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Review Article

A short review on structure and role of cyclic-3',5'-adenosine monophosphate-specific phosphodiesterase 4 as a treatment tool

Nahid Eskandari[1,2], Omid Mirmosayyeb[1], Gazaleh Bordbari[1], Reza Bastan[3], Zahra Yousefi[1], Alireza Andalib[1]

[1]Department of Immunology, Isfahan University of Medical Sciences, Isfahan, Iran

[2]Applied Physiology Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

[3]Department of Human Vaccines, Razi Serum and Vaccine Research Institute, Karaj, Alborz, Iran

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Corresponding author:
Dr. Nahid Eskandari,
E-mail: neskandari@med.mui.ac.ir

ABSTRACT

Cyclic nucleotide phosphodiesterases (PDEs) are known as a super-family of enzymes which catalyze the metabolism of the intracellular cyclic nucleotides, cyclic-3',5'-adenosine monophosphate (cAMP), and cyclic-3',5'-guanosine monophosphate that are expressed in a variety of cell types that can exert various functions based on their cells distribution. The PDE4 family has been the focus of vast research efforts over recent years because this family is considered as a prime target for therapeutic intervention in a number of inflammatory diseases such as asthma, chronic obstructive pulmonary disease, and rheumatoid arthritis, and it should be used and researched by pharmacists. This is because the major isoform of PDE that regulates inflammatory cell activity is the cAMP-specific PDE, PDE4. This review discusses the relationship between PDE4 and its inhibitor drugs based on structures, cells distribution, and pharmacological properties of PDE4 which can be informative for all pharmacy specialists.

Keywords: Cyclic-3',5'-adenosine monophosphate; inhibitors; phosphodiesterase; phosphodiesterase 4

INTRODUCTION

The cyclic nucleotide phosphodiesterases family (PDEs)[1,2] is large and complex, and it is derived from separate gene families. At present, at least 11, and possibly 12 families have been identified that catalyze the hydrolysis of cyclic-3',5'-adenosine monophosphate (cAMP),[3,4] cyclic-3',5'-guanosine monophosphate (cGMP)[5,6] or both. For many years, PDEs have been identified by their catalytic and regulatory properties. These isoenzymes differ in their substrate specificity, kinetic properties, responsiveness to endogenous regulatory molecules (Ca^{2+}/calmodulin, cGMP), and susceptibility to inhibition by various compounds.[7] One obvious difference between members of various PDE families is the range of differing K_m (Michaelis–Menten constant) values that these enzymes show for cAMP. However, it is pertinent to note that of the 12 PDE families, only eight are able to hydrolyze cAMP. Thus, these families will be further distinguished according to their structure, and their ability to hydrolyze either cAMP (PDE4, PDE7, and PDE8),[8,9] or cGMP (PDE5, PDE6, and PDE9),[10,11] or both (PDE1, PDE2, PDE3, PDE10, PD11, and PDE12)[12-14] [Table 1].

The distribution of PDE isoforms can vary among tissues and this has led to an appreciation that tissue-selective effects may be obtained by targeting the prominent PDE isoform in a given tissue.[15] The therapeutic benefit of such an approach is being realized with the emergence of drugs

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Table 1: Human cyclic nucleotide PDE isozyme families[8-14]

