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PHOSPHODIESTERASE INHIBITORS: THEIR ROLE AND IMPLICATIONS

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ABSTRACT: Phosphodiesterase (PDE) isoenzymes catalyze the inactivation of intracellular mediators of signal transduction such as cAMP and cGMP and thus have pivotal roles in cellular functions. PDE inhibitors such as theophylline have been employed as anti-asthmatics since decades and numerous novel selective PDE inhibitors are currently being investigated for the treatment of diseases such as Alzheimer's disease, erectile dysfunction and many others. This review attempts to elucidate the pharmacology, applications and recent developments in research on PDE inhibitors as pharmacological agents.

Keywords: Phosphodiesterases, Phosphodiesterase inhibitors.

INTRODUCTION

cAMP and cGMP are intracellular second messengers involved in the transduction of various physiologic stimuli and regulation of multiple physiological processes, including vascular resistance, cardiac output, visceral motility, immune response [(1)], inflammation [(2)], neuroplasticity, vision [(3)], and reproduction [(4)]. Intracellular levels of these cyclic nucleotide second messengers are regulated predominantly by the complex superfamily of cyclic nucleotide phosphodiesterase (PDE) enzymes. Cyclic nucleotide phosphodiesterases (PDEs) comprise a superfamily of metallophospho-hydrolases that specifically cleave the 3′, 5′-cyclic phosphate moiety of cAMP and/or cGMP to produce the corresponding 5′-nucleotide. PDEs are critical determinants for modulation of cellular levels of cAMP and/or cGMP by many stimuli [(10)] .Thus, the ubiquitously present PDEs play a pivotal role in regulating cell signalling via the breakdown of cAMP and cGMP [(5)].

PDE inhibitors are therapeutic agents which target PDE isoenzymes and inhibit the metabolism of the secondary messengers (cAMP, cGMP) thus, prolonging the biological effect determined by the type of cell involved.

Both non selective and selective inhibitors of PDE are currently being explored as possible treatments for a variety of conditions such as sexual dysfunction, Alzheimer's disease, COPD and other aliments. By inhibiting specifically the up-regulated PDE isozyme(s) with newly synthesized potent and isoezyme selective PDE inhibitors, it may possible to restore normal intracellular signaling selectively, providing therapy with reduced adverse effects [(9)].

AN OVERVIEW OF THE PHOSPHODIESTERASE SUPER FAMILY

The PDE super family is large, complex and represents 11 gene families (PDE1 through PDE11). Each of the PDE families contains one to four genes, and many genes generate multiple isoforms. All the members of the PDE superfamily differ in various aspects such as localization or tissue distribution, mode of regulation and inhibitor specificity [(6)]. The PDES are found in the cytosol, plasma membranes, endoplasmic reticulum, nuclear membranes and the cytoskeleton [(7, 8)]. PDEs are regulated by intracellular cyclic nucleotide concentrations, phosphorylation, interaction with regulatory proteins, subcellular compartmentalization, and binding of Ca2þ/calmodulin, as well as by changes in gene expression [(6)]. PDE3, PDE4, and PDE7 and PDE8 hydrolyze only cAMP (cAMP-PDE). PDE5, PDE6 and PDE9 hydrolyze only cGMP (cGMP-PDE), and isozymes PDE1 and PDE2 accept both nucleotides as a substrate [(6, 12)].

Table 1: Phosphodiesterases superfamily [6, 9]

PDE FAMILY	SUBSTRATE	REGULATIONS	INHIBITORS	CLINICAL APPLICATIONS
PDE 1	cAMP/cGMP	Ca2þ/calmodulin activated	Vinpocetine Nicardipine 8-MeOM-IBMX Nimodipine	Dementia, memory loss
PDE 2	cAMP/cGMP	Stimulated/ activated by cGMP	EHNA	Sepsis Acute respiratory distress syndrome Memory loss
PDE 3	cAMP/cGMP	cGMP-inhibited	Lixazinone Cilostamide Milrinone Cilostazol Dihydro-pyridazinone	Glomerulonephritis Congestive heart failure Intermittent claudication Thrombosis Pulmonary hypertension
PDE 4	cAMP	cGMP-insensitive. Phosphorylated by PKA Phosphorylated by ERK	Rolipram Denbufylline Cilomilast Roflumilast	Glomerulonephritis Asthma, COPD a Bipolar depression Autoimmune encephalomyelitis Organ transplantation
PDE 5	cGMP	PKA/PKGphosphorylated Binds cGMP	Sildenafil (Viagra) Zaprinast Dipyridamole Ariflo Vardenafil Tadalafil	Chronic renal failure Salt retention in nephritic syndrome Pulmonary hypertension Erectile dysfunction Organ transplantation
PDE 6	cGMP	Transducin-activated	Zaprinast Dipyridamole Vardenafil Tadalafil	Selective PDE6 inhibitors are few and have little applications due to adverse effects on vission.
PDE 7	cAMP	Rolipram-insensitive	Dipyridamole Thiadiazole	Airway and immunological diseases.
PDE 8	cAMP	Rolipram-insensitive IBMX-insensitive	Dipyridamole	Immunological applications.
PDE 9	cGMP	IBMX-insensitive	Zaprinast	Possible hypoglycemic effects
PDE 10	cAMP/cGMP	Unknown	Dipyridamole Papaverine	Treatment of Schizophrenia and other neuro-pyschiatric disorders.
PDE 11	cAMP/cGMP	Unknown	Tadalafil Zaprinast Dipyridamole	Proposed improvement of human testicular functions.

