatment of patients with active psoriatic arthritis (PsA) and also for patients with moderate to severe plaque psoriasis (PsO).Apremilast inhibits all four sub-families of PDE4, i.e., PDE4A1, PDE4B1, PDE4B2, PDE4C1 and PDE4D2. Both PsO and PsA are characterized by overproduction of pro-inflammatory mediators leading to dysregulated inflammatory process; and PDE4 plays a significant role in this chronic inflammatory process. Higher concentration of cAMP, the catalytic substrate of PDE4, within the immune cells reduces the synthesis of pro-inflammatory factors such as tumour necrosis factor-α (TNF-α), interferon-γ (IFN- γ), interleukin-17 (IL-17) and IL-23 whereas stimulates the production of anti-inflammatory cytokines such as IL-10 and transforming growth factor-β (TGF-β).

Ease of oral administration and direct modulation of intracellular signaling rather than binding to single inflammatory mediator along with favourable safety profile are some of the advantages of apremilast over the currently approved biologics for PsO. Apremilastwas not associated with reactivation of tuberculosis nor with risk of development of cancers; liver or kidney function monitoring was also not necessary.[26]

PDE3

The bipyridine derivatives inamrinone (amrinone) and milrinone are a class of cGMP-inhibited cAMP-specific PDE3-Is used for short-term management of congestive heart failure (CHF). These drugs cause elevated concentrations of cAMP within cardiac and vascular smooth muscle cells resulting in enhanced myocardial contractility and dilatation of both resistance (arteries & arterioles) and capacitance (veins &venules) vessels. Hence, they are aptly termed as *"inodilators"*- improving cardiac output through positive inotropism and decreasing both preload and afterload by direct vasodilatation. They are administered intravenously for severe and refractory acute CHF as an add-on therapy to digitalis, diuretics and other vasodilators. Chronic administration of these drugs has increased mortality rates due to ventricular arrhythmias and cardiac arrest; hence, they are contraindicated for long-term use.[4]Milrinone and sildenafil are also being tried for persistent pulmonary hypertension of the newborn.

Cilostazol (Pletal; Otsuka), another PDE3-I,is indicated for the reduction of symptoms of intermittent claudication seen in PADs. It has specific antiplatelet (anti-aggregatory) and vasodilatory properties by increasing intracellular cAMP in platelets and vascular smooth muscles. Consumed at an oral dose of 100 mg twice daily patients report an increase in the walking distance.[20]Cilostazol is currently being tested for mild to moderate Alzheimer's disease (AD) patients as a cognition enhancer along with donepezil.[27]Additional cardioprotective effects of cilostazol are exposed by its use in controlling arrhythmias in conditions such as Brugada syndrome and other cases of bradyarrhythmias.

Anagrelideis used in the management of thrombocythemia, secondary to myeloproliferative disorders, to decrease elevated platelet count and the risk of thrombosis and thrombohemorrhagic events.[20]

The PDE3B sub-class of PDE3 has been found to be abundantly expressed in various hypothalamic sites and also it is hypothesized that the PDE3B pathway could be involved in modulating leptin-induced anorexia and loss of body weight. PDE3B isoforms have been associated with regulation of energy homeostasis. Studies on these isoforms are pursued vigorously so as to make them as possible targets of diseases with deranged energy homeostasis such as, obesity and type 2 diabetes mellitus.[28]

Other PDEs

Methylxanthines such as theophylline, aminophylline (theophylline ethylenediamine), enprofylline and doxofylline are non-selective PDE-Is used as bronchodilators in the management of bronchial asthma and COPD. Except for PDE8 and PDE9 families, methyxanthines inhibit all other families of PDEs. With a narrow therapeutic margin these theophylline groups of drugs tend to stimulate the heart and central nervous system (CNS) producing arrhythmias and seizures, respectively, at plasma concentrations at or marginally above the upper level of the therapeutic range.

Caffeine another naturally occurring methylxanthine is used along with ergot alkaloids for the treatment of migrainous headaches. Caffeine acts by enhancing constriction of the cranial blood vessels and by increasing gastrointestinal absorption of ergotamine. As a psychostimulant caffeine is also used to allay fatigue and drowsiness.

Pentoxifylline (Trental; Sanofi Aventis), a xanthine derivative causing favourablehemorheological actions in patients with intermittent claudication symptoms, has also found to exert a weak PDE inhibitory action.

Levosimendan, a novel drug purported to sensitize the myocardium (troponin system) to calcium, also inhibits PDEs leading to both inotropic and vasodilatory action in heart failure patients.