Family	Subtypes	Isoforms	Characteristics	K_m (μM) cAMP/cGMP	Primary tissue distribution	Examples of Inhibitors
PDE1 (3)	PDE1A	PDE1A1 PDE1A2	Ca^{2+}/calmodulin-stimulated	1–30/3	Heart, brain, lung, smooth muscle	Ks-505a, methoxymethyl IBMX, Vinpocetine
	PDE1B	-				
	PDE1C	PDE1C1				
PDE2 (1)	PDE2	PDE2A1 PDE2A2 PDE2A3	cGMP-stimulated	50/50	Adrenal gland, heart, lung, liver, platelets	EHNA
PDE3 (2)	PDE3A PDE3B		cGMP-inhibited, cAMP-selective	0.2/0.3	Heart, lung, liver, platelets, immunocytes	Cilostamide Enoximone Milrinone Siguazodan
PDE4 (4)	PDE4A	PDE4A1A PDE4A4B PDE4A5	cAMP-specific, cGMP-insensitive	4/>3000	Kidney, brain, liver, lung, immunocytes	CDP840 Rolipram SB 207499 Tibenelast
	PDE4B	PDE4B1 PDE4B2A PDE4B2B PDE4B3				
	PDE4C	-				
	PDE4D	PDE4D1 PDE4D2 PDE4D4 PDE4D5 PDE4D6 PDE4D7 PDE4D8 PDE4D9				
PDE5 (1)	PDE5	-	cGMP-specific	150/1	Lung, platelets, smooth muscle	Dipyridamole MY-5445 Zaprinast
PDE6 (3)	PDE6A PDE6B PDE6B	- - -	cGMP-specific	60/>100	Photoreceptors	Dipyridamole Zaprinast
PDE7 (2)	PDE7A PDE7B	PDE7A1 PDE7A2 -	cAMP-specific, high-affinity	0.2/>100	Skeletal muscle, heart, kidney, brain, pancreas, T-lymphocyte	BRL 50481
PDE8 (2)	PDE8A PDE8B	PDE8A1 PDE8A2 -	cAMP-selective, IBMX-insensitive	0.06/124	Testes, eye, liver, skeletal muscle, heart, kidney, ovary, T-lymphocytes	None
PDE9 (1)	PDE9A	PDE9A1 PDE9A2	cGMP-specific, IBMX-insensitive	230/0.17	Kidney, liver, lung, brain	None
PDE10 (1)	PDE10A	-	cGMP-sensitive, cAMP-selective	0.05/3.0	Testes, brain	None
PDE11 (1)	PDE11A	-	cGMP-sensitive, dual specificity	0.7/0.5	Skeletal muscle, kidney, liver, salivary gland, testes	None

K_m (Michaelis–Menten constant): The substrate concentration required to achieve 50% of V_{max}, V_{max} (velocity): Maximum reaction rate at saturation. cAMP=Cyclic-3',5'-adenosine monophosphate, cGMP=Cyclic-3',5'-guanosine monophosphate, PDE=Phosphodiesterase

such as sildenafil, a PDE5 inhibitor, which is used to treat erectile dysfunction.[16] Progress in the context of inflammatory disease has also been made with the realization that the main isoform of PDE regulating inflammatory cell activity is PDE4.[17] As a consequence, cilomilast and roflumilast, selective PDE4 inhibitors, have been developed and have displayed some promise in targeting respiratory diseases.[18] The aim of this review will be discussed on the relationship between PDE4 and inhibitor drugs based on structures, cells distributions, and pharmacological properties of PDE4 and its isoform.

PHOSPHODIESTERASE 4

The PDE4 family has been identified as the most diverse one of all the PDE families. The enzymes of this family are widely distributed throughout different tissues, being present in all major organs such as the brain, and also very abundant in airway immune and

inflammatory cells of lung diseases such as asthma.[19] PDE4 consists of four gene products, PDE4A, PDE4B, PDE4C, and PDED, and several N-terminal splice variants which are found differentially expressed among tissues and cells.[20] This group of enzymes are characterized by high affinity for cAMP and insensitivity to cGMP and calmodulin.[21] PDE4 is inhibited selectively by a number of PDE inhibitors of which rolipram is, perhaps, the most widely-known.

The PDE4 family is the focus point of vast research efforts over recent years because this family is considered to be a prime target for therapeutic intervention in a number of inflammatory diseases such as asthma, chronic obstructive pulmonary disease (COPD), and rheumatoid arthritis. This is because the major isoform of PDE that regulates inflammatory cell activity is the cAMP-specific PDE, PDE4. To this end, a wide variety of PDE4-selective inhibitors has been developed with a view to treating inflammatory diseases.