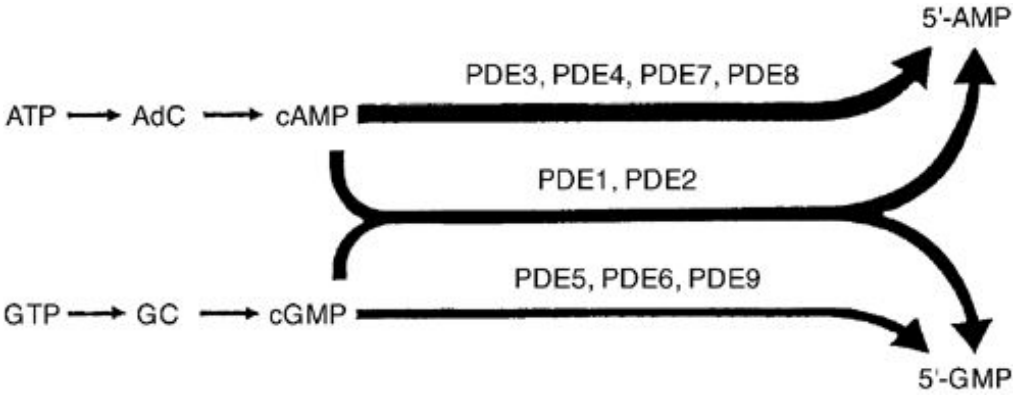

Fig1: General scheme of cyclic 3',5'-nucleotide metabolism[12].

- **General structure of phosphodiesterases isoenzyme.**

The general structure of the PDE super family consists of the basic features which include a catalytic core, regulatory region between the amino acid terminal and the catalytic core and lastly an amino acid terminal which imparts isoform specificity. In particular, the size of the N-terminal domain is substantially different in various PDE types.

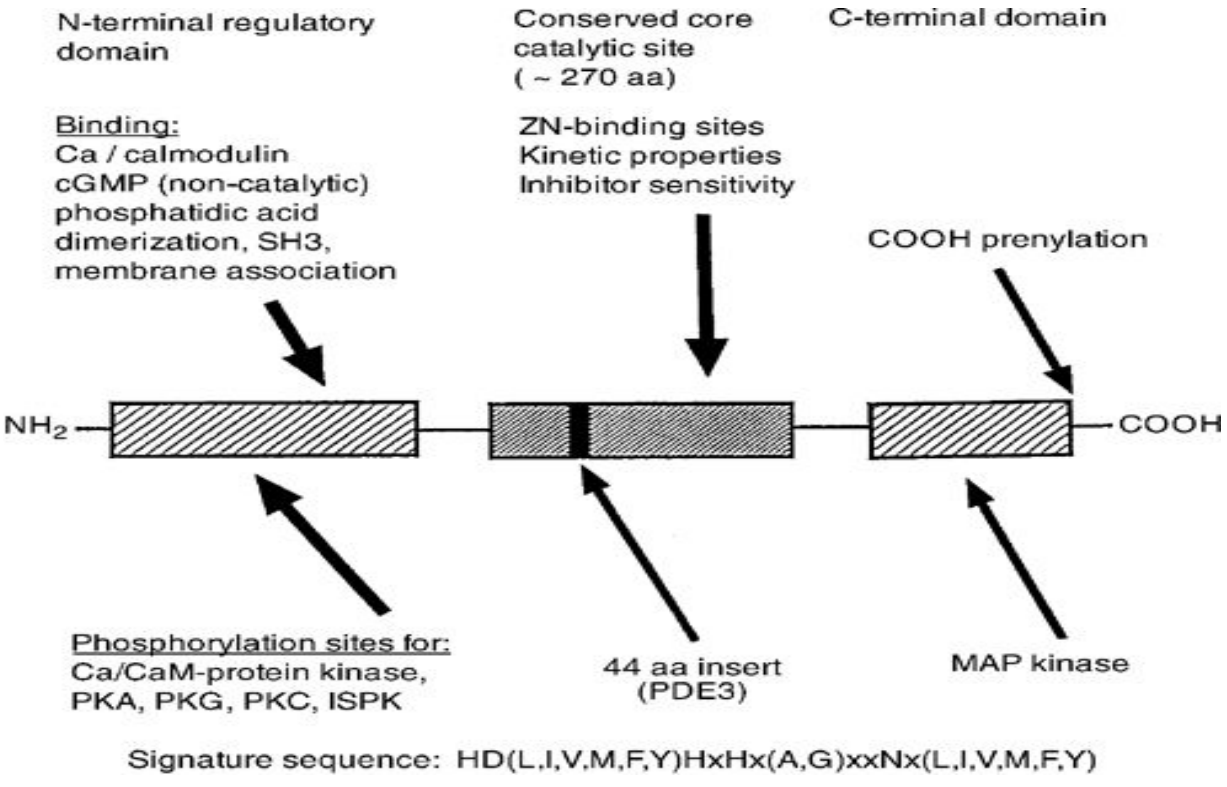

Fig 2: General Structure. Abbreviations: PKA, protein kinase A; PKG, protein kinase G; PKC, protein kinase C; PDE3IK, insulin-dependent PDE3B protein kinase[12]