The antiplatelet agent dipyridamole is also associated with its PDE inhibitory action (PDE5A-I) apart from its inhibitory action on adenosine uptake. Initially indicated for thromboembolic prophylaxis (in combination with warfarin) in patients with prosthetic heart valves, dipyridamole is also used for myocardial perfusion imaging (scintigraphy) in ischemia testing. It non-selectively dilates all resistance vessels including both ischemic and non-ischemic regions of the myocardium distorting autoregulation and causes the dreadful 'coronary-steal' phenomenon precluding its use in typical angina. However, in combination with low-dose aspirin, dipyridamole is used for secondary prophylaxis of stroke or transient ischemic attack (TIA).[10]

Papaverine, a synthetic derivative of opium, is indicated for relieving arterial spasms. Its smooth muscle spasmolytic action is attributed to inhibition of PDEs (PDE10). Papaverine alone or along with phentolamine is injected intracavernously for ED (off-label).

Despite identification of 11 distinct PDE families, therapeutic drugs against PDEs were clinically successful only with three families of PDEs so far.

Novel insights& future perspectives

As the PDEs were highly conserved across the species, it was postulated that modified PDE-Is could function as anti-parasitic agents especially where the PDE isoform is vital for the parasitic survival. This novel approach called as the "inverted magic bullet paradigm" deals with exploring those highly conserved therapeutic targets between the humans and the infectious microbes. From the above discussion, it is obvious that almost all classes of PDEs are potential therapeutic targets which are already studied in depth. Hence, development of inhibitors for parasitic homologues of PDEs, with the aid of medicinal chemistry, would be high yielding in certain parasitic diseases caused by *Trypanosoma brucei*(TbrPDEB1 & TbrPDEB2) and *Leishmaniadonovani*(LmjPDEB1).[29]

PDE5-Is are tried in the management of muscular dystrophies such as Duchenne and Becker Muscular Dystrophy (DMD & BMD). Enhanced concentration of cGMP within the cardiac, respiratory and skeletal muscles relieves impaired blood flow to muscle tissues.[30]

The importance of cAMP in learning, memory and cognitive function had led to novel exploration of modulating the cAMP signaling pathway as a therapeutic modality for neurological disorders such as AD, Huntington's disease (HD), depression and schizophrenia. As almost all isoforms of PDEs are present in the brain, vigorous research on selective PDE-Is for various neurological disorders are undertaken.Recently, it has been viewed that inhibition of PDE4, more specifically the PDE4D isoform enhances memory and cognition function and also promotes neuroprotection and neuro-regeneration.One such potential therapeutic agent HT-0712, a PDE4-I, was found to enhance hippocampus-related memory and improve cognition in mice models. As evidence of higher expression of cAMP-specific PDE mRNAs exist in AD patients, inhibiting these enzymes could be effective in halting the progress of AD and other age-associated memory impairments in humans.More than 10 PDE10A and around 25 PDE4 selective inhibitors are currently under pre-clinical and clinical trials for conditions such as schizophrenia, HD and other cognitive dysfunctiondisorders. A highly selective and potent PDE10-I, PF-2545920 and ITI-214 (PDE1-I) are being envisaged as drugs for schizophrenia.[3,27,31] PDE4-Is (e.g., MK-0952) and PDE9-Is (e.g., PF-04447943) are also currently evaluated for their cognition enhancement properties.[27]

Very recently, a clinical trial published in the reputed Brain journal reported that brain PDE10A levels are inversely related to disease progression and severity in Parkinson's disease (PD); making PDE10A as a promising biomarker in PD.[32]

An *In vitro* study in human autosomal dominant polycystic kidney disease (ADPKD) tissues and cells revealed that PDE4 as the major intracellular regulator of cAMP and Cl^--dependent fluid secretion in kidneys. It was also reported that PDE1 inhibition resulted in a mitogenic effect causing ADPKD cell proliferation. These findings corroborate the earlier discussion on specific subcellular compartmentalized expression of PDEs, in this case within renal epithelial cells.[33]

Another *in vitro* study in adult rat ventricular myocytes showedthat diverse isoforms of PDEs differentially regulate the cardiac excitation-contraction coupling. The results divulged that PDE2 and PDE3 were major modulators of basal cardiac muscle contraction whereas PDE4 was a major determinant of a prior stimulated heart, like with β-adrenergic stimulation.[34]Similarly, it was deciphered that, PDE3 and PDE4 inhibition resulted in cardiac hypertrophy whereas PDE2 inhibition was anti-hypertrophic. Hence, it was clear that distinct pools of cAMP affect cardiac myocyte growth differently. Therefore, PDE2-Is could function as therapeutic agents against heart failure.[35]

Celegene, the company which acquired marketing approval for apremilast, is currently actively exploring the same compound for use in rheumatoid arthritis, Bechet's disease and ankylosing spondylitis. Apremilast is also being extensively tested for cutaneous sarcoidosis, lichen planus, prostatitis, osteoarthritis, rosacea and vulvodynia. CC-11050 and AN2728 are other PDE4-Is under clinical trials for the management of lupus and paediatric atopic dermatitis.[26,36]