MOLECULAR BIOLOGY OF PHOSPHODIESTERASE 4

At present, the PDE4 family represents the largest PDE family, which is characterized by exhibiting a high affinity for cAMP, very weak affinity for cGMP, being insensitive to modulation by cGMP and Ca^{2+}/caM, and being potently and specifically inhibited by rolipram and Ro-2017. In human, rat, and mouse cells, the PDE4 family is encoded by four separate human PDE4 genes (PDE4A, B, C, and 4D) located on three chromosomes (chromosomes 19, 1, and 5) that are each characterized by unique N-terminal regulatory regions. Products of PDE4 genes have been shown to have more than 80% sequence identify over their catalytic regions.[22] Several studies have investigated the cellular/tissue distribution of PDE4 subtypes. Each gene has a distinct pattern of expression in tissues and cells. For example, the message for PDE4C is abundant in neuronal tissue, testis, and skeletal muscle (with PDE4A, PDE4B, PDE4D) but it is absent from immune and inflammatory. Message for PDE4A appears to be distributed ubiquitously, and a message for PDE4B is widely expressed in lung, heart, brain, and skeletal muscle, but not in the placenta, liver, kidney, or pancreas. PDE4D is particularly active in the lung, T-cells, and cerebellum.[23] Sequence analysis has identified several highly conserved domains in PDE4 subtypes. The catalytic domain (approximately 300 amino acids) is highly conserved in all PDE isozymes. This family also contains two upstream conserved regions (UCR), that is, UCR1 (55 amino acid) and UCR2 (97 amino acid) toward the N-terminus, which are unique to PDE4. UCR1 is separated from UCR2 by a linker region 1 (LR1) of approximately 33 residues and UCR2 is separated from the catalytic domain by LR2, which is approximately 28 residues. Houslay's group and others have suggested UCR1, and UCR2 may interact to form a regulatory module that may influence catalytic activity, sensitivity to drugs, and regulatory effects of phosphorylation. For example, UCR1 contains a protein kinase A (PKA) phosphorylation site allowing PDE4 to be activated by PKA, thereby providing an important part of cellular desensitization for cAMP signaling by increasing cAMP degradation.

SPLICE VARIANTS

Further variants of PDE4 are generated by alternative splicing in at least three of the four genes [Figure 1]. From the characterization of PDE4 mRNAs and their corresponding protein products, it has been established that ~20 different PDE4 isoenzymes or variants and at least 35 different PDE4 proteins are expressed in mammalian cells.[24-27] Splice variant isoforms of PDE4 are distinguished by the structure in the N-terminal domain. The alternative mRNA splice variants of PDE4A, PDE4B, PDE4D generally occur at one of two consensus regions within the 5′-end of the transcripts, although an insert occurring in the catalytic domain of PDE4A has also been reported.[28] Depending on the presence of UCR1 and UCR2, PDE4 splice variants can be divided into three major subgroups: (a) "Long forms," those that contain UCR1 and UCR2 (b) the "short forms," those which lack UCR1 and have an intact UCR2, and (c) the "super-short form," with an N-terminally truncated UCR2 [Figure 1]. UCR1 is encoded by three exons,[2-4] and the long form splice junction is located immediately 5′ to exon 2. Analyses of the human PDE4 genes show that six exons encode the catalytic unit of PDE4 isoenzyme.

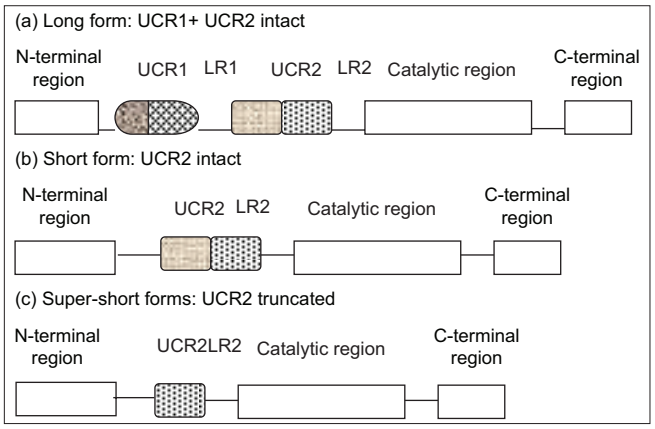

Figure 1: Diagram showing long (a), short (b) and super-short variants (c) of phosphodiesterase 4 generated by alternative splicing. UCR: Upstream conserved region; LR = Linker region

PDE4, like other PDEs, may exist as a dimer. Since the UCR module mediates dimerization, only long form PDE4 splice variants containing UCR1 and UCR2 are dimerized[29] [Figure 1].

These combined results suggest that alternative splicing of PDE4 mRNA, particularly in the region involving the UCRs and the amino terminus, may affect a number of properties of the PDE4s, including cellular distribution, subcellular localization, catalytic activity, inhibitor binding, and regulation by phosphorylation.