PHOSPHODIESTERASE AND ITS INHIBITORS

1. PDE1 and its inhibitors

Overview:

The PDE1 family was the first eluted fraction isolated by chromatography from vascular smooth muscle. This PDE1 fraction was specifically activated by Ca2+/CaM, and thus named CaM-PDE (Calcium calmodulin dependent PDE) [13]. The cooperative binding of four Ca^{2+} to calmodulin is required to fully activate CaM-PDE [14]. Three genes (PDE1A, PDE1B,and PDE1C) with various splice variants constitute PDE1 family. These soluble enzymes are homodimerics. In this unique CaM-sensitive PDE family, sensitivity to calcium and calmodulin varies from one variant to the other. [16,17].

Localization and distribution:

Most PDE1s are cytosolic; however there are instances of some being localized to sub cellular regions.PDE1A is highly expressed in the brain. In human spermatozoa, PDE1A is tightly associated to calmodulin and is permanently activated [18].

PDE1B1 mRNA is found predominantly in the human brain at the level of neuronal cells of the cerebellum, hippocampus, caudate, and Purkinje cells, its expression is correlated with brain regions having extensive dopaminergic innervation and D1 dopamine receptor mRNA [19]. PDE1B1 is also found in the heart and skeletal muscle [20].

PDE1C1 mRNA is mainly expressed in the brain and the heart [20] and seems to be the major type highly expressed in the mouse cerebellar granular cells [21]. PDE1C2 represents the high-affinity CaM-PDE in olfactory epithelium [22] and PDE1C1 and PDE1C4/5 mRNA are present in the testis [21].

Pharmacology:

PDE1 has been implicated to play a role in a number of physiological and pathological processes. PDE1A most likely serves to regulate vascular smooth muscle contraction and has been found to be up-regulated in rat aorta in response to chronic nitroglycerin treatment. There is also a possibility that it plays a role in sperm function. PDE1B mRNA is induced in PHA or anti-CD3/ CD28-activated human T-lymphocytes and participates in IL-13 regulation implicated in allergic diseases [23]. Studies performed in permanent cell lines suggest that the inhibition of PDE1B1 may induce apoptosis in human leukemic cells [24]. PDE1B knockout mice exhibit exaggerated locomotor hyperactivity in response to dopamine agonist and display impaired spatial learning [25].PDE1C is believed to be a major regulator of smooth muscle proliferation [26] and is highly expressed in proliferating smooth muscle cells. The induction of PDE1C promotes arterial smooth muscle cell proliferation [27], and down-regulates glucose-induced insulin secretion [28]. Hence, it is speculated that inhibition of PDE1C could produce beneficial effects due to its putative inhibition of smooth muscle cell proliferation, an event that contributes importantly to the pathophysiology of atherosclerosis. Other likely roles of PDE1C are in olfaction, regulation of sperm function and neuronal signaling [15].

PDE1 inhibitors:

Vinpocetine (Brand names: Cavinton, Intelectol; Chemical name: ethyl apovincaminate)

Vinpocetine is a semisynthetic derivative of vincamine, which is extracted from the periwinkle plant. It increases cerebral blood flow and is said to improve memory.It is an inhibitor of PDE1 with an IC_{50} of approximately 10^{-5} M. The substance is widely sold as a supplement. However, there appears to be some controversy over the possibility of adverse reaction, and so in some cases low initial dose is recommended. There is also an isolated claim of agranulocytosis [15].Vinpocetine is included in many performance-enhancing supplements to improve delivery of nutrients to muscles after physical workouts. Phosphodiesterases (PDEs) have come into focus as interesting potential targets for PDE inhibitor-based anti-parasitic drugs, Genomes of the various agents of human malaria, most notably Plasmodium falciparum, all contain four genes for class 1 PDEs, hinting PDE1 as possible anti-malarial targets[91].Recently, a new PDE1 inhibitor, IC224 , was developed by ICOS Corporation [29]. According to these few data, if IC224 similarly inhibits basal and calmodulin-activated PDE1 subtypes, this compound would be very helpful to characterize PDE1 activity and to clearly investigate the various roles of PDE1 in pathophysiology.

