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Classification of Phosphodiesterases and the Therapeutic Effects of their Inhibitors (Review)

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Abstract*—Phosphodiesterase (PDE) is an enzyme that catalyses the hydrolysis of phosphodiester bonds. The enzyme is also takes responsibility for the hydrolysis of cyclic 3',5'adenosine monophosphate (cAMP) and 3',5'cyclic guanosine monophosphate (cGMP). The PDE enzymes in mammals are classified into 11 families, namely PDE1-PDE11. The classification is on the basis of amino acid sequences, substrate specificities, regulatory properties, pharmacological properties, tissue distribution. Various PDE of the same family are related with regards to functionality but differs in their specificities for substrates. Some are hydrolases with selective preferences for cAMP (PDE4, 7 and 8), while the selective preference for some others is for cGMP (PDE5, 6 and 9). Some have the ability to hydrolyse both cAMP and cGMP (PDE1, 2, 3, 10 and 11). cAMP, and cGMP both has important roles in the regulation of inotropic mechanisms in the human myocardium. However, cAMP greatly affects other tissues, and different phosphodiesterase isoenzymes are found in many other tissues. Drugs with inhibitory effects on phosphodiesterase (thus reducing the breakdown of cAMP) have a therapeutic action on the heart, lung, and vasculature as well as on platelet function and inflammatory mechanisms. Inhibitors like these are commonly used as "biochemical tools" to study of role which cyclic nucleotides plays in the cell, but they also may be useful to investigate the structural and functional activities of PDE. As therapeutic agents, they can also be utilized in controlling the pathophysiological changes of responses generated by the cyclic nucleotides in the central nervous system (CNS), cardio-vascular, lung, digestive tract and respectively. PDE enzymes are often targets for inhibition by pharmacological processes due to their unique tissue distribution, structural and functional properties and the inflammatory process. The effect of many of these drugs is evident in more than one isoenzyme, and many tissues possess more than one isoenzyme. As a result, phosphodiesterase inhibitors (PDEI) can have a multiplicity of effects. For example, theophylline has effects on the lung, as well as cardiac and vascular effects; amrinone affects cardiac, vascular and platelet functions. The PDE inhibition, change the intracellular response to extra cellular signals by affecting the processes by the the cyclic nucleotides.*

***Keywords*—** *Phosphodiesterase; Hydrolysis; Isoenzymes; Central Nervous System: Theophylline.*

I. INTRODUCTION

Phosphodiesterases are enzymes causes the hydrolysis of the cyclic nucleotides adenosine 3', 5'-cyclic monophosphate (cAMP) and guanosine 3', 5'-cyclic monophosphate (cGMP), in their inactive form (5'nucleotide) and, therefore they play an important part in the cellular enzyme systems [5]. While an inhibitor for phosphodiesterase is drug that aids the blockage of one or more subtypes of the enzyme phosphodiesterase (PDE), thereby causing a prevention in the inactivation of the intracellular second messengers cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) by the respective PDE subtypes [11].

The PDE super family is large, complex and stands for 11 gene families (PDE1 through PDE11). Each family contains one to four genes, and many genes generate series of isoforms [22]. All the PDE superfamily members are different in various aspects such as amino acid sequences, substrate specificities, localization or tissue distribution, mode of regulation and inhibitor specificity [18]. The PDEs are localized in the cytosol, plasma membranes, endoplasmic reticulum, nuclear membranes and the cytoskeleton [1]. PDEs are regulated by intracellular cyclic nucleotide concentrations, phosphorylation, interaction with regulatory proteins, and binding of Ca2+/calmodulin, and also by changes in the expression of genes (Dessager, 2005). PDE3, PDE4, 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 [25].

Phosphodiesterase and its Inhibitors

PDE1

The PDE1 family was the first eluted fraction isolated by chromatography from vascular smooth muscle. This PDE1 fraction was activated specifically by Ca2+/CaM, and thus named CaM-PDE (Calcium calmodulin dependent PDE). The cooperative binding of four Ca^{2+} to calmodulin is required to completely activate CaM-PDE. PDE1s are mostly localized cytosol, however there are instances of some being localized to sub-cellular regions. In human spermatozoa, PDE1 is associated strongly to calmodulin and is permanently activated. PDE1 is predominantly detected in the human brain at the level of neuronal cells of the cerebellum, heart and skeletal muscle. PDE1 has been known to have a role in a number of physiological and pathological processes. PDE1 mostly serves in the regulation of the contraction of the vascular smooth muscle and has been found to be up-regulated in rat aorta in response to chronic nitroglycerin treatment [17]. Other possible roles of PDE1 are in olfaction, regulation of sperm function and neuronal signalling.

PDE1 Inhibitor

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. The substance is widely sold as a supplement [10].

PDE2

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. PDE2 protein is mainly present in adrenal medulla, heart, rat ventricle, brown adipose tissue, liver, and brain. PDE2 has a functional role in the heart since it was shown that PDE2 regulates basal calcium current in human atrial myocytes. Since nitric oxide (NO) increases cGMP levels by stimulating particulate guanylyl cyclase, PDE2 activation can mediate functional response to NO in permanent cell line [2].