ROLE OF N-TERMINAL DOMAIN IN PHOSPHODIESTERASE 4

The PDE4 family members differ in intracellular and subcellular localization. These localizations of PDE4 subtypes are determined by amino acid sequences at the extreme N-terminus of PDE4 subtypes. PDE4A1 (RD1) is unique among members of the PDE4 family in being totally membrane associated. RNPDE4A1 (rat PDE4), when expressed in COS-1 or COS-7 cells is found to be associated with membranes, particularly the Golgi fraction. The N-terminal end participates in the membrane-bound localization of PDE4. Removal of the first 67 nucleotides from RD1, a cDNA encoding a rat homolog of human PDE4A, generated an expressed protein that is primarily cytosolic, whereas the full-length protein is membrane-bound. The expression of PDE4A1 appears to be restricted entirely to the brain. Thus, it is tempting to suggest that it may play a particular role in memory and learning.[30]

Human PDE4A4B and PDE4A5 are homologs of rat PDE4A. These two show different intracellular localization to the related short form PDE4A1, despite having identical catalytic units. These enzymes appear to be found at ruffles and cortical regions at the cell margin as well as having perinuclear localization. PDE4A5, and also PDE4D4 have SH3-binding domains (SRC homology 3 family tyrosyl kinases) in the N-terminal region which have the potential to interact with cytoskeletal proteins, as well as with proteins involved in signaling. Recent study has shown that an isoform (s) of PDE4 from mast cells which are likely to interact with SH3-domains, may be important for localization of PDEs in the cell as well as for cross-talk with other signaling pathways.[31] Analyses of other PDE4 isoforms have demonstrated that the PDE4B2 short forms are soluble species when expressed in COS cells. However, this does not exclude the possibility that these enzymes may be complex to other cytosolic proteins, to form low-affinity complexes with particulate-associated proteins that dissociate upon cellular disruption, or show different distribution in particular cell types dependent on the cell-specific expression of appropriate anchor proteins. Short forms of PDE4D are essentially cytosolic, whereas long forms are found distributed in cytosolic and membrane fractions.[32]

REGULATION OF PHOSPHODIESTERASE 4 ACTIVITY

Some isoenzymes of PDE4 can be up-regulated over the short and longer term. In short-term activation, long isoforms of PDE4 are regulated by phosphorylation, association to a protein or endogenous mediators, as well as by proteolysis. For example, hormonal stimulation activates PKA by increasing cAMP levels, and PKA-mediated phosphorylation of UCR1 allows rapid changes in PDE4 activity. The increase in PDE4 activity, hydrolyses cAMP resulting in a return of cAMP levels to the basal state. PKA phosphorylation of PDE4 long forms may lead to dimerization and may stabilize PDE4 in a high-affinity conformation. Furthermore, the association of PDE4A5 and PDE4D4 isoforms with SH3-domains of SRC family tyrosine kinases in cells with LR2 of PDE4A alters the conformation of the catalytic domains.[33] PDE4 from monocytes and thymocytes is activated by phosphatidic acid (PA), which has been described as a T-cell mitogen in that levels are often elevated following activation of inflammatory cells. PA also stimulates lipid signaling pathways and activates long forms of PDE4A5, PDE4B1, and PDE4D3. It has been suggested that PA binding to UCR1 alters the conformation of the hydrophobic region triggering enzyme activation. PA binding sites are at the N-terminus.[34] Prolonged accumulation of cAMP induces long-term regulation of PDE4. Swinnen *et al*., have shown that dibutyryl-cAMP induces upregulation only of the short forms of PDE4D (PDE4D1 and PDE4D2) in rat sertoli cells, which suggests that the PDE4D gene has two distinct transcriptional units. The unit controlling short forms of PDE4D is up-regulated by cAMP. In the same way, in human monocytic U937 cells, increased cAMP enhances PDE4A and PDE4B2, whereas expression of PDE4D is decreased.[35]

PDE4 conformers

In addition to a regulatory role of UCR domains, interactions may affect the conformational state of PDE4, and this could influence how well inhibitors bind to PDE4. It has been shown that interactions between UCR1 and UCR2 generate conformational changes in the PDE4 catalytic unit, which are linked to changes in phosphorylation by PKA or extracellular signal-regulated kinases and to consequential responses upon binding of certain inhibitors, such as rolipram.[36] Both low- and high-affinity binding