2. PDE2 and its inhibitors

Overview:

The PDE2 family is encoded by a single gene (PDE2A) and has three splice variants, PDE2A1, PDE2A2, and PDE2A3. PDE2 enzymes are mainly purified from bovine hearts, adrenal tissues and brain cortex and characterized in the platelets and endothelial cells. Studies performed on purified PDE2 clearly showed that PDE2 hydrolyzes both cAMP and cGMP and is allosterically regulated by cAMP and cGMP with positive cooperative kinetics, with cGMP being preferred both as substrate and effector [30]. In the presence of cGMP, the rate of cAMP hydrolysis is increased by 6-fold [31]. PDE2 is thought to play a major feedback role by restoring the basal level in cyclic nucleotides in response to hormonal stimulation in the adrenal gland [32].

Localization and distribution:

PDE2 may cytosolic or associated to functional membrane structures: plasma membrane, sarcoplasmic reticulum [33], Golgi [34], as well as nuclear envelope [35,36]. PDE2A1 is cytosolic whereas PDE2A2 and PDE2A3 are membrane bound. It has been suggested that different localization of PDE2A2 and PDE2A3 is due to a unique N-terminal sequence, which is absent in PDE2A1.PDE2

protein is mainly present in adrenal medulla, heart, rat ventricle [37], brown adipose tissue [38], liver, and brain. Brain PDE2 is localized in the olfactory epithelia [39], in olfactory sensory neurons [40], bulb and tubercle, hippocampus pyramidal, and granule cells [41,42]. PDE2 proteins and mRNAs were characterized in bovine [43], human [44, 45] endothelial cells, media layer of the main pulmonary artery [46], and macrophages [47], Furthermore, in the same species, endothelial PDE2 distribution varies according to tissue localization [44].

Pharmacology:
PDE2 has a functional role in the heart since it was shown that PDE2 regulates basal calcium current in human atrial myocytes [48]. Since nitric oxide (NO) increases cGMP levels by stimulating particulate guanylyl cyclase, PDE2 activation can mediate functional response to NO in permanent cell line [49] as well as in rat cardiac fibroblasts [50] and participate in the regulation of endothelial permeability. In endothelial cells, PDE2A is up-regulated during phenotype changes [51] as well as under stimulation by vascular endothelium growth factor (VEGF) [45], indicating PDE2 participation in endothelial cell proliferation. Also PDE2 mRNA and proteins are increased in brown adipose tissue of obese [38].

PDE2 inhibitors:
EHNA
EHNA (erythro-9-(2-hydroxy-3-nonyl) adenine), a selective PDE2 inhibitor was the first to be developed. The core structure of EHNA resembles cAMP , but has a bulky hydrophobic carbon side chain replacing the phospho-ribose moiety in cAMP[39]. Inhibition of PDE2 by EHNA potentiates NMDA (N-metyl-D-aspartate) receptor activated increase in cGMP, but has no effect on cAMP concentrations [52,20]. Also EHNA is a potent inhibitor of adenosine deaminase.This dual inhibition leads to the accumulation of the two inhibitory metabolites, adenosine and cGMP, which may act in synergy to mediate diverse pharmacological responses including anti-viral, anti-tumour and anti-arrhythmic effects [53]. EHNA has been used to study implication of PDE2 in calcium control in cardiac myocytes and has shown to be effective to reverse hypoxic pulmonary vasoconstricion in perfused lung models. Thus the two major applications of EHNA are to serve as a lead structure for the rational design of more selective and potent PDE2 inhibitors and to define some of PDE's biological targets [39].

3. PDE3 and its inhibitors

Overview:
This enzyme was firstly named cAMP-PDE, PDE III, or PDE IV, according to its elution order, and then cGI-PDE and was regarded as the new cardiotonic drug target in eighties. PDE3 is characterized by its high affinity for cAMP and its capacity to hydrolyze both cAMP and cGMP. The PDE3 enzyme was initially found mainly in the heart, liver, platelet, and adipocyte. Beavo's and Manganiello's teams first purified PDE3 from the heart and platelet to homogeneity [54, 55,56]. PDE3 cloning reveals 2 genes (PDE3A and PDE3B) with various splices constitute that PDE3 family. Both PDE3 isoforms are structurally similar, containing an NH2-terminal domain important for the localization of the enzyme to particulate fraction and catalytic domain at the carboxy terminus end.

Localization and distribution:
PDE3 could be either cytosolic or membrane bound. It was shown to be associated to plasma membrane [57,58], sarcoplasmic reticulum [33], Golgi apparatus [34], as well as associated to nucleus envelope [36]. PDE3A is mainly present in the heart, platelet, vascular smooth muscle, and oocyte, whereas PDE3B is mainly associated to adipocytes, hepatocytes, and spermatocytes.

Pharmacology:
PDE3 plays a major role in cardiac contraction by modulating Ca^{2+} entry consecutively to cAMP-dependent phosphorylation of voltage-gated Ca^{2+} channel [59]. Furthermore, PDE3 inhibition was shown to be the mechanism by which NO stimulates renin secretion from the kidney [60]. Molecules that inhibit PDE3 were originally investigated for the treatment of heart failure, but, because of unwanted arrhythmic side-effects, they are not studied for that indication any longer. Nonetheless, the PDE3 inhibitor milrinone is approved for use in heart failure.