The co-administration of a long acting β_2–adrenoceptor agonist (LABA) and a PDE4-I has been found to be more effective than either drugs alone for the control of COPD symptoms. The synergistic potential of such a combination made the advent 'bifunctional ligands' comprising two pharmacophores of LABA and PDE4-I connected covalently by an optimally structured spacer or linker. Gilead Sciences is in the process of making one such bifunctional ligand named as "GS-5759" consisting of an active quinolinone head group of LABAs indacaterol and carmoterol and a highly potent PDE4-I GSK 256066 with a 'pent-1-yn-1yl benzene' spacer. Hence, by providing an accentuated cAMP response by simultaneous β_2–adrenoceptor stimulation and PDE4 inhibition by a single therapeutic molecule will evoke augmented suppression of the inflammatory cascade in individual immune cells along with bronchodilation. Gilead is planning to combine this inhalational GS-5759 compound with a glucocorticoid as a part of 'triple combination chemotherapy' in a single inhaler device.[6,37]

Following preliminary evidence of PDE5-Is inducing malignant melanoma (MM), Loeb *et al*[38], in June 2015, reported a case-control study comprising of 4,065 melanoma cases and 20,325 controls. They concluded that there was a significant association

between the use of PDE5-Is and the risk of development of MM (OR: 1.21; 95% CI: 1.08 to 1.36) and basal cell carcinoma (OR: 1.19; 95% CI: 1.14 to 1.25).

Recently, co-crystallography, divulged three different conformers of PDE4 viz., an open conformer, a symmetrical closed conformer and an asymmetric dimer. These conformers can act as more specific and selective targets for PDE4 inhibition.[39]

PDE10A-Is areinvestigated for their ability as positron emission tomography (PET) radioligands for functional imaging of the brain.*In vitro* studies on human breast cancer cell lines have shown that PDE8A-Is like PF-04957325 could be a possible therapy for controlling metastasis of breast carcinoma.

Due to the complexity of some of the diseases,PDE-Is that target multiple isoforms like dual PDE-Is are under investigation.Simultaneously targeting at multiple isoforms of PDEs localized at different cellular and subcellular levels could theoretically exert additive or synergistic beneficial effects and lead to more effective pharmacotherapy. PDE8/4-Is, PDE1/4-Is, PDE4/7-Is, PDE4/5-Is and PDE3/4-Is are some of the dual PDE-Is tested for their clinical properties.[3,25]

Resveratrol, a polyphenol found in red wine, has been purported to protect against cardiovascular complications of metabolic disorders like obesity, type 2 diabetes mellitus by non-specific inhibition of PDEs.[40]

Disruption of the cellular effectors or signalosomes, which are cyclic nucleotide effectors containing confined macromolecular complexes generated through protein-protein interactions, is being exhaustively studied. Therefore, small-molecule drugs or peptide-based drugs ('disruptor peptides') antagonizing discrete signalosomes can be speculated to be effective in controlling unique pathophysiological processes.[3]

These newer and novel understandings of the PDE system will make way for discovery and development of more efficacious therapeutic targets against intracellular signaling pathways (**Table 3**).

Table 3: Clinical trial status* of PDE-Is[3,30]

PDE-I	Clinical indication	Phase of clinical trial
Sildenafil	Schizophrenia	IV
	Heart failure	III
	Impaired glucose tolerance	III
	Endocrine dysfunction in patients with diabetes	III
Tadalafil	Diabetic cardiomyopathy	IV
	Becker muscular dystrophy	IV
	Aortic stenosis	IV
	Head and neck cancer	IV
	Lung disease	III
Udenafil	Raynaud's phenomenon	III
Apremilast	Ankylosing spondylitis	III
Cilostazol	Dementia	IV
	Atherosclerotic events	IV
	Restenosis	IV
	Ischemic stroke	IV
Enoximone	Heart failure	III

*Only those drugs in phase III and phase IV as reported in the http://www.clinicaltrials.gov

Conclusion

Though there are more than 100 isoforms of PDEs identified, each of them exerts distinctive, non-overlapping and non-redundant physiological effects on the human body.So, there exists a myriad of unique PDE isozymes that can be targeted to evoke multitude of beneficial biological responses. However, the number of therapeutically useful PDE-Is are comparatively very few.

Having said so, some of the novel PDE-Is mentioned in this review are the best examples of *"translational research"* in medicinal practice.
Progress in the field of biochemistry will throw more elaborate and conclusive viewpoints on the structure and function of PDEs; thereby, providing a solid foundation for development of more efficient therapeutic modulators of the PDE enzyme system.
Hence, lying at the cross-roads of cellular transduction, PDEs act as "gate-keepers"patrolling the basic essence of life.

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