sites for rolipram may exist, and these reflect discrete conformations of PDE4. However, high-affinity binding by rolipram requires both N-terminal and catalytic domains and occurs in the presence of Mg^{2+}, while the low-affinity binding state needs only the presence of the catalytic domain without the need for Mg^{2+}. Moreover, the activity of the high-affinity form of PDE4 is inhibited by low concentrations of rolipram, whereas the low-affinity binding site needs much higher concentrations of rolipram.[37] There is evidence for different cell/tissue distribution of high-affinity sites for rolipram. High-affinity sites are found in the brain and the form with which rolipram interacts with relatively low-affinity is found in peripheral tissue. Moreover, it has been suggested that the potency of rolipram against PDE4 is a sensitive monitor of the conformation of PDE4 expressed. The IC_{50} (the concentration of inhibitor to inhibit 50% of substrate activity) value of rolipram against a particular isoform of PDE4 depends on the cellular environment in which the enzyme is expressed and the intracellular compartment to which it is targeted.[38,39] Unlike rolipram, the potency of other inhibitors such as piclamilast (RP 73401) against PDE4 is for more consistent, suggesting that the two inhibitors interact differently with the enzyme.[40] Consideration of these multiple conformations of PDE4 is relevant for drug design because it has been proposed that the low-affinity conformation is associated with therapeutic effects of PDE4 inhibitors, whereas the high-affinity conformation correlates with central nervous system and the gastric side effects of PDE4 compounds. Therefore, efforts to target the low-affinity forms of PDE4 have been proposed as a mechanism to develop more selective anti-inflammatory agents.[41]

Phosphodiesterase 4 inhibitors

In the last decade, a large number of selective and potent PDE inhibitors have been synthesized and developed by pharmaceutical companies with some success.[42] For example, the PDE5 inhibitor sildenafil, has been introduced for the treatment of erectile dysfunction, and the PDE3 inhibitor cilostamide has been used in cardiovascular diseases. PDE4 is recognized as the predominant isoform of PDE in the majority of inflammatory cells and has been implicated in inflammatory airway diseases such as allergic asthma and COPD. As such PDE4-selective inhibitors are emerging to treat respiratory diseases.[43]

The first generation of PDE4-selective inhibitors, such as rolipram and Ro-201724, had been used for many years as research tools to investigate the role of PDE4. Rolipram has been shown to be effective at inhibiting a wide range of inflammatory cells *in vitro* including macrophages, eosinophils, lymphocytes, basophils, and neutrophils, all of which have been implicated in airway diseases. Furthermore, the ability of rolipram to induce relaxation of isolated bronchus gave rise to the hope that PDE4 inhibitors could possess both anti-inflammatory and bronchodilator activity.[44,45]

A number of pharmaceutical companies went on to develop potent second generation PDE4 inhibitors with the hope of a wider therapeutic ratio, particularly with respect to overcoming the nausea and vomiting that was commonly seen with first generation drugs. PDE4-selective drugs, such as roflumilast and cilomilast, have emerged as potential drugs for respiratory diseases.[46,47] Roflumilast and cilomilast display a small inhibitory effect on the response to allergen challenge in asthma. Moreover, both drugs attenuate exercise-induced bronchoconstriction in asthma. Roflumilast and cilomilast have been shown to cause significant improvement in forced vital capacity flow (forced expiratory volume) in COPD. These two drugs are emerging as being of greater benefit to treat COPD than asthma.[48]

To improve the therapeutic ratio and safety of PDE4 inhibitors, dual-specificity inhibitors of PDE have been developed which could be more effective than inhibition of single PDE isoforms. Interest in PDE3 as a target for the treatment of asthma and COPD has emerged from the finding that selective inhibitors promote bronchodilation in humans.[19] As PDE3 and PDE4 inhibitors induce relaxation of airways smooth muscle, a combination of PDE3 and PDE4 inhibitors should exhibit both anti-inflammatory and bronchodilatory activity and so have superior efficacy over compounds that only block PDE4.[49,50] Several dual-specificity inhibitors have been developed and evaluated in humans such as zardaverine and pumafentrine. The compound in most advanced clinical development for both asthma and COPD is pumafentrine.[51]

In addition, the expression of PDE7 in inflammatory cells has been recognized. While inhibition of this enzyme alone does not suppress inflammatory cell activities, combined use of PDE4 with PDE7 inhibitors provides a greater inhibition than targeting PDE4 alone, and may provide more effective anti-inflammatory activity. Dual-specificity inhibitors may prove to be more effective than single-specificity inhibitors in the treatment of respiratory diseases.[52]

CONCLUSION

Overall, to improve the therapeutic efficacy and safety of PDE4 inhibitors, dual specificity PDE inhibitors may develop to avoid the problem of side effects particularly with nausea and vomiting in the treatment of diseases.

AUTHORS' CONTRIBUTION

NE contributed in the conception of the work, conducting the study, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. OM, GB, ZY, RB and AA contributed in the conception of the work, and agreed for all aspects of the work.

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Conflicts of interest
There are no conflicts of interest.

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