PDE3 inhibitors:
Milrinone
Milrinone potentiates the effect of cyclic adenosine monophosphate (cAMP). It also enhances relaxation of the left ventricle by increasing Ca^{2+}-ATPase activity on the cardiac sarcoplasmic reticulum which increases calcium ion uptake. It has positive inotropic, vasodilating and minimal chronotropic effects. It is used in the management of heart failure only when conventional treatment with vasodilators and diuretics has proven insufficient due to the potentially fatal adverse effects of milrinone, including ventricular arrhythmias. One negative side to the use of milrinone is the prolonged half-life (2.5 hrs). This can result in a prolonged weaning and possible adverse outcomes from stopping this medication rapidly.

Other PDE3 inhibitors such as Amrinone (Trade name: Inocor), Enoximone (Trade name: Perfan) also have applications in treatment of congestive heart failure due their ionotropic effects. However, these drugs have shown increased mortality in controlled studies and therefore are used only if the benefits outweigh the risks.Recently, dihydropyridazinone was conceived by Merck and Co as the first orally active, potent, and selective PDE3B inhibitor [61]. These relatively selective subtype PDE3 inhibitors open the possibility to conceive

more specific PDE3 subtype devoid of interactions with other family and also represent new pharmacological tools that will allow to discriminate the respective functions of PDE3A (mainly implicated in cardiovascular function and fertility) and of PDE3B (mainly implicated in biolysis).More recent suggest that NT-702 (parogrelil hydrochloride, NM-702, 4-bromo-6-[3-(4-chlorophenyl) propoxy]-5-[(pyridine-3-ylmethyl) amino] pyridazin-3(2H)-one hydrochloride), a selective PDE3 inhibitor has an anti-inflammatory effect as well as a bronchodilating effect and might be useful as a novel potent therapeutic agent for the treatment of bronchial asthma, a new type of agent with both a bronchodilating and an anti-inflammatory effect [(62)].

4. PDE4 and its inhibitors

Overview:

PDE4 enzymes are cAMP-specific PDEs and were previously named cAMP-PDE, ROI-PDE, and PDE IV. PDE4 are inhihibited by rolipram but are insensitive to cGMP thus, differentiating them from PDE3.Presently, the PDE4 family represents the largest PDE family, constituted by 4 genes (PDE4A, PDE4B, PDE4C, and PDE4D) with various alternative mRNA splices encoding long PDE4 and short PDE4 isozymes, at least 35 different PDE4 proteins. [(63-66)]

Localization and distribution:

PDE4 being diverse have a complex localization and isoforms may be found in cytosol or associated with cellular membranes. They are mainly found in the brain, inflammatory cells, smooth muscle and cardiovascular tissues and are nearly absent in platelets.

Pharmacology:

PDE4D-deficient mice are characterized by delayed growth as well as reduced viability and female fertility [(67)]. PDE4D knockout mice have an antidepressant-like profile, suggesting that PDE4D-regulated cAMP signaling may play a role in the pathophysiology and pharmacotherapy of depression [(68,69)]. Also PDE4-D deficient mice do not develop airway hyperreactivity to cholinergic stimulation despite an apparently normal lung inflammatory infiltration in response to antigen, providing a rationale for developing new therapies for asthma [(70)]. However, the deletion of PDE4D specifically results in a behavior correlated to emesis, supporting the hypothesis that the inhibition of PDE4D is likely responsible for emesis induced by PDE4 inhibitors [(71)]. This latter PDE4D function will deserve the use of specific inhibitor of PDE4D as an anti-inflammatory compound. Opposite to PDE4D, PDE4B was shown to be essential for LPS-activated TNFα responses since, in deficient PDE4B mice, lipopolysaccharide (LPS) stimulation failed to induce TNFα secretion and mRNA accumulation [(72)], indicating that a PDE4B inhibitor would be an anti-inflammatory drug without emetic adverse effects.

PDE4 inhibitors:

Rolipram

Rolipram, an antidepressant compound, was shown to be a potent PDE4 inhibitor in brain homogenates. The high selectivity of rolipram, for PDE4 was demonstrated on vascular purified PDE. Therefore, rolipram became an archetype to synthetize new potent and selective PDE4 inhibitors.

Denbufylline

A xanthine derivative, is also selective for PDE4, but inhibits PDE5 at 10-fold higher concentration. Since PDE4 was characterized as a new target to design anti-inflammatory drugs, many pharmaceutical companies started to develop rolipram analogues for asthma and COPD. However, the rolipram analogues, due to their adverse emetic effect, failed in clinical studies.