PDE2 Inhibitor

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. Inhibition of PDE2 by EHNA potentiates NMDA (Nmetyl-D-aspartate) receptor activated increase in cGMP, but has no effect on cAMP concentrations. 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 antiarrhythmic effects [32].

PDE3

This enzyme was firstly named cAMP-PDE, PDE III. 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. PDE3 could be either cytosolic or membrane bound. It was shown to be associated to plasma membrane, sarcoplasmic reticulum, Golgi apparatus, as well as associated to nucleus envelope. PDE3 plays a major role in cardiac contraction by modulating Ca2+ entry consecutively to cAMP-dependent phosphorylation of voltage-gated Ca2+ channel [12]. Furthermore, PDE3 inhibition was shown to be the mechanism by which NO stimulates renin secretion from the kidney. 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 [12].

PDE3 Inhibitor

Milrinone Milrinone potentiates the effect of cyclic adenosine monophosphate (cAMP). It also enhances relaxation of the left ventricle by increasing Ca2+-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. 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 [8]. However, these drugs have shown increased mortality in controlled studies and therefore are used only if the benefits outweigh the risks.

PDE4

PDE4 enzymes are cAMP-specific PDEs and were previously named cAMP-PDE. PDE4 are inhihibited by rolipram but are insensitive to cGMP thus, differentiating them from PDE3. They are mainly found in the brain, inflammatory cells, smooth muscle and cardiovascular tissues and are nearly absent in platelets [31].

PDE4 Inhibitor

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. The inflammatory pathology field, PDE4 inhibition decreases the expression of mucin gene in human airway epithelial cells and reduces the parainfluenza 3-virus induced airway influx of macrophages, eosinophils, and neutrophils [7].

PDE5

The PDE5 member of PDEs is also named as cGMP PDE. 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. It is specifically inhibited by compound MandB 22948, presently named zaprinast, the archetype for PDE5 inhibitor, and insensitive to rolipram. 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 [6]. The high level of PDE5 encountered in the lung, as well the observation that PDE5 was activated in pulmonary hypertension, 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.

PDE5 Inhibitor

Zaprinast is the first characterized selective PDE5 inhibitor. Later more PDE5 inhibitors were developed and these were mainly indicated for erectile dysfunction.

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 [15], effective in hypoxia-induced

pulmonary hypertension in rat, and improve endothelium-dependent vasodilatation in smokers.

PDE6

PDE6 plays a major role in phototransduction [30]. 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. 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 cyclic nucleotide –gated (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 [14].

PDE6 Inhibitor

PDE5 and PDE 6, are structurally related, so compounds inhibiting PDE5 also interact with PDE6 [3]. 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.

PDE7

PDE7 enzymes are cAMP specific and insensitive to rolipram. 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 PDE7 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 [24].

PDE8

PDE8 has the highest expression in the testis, followed by the eye, liver, skeletal muscle, heart, kidney, ovary, and brain, in decreasing order. Findings suggest that PDE8 is highly up regulated during CD3/CD28 T- lymphocyte stimulation suggesting that PDE8 may be involved in T cell activation [16]. Findings suggest that PDE8 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.

PDE9

PDE9 enzymes are cGMP specific members of PDE superfamily. Human PDE9 is expressed in the spleen, small intestine, and brain [23]. Not many studies clearly elucidate a specific function for PDE9. The pattern of PDE9 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 behavioural state regulation and learning [19].

PDE10

PDE10 a more recent member is thought to be encoded by PDE10A gene. PDE10A transcripts are particularly abundant in the brain, thyroid, and testis [26]. The PDE10 family was recently shown to be associated to the progressive neurodegenerative disease, and Huntington's disease [27].

PDE 11

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 [29].

Inhibitors of PDE7,8,9,10 and 11

There are very few selective inhibitors known for these new families discovered by cloning, since their design is only beginning. Thiadiazoles, a new structural class of potent and selective PDE7 inhibitors, acting in the nanomolar range, was discovered by Pfizer [20]. For the last PDE families, only their differential sensitivity to known inhibitors was reported. PDE8A, insensitive to IBMX (3-isobutyl-1-methylxanthine), is inhibited by dipyridamole [13]. PDE9A is only sensitive to zaprinast. PDE10A is also inhibited by dipyridamole. 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 [21]. 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 [28]. Newer studies revealed PDE9 inhibitors as potential antidiabetic agents originated from knock out (KO) studies in mice. Mice developed a phenotype that included reduced insulin resistance, reduced weight gain, and lower fat mass. 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 [6].

II. CONCLUSION

PDE inhibitors are being investigated in a wide range of diseases including PDE2 inhibitors in sepsis, PDE5 inhibitors to treat sexual dysfunction in females and cardiovascular diseases. However potent PDE selective drugs have begun to make an impact in treatment of diseases and worldwide success of sildenafil in treatment of erectile dysfunction is evidence of the effect of such drugs can have.

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