Benzyladenine derivatives [(73)] were synthesized as potent and selective inhibitors being effective in vivo per oral administration. In the inflammatory pathology field, PDE4 inhibition decreases the expression of mucin gene in human airway epithelial cells [(74)] and reduces the parainfluenza 3-virus induced airway influx of macrophages, eosinophils, and neutrophils. In central nervous system, rolipram treatment improves deficits in both long-term potentiation (LTP) and contextual learning in the double transgenic mice for amyloid precursor protein, suggesting that PDE4 inhibitor would be effective in Alzheimer disease. Furthermore, PDE4 inhibition will be a new approach for schizophrenia and defective long-term memory such as Rubinstein-Taybi syndrome. Specific PDE4A4 subtype inhibitor would be relevant as an anti-inflammatory target for COPD, since overexpressions of PDE4A4 were found in peripheral blood monocytes of smokers[(75)].

Due to the broad anti-inflammatory and immunomodulatory actions of phosphodiesterase (PDE) 4 inhibitors, it has been proposed that PDE4 inhibitors might be efficacious for skin disorders such as atopic dermatitis. KF66490 is a newly developed PDE4 inhibitor that inhibits PDE4B (IC_{50} = 220 nM) and the production of tumor necrosis factor (TNF)-α by mouse peritoneal exudated cells stimulated with lipopolysaccharide. Furthermore, KF66490 produced less potent emetic effects than the first generation PDE4 inhibitor, rolipram. The present studies suggest that KF66490 has excellent potential as an oral medicine for the treatment of atopic dermatitis.[(76)] These data point out that another generation of PDE4 inhibitors targeting specifically PDE splices variants would be relevant for specific pathology with minimal adverse effects.

5. PDE5 and its inhibitor

Overview:

The PDE5 member of PDEs is also named as cGMP PDE, cGMP-binding or cGMP- specific phosphodiesterase (cG-BPDE), or PDE V. In human,

bovine, and rat vascular smooth muscle, PDE5 was purified and characterized as a cytosolic PDE isozyme that specifically hydrolyzes cGMP without being activated by Ca/calmodulin and specifically inhibited by compound MandB 22948, presently named zaprinast, the archetype for PDE5 inhibitor, and insensitive to rolipram. The PDE5 enzymes are derived mainly from single gene in the human corpus cavernosum PDE5A and have two 5` splice variants PDE5A1 and PDE5A2.

Localization and distribution:
PDE5A mRNA was shown to be expressed in aortic smooth muscle cells, heart, placenta, skeletal muscle, pancreas, and, to a much lesser extent, in the brain, liver, and lung.

Pharmacology:
PDE5 was firstly implicated in vasorelaxation, since the specific inhibition of PDE5 by zaprinast was shown to induce an increase in cGMP associated with a vasorelaxing effect. The potentiation of a PDE5 inhibitor relaxing effect obtained on the aorta containing functional endothelium or treated with NO donors suggested that PDE5 mediates the NO/cGMP relaxing effect. In that way, new PDE5 inhibitors derived from zaprinast were designed as antihypertensive compounds or coronary vasodilators; unexpectedly, during clinical studies, sildenafil ameliorated erectile dysfunction, pointing out PDE5 as a new target for treatment of erectile dysfunction and increasing the development of PDE5 inhibitors. The high level of PDE5 encountered in the lung, as well the observation that PDE5 was activated in pulmonary hypertension [(70)], has contributed to propose also PDE5 as a new target for the treatment of pulmonary hypertension and respiratory distress. Also it was recently shown that PDE5 inhibition in the brain improves early memory consolidation of object information [(77)].

PDE5 inhibitors:
Zaprinast is the first characterized selective PDE5 inhibitor. . Later more PDE5 inhibitors were developed and these were mainly indicated for erectile dysfunction. Currently, three PDE-5 inhibitors have been approved by the U.S. Food and Drug Administration (FDA) for use in the United States: sildenafil citrate, tadalafil, and vardenafil hydrochloride trihydrate [(78)].

Sildenafil
Sildenafil citrate, sold under the names Viagra (Pfizer), Revatio and under various other names, is a drug used to treat male erectile dysfunction (impotence). Sildenafil was shown to induce neurogenesis and promote functional recovery after stroke in rat [(68,69)], to be effective in hypoxia-induced pulmonary hypertension in rat, and to improve endothelium-dependent vasodilatation in smokers.

Tadalafil
Tadalafil is an orally administered drug used to treat male erectile dysfunction (impotence) under the brand name Cialis. Tadalafil is now approved for the treatment of pulmonary arterial hypertension. Tadalafil has more recently shown a potential anti-inflammatory effect in vitro on the inflammatory response of endothelial cells stimulated by myeloperoxidase-modified low-density lipoprotein (Mox-LDL) or tumor necrosis factor alpha (TNF-α)[(81)].

Vardenafil
Vardenafil l is closely related in both function and marketing to sildenafil and tadalafil. Structurally, the vardenafil molecule differs from sildenafil by only a methyl group and the position of one nitrogen atom in its structure. It has a relatively short effective time as compared to sildenafil [(80)].Sildenafil and vardenafil also enhance early memory consolidation of object information [(77)]. Some studies indicate that vardenafil and tadalafil, PDE5/6 inhibitors, are able to induce caspase-dependent apoptosis in B-chronic lymphocytic leukemia cells. T0156, a newly developed potent inhibitor of PDE5 has recently shown to prevent nitroglycerin tolerance in vascular smooth muscle cells [(89)].

6. PDE6 and its inhibitors

Overview:
After showing that retinal PDE was the main site for light regulation of cyclic GMP metabolism, PDE6, a CGMP specific PDE was purified from frog retinal rods outer segments. In humans PDE6 enzymes are found mainly in the retinal rods and cones are derived from different genes.

Pharmacology:
PDE6 plays a major role in phototransduction. The PDE6 cascade activation is initiated when the protein rhodopsin absorbs a photon. Each activated rhodopsin activates thousands of transducin (a G-protein) by catalyzing the exchange of GDP for GTP. Transducin Tα subunit, with GTP bound activates the catalytic PDEah subunits by displacing γ subunits from the active site of the enzyme, thus allowing cGMP hydrolysis. The main function of the rod PDE is to rapidly reduce the steady-state concentration of cGMP in response to light stimulus. This decrease in cGMP concentration causes the closure of CNG cationic channels and generates cell membrane hyperpolarization. This initial signal is transmitted via second-order retinal neurons to the optic nerve and to the brain. Numerous visual alterations have been related to mutations affecting the various protein subunits of the rod and cone PDEs. More recently, another functional role was shown for PDE6 since, in chick pineal gland cells, rod and cone forms of PDE6 are expressed and play a role in the inhibition by light of melatonin synthesis.

PDE6 inhibitors:
PDE5 and PDE6, being structurally related, compounds inhibiting PDE5 also interact with PDE6 (82). Zaprinast and dipyridamole inhibit PDE6 as potently as PDE5. Due to the adverse vision effects of PDE6 inhibitors and the specific localization of PDE6, in the retina, there is no pharmaceutical investment on PDE6 inhibitors.

7. PDE7
Overview:
PDE7 enzymes are cAMP specific and insensitive to rolipram. They are encoded by two genes PDE7A and PDE7B. PDE7A has many splice variants of which .PDE7A1 protein was greatest in T-cell lines, peripheral blood T-lymphocytes, epithelial cell lines, airway and vascular smooth muscle cells, lung fibroblasts, and eosinophils but was not detected in neutrophils. In contrast, the PDE7A2 protein, identified in human cardiac myocytes, was not found in any of the other cell types investigated [(83)]. PDE7A expressed in human Blymphocytes is up-regulated by cAM. PDE7B mRNA was abundantly expressed in the brain and heart, followed by skeletal muscle and pancreas.

Pharmacology:
PDE7 inhibitors and some genetic knockout technologies have been used to probe the function of PDE7 in cells and whole animals. Initial studies suggested that PDE7A could be induced in T lymphocytes in response to activation of the T-cell receptor. Increased PDE7 correlated with a decrease in cAMP, increased interleukin-2, and increased proliferation. However, phosphodiesterase 7A-deficient mice have functional T-cells, supporting the notion that PDE7A is not essential for T-cell activation.

PDE7B1 but not other splice variants, was activated by D1 agonist through the cAMP/cAMPdependent protein kinase cAMP response element binding protein pathway in striatal neurons, indicating a role for PDE7B1 in memory function [(84)].
Phosphodiesterase 7A (PDE7A) has been suggested to be involved in activation of T lymphocytes.Recent studies on ASB16165, which has an IC50 value of 15 nM for human PDE7A showed that it suppressed IL-12-induced IFN-γ production by T lymphoblasts which have been prepared by stimulating mouse T cells with anti-CD3 antibody thus indicating that PDE7 inhibitors such as ASB16165 will be beneficial for the patients with immunological disorders [(85)].

8. PDE8
Overview:
PDE8 enzymes are encoded mainly by two genes PDE8A and PDE8B. PDE8 mRNA has highest the expression in the testis, followed by the eye, liver, skeletal muscle, heart, kidney, ovary, and brain, in decreasing order. PDE8As 1–5 splice variants of PDE8A were cloned from testis. In all tissues, the expression levels of PDE8A1 are much higher than that of PDE8As 2–5. PDE8A1 is induced in response to a combination of T cell receptor and co-stimulatory receptor pathway activation. The mRNA encoding PDE8B is expressed specifically and abundantly in the thyroid gland. There are at least four PDE8B variants. RT-PCR analysis revealed that while PDE8B1 is the most abundant variant in the thyroid gland, PDE8B3 is the most abundant form in the brain [(86)].

Pharmacology:
Findings suggest that PDE8A1 is highly up regulated during CD3/CD28 T- lymphocyte stimulation suggesting that PDE8A may be involved in T cell activation. The existence of the PAS and REC domains and the comparison of function of these domains in other proteins suggest that the PDE8s may serve as environmental sensors for regulation of cAMP in the cell.

9. **PDE9**
Overview:
PDE9 enzymes are cGMP specific members of PDE superfamily and are encoded by a single gene PDE9A with several variants. Human PDE9A mRNA is expressed in the spleen, small intestine, and brain. A new human splice variant, PD9A5, highly expressed in immune tissues and being exclusively in the cytoplasm, was characterized, whereas PDE9A1 is localized in the nucleus only. More than 20 variants have been observed, indicating that the PDE9A gene appears to have a complex regulation of expression.

Pharmacology:
Not many studies clearly elucidate a specific function for PDE9A. The pattern of PDE9A mRNA expression in the brain closely resembles that of soluble guanylyl cyclase, suggesting a possible functional association in the regulation of cGMP levels that may play an important role in behavioral state regulation and learning

10. PDE10
Overview:
PDE10 a more recent member is thought to be encoded by PDE10A gene. PDE10A transcripts are particularly abundant in the brain (putamen and caudate nucleus), thyroid, and testis. PDE10A2, a novel alternative splice variant of human PDE10A, having a putative phosphorylation site by PKA, was characterized as a major form in human tissues. Although the recombinant PDE10A1 protein is not phosphorylated, recombinant PDE10A2 is preferentially phosphorylated by PKA in its unique-N terminus, opening a new regulation way of its potential physiological roles, especially in the striatum (PDE10A2 is more abundant in membrane fractions than in cytosolic fractions of rat striatum, and immunocytochemical analysis showed that PDE10A2 (87 kDa) is localized in the Golgi apparatus of transfected cells.

Pharmacology:
The PDE10 family was recently shown to be associated to the progressive neurodegenerative disease Huntington's disease (HD), since PDE10A2 mRNA decreases prior to the onset of motor symptoms in transgenic HD mice expressing exon 1 of the human Huntington gene [87]. These first data showing that PDE10A seems to be implicated in neuropathophysiology do not exclude the possible role of PDE10A in other tissues such as thyroid, kidney, and testis.

11. PDE 11

Overview:
Most recently discovered PDE member, PDE11 is encoded by a single gene PDE11A discovered till now. The PDE11A gene, which undergoes tissue-specific alternative splicing that, generates structurally and functionally distinct genes products, may have tissue-selective functions that remain to be elucidated.

Pharmacology:
Recent studies with a PDE11 knockout mouse model suggest that PDE11 may be important for sperm development and function. Ejaculated sperm from knockout mice displayed slightly lower sperm concentration and decreased viability compared with controls, and the sperm had a lower rate of forward progression. However, the animals were fertile[5].

PDE7/8/9/10/11 inhibitors

There are very few selective inhibitors known for these new families discovered by cloning, since their design is only beginning. IC242 inhibits PDE7A. Recently, BRL 50481 was discovered as a PDE7 inhibitor (Ki=180 nM), with an acceptable in vitro selectivity [83]. Thiadiazoles, a new structural class of potent and selective PDE7 inhibitors, acting in the nanomolar range, was discovered by Pfizer. For the last PDE families, only their differential sensitivity to known inhibitors was reported. PDE8A, insensitive to IBMX, is inhibited by dipyridamole. PDE9A is only sensitive to zaprinast. PDE10A is also inhibited by dipyridamole, with IC50 values of 1.2 and 0.45 AM for the inhibition of cAMP and cGMP hydrolysis. The search for novel mechanisms to treat schizophrenia has led to investigate the striatal enriched dual cAMP/cGMP phosphodiesterase PDE10. Studies reveal that PDE10 inhibitors may have applications in treatment of schizophrenia.[90]

PDE11A3 is found in the testis while PDE11A4 is found in the prostate and various studies have indicated their role in improvement of testicular functions [91].PDE11A variants are sensitive to dipyridamole, with an IC50=0.8 to 1.8 AM, and to zaprinast, with an IC50=5 to 28 AM. Newer studies revealed PDE9 inhibitors as potential antidiabetic agents originated from knock out (KO) studies in mice. When placed on a high fat diet, these mice developed a phenotype that included reduced insulin resistance, reduced weight gain, and lower fat mass. [88] Various studies indicate the potential role of these newer PDE families thus paving way for new drug development with the more recent PDEs as their targets.

CONCLUSION

Non-selective PDE inhibitors including theophylline and papaverine have been used therapeutically for over 70 years for a range of diseases. However, it is only in the last 10 years, that potent PDE selective drugs have begun to make an impact in the treatment of various diseases and the worldwide success of sildenafil in treating erectile dysfunction is a an evidence of the same. Selective PDE inhibitors are being investigated in a wide range of diseases including the use of PDE2 inhibitors in sepsis; PDE5 inhibitors to treat sexual dysfunction in females, cardiovascular disease and pulmonary hypertension; and PDE4 inhibitors to treat asthma, COPD, allergic rhinitis, psoriasis, multiple sclerosis, depression, Alzheimer's disease and schizophrenia.

As the study on the physiological roles of the individual PDE isoforms progresses, there is a parallel development of more selective inhibitors of these enzymes, and as a result it is likely that better therapeutically active drugs will emerge. A specific inhibitor for each form of PDE could pave the way for basic research on PDE regulation and provide for eventual therapeutic application to control abnormal function.

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